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THÈSE DE DOCTORAT

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Amélioration de la prise en charge diagnostique des infections fongiques systémiques

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Là où il y a une volonté, il y a un chemin.

William Hazlitt (1822)

Résumé

Les infections fongiques systémiques (IFS) sont un problème de santé publique car elles concernent des patients à risque en constante augmentation et leur incidence a augmenté ces dernières années. Ces infections opportunistes sont majoritairement dues à *Candida* sp. et à *Aspergillus* sp., et plus rarement aux mucorales, *Fusarium* sp. ou *Scedosporium* sp.. Certains champignons, comme *Trichosporon* sp. ou *Saprochaete* sp., ont émergé comme agents d'épidémies nosocomiales.

La performance du diagnostic biologique des IFS reste insuffisante. Le développement des méthodes basées sur la détection d'acides nucléiques, n'ont pas supplanté la culture mycologique qui reste le « gold standard » du diagnostic des IFS. La difficulté d'extraire efficacement l'ADN fongique est une des principales limites à dépasser. D'autres outils diagnostiques basés sur la détection de biomarqueurs spécifiques des champignons, comme l'antigène galactomannane pour le diagnostic de l'aspergillose invasive, ont des performances sub-optimales, dépendant des populations de patients, des maladies sous-jacentes, de l'utilisation de prophylaxie antifongique et de l'agent pathogène en cause. Il en résulte une létalité à 3 mois des IFS d'environ 40% en France. Le diagnostic précoce est un axe majeur de progrès, dans le but d'améliorer la prise en charge des patients et réduire la létalité.

Dans la première partie, nous avons réalisé un répertoire des champignons filamenteux isolés chez l'Homme. Une revue méthodique et exhaustive de la littérature nous a permis d'inventorier 565 espèces de champignons filamenteux d'intérêt clinique et de décrire les sites anatomiques et le cadre nosologique de ces infections. Une meilleure interprétation des résultats d'identification de ces champignons au laboratoire, basée sur les données de ce répertoire, participera à améliorer la prise en charge des patients concernés.

La seconde partie de notre travail visait à optimiser le diagnostic des infections fongiques invasives à levure. Pour cela, nous avons d'abord sélectionné les techniques les plus performantes pour extraire l'ADN des cinq principales espèces de levures d'intérêt clinque : *Candida albicans*, *C. glabrata*, *C. tropicalis*, *C. parapsilosis* et *C. krusei*. Après avoir comparé 11 techniques d'extraction d'ADN, nous en avons retenu trois dont la performance était adéquate : NucliSENS™ EasyMAG™ (BioMérieux), EZ1™ DNA Blood 200 µL Kit avec prétraitement (Qiagen), et EZ1™ DNA Tissue Kit avec prétraitement (Qiagen). Ensuite, nous avons cherché à améliorer la détection des levures à partir d'échantillon liquide par une méthode

de capture des *Candida* sp. en solution au moyen de billes magnétiques couplées à des anticorps spécifiques suivie par une détection moléculaire ou antigénique. Ces travaux étant toujours en cours, seuls les résultats préliminaires seront présentés. Enfin, nous nous sommes intéressés au diagnostic des infections invasives à levures rares, pour lesquelles les outils diagnostiques spécifiques sont inexistants. Nous avons ainsi appliqué au diagnostic et suivi des trichosporonoses invasives le dosage d'antigène de *Cryptococcus neoformans* après avoir décrit une réaction croisée avec *Trichosporon japonicum*.

La dernière partie s'intéresse à l'identification des sources potentielles des infections fongiques invasives. Les micromycètes pouvant être responsables d'épidémies nosocomiales, il est important de connaître leurs réservoirs et modes de transmission. Nous avons ainsi investigué une épidémie d'infection à *Saprochaete clavata* dans un centre de cancérologie. Le typage des isolats cliniques et environnementaux nous a permis d'identifier pour la première fois les lave-vaisselles comme réservoir et la vaisselle, contaminée pendant le lavage, comme vecteur passif de ces champignons.

Mots clés : levures, champignons filamenteux, infections fongiques systémiques, *Candida*, *Trichosporon*, *Saprochaete*.

Résumé

Abstract

Invasive fungal infections (IFI) are a public-health issue because they concern patients at risk, which is constantly increasing and their incidence has risen in recent years. These opportunistic infections are predominantly caused by *Candida* sp. and *Aspergillus* sp., and more rarely to mucorales, *Fusarium* sp. or *Scedosporium* sp.. Some fungi, such as *Trichosporon* sp. or *Saprochaete* sp., have emerged as agents of nosocomial epidemics.

The performance of biological diagnosis of IFI remains insufficient. The development of methods based on the detection of nucleic acids has not supplanted mycological culture, which remains the "gold standard" of IFI diagnosis. The difficulty of extracting fungal DNA efficiently is one of the main limitations to overcome. Other diagnostic tools based on the detection of fungal-specific biomarkers, such as galactomannan antigen for the diagnosis of invasive aspergillosis, have suboptimal performance, depending on patient populations, underlying diseases, use of antifungal prophylaxis, and the pathogen involved. As a result, the overall 3-month IFIs fatality rate is about 40% in France. Early diagnosis is a major improvement target that would allow enhance the patients' management and curb this fatality risk.

In the first part, we listed all filamentous fungi of clinical interest. After a systematic and comprehensive review of the literature allowed us to inventory 565 species of filamentous fungi of clinical interest and to describe the anatomical sites and nosological entities associated with these fungi. A better interpretation of the results of identification of these fungi in the laboratory, based on the data of this inventory, will contribute to improve the management of the patients concerned.

The second part of our work aimed at optimizing the diagnosis of invasive fungal yeast infections. Therefore, we first selected the most efficient techniques to extract DNA from the five main yeast species of clinical interest: *Candida albicans*, *C. glabrata*, *C. tropicalis*, *C. parapsilosis* and *C. krusei*. After comparing 11 DNA extraction techniques, we selected three which exhibited adequate performances: NucliSENS™ EasyMAG™ (BioMérieux), EZ1™ DNA Blood 200 µL Kit with pretreatment (Qiagen), and EZ1™ DNA Tissue Kit with pretreatment (Qiagen). Next, we aimed to improve the detection of yeasts from liquid suspensions by capturing *Candida* sp. in solution using magnetic beads coupled to specific antibodies followed by molecular or antigenic detection. As this work is still in progress, only

preliminary results will be presented. Finally, we are interested in the diagnosis of rare invasive yeast infections, for which specific diagnostic tools are inexistent. We have applied the *Cryptococcus neoformans* antigen assay to the diagnosis and follow-up of invasive trichosporonosis after having described a cross-reaction with *Trichosporon japonicum*.

The last part focuses on identifying potential sources of invasive fungal infections. Micromycetes can be responsible for nosocomial epidemics, so it is important to know their reservoirs and modes of transmission. We have investigated a *Saprochaete clavata* infection outbreak in a cancer center. We typed both clinical and environmental isolates and identified for the first time dishwashers as the reservoir, and dishes, which were contaminated during washing, as passive vectors of these fungi.

Keywords : yeast, molds, invasive fungal infection, *Candida*, *Trichosporon*, *Saprochaete*.

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Enfin, comme l'a si bien dit Henry Miller, « La science cherche encore, l'amour a trouvé ».

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Mes parents, qui continuent à me soutenir dans mes projets.

Mon frère et ma sœur, qui se disent « ses études s'achèvent enfin ».

Rémi, pour la vie que nous partageons.

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Abréviations

ADN	Acide désoxyribonucléique
APHM	Assistance publique des hôpitaux de Marseille
ARN	Acide ribonucléique
BiAc	Couple Billes magnétiques/Anticorps
CDC	Centre pour le contrôle et la prévention des maladies
CNRMA	Centre national de référence des Mycoses invasives et des antifongiques
COVID-19	Maladie à coronavirus
Ct	Cycle treshold
ECMM	Confédération européenne de mycologie médicale
ELISA	Enzyme-linked-immuno-sorbent-assay
EORTC	Organisation européenne pour la recherche et le traitement du cancer
ESCMID	Société européenne de microbiologie clinique et de maladies infectieuses
IFS	Infections fongiques systémiques
IgG	Immunoglobuline type G
IgM	Immunoglobuline type M
OMS	Organisation Mondiale de la Santé
PCR	Réaction par polymérisation en chaîne
UFC	Unités Formant Colonies

Introduction

Les infections fongiques systémiques (IFS) sont principalement des infections opportunistes dont l'incidence annuelle est estimée à 1,9 million de patients dans le monde (1). Ces dernières années, un changement dans l'épidémiologie des IFS a été observé, de nouveaux facteurs de risque sont apparus et le nombre de patients à risque de développer ces infections ne cesse d'augmenter, donnant lieu à l'augmentation de l'incidence des IFS dans plusieurs pays (2,3). En France, cette incidence est passée de 2,16 cas par 10 000 jours d'hospitalisation en 2012 à 2,36 cas par 10,000 jours d'hospitalisation en 2018 (4). Devant cette menace fongique, l'Organisation Mondiale de la Santé (OMS) a récemment publié une liste de 19 agents fongiques pathogènes pour l'Homme, prioritaires en recherche et développement (Figure 1) (5). Cette liste inclue les trois principaux agents responsables d'IFS : *Candida* spp. (responsable des candidémies), *Aspergillus* spp. (responsable de l'aspergillose invasive) et *Pneumocystis jirovecii* (responsable de la pneumocystose).

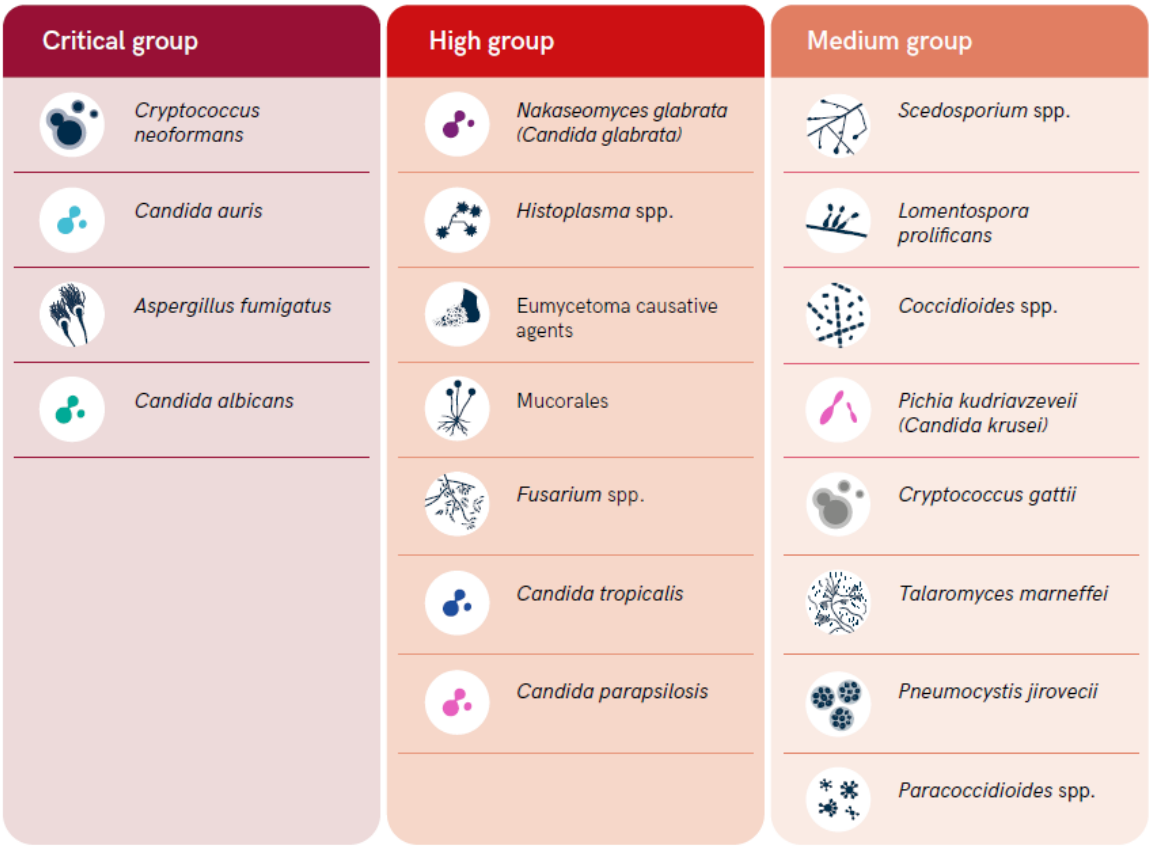

Figure 1. Liste des agents pathogènes fongiques prioritaires selon l'OMS (5)

Les levures du genre *Candida* sont les agents pathogènes les plus courants à l'origine des infections fongiques invasives (4). Ces pathogènes opportunistes appartiennent à la communauté microbienne commensale des muqueuses digestives et vaginales chez l'Homme (6–8). Lors de situations à risques, telles qu'un séjour prolongé dans une unité de soins intensifs, une chirurgie abdominale, des maladies hématologiques malignes, la présence de cathéter intraveineux, la nutrition parentérale ou l'administration d'un traitement antibactérien à large spectre (9,10), les patients peuvent développer des candidoses invasives. La candidémie, infection systémique par une levure du genre *Candida*, est définit par l'identification d'un *Candida* dans au moins une hémoculture. C'est la situation la plus fréquente de candidose invasive et la septième cause d'infections sanguines nosocomiales en Europe (11). Cinq espèces de *Candida* sont responsables de 90% des infections chez l'Homme : *C. albicans*, *C. glabrata*, *C. krusei*, *C. tropicalis*, et *C. parapsilosis* (12,13).

Les champignons filamenteux du genre *Aspergillus* arrivent en deuxième position des agents pathogènes opportunistes les plus courants à l'origine des infections fongiques invasives (4). Les *Aspergillus* sont des espèces saprophytes dans l'environnement, que l'on retrouve dans l'air ou la terre. Ces moisissures se développent sur des matières mortes ou en décomposition, et leurs spores sont aéroportées. Les individus vont se contaminer par inhalation des conidies (spores asexués) aéroportées (14,15). Elles se retrouvent alors dans l'appareil respiratoire : sinus, bronches, alvéoles pulmonaires. Dans l'organisme, la spore du champignon est normalement expectorée ou détruite par les défenses immunitaires, avant l'envahissement des tissus. Lorsque le système immunitaire n'a pas les capacités de la détruire, la colonisation peut aboutir au dépôt de ces conidies dans les bronchioles ou les espaces alvéolaires, et évoluer vers une aspergillose pulmonaire invasive (16). Les facteurs de risque de développer ces infections incluent la neutropénie prolongée, l'utilisation de corticostéroïdes à forte dose, les hémopathies, les transplantations d'organes solides, les maladies pulmonaires obstructives chroniques, les maladies du foie, la malnutrition, les brûlures, et le diabète (17,18). *Aspergillus fumigatus* et *Aspergillus flavus* sont les espèces les plus fréquemment identifiées au sein du genre *Aspergillus*. Parmi les autres moisissures, les *Fusarium* spp. et les mucorales sont plus rarement à l'origine d'infections fongiques invasives, mais sont estimées respectivement à 2,3% et 2,9% des épisodes d'IFS en France (4).

D'autres espèces responsables d'IFS ont émergé ces dernières années, telles que *Saprochaete* spp. et *Trichosporon* spp. (19–22). L'ensemble de ces IFS restent sous-

diagnostiquées. En effet, des études de cohortes autopsiques de patients à risque de développer ces infections ont rapporté jusqu'à 75% de diagnostic post-mortem (23,24).

La définition actuelle du diagnostic de certitude des infections fongiques invasives repose depuis plusieurs années sur la mise en évidence microbiologique et/ou histopathologique du pathogène à partir de site stérile (25). En pratique clinique, le diagnostic actuel repose essentiellement sur des techniques directes. Ces techniques comprennent l'histopathologie et la culture fongique qui reste le « gold standard » diagnostic des IFS malgré un manque de sensibilité rapporté et un délai d'incubation long (26). La culture permet une identification précise du champignon en cause et la réalisation d'antifongigramme nécessaire à l'obtention de données de sensibilité *in vitro* aux antifongiques. Ces dernières années, les techniques moléculaires ont rejoint le panel d'outils diagnostiques directs avec le développement de plusieurs kits de test de réaction par polymérisation en chaîne (PCR) dites maison ou commercialisés (27). D'autres techniques les plus couramment utilisées pour aider au diagnostic des IFS reposent sur la détection à partir d'échantillons cliniques d'antigènes spécifiques aux champignons. Ainsi, la détection de l'antigène galactomannane pour le diagnostic de l'aspergillose invasive, le bêta-(1-3)-D-glucane pour l'ensemble des infections fongiques et l'antigène capsulaire de *Cryptococcus neoformans* pour les infections à cryptocoque permettent d'aider à la prise de décision diagnostique. Cependant, la performance de ces tests est inégale suivant les populations de patients à risque, les maladies sous-jacentes, l'utilisation de prophylaxie antifongique et de l'agent pathogène en cause (28–30). La mortalité globale à 3 mois des IFS est estimée à environ 40% dans une étude française récente, mais elle varie significativement en fonction de l'infection diagnostiquée et de l'espèce en cause (4). Un diagnostic précoce est essentiel pour une prise en charge appropriée des patients et une réduction de la mortalité. L'amélioration du diagnostic de ces infections comprend l'amélioration des outils existants et le développement de nouveaux outils performants.

Cette thèse d'Université se divise en deux parties : la première concerne la description des infections fongiques à moisissures et la seconde l'amélioration du diagnostic des infections fongiques systémiques à levures.

Parmi les infections à moisissures, bien que le genre *Aspergillus* reste très majoritairement en cause, d'autres agents pathogènes fongiques peuvent être responsables d'infections invasives, tels que les Mucorales, *Fusarium* ou *Scedosporium* (4,31). Les champignons étant ubiquitaires dans l'environnement, il est parfois difficile de trancher entre colonisation ou réelle infection. De plus, la multiplication des outils moléculaires tels que la

métagénomique, le séquençage de nouvelle génération ou les PCR pan-fongique permettent de détecter une grande variété de pathogènes à partir de prélèvements biologiques humains, conduisant à des difficultés d'interprétation (32–34). Par conséquent, il est nécessaire de connaître les spectres des infections fongiques par espèce afin d'aider à l'interprétation d'un résultat positif. Pour répondre à cette problématique, nous avons réalisé une revue de la littérature scientifique afin d'obtenir une bibliographie la plus exhaustive possible sur les infections fongiques superficielles et profondes à moisissures. Nous avons ainsi établi un répertoire fongique comprenant l'ensemble des moisissures ayant été au moins une fois isolée chez l'Homme (**Article 1**). Dans cette revue, nous avons détaillé les localisations par système des champignons décrits et avons repris l'évolution de la taxonomie au cours du temps depuis les années 1950.

Dans un second temps, nous nous sommes intéressés à l'amélioration du diagnostic des infections systémiques à levure. Les espèces de *Candida* figurant parmi les cinq principaux agents pathogènes associés aux infections sanguines dans le secteur des soins de santé (35), nous nous sommes particulièrement focalisés sur le diagnostic des candidémies. Ces dernières années, dans un objectif de mutualisation au sein des laboratoires cliniques, les outils moléculaires doivent être capables de détecter un éventail complet de micro-organismes (tels que les virus, les bactéries et les champignons) impliqués dans les infections systémiques. Le diagnostic de la candidémie est donc intégré dans une approche syndromique du diagnostic des infections systémiques (36). Le choix d'une méthode d'extraction de l'ADN appropriée est une étape critique dans le flux de travail du laboratoire de diagnostic. Par conséquent, il nous a paru essentiel d'évaluer l'efficacité de ces méthodes. Pour cela nous avons évalué l'efficacité de onze protocoles automatisés d'extraction d'ADN sur des échantillons de sang humain artificiellement infectés par *Candida*. Nous avons comparé la performance de ces protocoles d'extraction sur les cinq principales espèces impliquées dans les candidémies (*Candida albicans*, *Candida glabrata*, *Candida parapsilosis*, *Candida tropicalis* et *Candida krusei*) (**Article 2**). Nous avons également élaboré une méthode de diagnostic fondée sur la capture en milieu liquide de *Candida* grâce à l'utilisation de billes magnétiques couplées à des anticorps spécifiques suivie par une détection par biologie moléculaire ou détection antigénique. Ce dernier travail est en cours et conduira à l'écriture d'une publication à visée internationale (**Article 3**). Les données préliminaires sont présentées dans cette thèse.

Les cliniciens peuvent également être confrontés à des infections systémiques à levures rares, et la démarche diagnostique peut se montrer complexe. Dans ce cadre, l'utilisation

détournée d'outil diagnostique existant peut avoir un intérêt. Nous pouvons citer comme exemple la réaction croisée entre antigène galactomannane et *Histoplasma* spp. qui est désormais intégrée dans le diagnostic de l'histoplasmose (37–40). Nous avons étudié une infection invasive à *Trichosporon japonicum* chez une patiente transplantée cardiaque hospitalisée dans un service de réanimation de l'Assistance Publique des Hôpitaux de Marseille. Les *Trichosporon* sont des organismes opportunistes émergents appartenant aux basidiomycètes dont le diagnostic de référence est la culture avec une croissance entre 48 et 72 heures sur milieu de Sabouraud. Il n'existe pas d'outil diagnostique direct spécifique de ces infections. Cependant, des réactions croisées entre certaines espèces du genre *Trichosporon* et la détection de l'antigène cryptocoque ou de l'antigène galactomannane ont déjà été décrites (41,42). Nous avons donc évalué l'intérêt de ces d'outils diagnostiques « détournés » pour le diagnostic des trichosporonoses (**Article 4**).

Dans un dernier temps, l'amélioration du diagnostic des infections à levures inclut aussi la compréhension des sources de ces infections. Dans cet objectif, nous avons en collaboration avec le Centre national de référence des Mycoses invasives et des antifongiques (CNRMA, Institut Pasteur) investigué une épidémie d'infections sanguines à *Saprochaete clavata* dans un centre marseillais de lutte contre le cancer afin d'identifier la source. Au total, durant l'année 2017, cette épidémie avait impliqué 8 patients hospitalisés dans 3 services différents et une source commune de contamination avait été suggérée. Au cours de cette investigation nous avons décrit les résultats de l'enquête environnementale et clinique autour de cette épidémie et identifié précisément la source de l'infection (**Article 5**).

Partie I: Répertoire fongique des infections à moisissures chez l'Homme élaboré à partir de la revue de la littérature

AVANT PROPOS

Les micromycètes appartiennent au règne des Fungi, un taxon comprenant entre 1,5 et 5,1 millions d'espèces fongiques (43). Parmi ces espèces fongiques, seules quelques centaines sont pathogènes pour l'Homme, et très peu sont capables d'affecter des personnes immunocompétentes (44). Cette liste est en constante évolution en raison de l'augmentation du nombre de patients à risque, des facteurs de risque (2,3) et de l'apparition de nouvelles méthodes diagnostiques dans le domaine de la biologie moléculaire dont le séquençage de nouvelle génération (43,45). Les champignons filamenteux ou moisissures se situent au sein du groupe des micromycètes pluricellulaires.

Les espèces du genre *Aspergillus* représentent la principale cause d'infections fongiques invasives à moisissures chez l'Homme (4). Le taux de mortalité globale associé à ces infections est estimé à 42,5% à 3 mois et peut atteindre 90% chez des populations à risque (4,46,47). Bien qu'à l'origine d'infections sporadiques dans la majorité des cas dû à leur omniprésence dans l'environnement, les *Aspergillus* spp. ont été associés à des épidémies nosocomiales lors de travaux de construction et de démolition (48–50). D'autres champignons filamenteux opportunistes rares ont vu leur incidence augmenter en raison de catastrophes naturelles (i.e. *Apophysomyces trapeziformis*) (51) ou d'épidémies nosocomiales (i.e. *Exserohilum rostratum*) (52). Récemment, une nouvelle population de patients à risque est apparue lors de la pandémie de COVID-19 (53,54). Le séquençage de nouvelle génération et la métagénomique ont un intérêt grandissant pour le diagnostic des infections fongiques (55,56). Il permet la détection d'agents pathogènes même dans des échantillons contenant des niveaux extrêmement faibles d'acides nucléiques (57,58) mais peut conduire à des difficultés d'interprétation (27,32), notamment en mycologie médicale où la détection de champignons environnementaux peu communs soulève des interrogations quant à leur pathogénicité. Il est donc essentiel de connaître les champignons impliqués en pathologie humaine et le profil des infections associées à ces pathogènes.

Article 1 : A Repertoire of Clinical Moulds

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A Repertoire of Clinical Moulds

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Summary

Humans are constantly exposed to micromycetes, especially filamentous fungi that are ubiquitous in the environment. In the presence of risk factors, mostly related to an alteration of the immunity, these fungi can then become opportunistic pathogens causing superficial, deep or disseminated infections. In recent years, the populations at risk of developing these infections are growing, as are the risk factors. With new molecular tools applied to medical mycology, and revisions in taxonomy, the number of fungi described in humans is rising. Some rare species are emerging, others more frequent are increasing. The aim of this review are to (i) inventory the filamentous fungi found in humans, and to (ii) provide details on the anatomical sites where they have been identified and the semiology of infections. Among the 239 890 fungi taxa and corresponding synonyms if any retrieved from the Mycobank and NCBI Taxonomy databases, we were able to identify 565 molds in human. These filamentous fungi were identified in one or more anatomical sites among systemic, central nervous system, ophthalmic system, heart, osteo-articular system, skeletal muscles, soft-tissue, endocrine glands, skin system, otorhinolaryngeal sphere, auditory system, dental, pulmonary system, urinary tract, genital tract, digestive system, hepatic and placenta. From a clinical point of view, this review allows to realize that some uncommon fungi isolated in non-sterile sites may be involved in invasive infections. It may present a first step in the understanding of the pathogenicity of filamentous fungi and the interpretation of the results obtained with the new molecular diagnostic tools.

Introduction

It is estimated that there are between 1.5 and 5 million fungal species on earth and about 100,000 species are currently described (1, 2). Of these species, only a few hundred have the capacity to infect humans (3). Humans are constantly exposed to potential fungal pathogens, as they are part of their normal flora and that of soil, water, and air (3). Moulds are a part of the vast kingdom of fungi alongside yeasts, mushrooms, polypores, plant parasitic rusts and smuts, microsporidia and *Pneumocystis*. Filamentous fungi are ubiquitous in the environment and can lead to opportunistic diseases presenting as superficial, invasive or disseminated infections. The number of described species is constantly increasing, probably due to the popularisation of DNA-based diagnostic tools, which now allows the distinction between close taxa and the identification of fungi, even in small quantities (1, 4). The taxonomy of fungi is also in constant evolution with the differentiation between sexual and asexual species and integrative taxonomy approaches combining genomics, morphology and ecology (1, 5). In recent years, a change in the epidemiology of invasive fungal diseases has been observed, new risk factors have emerged, and the number of patients at risk of developing these infections is also increasing (6, 7). Medical mycology is, therefore, a constantly evolving dynamic. *Aspergillus*, *Penicillium*, mucorales and dematiaceous fungi are the main filamentous fungi taxa involved in human disesases. Current reviews mainly focused on these taxa (3, 8, 9). However, other, rarer species of moulds can emerge in specific infection sites, such as *Paecilomyces variotii* or *Purpureocillium lilacinum* in sino-pulmonary fungal infections, and should not be overlooked (10).

In this review, we offer an overview as of 16 June 2020, of the filamentous fungi identified in humans by culture and nucleotide analyses associated or not with histopathology. We have also provided information on the organs where these micromycetes are isolated and on the semiology of the infections. We have chosen to divide our review into two approaches. First, we describe the taxa of interest and indicate their preferred site of infection. We then described which filamentous fungi were involved at each major anatomical sites.

Materials and method

Systematic literature review and database creation

First, all fungi names and synonyms were collected on both Mycobank (https://www.mycobank.org/) and NCBI Taxonomy (https://www.ncbi.nlm.nih.gov/taxonomy) that represent the state of the art of the taxonomy

of microfungi, updated on 15 November 2019. From Mycobank, the downloaded fungi taxon names and synonyms were provided in the worksheet https://www.MycoBank.org/images/MBList.zip. From NCBI Taxonomy, the query used was Fungi[subtree] AND species[rank] AND specified[prop]. Python script using Biopython package (11) was also implemented to fetch synonyms from NCBI Taxonomy. We have aggregated and deduplicated these two fungi name listings in order to obtain a single a list of 239,890 fungi taxa and corresponding synonyms, if any. For each fungus name in the list, we used a python script and Biopython package (11) to query PubMed to find bibliographic references that mention the fungi name or its synonyms, associated with the term "human" in the article title (TI), abstract (AB), author-supplied keywords (Other Term (OT)) or in the Medical Subject Headings (MeSH) terms. The syntax of the queries was dynamically built using this pattern (fungi_name_or_synonyms [TIAB] OR fungi_name_or_synonyms[OT] OR fungi_name_or_synonyms[MeSH]) AND ("Human"[TIAB] OR "Human"[OT] OR "Human"[MeSH]). Based on the query performed on 15 November 2019, 7,428 fungi taxa were found with at least one PubMed reference.

An MS Access® database (Access 2013, Microsoft) was set up on 16 June 2020, with these 7,428 recorded fungi names. Using this relational database management system, a link for each taxa corresponding to the query described above gave access to the relevant PubMed references and made it possible to study them one by one in order to identify and collect the relevant information.

Manual database incrementation

In the Accessdatabase (MS Access 2013 ™, Microsoft), each of the 7,428 fungi had a record from page linked to the PubMed relevant references. For each PubMed references, an analysis of the title and/or abstract and/or whole paper was performed manually to ensure that it was isolated from humans. This process was time consuming. Only references present in PubMed before 16 June 2020 were taken into account in order to have the same PubMed content for each fungal species.

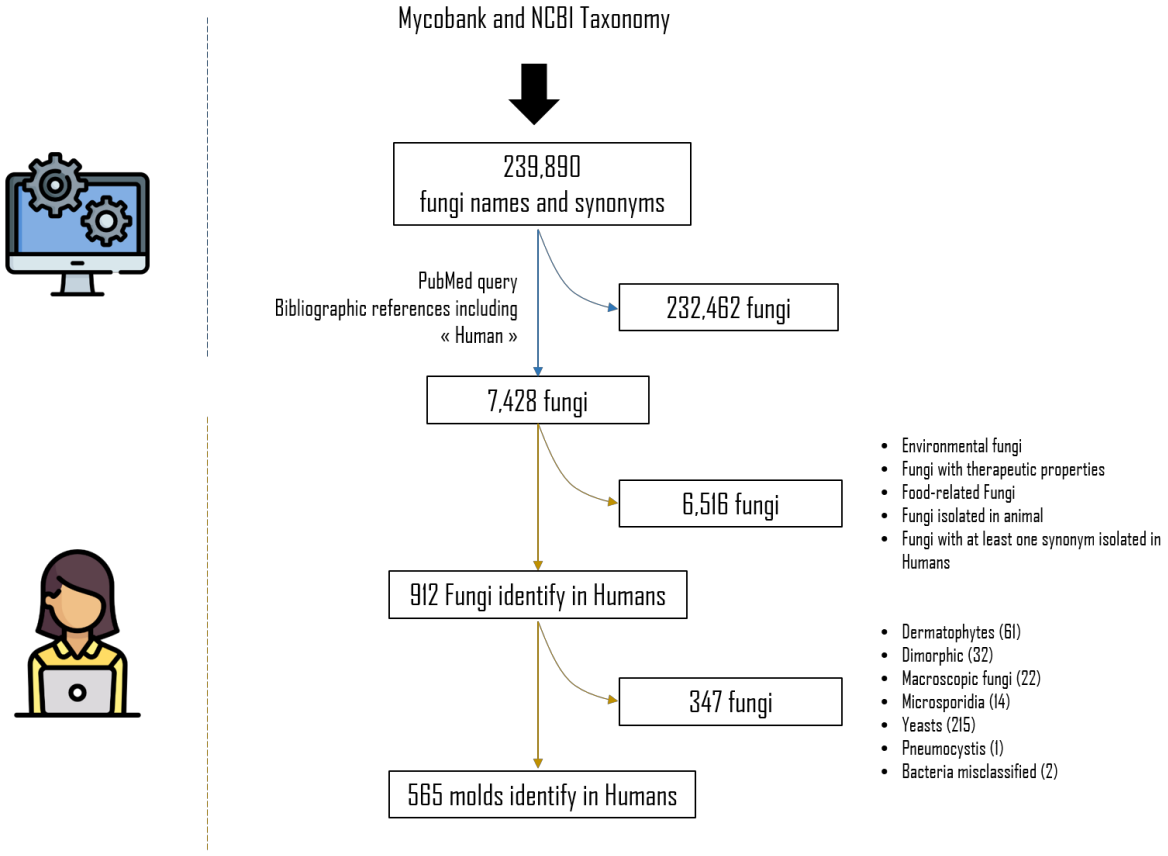
After analysis, 6,516 fungal taxa that were ultimately not found in humans were excluded. They included fungi of food, therapeutic, or environmental interest, or those involved in domesticated animal diseases. Synonyms, when not isolated from humans but associated with a species involved in humans were also excluded. Yeasts, microsporidia, dimorphic fungi, dermatophytes and *Pneumocystis* isolated in humans were also excluded.

We analysed the titles and/or abstracts and/or full paper and/or supplementary data, when available, of 565 mould fungal names and synonyms isolated in humans to complete information on the anatomical site involved and the semiologies of the associated infection by filling in the PubMed Unique Identifier (PMID) of the publication concerned. Only identifications by direct diagnosis were taken into account, including culture (followed by morphological, Matrix Assisted Laser Desorption Ionization Time of Flight (MALDI -TOF) mass spectrometry,or DNA sequence-based identification) associated or not with histopathological findings and Polymerase Chain Reaction (PCR). Publications reporting a species-level diagnosis based solely on histopathological examination or indirect methods results were excluded. The date of first publication, last publication and the name used were also reported in the software.

The anatomical sites included: systemic (isolated from blood, bone-marrow, blood vessels/arteries or lymph nodes), central nervous system (isolated from cerebrospinal fluid or brain biopsy); ophthalmic systems (isolated from ocular samples such as vitreous humour, corneal scrapings or lacrimal fluid); heart (isolated from cardiac specimens, *e.g*. valve or pericardial fluid); osteo-articular system (isolated from joint or bone samples); skeletal muscles (isolated from muscles); soft-tissue (isolated from soft-tissue); endocrine glands (isolated from adrenal, pituitary gland or thyroid); skin system (isolated from cutaneous or subcutaneous samples); otorhinolaryngeal sphere (isolated from nasal specimens including sinuses, and throat specimens including mouth, tongue, oesophagus, larynx, pharynx, trachea); auditory system (isolated from ear samples); dental (isolated from tooth root, dental pulp or anatomical structures directly in contact with the tooth in case of periodontitis, gingivitis, implant infection or abscess); pulmonary (isolated from the upper respiratory tract, *e.g*. sputum, lower respiratory tract, *e.g*. bronchoalveolar lavage fluid, lung biopsy, bronchial brushing, bronchoscopic needle aspiration and bronchial aspirate, pleural fluid and mediastinal specimen); breast; urinary tract (isolated from the urinary tract including kidneys, ureters, bladder, urinary meatus, and the prostate); genital (isolated from genitalia or related body fluids, both male and female, including penile and urethral samples); digestive system (isolated from stool samples or organs of the digestive system including peritoneum, intestines, pancreas, spleen, gallbladder or appendix, but excluding the liver); hepatic (liver biopsy); pregnancy (isolated from the placenta or foetus). When information on the anatomical site was provided, we added a degree of accuracy by specifying the semiology of the associated infection (e.g. rhinosinusitis for otorhinolaryngeal sphere) by filling in the PMIDs (Table 2). In brief, for each fungus with publications reporting

having been identified in humans, the current name and dates of the first and last publications were completed by filling in a list of PMID for each anatomical site and the semiology of the associated infection.

Figure 1. Systematic literature review flowchart.



Data analysis

The MS Access® database (Access 2013, Microsoft) was converted into two Excel files (Excel 2013, Microsoft). In the first file, the number of PMIDs per taxa was calculated by the anatomical site where the fungi were isolated (Table 1). In the second, the number of PMIDs per fungus was given for six major fungal categories (i.e., *Aspergillus*, Dematiaceous, *Fusarium*, Mucorales, *Penicillium* and *Pseudallescheria*/*Scedosporium*) according to the infections associated with the isolation of the fungus, as stated by the authors of the article (Table 2). If more than one case was described in a publication with the same anatomical site, it counted as one publication because of a single PMID.

Taxonomy

Taxa were organised at the high-level classification into sections or species complex based on https://www.aspergilluspenicillium.org/ for *Aspergillus* spp. (12) and *Penicillium* spp. (13, 14), and relevant publications for *Fusarium* spp. (15, 16). Mucorales and dematiaceous fungi were classified by genus (17).

Synonyms

We referred to the "current name" in Mycobank to identify the current name and the synonyms. The current name/synonym association was then checked by querying the PubMed database.

Figures

All figures were produced using the online tool, Wordart (https://wordart.com/). The size of the name of each species was proportional to the number of times it occured in the database.

Results

Fungal location by focusing on the predominant genus

Our bibliographical research identified 565 fungal species of 192 genera, which had been reported at least once in human. The list of theses taxa is detailled in Table 1, with their former and current scientific name if applicable. Briefly, there were 199 dematiaceous fungi, 76 *Aspergillus* spp., 26 spp., *Penicillium* spp., 34 *Fusarium* spp., 44 mucorales, 13 of to the *Scedosporium*/*Lomentospora* complex, and 173 to other mould species distributed in 114 genera. The results obtained for each of these taxa will be presented below. Regarding the publications reporting the isolation of these micromycetes at all anatomical sites (a publication can be counted multiple times due to the possible report of multiple anatomical sites in the same publication), the leading genus was *Aspergillus* (total: 4,385). The *Fumigati* and *Flavi* sections were the most recorded into this genus (total: 2,671 and 865, respectively). In second place come the dematiaceous fungi (total: 1,957) followed by the *Scedosporium*/*Lomentospora* complex (total: 1,220), Mucorales (total: 1,088) and *Fusarium* (total: 708). *Penicillium* were rarely isolated in human infections (total: 164).

Table 1. Number of publications found by anatomical site and species. In the same publication (PMID) several anatomical sites of isolation could be found.

Name	Current name	Year of 1st publication	Year of last publication	Systemic	CNS	Ocular	Auditory system	Dental and gums	ORL sphere	Pulmonary	Breast	Heart	Digestive system	Liver	Urinary tract	Genital	OA system	Skeletal muscles	Soft-tissue	Skin system	Endocrine gland	Placental infection	Total
***Aspergillus* spp.**				**181**	**271**	**299**	**191**	**4**	**526**	**1667**	**7**	**197**	**125**	**41**	**121**	**9**	**233**	**21**	**44**	**412**	**35**	**1**	**4385**
Section *Aspergillus*					**1**	**1**			**1**	**2**	**1**		**1**							**2**			**9**
Aspergillus chevalieri		1994	1994																	1			1
Aspergillus cibarius		2014	2014			1																	1
Aspergillus glaucus		1990	2020		1				1	2	1									1			6
Aspergillus ruber		2014	2014										1										1
Section *Candidi*					**1**		**1**		**1**	**3**										**6**			**12**
Aspergillus candidus		1962	2015		1		1		1	3										6			12
Section *Circumdati*						**1**	**1**		**1**	**2**							**1**		**1**	**4**			**11**
Aspergillus melleus		2015	2020						1											1			2
Aspergillus ochraceopetaliformis		2009	2009																	1			1
Aspergillus ochraceus		1987	2019			1				2							1		1				5
Aspergillus persii		2010	2010																	1			1
Aspergillus sclerotiorum		2004	2004				1																1
Aspergillus subramanianii		2018	2018																	1			1
Section *Clavati*										**1**		**1**	**1**							**2**			**5**
Aspergillus clavatus		1986	2019							1		1	1							2			5
Section *Cremei*						**1**	**1**																**2**
Aspergillus stromatoides		1987	1987			1																	1
Aspergillus wentii		2011	2011				1																1
Section *Flavi*				**32**	**43**	**93**	**47**		**166**	**190**	**1**	**45**	**22**	**7**	**33**	**1**	**58**	**5**	**14**	**104**	**4**		**865**
Aspergillus alliaceus		2007	2010							2													2
Aspergillus caelatus		2019	2019							1													1
Aspergillus effusus		2019	2019			1																	1
Aspergillus flavus		1956	2020	32	41	81	47		162	174	1	44	21	7	33	1	58	5	14	98	4		823
Aspergillus flavus complex		2006	2020			3				3													6
Aspergillus minisclerotigenes		2014	2014						1														1

Aspergillus nomius		2009	2020			1				3										1		5
Aspergillus oryzae		1976	2018		2	3			2	5		1	1									14
Aspergillus tamarii		1992	2020			4			1	1										5		11
Aspergillus tanneri		2012	2012							1												1
Section *Flavipedes*										**2**							**1**					**3**
Aspergillus flavipes		1979	1999							2							1					3
Section *Fumigati*				**116**	**194**	**140**	**46**	**1**	**293**	**1225**		**118**	**71**	**30**	**73**	**7**	**133**	**14**	**21**	**161**	**28**	**2671**
Aspergillus felis		2013	2019				0		1	4										1		6
Aspergillus fischeri	*Neosartorya fischeri*	1973	1998							2												2
Aspergillus fumigates	*Aspergillus fumigatus*	2010	2020		1	2				6		2										11
Aspergillus fumigatus		1945	2022	114	190	134	46	1	287	1180		113	67	29	73	7	131	14	21	156	27	2590
Aspergillus fumigatus complex		2012	2020	0	1	1			1	10							1			1		15
Aspergillus lentulus		2006	2020							14												14
Aspergillus novofumigatus		2013	2013							1												1
Aspergillus thermomutatus		1994	2020	1					1	1			1				1			2		7
Aspergillus udagawae		2012	2017							1		1										2
Aspergillus viridinutans		2009	2014	1		1				4												6
Neosartorya aureola		2011	2017						1													1
Neosartorya fischeri	*Aspergillus fischeri*	1990	1997		1	1				1		2	1	1						1	1	9
Neosartorya hiratsukae		2002	2010		1				1				2									4
Neosartorya laciniosa		2013	2013						1													1
Neosartorya udagawae		2009	2011			1				1												2
Section *Nidulantes*				**3**	**11**	**9**	**5**	**1**	**10**	**31**	**1**	**2**	**4**		**3**	**1**	**14**	**2**	**4**	**35**		**136**
Aspergillus amoenus		2016	2016						1	1												2
Aspergillus creber		2016	2016							1										1		2
Aspergillus delacroxii		2015	2015	1								1										2
Aspergillus hongkongensis		2016	2016																	1		1
Aspergillus nidulans		1963	2021	1	9	3	2		6	20	1	1	1		3		11	2	4	19		83
Aspergillus nidulans var. echinulatus	*Aspergillus delacroxii*	1988	1988							1							1					2
Aspergillus protuberus		2015	2019			1										1				1		3
Aspergillus quadrilineatus		1992	1992						1													1
Aspergillus sydowii		1989	2019	1		2	1		1	2			1				1			7		16

Aspergillus tabacinus	2016	2016							1													1
Aspergillus unguis	2016	2016																	1			1
Aspergillus versicolor	1950	2021		2	3	2	1	1	5			2				1			5			22
Section *Nigri*			**17**	**4**	**36**	**79**	**1**	**33**	**112**	**4**	**12**	**12**	**1**	**4**		**9**		**4**	**58**		**1**	**388**
Aspergillus aculeatus	1984	1984						1				1										2
Aspergillus awamori	1992	2020				1		1	2										1			5
Aspergillus brasiliensis	2010	2020			1														1			2
Aspergillus foetidus	1992	1992																	1			1
Aspergillus luchuensis	2010	2010				1																1
Aspergillus niger	1965	2020	14	4	33	72	1	28	102	2	12	10	1	4		8		4	47		1	343
Aspergillus niger complex	2002	2014			1				1	1												3
Aspergillus tubingensis	2009	2020	3		1	5		2	7			1				1			6			26
Aspergillus uvarum	2015	2015																	1			1
Aspergillus welwitschiae	2016	2019						1	1										1			3
Section *Restricti*			**1**	**1**	**2**				**4**		**3**	**2**		**1**								**14**
Aspergillus conicus	2013	2013			1																	1
Aspergillus gracilis	2019	2019										1										1
Aspergillus penicillioides	2016	2018		1	1				1		1	1		1								6
Aspergillus restrictus	1960	1993	1						3		2											6
Section *Terrei*			**11**	**13**	**15**	**11**	**1**	**16**	**82**		**16**	**11**	**3**	**7**		**16**			**35**	**3**		**240**
Aspergillus carneus	2016	2016										1										1
Aspergillus niveus	1984	2008				1			1													2
Aspergillus terreus	1948	2021	11	13	15	10	1	16	81		16	10	3	7		16			35	3		237
Section *Usti*			**1**	**2**	**1**			**3**	**10**			**1**				**1**			**3**			**22**
Aspergillus calidoustus	2008	2021	1	1	1			3	9			1				1			2			19
Aspergillus granulosus	1995	2009		1															1			2
Aspergillus pseudodeflectus	2018	2018							1													1
Unknown Section				**1**				**2**	**3**										**2**			**8**
Aspergillus amstelodami	1983	2008		1				1														2
Aspergillus repens	1989	1989						1														1
Aspergillus sublatus	2014	2019							3													3
Aspergillus tetrazonus	2004	2015																	2			2
***Penicillium* spp.**			**36**	**3**	**8**	**5**	**1**	**17**	**49**	**1**	**3**	**11**	**2**	**1**		**4**			**23**			**164**

Section *Biverticillata*				**1**						**1**							**1**						**3**
Penicillium piceum		2001	2006	1						1							1						3
Section *Brevicompacta*									**1**	**1**			**1**										**3**
Penicillium brevicompactum		1996	2013						1	1			1										3
Section *Canescentia*							**3**																**3**
Penicillium nigricans		1992	2002				3																3
Section *Chrysogena*				**2**	**2**	**2**	**1**		**5**	**5**	**1**	**2**	**2**							**2**			**24**
Penicillium chrysogenum		1974	2016	2	1	2			3	3		1	2							2			16
Penicillium notatum		1978	2016				1		1	1													3
Penicillium rubens		2016	2020		1				1	1	1	1											5
Section *Citrina*						**5**	**1**	**1**	**3**	**4**		**1**											**15**
Penicillium citrinum		1976	2020			4	1		3	4		1											13
Penicillium implicatum		2010	2010			1		1															2
Section *Exilicaulis*				**1**						**3**													**4**
Penicillium corylophilum		2002	2011							2													2
Penicillium decumbens		1990	1992	1						1													2
Section *Fasciculata*										**1**													**1**
Penicillium verrucosum		2001	2001							1													1
Section *Lanata-Divaricata*																				**1**			**1**
Penicillium vitale	*Penicillium simplicissimum*	1992	1992																	1			1
Section *Penicillium*						**1**			**1**	**1**													**3**
Penicillium digitatum		2013	2013							1													1
Penicillium expansum		1978	2000			1			1														2
Section *Ramigena*										**1**													**1**
Penicillium capsulatum		2013	2013							1													1
Section *Robsamsonia*													**1**										**1**
Penicillium dipodomyicola		2013	2013										1										1
Section *Roquefortorum*									**1**														**1**
Penicillium roqueforti		2018	2018						1														1
Section *Talaromyces*				30	1				4	25			5	2	1		3			19			90
Talaromyces (Penicillium) marneffei		2015	2020	30	1				4	24			5	2	1		3			19			89
Talaromyces purpurogenus		2016	2016							1													1
Section *Tachyspermi*				1					1	3			1							1			7

Talaromyces amestolkiae		2016	2017							2													2
Talaromyces atroroseus		2020	2020	1					1	1			1								1		5
Unclassified into a Section				1					1	4			1										7
Penicillium emersonii		1999	1999							1													1
Penicillium lilacinum	*Purpureocillium lilacinum*	1972	1972							1													1
Penicillium purpurogenum		1998	1998							1													1
Penicillium rugulosum	*Talaromyces rugulosus*	1999	1999						1														1
Talaromyces eburneus		2010	2010	1						1			1										3
***Fusarium* spp.**				**134**	**8**	**191**	**1**	**1**	**34**	**41**	**1**	**12**	**17**	**4**	**14**	**1**	**15**	**1**	**4**	**228**	**1**		**708**
F. chlamydosporum species complex (FCSC)				**1**		**1**			**1**						**1**					**1**			**5**
Fusarium chlamydosporum		1985	2020	1		1			1						1					1			5
F. dimerum species complex (FDSC)				**2**		**7**			**1**			**1**	**1**						**1**	**4**			**17**
Fusarium dimerum		1972	2018	2		6			1			1	1						1	4			16
Fusarium penzigii		2016	2016			1																	1
F. incarnatum - F. equiseti species complex (FIESC)				**1**		**5**			**1**	**1**		**1**								**3**			**12**
Fusarium equiseti		2007	2007			1																	1
Fusarium incarnatum		2014	2020	1		4			1	1		1								3			11
F. oxysporum species complex (FOSC)				**21**	**2**	**24**			**5**	**10**		**3**	**4**	**3**	**3**		**3**			**53**			**131**
Fusarium oxysporum		1958	2021	21	2	24			5	10		3	4	3	3		3			53			131
F. sambucinum species complex (FSAMSC)						**2**														**1**			**3**
Fusarium nivale	*Microdochium nivale*	1966	1966			1																	1
Fusarium sporotrichioides		2017	2017			1														1			2
F. solani species complex (FSSC)				**81**	**6**	**138**			**20**	**24**	**1**	**6**	**9**		**8**	**1**	**9**	**1**	**3**	**138**	**1**		**446**
Fusarium keratoplasticum		2015	2019	7		8			2	1					1		1			9			29
Fusarium lichenicola		2003	2020			3														2			5
Fusarium metavorans		2018	2018							1													1
Fusarium petroliphilum		2013	2019	6		2			1											3			12
Fusarium proliferatum		1988	2020	6		3			3	2			1		3				1	14			33
Fusarium pseudensiforme		2020	2020							1													1
Fusarium riograndense		2018	2018						1														1
Fusarium solani		1970	2020	55	5	102			10	15	1	5	8		4	1	7	1	2	94	1		311
Fusarium solani complex		2005	2019	6	1	18			2	3		1								14			45

Neocosmospora tonkinensis		2018	2018			1																1
Neocosmospora vasinfecta	*Fusarium neocosmosporiellum*	1993	2008	1		1			1	1							1			2		7
F. fujikuroi species complex (FFSC)				**2**		**1**														**2**		**5**
Fusarium musae		2015	2016	2		1														2		5
Gibberella fujikuroi species complex (GFSC)				**25**		**12**	**1**	**1**	**6**	**6**		**1**	**3**	**1**	**2**		**3**			**25**		**86**
Fusarium acutatum		2006	2015																	2		2
Fusarium moniliforme		1977	2013	7		6	1	1	1	2		1	2	1	2		2			11		37
Fusarium napiforme		1993	2018	2						2										2		6
Fusarium nygamai		1996	2015	3																		3
Fusarium ramigenum		2016	2016							1												1
Fusarium sacchari		2000	2020	1		4														1		6
Fusarium subglutinans		2010	2013																	2		2
Fusarium temperatum		2014	2014			1																1
Fusarium thapsinum		2004	2004	1																		1
Fusarium verticillioides		1995	2018	11					5	1			1				1			7		26
Fusarium verticillioides complex		2013	2013			1																1
Unclassified into complex				**1**		**1**														**1**		**3**
Fusarium andiyazi		2014	2014	1																		1
Fusarium langsethiae		2015	2015			1																1
Fusarium roseum	*Fusarium sambucinum*	1987	1987																	1		1
Mucorales				**45**	**40**	**18**	**13**		**163**	**189**	**1**	**26**	**69**	**34**	**50**	**6**	**25**	**24**	**50**	**321**	**14**	**1088**
Absidia				**4**	**8**	**4**	**6**		**11**	**22**		**4**	**5**	**3**	**4**	**1**	**1**	**2**	**6**	**29**	**2**	**112**
Absidia coerulea		1988	1988							2												2
Absidia corymbifera	*Lichtheimia corymbifera*	1982	2015	4	8	4	5		11	20		4	5	3	4	1	1	2	6	29	2	109
Absidia Lichtheimii		1968	1968				1															1
Actinomucor				**1**					**2**	**2**			**1**	**1**	**1**			**1**		**3**	**1**	**13**
Actinomucor elegans		2001	2020	1					2	2			1	1	1			1		3	1	13
Apophysomyces				**3**	**1**	**1**			**23**	**3**		**1**	**5**	**1**	**14**		**7**	**9**	**19**	**54**		**141**
Apophysomyces elegans		1985	2020	2	1	1			19	3		1	3	1	12		7	7	12	41		110
Apophysomyces trapeziformis		2012	2014	1														2	5	4		12
Apophysomyces variabilis		2011	2020						4				2		2				2	9		19
Cunninghamella				**10**	**4**				**6**	**46**		**9**	**5**	**3**	**3**		**3**	**1**	**3**	**12**	**4**	**109**

Cunninghamella bertholletiae		1979	2019	10	4				5	44		9	5	3	3		3	1	3	11	4		105
Cunninghamella blakesleeana		2012	2012							1													1
Cunninghamella echinulata		2009	2013						1	1										1			3
Lichtheimia				**10**	**10**	**5**	**4**		**11**	**30**		**5**	**15**	**5**	**8**	**1**	**3**	**1**	**3**	**47**	**4**		**162**
Lichtheimia corymbifera		1982	2019	9	9	4	4		8	22		3	9	3	6	1	3	1	3	37	2		124
Lichtheimia hongkongensis		2010	2010						1				1							1			3
Lichtheimia ornata		2018	2020						1											1			2
Lichtheimia ramosa		2010	2019	1	1	1			1	8		2	5	2	2					8	2		33
Mucor				**9**	**1**	**2**	**1**		**9**	**8**		**1**	**10**	**5**	**3**	**1**	**2**	**2**		**32**			**86**
Mucor circinelloides		1987	2019	3	1				2	4			1		2	1	2			7			23
Mucor ellipsoideus	*Mucor ardhlaengiktus*	2011	2011										1										1
Mucor hiemalis		1986	2015						1											4			5
Mucor indicus		1990	2019	2		1				1		1	7	5	1			2		5			25
Mucor irregularis		2011	2019			1			3	1										12			17
Mucor lusitanicus		1990	1990																	1			1
Mucor mucedo		1966	2013	1			1																2
Mucor pusillus	*Rhizomucor pusillus*	1952	1981						1	1										1			3
Mucor racemosus		2011	2015						1				1										2
Mucor ramosissimus		1993	1993																	1			1
Mucor velutinosus		2011	2018	3					1	1										1			6
Mycocladus					**1**					**1**					**1**					**1**			**4**
Mycocladus corymbifera	*Lichtheimia corymbifera*	2013	2013		1					1					1					1			4
Rhizomucor				**2**	**5**				**12**	**20**		**3**	**4**	**7**	**5**	**1**	**1**			**16**	**2**		**78**
Rhizomucor miehei		1999	2017							2		0	1	1						1			5
Rhizomucor pusillus		1983	2017	2	5				9	18		3	3	6	5	1	1			6	2		61
Rhizomucor variabilis	*Mucor irregularis*	2009	2018						3											9			12
Rhizopus				**6**	**10**	**4**			**80**	**54**	**1**	**2**	**21**	**9**	**10**	**2**	**6**	**4**	**13**	**75**			**297**
Rhizopus arrhizus		1975	2020		4				19	8					1			2	3	16			53
Rhizopus azygosporus		1996	2005										1	1	1					1			4
Rhizopus delemar		2014	2014																	1			1
Rhizopus homothallicus		2010	2019						2	4									0	2			8
Rhizopus microsporus		1988	2020	1	1	1			15	19			15	4	5	1	2	1	5	20			90

Rhizopus oligosporus	*Rhizopus microsporus var. oligosporus*	1989	1989																1			1
Rhizopus oryzae	*Rhizopus arrhizus*	1955	2020	3	5	3			38	21	1	2	4	4	3	1	4	1	4	27			121
Rhizopus pusillus		2019	2019																	1			1
Rhizopus rhizopodiformis		1978	2008	1					3	2									1	6			13
Rhizopus schipperae		1999	1999						1				1										2
Rhizopus stolonifer		1996	2018	1					2											3			3
Saksenaea						**2**	**2**		**6**	**2**		**1**			**1**		**2**	**4**	**6**	**45**	**1**		**72**
Saksenaea erythrospora		2011	2018			1													3	4			8
Saksenaea vasiformis		1976	2020			1	2		6	2		1			1		2	4	3	41	1		64
Syncephalastrum									**2**	**1**			**3**							**6**			**12**
Syncephalastrum racemosum		2005	2020						2	1			3							6			12
Thermomucor																				**1**			**1**
Thermomucor indicae-seudaticae		1993	1993																	1			1
Dematiaceous fungi				**71**	**181**	**160**	**16**	**5**	**124**	**156**	**3**	**29**	**45**	**9**	**12**	**1**	**71**	**8**	**15**	**1046**	**5**		**1957**
Alternaria				**1**	**2**	**15**	**1**		**15**	**5**			**4**		**2**		**2**		**2**	**84**			**133**
Alternaria alternata		1976	2019	1	1	13			13	4			3		1		2		2	40			80
Alternaria chlamydospora		1990	2001																	4			4
Alternaria dennisii		2016	2016																	1			1
Alternaria humicola		1984	1985				1													1			2
Alternaria infectoria		1998	2020		1	1			2	1										25			30
Alternaria longipes		1995	1995																	1			1
Alternaria malorum		2012	2013																	1			1
Alternaria rosae		2017	2017																	1			1
Alternaria tenuis	*Alternaria alternata*	1960	1970												1					1			2
Alternaria tenuissima		1986	2020			1							1							8			10
Alternaria triticina		2014	2014																	1			1
Exophiala				**25**	**7**	**20**	**2**	**2**	**9**	**44**	**1**	**3**	**9**	**3**	**1**	**1**	**11**	**1**	**3**	**168**	**1**		**311**
Exophiala asiatica		2009	2009		1				1														2
Exophiala bergeri		2016	2016																	1			1
Exophiala castellanii		1994	1994									1											1
Exophiala dermatitidis		1984	2020	17	5	6	1		5	38	1	1	6	3			1	1	2	9	1		97
Exophiala equina		2013	2013																	1			1

Exophiala hongkongensis		2013	2013																1			1
Exophiala jeanselmei		1981	2020	3	1	10		2	1	3		1	3			1	3			85			113
Exophiala lecanii-corni		1994	2018																	5			5
Exophiala mansonii	*Exophiala jeanselmei var. castellanii*	1986	1989														1			2			3
Exophiala moniliae		1981	1984																	2			2
Exophiala oligosperma		2003	2020	1			1		1	2							1		1	16			23
Exophiala phaeomuriformis		2017	2018			3																	3
Exophiala pisciphila		1991	1991																	1			1
Exophiala polymorpha		2015	2015																	1			1
Exophiala salmonis		2006	2012																	2			2
Exophiala spinifera		1983	2020	4					1	1						1				29			40
Exophiala werneckii		1980	2000			1														7			8
Exophiala xenobiotica		2009	2016														1			6			7
Cladophialophora				**1**	**51**	**2**			**1**	**8**						**1**		**6**		**1**	**65**			**136**
Cladophialophora (Cladosporium) carrionii		1979	2021			1				1						1				38			41
Cladophialophora ajelloi	*Cladophialophora carrionii*	1982	1982																	1			1
Cladophialophora arxii		2009	2015							1							1						2
Cladophialophora bantiana		1996	2020	1	51	1			1	5							5		1	19			84
Cladophialophora boppii		2009	2020							1										5			6
Cladophialophora devriesii		2006	2006																	1			1
Cladophialophora saturnica		2009	2009																	1			1
Scopulariopsis				**6**	**3**	**7**	**4**		**6**	**16**		**7**	**3**	**1**	**3**		**1**		**1**	**80**	**2**		**140**
Scopulariopsis acremonium		1998	2009		1				2	1		1	1		1						1		8
Scopulariopsis alboflavescens		2018	2018							1										1			2
Scopulariopsis brevicaulis		1951	2020	6		7	4		3	7		5	1		1		1		1	72			108
Scopulariopsis brumptii		1975	2017		2					6		1	1	1	1					5	1		18
Scopulariopsis candida		1994	2015						1	1										2			4
Curvularia					**6**	**19**	**1**		**21**	**10**		**1**	**8**				**3**		**2**	**28**			**99**
Curvularia australiensis		2015	2015			1																	1
Curvularia borreriae		2013	2013			1			1	1			1										4
Curvularia brachyspora		1992	1997			1														1			2
Curvularia clavata		1999	2009		1				1											1			3

Curvularia geniculata	1964	2014		1	3	1		1	1			2							1			10
Curvularia hominis	2018	2018			1																	1
Curvularia Inaequalis	2005	2013						2				1							1			4
Curvularia lunata	1970	2019		3	11			13	6		1	4				3		2	20			63
Curvularia pallescens	1977	1995		1					1										2			4
Curvularia senegalensis	1991	1999			1			1											1			3
Curvularia spicifera	2017	2017						1														1
Curvularia tuberculata	2019	2019						1	1										1			3
Phialemonium			**3**		**1**				**2**		**2**	**2**				**1**			**5**			**16**
Phialemoniopsis endophytica	2017	2017																	1			1
Phialemoniopsis hongkongensis	2014	2014																	1			1
Phialemonium dimorphosporum	1993	1999	1																1			2
Phialemonium obovatum	1986	2012	2		1				2		2	2				1			2			12
Exserohilum			**1**	**10**	**10**			**11**	**1**							**2**			**14**			**49**
Exserohilum longirostratum	1994	2006			1														1			2
Exserohilum mcginnisii	1986	2018			2			1														3
Exserohilum rostratum	1986	2020	1	10	7			10	1							2			13			44
Microascus			**1**	**1**				**1**	**8**		**2**								**8**			**21**
Microascus cinereus	1980	2013		1				1	2		1								4			9
Microascus cirrosus	1992	2018	1						4		1								3			9
Microascus ennothomasiorum	2019	2019																	1			1
Microascus trigonosporus	2004	2015							2													2
Bipolaris			**5**	**8**	**18**	**1**		**22**	**9**	**1**	**2**	**3**	**2**	**2**			**1**		**15**	**2**		**91**
Bipolaris australiensis	1986	2017	1	1	3			1	1		1								2			10
Bipolaris cynodontis	2012	2015			1			1	1										2			5
Bipolaris hawaiiensis	1986	2019		2	8			5	2			1							4			22
Bipolaris oryzae	2016	2016			1																	1
Bipolaris papendorfii	2005	2005			1																	1
Bipolaris spicifera	1984	2015	4	5	4	1		15	5	1	1	2	2	2			1		7	2		52
Chaetomium			**1**	**4**	**2**	**1**		**1**	**6**		**1**								**16**			**32**
Chaetomium atrobrunneum	1998	2019		2	1				1										3			7
Chaetomium brasiliense	2011	2011				1																1
Chaetomium funicola	2007	2007																	1			1

Chaetomium globosum		1988	2020	1	1	1				4									12			19
Chaetomium homopilatum	*Humicola homopilata*	1997	1997						1													1
Chaetomium perlucidum		2003	2003		1					1		1										3
Cladosporium				**1**	**34**	**4**		**2**	**7**	**8**			**1**							**31**			**88**
Cladosporium bruhnei		1994	2014						1				1							1			3
Cladosporium castellanii		2005	2005																	1			1
Cladosporium cladosporioides		1975	2019		1	3		1	3	6										9			23
Cladosporium devriesii	*Cladophialophora devriesii*	1984	1990																	2			2
Cladosporium herbarum		1994	2012						3											1			4
Cladosporium langeronii		2018	2018																	1			1
Cladosporium macrocarpum		2011	2011		1																		1
Cladosporium oxysporum		1999	2006																	2			2
Cladosporium sphaerospermum		2003	2019	1	2	1		1		1										2			8
Cladosporium trichoides	*Cladophialophora bantiana*	1952	2020		30					1										6			37
Cladosporium werneckii		1964	1978																	6			6
Ochroconis				**1**	**2**	**1**				**6**			**2**				**2**			**9**			**23**
Ochroconis constricta		2014	2014																	1			1
Ochroconis cordanae		2014	2014																	1			1
Ochroconis gallopava		1986	2018	1	2	1				4			2				2			3			15
Ochroconis mirabilis		2014	2016							1										2			3
Ochroconis musae		2018	2018																	1			1
Ochroconis olivacea		2014	2014							1													1
Ochroconis tshawytschae		2012	2012																	1			1
Phaeoacremonium				**4**	**2**	**3**				**3**		**1**					**6**	**1**	**1**	**29**			**50**
Phaeoacremonium aleophilum		2003	2011																	2			2
Phaeoacremonium fuscum		2015	2015																	1			1
Phaeoacremonium inflatipes		1998	2014	1		1														2			4
Phaeoacremonium krajdenii		2006	2006																	1			1
Phaeoacremonium parasiticum (Phialophora parasitica)		1983	2019	3	2	2				3		1					5	1	1	18			36
Phaeoacremonium rubrigenum		1999	2012																	3			3
Phaeoacremonium sphinctrophorum		2016	2016																	1			1
Phaeoacremonium venezuelense		2006	2012														1			1			2

Rhinocladiella					**15**	**1**	**2**							**1**			**1**			**8**			**28**
Rhinocladiella aquaspersa		1983	2019				2													4			6
Rhinocladiella atrovirens		1989	1989		1																		1
Rhinocladiella basitona		2013	2015			1														1			2
Rhinocladiella mackenziei		2009	2020		14									1			1						16
Rhinocladiella similis		2017	2020																	3			3
Fonsecaea				**1**	**9**	**6**	**3**		**4**	**5**				**1**			**2**		**1**	**139**			**171**
Fonsecaea compactum	*Fonsecaea pedrosoi*	1983	1989																	2			2
Fonsecaea compactum/compacta	*Fonsecaea pedrosoi*	1983	2007																	5			5
Fonsecaea monomorpha		2005	2019		2	1														10			13
Fonsecaea nubica		2010	2020				1													14			15
Fonsecaea pedrosoi		1973	2020	1	7	5	2		4	5				1			2		1	107			135
Fonsecaea pugnacius		2015	2015																	1			1
Phialophora						**9**	**1**		**2**		**1**	**3**					**8**		**1**	**61**			**86**
Phialophora americana		2019	2019																	1			1
Phialophora cyanescens	*Neocosmospora cyanescens*	1984	1993														1			3			4
Phialophora gougerotii	*Sporotrichum gougerotii*	1967	1983																	6			6
Phialophora hoffmannii		1982	1982																	1			1
Phialophora jeanselmei	*Exophiala jeanselmei*	1964	1979																	4			4
Phialophora mutabilis	*Coniochaeta mutabilis*	1973	1991			1						2											3
Phialophora pedrosoi	*Fonsecaea pedrosoi*	1951	1994																	6			6
Phialophora repens	*Pleurostoma repens*	1975	1996																	3			3
Phialophora reptans		2011	2011																	1			1
Phialophora richardsiae		1968	2004			1						1					5		1	11			19
Phialophora verrucosa		1968	2019			7	1		2		1						2			25			38
Phoma						**1**				**1**										**6**			**8**
Phoma cava	*Neocucurbitaria cava*	1997	1997																	1			1
Phoma exigua		2006	2006							1													1
Phoma glomerata		2008	2008			1																	1
Phoma herbarum		2010	2010																	1			1
Phoma hibernica	*Phoma herbarum*	1970	1970																	1			1
Phoma minutella		1987	1987																	1			1
Phoma minutispora	*Westerdykella minutispora*	1984	1984																	1			1

Phoma Sorghina	*Phyllosticta sorghina*	1989	1989																1			1
Madurella					**1**				**2**	**1**			**1**				**15**	**4**		**94**			**118**
Madurella fahalii		2012	2014																2			2
Madurella mycetomatis		1985	2020		1					1			1				13	4		63			83
Madurella mycetomi		1956	2013						2								2			25			29
Madurella pseudomycetomatis		2010	2020																	3			3
Madurella tropicana		2012	2012																	1			1
Other Genus				**20**	**26**	**41**		**1**	**22**	**23**		**7**	**12**	**1**	**3**		**11**	**1**	**3**	**186**			**357**
Achaetomium strumarium		2018	2018																	1			1
Acrophialophora fusispora		1983	2020		3	5			1	5													14
Acrophialophora levis		2015	2019		1					2										1			4
Anthopsis deltoidea		1984	1984														1						1
Arnium leporinum		1984	1984									1			1		1						3
Arthrinium arundinis		2017	2018																	4			4
Arthrinium phaeospermum		1989	1989																	1			1
Ascotricha chartarum		1996	2019						1	1													2
Aureobasidium mansoni	*Exophiala jeanselmei var. castellanii*	1989	1998		3															1			4
Aureobasidium melanogenum		2016	2016																	1			1
Aureobasidium proteae		2012	2012		1																		1
Aureobasidium pullulans		1971	2019	11		5		1	1	3			6		1					13			41
Cladorrhinum bulbillosum		2011	2011			1																	1
Coniothyrium fuckelii		1987	1987											1									1
Corynespora cassiicola		1969	2019			1														8			9
Cyphellophora pluriseptata		1986	2002																	2			2
Drechslera dematioidea		2005	2005																	1			1
Drechslera hawaiiensis	*Curvularia hawaiiensis*	1973	1999		2				2	1							2						7
Drechslera rostrata		1986	1986																	1			1
Drechslera spicifera	*Curvularia spicifera*	1975	1988		2	1			3				2				1			1			10
Dichotomophthoropsis nymphaearum		1990	1990			1																	1
Hormodendrum pedrosoi	*Fonsecaea pedrosoi*	1961	1978							1										2			3
Hormonema dematioides		1990	1998	1									1							1			3
Hortaea werneckii		2005	2019	1									1							13			15

Lasiodiplodia theobromae		1976	2019	1		12			4	1									3			21
Lecythophora hoffmannii	*Coniochaeta hoffmannii*	1997	1997						1													1
Lecythophora mutabilis		1985	2011	1		4						2	1						1			9
Leptosphaeria senegalensis		1960	2006																4			4
Macrophomina phaseolina		2008	2020			2										1		1	1			5
Microsphaeropsis arundinis		2004	2019															2	5			7
Microsphaeropsis olivacea		1999	2001			1													1			2
Myceliophthora thermophila		1992	2011	2	1					3		3	1		1		1		1			13
Mycoleptodiscus indicus		1995	2012													2	1		3			6
Neoscytalidium dimidiatum		2009	2019		0				1	1									17			19
Neotestudina rosatii		1968	1982																3			3
Nigrospora oryzae		2014	2014																1			1
Nigrospora sphaerica		2009	2020			1													2			3
Oidiodendron cerealis		1969	1969																1			1
Papulaspora equi		2014	2020			2																2
Phaeosclera dematioides		1987	1996						1										3			4
Phomopsis bougainvilleicola		2013	2013													1						1
Phomopsis longicolla		2011	2011																1			1
Piedraia hortae		1978	1997																4			4
Pleurophomopsis lignicola		1995	2004						1										3			4
Pleurostomophora richardsiae		2012	2019	1					1										4			6
Pseudochaetosphaeronema larense		1987	2014																3			3
Pseudochaetosphaeronema martinelli		2015	2015																1			1
Pseudomicrodochium fusarioides	*Cyphellophora fusarioides*	1991	1991							1												1
Pyrenochaeta unguis-hominis		1980	2020																3			3
Scytalidium cuboideum		2013	2013						1	1												2
Scytalidium dimidiatum		1993	2015	2	2	1			3	1									29			38
Scytalidium hyalinum		1977	2018			1													21			22
Scytalidium lignicola		1983	2020			1													4			5
Sphaeropsis subglobosa		1991	1991			1																1
Taeniolella boppi	*Cladophialophora boppii*	1983	1983																2			2
Tetraploa aristata		1990	2013							1									1			2

Thermomyces lanuginosus		1991	1991									1										1
Ulocladium atrum		2006	2006			1																1
Ulocladium botrytis		2004	2010						1											1		2
Ulocladium chartarum		1981	2003																	2		2
Veronaea botryosa		2003	2018			0														10		10
Xylohypha bantiana	*Cladophialophora bantiana*	1989	2013		11					1							1					13
***Scedosporium*/*Lomentospora* complex**				**98**	**126**	**132**	**27**	**1**	**73**	**253**	**1**	**50**	**35**	**17**	**44**	**3**	**110**	**4**	**21**	**209**	**16**	**1220**
Allescheria boydii	*Pseudallescheria boydii*	1948	1996		8	9			2	17		2			1		4			12	2	57
Lomentospora prolificans		2015	2020	7	4	4				10		1	0	0	0		4		2	2		34
Monosporium apiospermum	*Scedosporium apiospermum*	1953	1993		3	8	1		2	8					2		3			12		39
Scedosporium apiospermum		1981	2020	18	40	56	15		25	86	1	13	7	3	6	1	42	1	14	96	3	427
Scedosporium aurantiacum		2005	2019	2	1	3	3		1	14							2			3		29
Scedosporium boydii		2014	2020		1	2				4		1								2		10
Scedosporium dehoogii		2017	2018																	2		2
Scedosporium inflatum		1990	2012	13	6	4	1		5	9		5	10	4	7		5	2	3	6	6	86
Scedosporium prolificans		1993	2017	45	17	13	2	1	11	46		16	13	8	15		21	1		16	2	227
Petriellidium boydii	*Pseudallescheria boydii*	1976	1997	1	7	6	1		5	9		3			1		5			10		48
Pseudallescheria angusta		2011	2019							1										1		2
Pseudallescheria boydii		1982	2017	12	39	27	4		22	48		9	5	2	12	2	24		2	47	3	258
Pseudallescheria minutispora		2013	2013							1												1
Others				**67**	**40**	**151**	**7**		**110**	**157**	**2**	**24**	**54**	**12**	**10**	**1**	**17**	**4**	**5**	**264**	**3**	**928**
Acremonium atrogriseum		2000	2000			1																1
Acremonium falciforme		1976	2019	1		3							2				1			10		17
Acremonium implicatum		2001	2012			1														1		2
Acremonium kiliense		1981	2017	5		6			1	2		1	2				2			8		27
Acremonium polychrorum		2004	2004						1													1
Acremonium potronii		1975	2015			1														1		2
Acremonium recifei		1979	2010			2														3		5
Acremonium sclerotigenum		2011	2014	1																2		3
Acremonium strictum		1984	2015	7	2	1				8		2	3	1	1		1			9		35
Albifimbria verrucaria		2020	2020	1		1																2
Amesia atrobrunnea		2019	2019																	1		1

Amylomyces rouxii		2018	2018						1													1
Aphanoascus fulvescens		1970	1992																3			3
Arachnomyces kanei		2002	2002																1			1
Arthrobotrys oligospora		1990	1990			1																1
Arthrographis kalrae		1997	2020	3	2	4			3	3		1					2		3			21
Arthropsis hispanica		2013	2013							1									1			2
Auxarthron ostraviense		2013	2013																1			1
Basidiobolus haptosporus		1978	2020						2				3					1	8			14
Beauveria alba	*Engyodontium album*	1984	1984			1																1
Beauveria bassiana		1997	2016			8			1	2									2			13
Botryodiplodia theobromae	*Lasiodiplodia theobromae*	1975	1976			1													1			2
Botryosphaeria dothidea		2020	2020																2			2
Carpoligna pleurothecii		2010	2010			1																1
Cephaliophora irregularis		1995	1995			1																1
Cephalosporium falciforme	*Neocosmospora (fusarium) falciformis*	1968	1983																2			2
Cephalosporium madurae	*Sarocladium kiliense*	1962	1966																2			2
Cephalosporium niveolanosum	*Sarocladium kiliense*	1960	1960			1																1
Cephalosporium serrae	*Gibellulopsis serrae*	1974	1974																1			1
Cephalotheca foveolata		2006	2006																1			1
Ceratocystis adiposa		2014	2014						1													1
Cercospora apii		1957	1957																1			1
Chalara ellisii		1999	1999														1					1
Chrysosporium articulatum		2015	2015							1												1
Chrysosporium georgii		2001	2001																1			1
Chrysosporium keratinophilum		2017	2017																1			1
Chrysosporium parvum		1973	2007	1		1				7							1		2			12
Chrysosporium tropicum		2007	2007						1													1
Chrysosporium zonatum		1999	2016							3							1					4
Cochliobolus hawaiiensis		2015	2015						1													1
Colletotrichum coccodes		2015	2015			1																1
Colletotrichum crassipes		2001	2001																1			1
Colletotrichum dematium		2004	2019			7																7

Colletotrichum gloeosporioides	1998	2020			10													1	4			15
Colletotrichum siamense	2019	2019																	1			1
Colletotrichum truncatum	2011	2020			3														1			4
Conidiobolus coronatus	1978	2021	3	1				47	2		1			1	1				3	1		60
Conidiobolus incongruus	1983	2010	1					4	2		1	1	1						3			13
Conidiobolus lamprauges	2011	2011							1			1	1	1								4
Coniochaeta polymorpha	2013	2013						1														1
Coniosporium epidermidis	2008	2012																	3			3
Coniothyrium fuckelii	1987	1987											1									1
Cryptendoxyla hypophloia	2014	2014																	1			1
Cryptostroma corticale	1962	1966							2													2
Cylindrocarpon destructans	1991	2011																	2			2
Cylindrocarpon lichenicola	1997	2012			5				1			1							5			12
Dactylaria constricta	1992	2012	1	2	1				3											1		8
Dactylaria gallopava	1990	2001		4					2			1	1	1					1			10
Daldinia eschscholtzii	2015	2015																	1			1
Diaporthe phaseolorum	2011	2019																1	2			3
Diaporthe phaseolorum (Phomopsis phaseoli)	2011	2011																	1			1
Didymella microchlamydospora	2019	2019							1													1
Edenia gomezpompae	2013	2013			1																	1
Emarellia grisea	2016	2016																	1			1
Emarellia paragrisea	2016	2016																	1			1
Emergomyces africanus	2017	2019	2																3			5
Emergomyces canadensis	2018	2018	1						1										1			3
Emergomyces orientalis	2017	2017							1										1			2
Emergomyces pasteurianus	2015	2020	1						2	1									5			9
Emericella nidulans	1983	2016		1	1			2	3													7
Emericella quadrilineata	2004	2015							1										2			3
Emericella rugulosa	2012	2012							1		1			1					1			4
Emmonsia crescens	1964	2012	1					1	17			2							1			22
Engyodontium album	1983	2016		1	1						2								1			5
Entomophthora coronata	1965	2006						10														10

Epicoccum nigrum		1997	2020			1			2														3
Eurotium amstelodami		2016	2016							1													1
Falciformispora senegalensis		2014	2014																	1			1
Fuscoporia ferrea		2010	2010							1													1
Geomyces pannorum		2003	2008																	3			3
Geosmithia argillacea		2010	2015		3				1	8						1				1			14
Glomerella cingulata	*Colletotrichum gloeosporioides*	1983	1983			1																	1
Graphium basitruncatum		2007	2017	2																1			3
Gymnascella dankaliensis		1989	2007																	2			2
Hendersonula toruloidea		1970	2004			1														34			35
Hongkongmyces pedis		2014	2014																	1			1
Hormographiella aspergillata		1996	2019		3	2			1	11			1							3			21
Hypocrea orientalis		2008	2008										1										1
Inonotus (Phellinus) tropicalis		2005	2017	1						1						1				2			5
Isaria farinosa		2013	2013										1										1
Knufia epidermidis		2019	2019																	1			1
Laetisaria arvalis		2018	2018			1																	1
Malbranchea pulchella		1951	1994						1											1			2
Medicopsis romeroi		2016	2019			1										1			1	7			10
Metarhizium anisopliae		1997	2017			4														1			5
Metarhizium pingshaense		2017	2017							1													1
Metarhizium robertsii		2017	2017			1			1														2
Microcyclosporella mali		2015	2015			1																	1
Monascus ruber		2010	2018										1							1			2
Mycotypha microspora		2018	2018										1										1
Myriodontium keratinophilum		1985	1985						1														1
Nannizziopsis obscura		2017	2017	1														1		1			3
Nattrassia mangiferae		1997	2010		3	4														7			14
Neocucurbitaria keratinophila		2019	2019			2														1			3
Neurospora sitophila	*Chrysonilia sitophila (asexual state)*	1997	1997							1													1
Nigrograna mackinnonii		2013	2020																	4			4
Onychocola canadensis		1994	2016																	17			17

Ophiostoma piceae		2009	2009		1					1													2
Ovadendron sulphureo-ochraceum		1995	1995			1																	1
Paecilomyces farinosus	*Cordyceps farinosa*	1994	1994			1																	1
Paecilomyces formosus		2016	2017							1										1			2
Paecilomyces fumosoroseus		2015	2015										1										1
Paecilomyces javanicus	*Cordyceps javanica (teleomorph)*	1984	1986	1	1							2											4
Paecilomyces marquandii		1979	2000																	2			2
Paecilomyces varioti	*Paecilomyces variotii (orthographic variant)*	1971	1998	1						2		3	2				1		1	1			11
Paecilomyces variotii		1981	2019	6	3	4	2		3	10	1	1	10	2	2		1			8			53
Paecilomyces viridis	*Metarhizium viride*	1975	1975			1																	1
Pallidocercospora crystallina		2019	2019																	1			1
Parathyridaria percutanea		2019	2019																	1			1
Pestalotiopsis clavispora		2013	2013			1																	1
Petromyces alliaceus		2007	2007				1			1													2
Phaeoisaria clematidis		2000	2000			1																	1
Phanerochaete chrysosporium		2014	2014							1													1
Phanerochaete sordida		2017	2017							1													1
Pleurostoma ootheca		2014	2014																	1			1
Pleurostoma richardsiae		2017	2019	1		1								1			0			2			5
Podospora austroamericana		2018	2018			1																	1
Polycytella hominis		1987	2006																	2			2
Polypaecilum insolitum	*Aspergillus insolitus*	1972	1972				1																1
Pseudopestalotiopsis theae		2019	2019			1																	1
Pseudotaeniolina globosa		2003	2003	1																			1
Purpureocillium lilacinum		2011	2020	1	1	9			3	3		2								9			28
Pythium insidiosum		1993	2021	15	1	36			2	1		1	1	1	1			1	1	4			65
Ramichloridium obovoideum		1988	1999		3																		3
Ramichloridium schulzeri	*Myrmecridium schulzeri*	1985	1985						1														1
Rasamsonia aegroticola		2015	2017			1				3		1			1			1		1	1		9
Rasamsonia argillacea		2010	2018	1						5													6
Rasamsonia piperina		2016	2019							1			1				1						3
Rhizoctonia solani		2012	2013			1														1			2

Rhytidhysterium rufulum		2014	2018																	4			4
Roussoella solani		2017	2017			1																	1
Setosphaeria holmii		2018	2018			1																	1
Stachybotrys atra		1999	1999							3													3
Stachybotrys chlorohalonata		2021	2021						1														1
Stachybotrys eucylindrospora		2014	2014			1																	1
Stemphylium lanuginosum	*Ulocladium lanuginosum*	1983	1983							1													1
Thamnostylum lucknowense		2012	2012						1														1
Thermoascus aurantiacus		1967	1967							1													1
Thermoascus crustaceus		2010	2019							2			4										6
Thermoascus taitungiacus		2001	2001										1										1
Thermothelomyces thermophila		2017	2017		1					1		1	1		1		1			1			7
Thielavia sepedonium	*Corynascus sepedonium*	1990	1990																	1			1
Thielavia subthermophila		2009	2011		1	1																	2
Tilletiopsis minor		1997	2018						1	1										1			3
Tintelnotia destructans		2018	2019			2																	2
Toxicocladosporium irritans		2011	2011																	1			1
Triadelphia pulvinata		2001	2013	1																1			2
Trichoderma bissettii		2014	2014						1	1							1			1			4
Trichoderma citrinoviride		1999	2008	1						1													2
Trichoderma harzianum		1996	2014	1	1	1			2	3			1							1			10
Trichoderma longibrachiatum		1995	2019	2	3		2		6	9		4	7	2						5			40
Trichoderma orientale		2014	2014	1					1	1			1							1			5
Trichoderma pseudokoningii		1995	2000		1					1			1							1			4
Trichoderma viride		1976	2005							2			2	1									5
Tritirachium oryzae		2010	2018																	4			4
Tritirachium roseum		1975	1975			1																	1
Truncatella angustata		2015	2015																	1			1
Ustilago echinata		2016	2016																	1			1
Ustilago esculenta		1996	2007							2													2
Valsa sordida		2006	2008						2	1													3
Verruconis gallopava		2014	2020		1		1		1	6										1			10
Westerdykella dispersa		2014	2018	1																1			2

Xanthothecium peruvianum		2017	2017																1			1
Xylaria enteroleuca		2006	2006						1													1
Total	-	-	-	632	669	959	260	12	1047	2513	15	341	356	119	252	21	475	62	139	2503	74	1	10450

CNS: Central nervous system; ORL : Oto-rhino-laryngological; OA: Osteo-articular

Aspergillus

Aspergillus species were the most frequent moulds isolated in human clinical samples. In this repertory, the 76 *Aspergillus* species identified at least once in humans belong to 13 sections of the 20 described (18), as follows: *Aspergillus*, *Candidi*, *Circumdati*, *Clavati*, *Cremei*, *Flavi*, *Flavipedes*, *Fumigati*, *Nidulantes*, *Nigri*, *Restricti*, *Terrei* and *Usti*. As reported in the literature, the filamentous fungus mostly isolated from humans is *Aspergillus fumigatus* (19) followed by *Aspergillus flavus*, *Aspergillus niger*, and *Aspergillus terreus*. The lung and respiratory tracts were the most common anatomical sites of infection with a total of 1,180 publications for *A. fumigatus*, 174 for *A. flavus*, 102 for *A. niger* and 81 publications for *A. terreus*. These fungi are indeed ubiquitous in the environment and are transmitted by the airways (20). To rule out potential misidentifications related to morphological identification, the results will be approached in terms of *Aspergillus* section (4). The anatomical site in second position and concerning all the sections was the skin system. Cutaneous aspergillosis can be primary and can affect immunocompetent patients or can be secondary in cases of disseminated infection, predominantly in immunosuppressed patients (21–23). The *Nigri* section has a tropism for the auditory system, with 79 publications, which has already been noted in the literature (24). Regarding the clinical presentations involving the central nervous system or heart, the *Fumigati* section were predominantly represented (194 and 118 publications, respectively). The *Fumigati*, *Flavi* and *Nigri* sections had a predominantly ocular involvement, with 140, 93, and 36 publications, respectively.

Fusarium

The genus *Fusarium* includes at least 200 species, grouped into about ten phylogenetic species complexes (15, 16, 25). In this review of the literature, only eight species complexes were found to have been isolated from humans: *F. chlamydosporum* species complex (FCSC), *F. dimerum* species complex (FDSC), *F. incarnatum - F. equiseti* species complex (FIESC), *F. oxysporum* species complex (FOSC), *F. sambucinum* species complex (FSAMSC), *F. solani* species complex (FSSC), *F. fujikuroi* species complex (FFSC), and *Gibberella fujikuroi* species complex (GFSC). Interestingly, looking at the *Fusarium* genus as a whole, three anatomical sites stand out: the ocular system (191 publications), the cutaneous system (228 publications) and systemic involvement (134 publications). This is consistent with the data in the literature reporting superficial cases, mainly keratitis and onychomycosis in immunocompromised or immunocompromised patients, and disseminated infcetions in immunocompromised patients

(26–29). Species belonging to the FSSC are predominantly represented, which has previously been shown to be the most virulent *Fusarium* species complex in animal models (30).

Penicillium

The genus *Penicillium* is ubiquitous in the environment and is rarely involved in human infections. In this literature review, only 164 publications reporting the isolation of these hyaline hyphomycetes in humans were found. One species has emerged since the 1990s and mainly affects human with acquired immunodeficiency syndrome (AIDS): *Talaromyces marneffei* (31, 32), and this is the predominant species within the genus *Penicillium* representing 54% of the publications (89/164). The three anatomical sites most affected by the members of the genus *Penicillium* are the pulmonary sphere (49 publications), the cutaneous system (23 publications) and systemic involvement (36 publications).

Mucorales

The order of the Mucorales (previously Zygomyces) includes the hyaline pauciseptated filamentous fungi group, comprising 11 genera: *Mucor*, *Lichtheimia*, *Rhizomucor*, *Rhizopus*, *Absidia*, *Syncephalastrum*, *Cunninghamella*, *Apophysomyces*, *Saksenaea*, *Actinomucor* and *Thermomucor*. All of these are implicated in human infections (33). In this literature review, *Rhizopus* was the genus mostly involved in human infections, with *Rhizopus oryzae* (121 publications) (plus its synonym and current appellation *Rhizopus arrhizus* (53 publications)) leading the way, followed by *Rhizopus microsporus* (90 publications). Mucorales can be classified according to the primary route of infection: airborne, by direct contact with contaminated devices, or by trauma (33, 34). Here, we compare the number of publications reporting isolation in the skin system versus the respiratory system including from the oto-rhino-laryngological sphere and pulmonary sphere. The genera *Apophysomyces* (54 versus 26 publications, respectively), *Mucor* (32 versus 17 publications, respectively) and *Saksenaea* (45 versus eight publications, respectively) are mostly isolated from the skin system, and therefore transmitted by contact. The genera *Cunninghamella* (12 versus 52 publications, respectively), *Rhizopus* (75 versus 134 publications respectively) and *Rhizomucor* (16 versus 32 publications, respectively) are mostly isolated from the oto-rhino-laryngological sphere and pulmonary system and are, therefore, airborne. *Absidia* (29 versus 33 publications respectively), and *Lichtheimia* (47 versus 42 publications respectively) had a less obvious tropism for one or the other of these two systems. The cutaneous or respiratory tropism can be explained by the differences found in the sporangia of these genera (34). In fact, the wet spores of the *Mucor*,

Apophysomyces and *Saksenaea* species are probably not primarily dispersed by the air and transmitted by trauma (34, 35). On the contrary, the sporangiospores of *Rhizopus* and *Rhizomucor* species are small (less than 4 µm in diameter), dry and therefore easily airborne (36, 37). This morphological hypothesis does not explain everything because, similar to *Mucor*, *Absidia* and *Lichteimia* have wet spores and yet they are reported equivalently in the lung and skin in this review.

Dematiaceous moulds

Dematiaceous fungi are also known as "black fungi", due to the predominance of melanin in their cell walls, which likely acts as a virulence factor. These darkly pigmented fungi are found on the soil surface where they live as saprophytes but also sometimes as parasites of plants (38). This review has highlighted 199 dematiaceous fungi species isolated from humans, belonging to 17 genera: *Alternaria*, *Exophiala*, *Cladophialophora*, *Scopulariopsis*, *Curvularia*, *Phialemonium*, *Exserohilum*, *Microascus*, *Bipolaris*, *Chaetomium*, *Cladosporium*, *Ochroconis*, *Phaeoacremonium*, *Rhinocladiella*, *Fonsecaea*, *Phialophora* and *Phoma*. The genus *Exophiala* is the most represented. Contamination most often takes place through infection of a wound by a telluric strain or during a transcutaneous traumatism by means of a plant (39). This explains the predominantly cutaneous location found in this review (1,046 publications). Among the cutaneous affections, melanised fungi are responsible for chromoblastomycosis, which mainly affect individuals performing soil-related tasks (40), phaeohyphomycosis (41), and eumycotic myetoma (42). Two synonyms species stand out for their tropism for the central nervous system, *Cladiophialophora bantiana* and *Cladosporium trichoides*, for which 51 publications and 30 publications, respectively, were found in this location.

Pseudallescheria/Scedosporium* complex species *(PSC)

Among the PSC, six genera were represented: *Allescheria*, *Lomentospora*, *Monosporium*, *Petriellidium*, *Pseudallescheria* and *Scedosporium*. These fungi are ubiquitous in the environment and can be found in soil, compost and polluted water (43). Three main species have been isolated in humans: *Scedosporium apiospermum* (427 publications), *Pseudallescheria boydii* (258 publications) and *Scedosporium prolificans* (227 publications). *Pseudallescheria boydii* is the teleomorph of *S*. apiospermum (685 publications), which also has a synanamorph called *Graphium eumorphum* (44). It should be noted that *S*. *prolificans* has neither a known sexual state, nor a synanamorph. *Pseudallescheria boydii* (258 publications) and *S*. *apiospermum* (427 publications) are found in all anatomical sites, with a greater prevalence in the pulmonary sphere (48 and 86 publications,

respectively) and the cutaneous system (47 and 96 publications, respectively). *Scedosporium prolificans* is found mainly in the pulmonary sphere (46 publications) and in systemic infections (45 publications).

Others

Among the moulds not classified in the five major genera, some present in the environment stand out for their ability to affect multiple organs, such as the members of the genus *Acremonium*, with *Acremonium strictum* (35 publications) and *Acremonium kiliense* (27 publications) in the lead, members of the genus *Paecilomyces*, with *Paecilomyces variotii* (53 publications), and members of the *Trichoderma* genus, with *Trichoderma longibrachiatum* in the lead (40 publications). Others have a cutaneous tropism. *Hendersonula toruloidea* is an opportunistic fungus which is almost exclusively responsible for skin infections (34 /35 publications). *Onychocola canadensis* is found only on the skin system (17 publications) and was mostly responsible for onychomycosis (16 publications). Finally, some rarely isolated species, were found exclusively in the ocular area, often secondary to trauma, such as *Arthrobotrys oligospora* (one publication), *Beauveria alba* (one publication), *Carpoligna pleurothecii* (one publication), *Cephaliophora irregularis* (one publication), *Cephalosporium niveolanosum* (one publication), *Colletotrichum coccodes* (one publication), *Colletotrichum dematium* (seven publications), *Edenia gomezpompae* (one publication), *Epicoccum nigrum* (1 publication), *Glomerella cingulate* (one publication), *Laetisaria arvalis* (one publication), *Metarhizium robertsii* (one publication), *Microcyclosporella mali* (one publication), *Paecilomyces viridis* (one publication), *Papulaspora equi* (one publication), *Pestalotiopsis clavispora* (one publication), *Phaeoisaria clematidis* (one publication), *Podospora austroamericana* (one publication), *Pseudopestalotiopsis theae* (one publication), *Roussoella solani* (one publication), *Setosphaeria holmii* (one 1 publication), *Stachybotrys eucylindrospora* (one publication), *Tintelnotia destructans* (one publication), and *Tritirachium roseum* (one publication).

Fungal location by focusing on the anatomical site

Within the 19 anatomical sites, the semiology of infection was detailed for the six major categories of fungi involved in human pathologies (Table 2).

Table 2. Anatomical sites and nosological framework of the different taxa. CNS: Central nervous system ; ORL: Oto-rhino-laryngological

	Aspergillus	Dematiaceous	*Pseudallescheria /Scedosporium*	Other	Mucorales	*Fusarium*	*Penicillium*	Total
Systemic	**193**	**87**	**105**	**56**	**46**	**137**	**54**	**678**
-	19		8		18	2		47
Aortitis	29	6	6	1	1	1		44
Blood (Fungemia)	91	63	78	24	13	127	31	427
Bone-marrow	5	1	2	4			13	25
Lymph nodes	17	13	7	6	2	2	10	57
Vasculitis	32	4	4	21	12	5		78
CNS	**287**	**200**	**133**	**38**	**41**	**8**	**3**	**710**
-	127	37	46	16	25	2	2	255
Brain abscess	78	112	59	13	8	3		273
Encephalitis	1	3	1	1				6
Mass	37	18	3	4	5			67
Meningitis	39	27	17	3	3	3	1	93
Meningo-encephalitis	5	3	7	1				16
Ocular	**320**	**166**	**147**	**155**	**18**	**206**	**9**	**1,021**
-	42	10	28	7	2	4	1	94
Blepharitis	1							1
Conjonctival ulceration	6	2	3	2				13
Dacryocystis	3		2	1	1			7
Endophtalmitis	86	44	43	19	4	41	3	240
Granuloma	5							5
Keratitis	157	107	63	121	10	160	5	623
Orbital	20	3	8	5	1	1		38
Auditory system	**192**	**16**	**29**	**8**	**12**	**1**	**6**	**264**
-	17	7	10	1	3			38
Implant-associated otomycosis		1	1		1			3
Otomycosis	175	8	18	7	7	1	6	222
Dental and gums	**5**	**5**	**1**			**1**	**1**	**13**
-	4	4				1	1	10
Abscess		1	1					2
Parodontitis	1							1
ORL sphere	**656**	**141**	**87**	**133**	**170**	**37**	**22**	**1,246**
-	5		1	1		1	1	9
Cervical lymphadenitis	1							1
Fungus ball	42	6	7	4		2	1	62
Laryngitis	18	1			1		2	22

Nasal	81	23	5	11	7	8	4	139
Oesophagitis	7	4	3	1	1	4	2	22
Oral mucosa	20	7	2	3	5	2		39
Pharyngeal abscess	3							3
Pharyngitis	4	2					3	9
Rhinitis		1						1
Rhino-facial	1	2	1	47	9			60
Rhino-orbital	51	7	6	11	30			105
Rhino-orbito-cerebral	20		1	9	59			89
Rhino-sinusitis	282	78	51	38	40	17	8	514
Sino-oral	11	3		3	8	1		26
Tongue	7	1			1			9
Tonsillitis		1						1
Tracheal	103	5	10	5	9	2	1	135
Pulmonary	**3,100**	**229**	**424**	**221**	**289**	**50**	**66**	**4,379**
-	206	15	25	13	19	9		287
Abscess	40	1	13	6	2	1		63
Halo-sign	22			1	1			24
Hypersensitivity/Allergy	105	8		3		1	3	120
Interstitial pneumonitis				2				2
Invasive	387	11	12	14	28	1	9	462
Lower respiratory tract	998	90	166	93	120	20	22	1,509
Lymph nodes	84	12	8	13	21	1	2	141
Mediastinal infection	37	2	2	3	4			48
Parenchymal cavity	331	10	46	8	28	1	6	430
Pleurisy	73	14	13	8	11	1	5	125
Pneumonia	125	20	28	23	24	8	6	234
Upper respiratory tract	692	46	111	34	31	7	13	934
Breast	**6**	**3**	**1**	**2**	**1**	**1**	**1**	**15**
-	1	3		1	1	1		7
Breast implant	2		1	1				4
Milk	2						1	3
Nipples	1							1
Heart	**218**	**32**	**54**	**24**	**27**	**13**	**3**	**371**
-	37	5	11	1	16	4		74
Implanted device endocarditis	55	16	12	10		2	1	96
Native valve endocarditis	66	6	17	6	4	5		104
Myocarditis	30	2	9	2	4	2		49
Pericarditis	22	3	3	5	2		2	37
Thrombus	8		2		1			11
Digestive	**149**	**52**	**43**	**56**	**89**	**22**	**11**	**422**
-	38	7	12	17	25	5	9	113
Abscess	2		1	1	1			5
Appendicitis	1			2	3			6
Biliary tract	5	3	2					10
Cholecystis	1							1

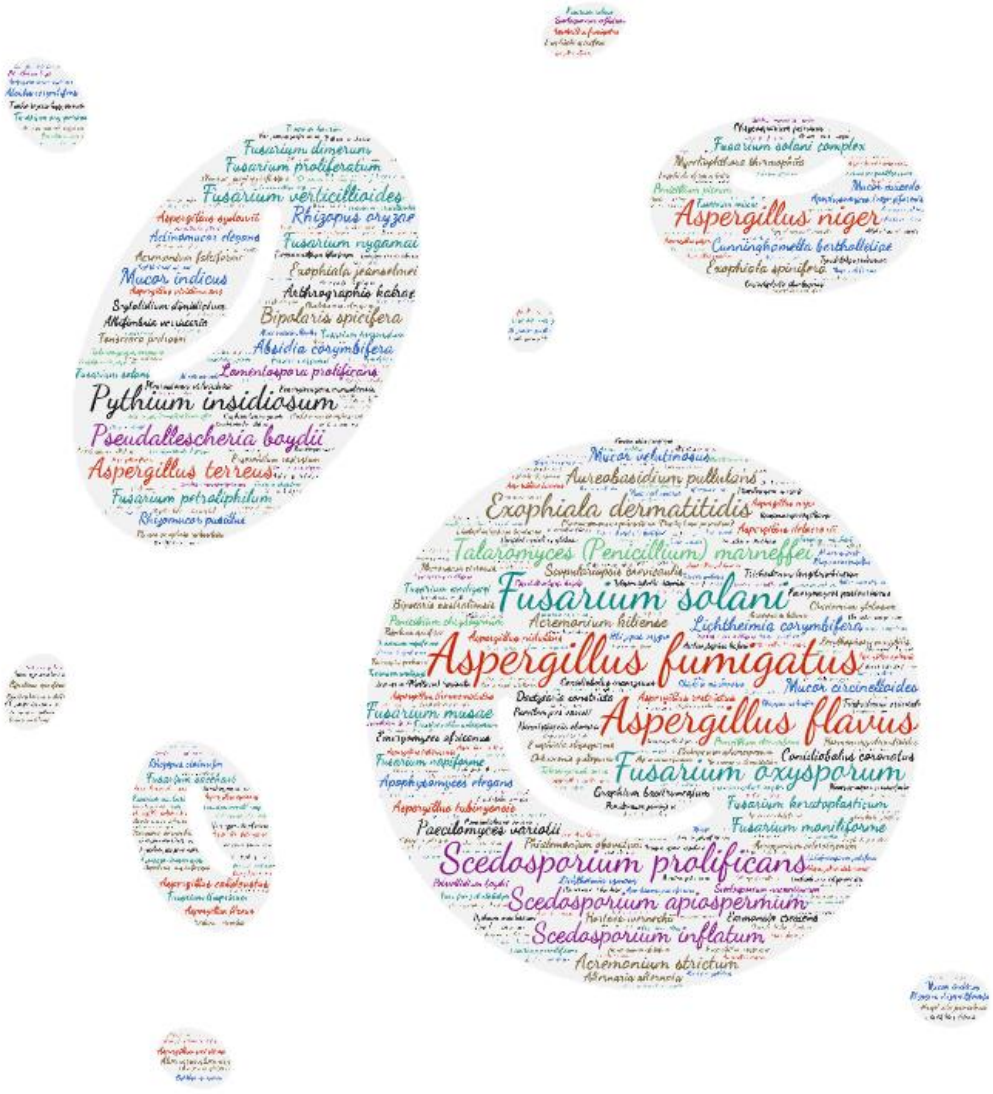
Colitis	2		2		1			5
Enteritis	2			1	6			9
Gastric	10	3	1	5	14	1		34
Mucosal necrosis	5	1			3	1		10
Pancreas	7		3		3	1		14
Peritonitis	43	34	7	27	15	8	1	135
Spleen	33	4	15	3	18	6	1	80
Liver	**43**	**9**	**17**	**11**	**34**	**4**	**2**	**120**
-	27	7	17	10	23	3	2	89
Abscess	11	1		1	11	0		24
Ascitis	3	1				1		5
Hepatitis	2							2
Urinary tract	**158**	**14**	**51**	**14**	**60**	**16**	**1**	**314**
-	3							3
Bladder	6	1			4			11
Kidney abscess	85	7	30	9	46	7	1	185
Mass	26		2		1			29
Prostatitis	7	1	1		1			10
Pyelonephritis	2	1	2	3	1			9
Urinary tract infection	1		3			1		5
Urine	28	4	13	2	7	8		62
Genital	**9**	**1**	**3**	**1**	**6**	**1**		**21**
-	3							3
Epididymic infection		1						1
Genital infection (External)			3		1			4
Glans necrosis	1				3	1		5
Ovarian abscess					1			1
Testis	4							4
Vaginitis	1			1	1			3
Osteo-articular system	**287**	**81**	**151**	**22**	**26**	**18**	**4**	**589**
-	38	8	18	4	9	2		79
Arthritis	6	13	28	3	2	5		57
Bursitis		1						1
Joint	26	7	21	1	4	1		60
Mass (including Mycetoma)	6	3						9
Osteomyelitis	147	34	55	12	9	5	4	266
Spondylodiscitis	56	3	9	1	1	1		71
Synovitis	8	12	20	1	1	4		46
Skeletal muscles	**21**	**8**	**4**	**8**	**19**	**1**		**61**
-	21	8	4	8	19	1		61
Soft-tissue	**44**	**12**	**21**	**12**	**44**	**4**		**137**
-	44	12	21	12	44	4		137
Skin system	**454**	**1,144**	**237**	**361**	**316**	**266**	**23**	**2,801**
-	43	78	17	5	25	5		173
Dermatitis	1	2		1	1	1	1	7
Intertrigos						1		1

Mycetoma	15	145	62	46		14		282
Onychomycosis	91	156	3	77	1	65	1	394
Subcutaneous	56	338	47	78	66	25		610
Superficial cutaneous	214	374	91	149	209	137	21	1,195
Tinea capitis	1	1						2
Tinea corporis		1		3				4
Tinea manuum		3						3
Tinea pedis		8		1				9
Ulcer	33	38	17	1	14	18		121
Endocrine gland	**40**	**6**	**18**	**4**	**14**	**2**		**84**
-		1	2					3
Adrenal	7	2			3	1		13
Parathyroid	1							1
Thymus					1			1
Thyroid	32	3	16	4	10	1		66
Placental infection	**1**							**1**
-	1							1
Total	**6,183**	**2,206**	**1,526**	**1,126**	**1,211**	**788**	**206**	**13,248**

Systemic

Regarding systemic localisation (comprising fungaemia, aortitis, vasculitis, lymph node infection and bone-marrow infection), there is a large majority of fungaemias. The genus *Aspergillus* was predominantly isolated from blood samples with *Aspergillus fumigatus* and *Aspergillus flavus* predominating (Figure 2). It should be noted that the genus *Fusarium* was in second place with *Fusarium solani*. *Aspergillus* is rarely isolated from blood cultures, and usually in the case of infective endocarditis. This predominance of *Aspergillus* detection in systemic infections is explained by the use of new detection tools, including PCR.

Figure 2. Wordcloud representing the species name involved in systemic infections. Using the online tool, Wordle (www.wordle.net), the size of the name of each species is proportional to the number of times it occurs in the repertory.



Central nervous system

Three genera are mostly represented in central nervous system infections: *Aspergillus*, dematiaceous fungi and *Pseudallescheria*/*Scedosporium* (Figure 3). The majority of infections occur as brain abscesses or meningitis. Figure 2 highlights several species of dematiaceous fungi. *Exserohilum rostratum* has been involved in iatrogenic meningitis outbreaks secondary to the use of contaminated injectable corticosteroids (45–50). *Cladophialophora bantiana* is a dematious mould that may infect immunocompetent patients (mainly farmers or residents of agricultural regions) whose reservoir and mode of transmission are still poorly known (51). Its neurotropism is highlighted by 51 publications concerning the CNS among a total of 84 in this review.It synonym, *Cladosporium trichoides* causing brain abscesses for which we have recovered 30 publications. Both are responsible for 81 publications reporting CNS involvement.

Figure 3. Wordcloud representing the species name isolated in the central nervous system. Using the online tool, Wordle (www.wordle.net), the size of the name of each species is proportional to the number of times it occurs in the repertory.

Ocular system

All categories of fungi can cause ocular damage. Indeed, as filamentous fungi are ubiquitous in the environment, this type of infection is frequently observed during injuries with plants. The genera *Aspergillus* with *Aspergillus fumigatus*, *Aspergillus flavus*, *Aspergillus terreus* and *Aspergillus niger* and *Fusarium* with *Fusarium solani* and *Fusarium oxysporum* are predominant (Figure 4). In most cases, it can be keratitis (623 publications) or endophthalmitis (240 publications). The most described species that can cause both keratitis and endophthalmitis are *Fusarium solani* (85 and 21 publications, respectively), *Aspergillus fumigatus* (63 and 44 publications, respectively), *Aspergillus flavus* (52 and 16 publications, respectively), *Scedosporium apiospermum* (35 and 13 publications, respectively), *Aspergillus niger* (12 and 13 publications, respectively) and *Pseudallescheria boydii* (10 and 10 publications, respectively). The other species mostly involved in keratitis are *Pythium insidiosum* (31 publications), *Fusarium oxysporum* (21 publications), *Lasiodiplodia theobromae* (11 publications), *Alternaria alternata* (10 publications) and *Colletotrichum gloeosporioides* (10 publications). It should be noted that ten species have been found in conjunctival infections: *Aspergillus flavus* (1), *Aspergillus fumigatus* (1), *Aspergillus niger* (4), *Cephalosporium niveolanosum* (1), *Exophiala jeanselmei* (1), *Fonsecaea pedrosoi* (1), *Monosporium apiospermum* (1), *Neocucurbitaria keratinophila* (1), *Pseudallescheria boydii* (1) and *Scedosporium apiospermum* (1).

Figure 4. Wordcloud representing the species name isolated in the ocular system. Using the online tool, Wordle (www.wordle.net), the size of the name of each species is proportional to the number of times it occurs in the repertory.

Auditory system

The genus *Aspergillus* is predominantly isolated from the auditory system, with *Aspergillus niger* in the lead, a member of the *Nigri* section known for it tropism for the auditory meatus (24). *Aspergillus flavus* and *Aspergillus fumigatus* come in second place (Figure 5). These fungi are responsible for fungal otomycosis in the majority of cases (52). Interestingly, 29 publications reported the identification of *Pseudallescheria*/*Scedosporium* complex in the auditory system, including 18 reporting otomycosis. *Scedosporium apiospermum* was predominant with ten publications reporting otomycosis, including two cases of malignant otitis externa (53, 54) and one otitis complicated with a temporomandibular arthritis (55). Other species stand out in Figure 4, such as *Absidia corymbifera*, which five publications report the involvement of in otomycosis, including one case of malignant otitis externa (56) and a few cases of *Penicillium* otomycosis (six publications).

Figure 5. Wordcloud representing the species name isolated in the auditory system. Using the online tool, Wordle (www.wordle.net), the size of the name of each species is proportional to the number of times it occurs in the repertory.

Oto-rhino-laryngeal system

It is not surprising that a majority of aspergillosis rhinosinusitis is recorded (282 publications). This anatomical site also includes rhino-orbital, rhino-facial and rhino-orbito-cerebral involvement, which explains the strong representation of mucorales (170 publications). *Rhizopus oryzae* (heterotypic synonym *Rhizopus arrhizus*) is largely in the majority in the mucorales (38 publications), which is not surprising, given that it is the most common Mucorale species worldwide (57). *Rhizopus oryzae* is responsible for variable oto-rhino-laryngeal system infections with a majority of rhino-orbito-cerebral infections (17 publications) followed by rhino-sinusitis (nine publications) and rhino-orbital involvement (seven publications). These rhino-orbito-cerebral lesions can also be caused by *Aspergillus fumigatus* (16 publications) and two other species of mucorales *Rhizopus arrhizus* (12 publications) and *Apophysomyces elegans* (11 publications). One fungus stands out, *Conidiobolus coronatus*, which is responsible for entomophthoromycosis and rhino-facial infections (Figure 6). *C. coronatus* is a widely distributed insect pathogenic fungus belonging to the class Zygomycetes and is rarely involved in human pathology. This mycosis is mainly tropical due to its tropism of plant detritus in very humid environments (58).

Figure 6. Wordcloud representing the species name isolated in the Oto-rhino-laryngeal sphere. Using the online tool, Wordle (www.wordle.net), the size of the name of each species is proportional to the number of times it occurs in the repertory.

Pulmonary system

The respiratory system is the anatomical site most affected by fungal infections (4,496 publications). It should be noted that no distinction was made between colonisation and allergic bronchopulmonary aspergillosis, which thus includes the respiratory isolation of *Aspergillus* sp.. Like Aspergilli, moulds are ubiquitous saprophytes in the environment. Their dispersion in the air is possible by the production of volatile spores which ensures their presence in both indoor and outdoor environments (59, 60). Human contamination thus occurs mainly by inhaling the airborne conidia with the lungs being the first to be exposed. We therefore note a wide variety of moulds responsible for lung damage (Figure 7).

Figure 7. Wordcloud representing the species name isolated in the pulmonary system. Using the online tool, Wordle (www.wordle.net), the size of the name of each species is proportional to the number of times it occurs in the repertory.

Cardiac involvement

Cardiac involvement was found among 13% (74/565) of the species described in this repertory. These species belonged to the major taxa *Aspergillus*, *Penicillium*, *Fusarium*, mucorales, dematiaceous fungi, and *Scedosporium*/*Lomentospora* complex, as well as the genera *Acremonium* sp, *Arthrographis* sp., *Conidiobolus* sp., *Emericella* sp., *Engyodontium* sp., *Paecilomyces* sp., *Purpureocillium* sp., *Pythium* sp., *Rasamsonia* sp., *Thermothelomyces* sp., and *Trichoderma* sp.. *Aspergillus fumigatus* was the most common species isolated (Figure 8). These infections are, however, rare with species belonging to the genus *Penicillium*, including only three publications (one endocarditis due to *Penicillium chrysogenum* (61) and two pericarditis due to *P*. *citrinum* and *P*. *rubens* (62, 63) being reported. Cardiac involvement was mainly in the form of endocarditis, whether native (105 publications) or on a prosthetic valve or implanted equipment (96 publications). Interestingly, in native valve endocarditis, mitral involvement seems to be the most frequent (41 publications) (Table 3). Pericarditis and myocarditis were described in 37 and 49 publications, respectively.

Figure 8. Wordcloud representing the species name isolated in the cardiac system. Using the online tool, World (www.wordle.net), the size of the name of each species is proportional to the number of times it occurs in the repertory.

Table 3. Details of cardiac sites affected by native valve endocarditis and associated species.

Current Name	Tricuspid	Mitral	Aortic	All 4 chambers	Mural endocardium	Pulmonary valve	Atrium	Ventricle	ND
Absidia corymbifera	1[64]								
Arnium leporinum		1[65]							
Arthrographis kalrae			1[66]						
Aspergillus clavatus			1[67]						
Aspergillus flavus	6[68–73]	9[68, 72, 74–80]	2[73, 81]						
Aspergillus fumigatus	4[82–85]	18[86–103]	10[99, 104–112]	2[113, 114]	2[115, 116]	2[115, 117]	3[118–120]		2[121, 122]
Aspergillus nidulans							1[123]		
Aspergillus niger		1[124]							
Aspergillus terreus	1[125]	2[125, 126]	1[127]	1[125]			1[126]		1[128]
Aspergillus udagawae		1[129]							
Cunninghamella bertholletiae			1[130]		1[131]				
Engyodontium album			1[132]						
Exophiala dermatitidis			1[133]						
Fusarium incarnatum									1[134]
Fusarium solani		1[135]	1[136]			1[137]			
Fusarium solani complex								1[138]	
Lomentospora prolificans			1[139]						
Myceliophthora thermophila							1[140]		1[141]
Paecilomyces javanicus			1[142]						
Phaeoacremonium parasiticum	1[143]	1[143]	1[143]						
Phialemonium obovatum	1[144]								1[145]
Pseudallescheria boydii	1[146]	3[147–149]				1[150]			
Saksenaea vasiformis		1[151]							
Scedosporium apiospermum		1[152]							
Scedosporium boydii									1[153]
Scedosporium inflatum									
Scedosporium prolificans	1(15[4])	2[155, 156]	2[157, 158]						1[159]
Trichoderma longibrachiatum							1[160]		
Total	16	41	24	3	3	4	7	1	8

ND: Not determined

Digestive system

Concerning the digestive system, the two main diseases are fungal peritonitis (135 publications) and spleen disease (80 publications) (Figure 9). Micromycetes are known to be responsible for peritonitis during peritoneal dialysis in between 1% and 3% of cases, which is indeed the situation we found in the majority of this review (161, 162). In this review, 77.8% of the publications reported peritonitis secondary to peritoneal dialysis (105/135). Other risk factors that emerged were prematurity (163–167) and solid organ transplantation, including the kidney (168–172), heart (173), small bowel (174) and liver (175, 176). Seven publications reported peritoneal involvement secondary to dissemination (173, 177–181). The species mostly found were *Aspergillus fumigatus* (18 publications), *Aspergillus niger* (nine publications) and *Paecilomyces variotii* (eight publications).

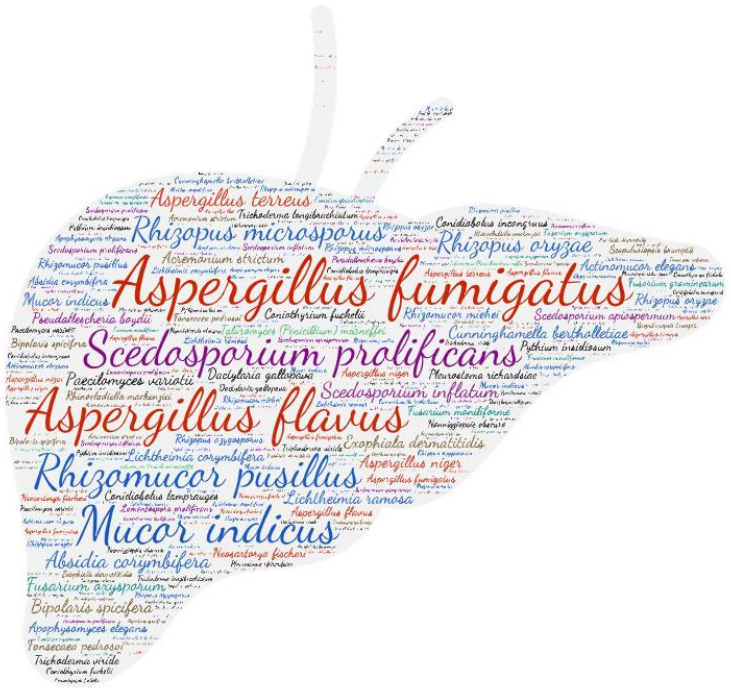
Splenic involvement was, in the majority of cases, secondary to haematogenous dissemination of the pathogen, with the exception of three situations where splenic abscesses were described. One case of a splenic abscess caused by *Aureobasidium pullulans* in a patient with lymphoma (182) and one caused by *Hortaea werneckii* in a patient with acute myeloid leukaemia (183) were both diagnosed post-mortem. The third case of splenic abscess caused by *Paecilomyces variotii* was described in a child with chronic granulomatous disease (184).

Figure 9. Wordcloud representing the species name isolated in the digestive system. Using the online tool, World (www.wordle.net), the size of the name of each species is proportional to the number of times it occurs in the repertory.

Liver involvement

In terms of hepatic involvement, there is great variability in the forms found, including abscesses, hepatitis, ascites and lesions secondary to hematogenous dissemination (Table 2). The genus *Aspergillus* is mostly represented (41 publications) with a predominance of *Aspergillus fumigatus* and *Aspergillus flavus*.

Figure 10. Wordcloud representing the species name isolated in the liver. Using the online tool, Wordle (www.wordle.net), the size of the name of each species is proportional to the number of times it occurs in the repertory.



Urinary tract

In terms of urinary tract involvement, the vast majority of infections are renal (185 publications) with a predominance of the genus *Aspergillus* (85 publications) (Figure 11). These infections may be primary or secondary to the haematogenous dissemination of the fungus.

Figure 11. Wordcloud representing the species name isolated in the urinary tract. Using the online tool, Wordle (www.wordle.net), the size of the name of each species is proportional to the number of times it occurs in the repertory.

Osteo-articular system

Among the osteoarticular diseases, osteomyelitis is the most frequent (266 publications). The second most common form is joint damage (164 publications), which includes arthritis (57 publications), bursitis (one publication), unspecified joint damage (60 publications) and synovitis (46 publications). Spondylodiscitis comes in third place (71 publications). Among the species involved in these infections are species of the genus *Aspergillus* (*Aspergillus fumigatus*, *Aspergillus flavus* and *Aspergillus terreus*), *Scedosporium*/*Lomentospora* complex (*Pseudallescheria boydii*, *Scedosporium prolificans*, *Scedosporium apiospermum*) and *Fusarium solani* (Figure 12)

Figure 12. Wordcloud representing the species name isolated in the osteo-articular system. Using the online tool, Wordle (www.wordle.net), the size of the name of each species is proportional to the number of times it occurs in the repertory.

Skin system

Concerning the involvement of the cutaneous system, four affections stand out: superficial cutaneous infection (1,195 publications), subcutaneous infection (610 publications), onychomycosis (394 publications) and mycetoma (282 publications). *Aspergillus fumigatus* is mostly represented (Figure 13) and is mainly responsible for superficial (84 publications), and subcutaneous (37 publications) infections. The subcutaneous infections are mainly due to dematiaceous fungi (338 publications), with *Exophiala jeanselmei* coming in first position (47 publications). Melanised fungi are responsible for chronic infections such as chromoblastomycosis, or phaeohyphomycosis, which can evolve towards an invasive character (42). Eumycotic mycetoma are subcutaneous infections that we have chosen to put aside, mainly due to the species *Madurella mycetomatis* (67 publications) and *Madurella mycetomi* (25 publications). Among the agents mainly responsible for onychomycosis, we found *Scopulariopsis brevicaulis* (50 publications), *Hendersonula toruloidea* (28 publications), *Fusarium oxysporum* (22 publications), *Scytalidium dimidiatum* (20 publications), *Aspergillus niger* (18 publications) and *Fusarium solani* (17 publications). These species are responsible for distal and lateral subungual onychomycosis, the most common type of onychomycosis mainly affecting the toenails (185).

Figure 13. Wordcloud representing the species name isolated in the skin system. Using the online tool, Wordle (www.wordle.net), the size of the name of each species is proportional to the number of times it occurs in the repertory.

Endocrine glands

Among the endocrine glands, the thyroid is the most frequently affected (66 publications/84). In the majority of cases, the disease is secondary to a systemic dissemination of the pathogen, diagnosed post-mortem (186). In fact, infiltration of the thyroid with *Aspergillus* organisms occurs in approximately 20% of autopsies in patients dying with disseminated disease (187). However, a few rare cases of primary thyroid infections have been reported with *Aspergillus fumigatus*. Two cases of thyroid supuration in lupus patients treated with corticosteroids (188, 189), two cases described in HIV patients (190, 191), and one case in a child with chronic granulomatous disease (192). *Scedosporium apiospermum* was reported causing multiple thyroid abscesses in a patient with cirrhosis and autoimmune haemolytic anaemia, presenting swelling in the neck (193).

Anatomical sites rarely involved

Dental location. Endodontic infections were rarely found in this review and manifested themselves in the form of root involvement (194, 195), granuloma (196, 197) or gingival infections (198, 199). No species seemed to have a particular tropism for this sphere.

Genital sphere. Interestingly, the male genital sphere seems to be mostly affected, with 71.4% of the publications (15/21) reporting testicular, epididymal or glans involvement. Only three publications reported vaginitis (200–202), two reported labial involvement (188, 203) and one tubo-ovarian abscess (204). No species seemed to have a particular tropism for this sphere.

Breast. A majority of *Aspergillus* was isolated from this particular site. It should be noted that the *Aspergillus glaucus* and *Aspergillus niger* complexes have already been isolated from milk samples (205, 206). Interestingly, four publications reported fungal infections following breast implant surgery, a situation that is not well-known in this field, due to *Aspergillus niger* (207, 208), *Paecilomyces variotii* (209) and *Scedosporium apiospermum* (210).

Placental infection. A single case of placental aspergillosis due to *Aspergillus niger* was found in this literature review (211).

Conclusions

The aim of this study was to establish as exhaustive a catalogue as possible of filamentous fungi identified in humans by culture and molecular biology, whether or not they were associated with histopathological findings. We found 565 filamentous fungi identified in humans for which we specified the organs where these fungi had been found, and the semiology of the infections. This repertory thus helps to understand the pathogenic potential of certain fungi and can also alert clinicians that the isolation of certain rare fungi, such as *Trichoderma longibrachiatum* from stool specimens, can lead to a disseminated infection. One of the limitations of this work, however, is the lack of distinction between colonisation and infection, mainly for fungi isolated from non-sterile sites (i.e., the cutaneous system, pulmonary system, digestive system, ENT sphere). Fungi isolated from sterile sites were considered as infections (i.e., the heart, liver, ocular system, CNS). The use of new powerful molecular tools, such as pan-fungal PCR, metagenomic and next generation sequencing, now means it is possible to detect pathogens even in samples containing extremely low levels of nucleic acids (212, 213) and to diagnose mixed infections (214). However, the application of these tools to medical mycology can lead to interpretation difficulties (215–217). This problem is particularly

encountered with filamentous fungi, which are ubiquitous in the environment, and for which it is sometimes difficult to distinguish between colonisation, infection and environmental contamination. It is now necessary to go further in our work for each anatomical site, in order to distinguish colonisations from infections, in order to help clinicians interpret positive results and to assist with the diagnostic management of patients. Similarly, we did not distinguish between diagnosis by molecular biology and macroscopic identification, although it has been shown that potential errors were found in macroscopic identifications, mostly between close species within a section or species complex (4). Therefore, in Table 1, we have chosen to group species by sections, sharing morphological similarities, in order to bypass this limitation.

Finally, one of the limitations of this publication is its temporality. As explain in the material and methods section, only references present in PubMed before 16 June 2020 were take into account, in order to have the same PubMed content for all fungi species. However, medical mycology is dynamic and new organisms constantly need to be accounted for by both clinicians and microbiology laboratories (218). Moreover, with the COVID-19 pandemic, a new risk factor has emerged (219). Publications reporting the detection of filamentous fungi in humans have multiplied, reporting, for example, an emergence of mucormycosis throughout the world (220, 221). It will therefore be necessary to regularly update this data.

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Partie II : Amélioration du diagnostic des infections fongiques systémiques à levures

AVANT PROPOS

Cette seconde partie se concentre sur les infections fongiques systémiques à levures et plus particulièrement à l'amélioration du diagnostic de ces infections. Deux axes vont être traités : l'amélioration du diagnostic des candidémies et l'utilisation d'outils existants pour le diagnostic de levuroses invasives rares à *Trichosporon* sp..

Les fongémies à levures, représentent la première cause d'infections fongiques invasives en France avec une incidence en augmentation, estimée à 1,19 par 10 000 jours d'hospitalisation en 2018 (4). Ces infections sont des complications des patients hospitalisés en service de chirurgie, d'hématologie et de réanimation et présentent une létalité élevée de plus de 40% (4,10,59,60). Des données rétrospectives similaires de létalité ont été observées à l'assistance publique des hôpitaux de Marseille (AP-HM) (données non publiées). Sur la période 2015-2019, 518 patients hospitalisés à l'AP-HM ont présenté au moins un épisode de candidémie. L'âge moyen était de 58 ± 22 ans [0-95] et la létalité était de 44,0%.

Obtenir un large consensus dans le diagnostic des infections à levures a longtemps été un défi (61,62). Il est donc impératif que des tests de diagnostic soient développés et utilisés pour donner des résultats précis, permettant de débuter rapidement un traitement efficace. Malheureusement, le diagnostic de ces infections demeure complexe, en raison de la symptomatologie non spécifique, de l'hétérogénéité des populations de patients à risque et de la performance insuffisante des outils diagnostiques mis à disposition.

Bien que l' hémoculture reste le gold standard diagnostic des candidémies, la sensibilité est limitée, allant de de 21% à 71%, et le délai de positivité dépasse les 48 heures (63,64). La sensibilité dépend, entre autres, des incubateurs et des milieux utilisés. Dans un travail préalable, nous avions comparé le nombre d'hémocultures positives à *Candida* par rapport au nombre d'hémocultures prélevées chez le patient candidémique pour deux incubateurs et leurs flacons d'incubation respectifs (Figure 2). Le BD Bactec™ FX (BD, New Jersey, USA) a été évalué sur une pèriode de 15 mois et le BacT/ALERT® (Biomérieux, Marcy-l'Étoile, France) sur une pèriode de 18 mois. Si l'on considère qu'un test diagnostique idéal correspond à une hémoculture positive pour chaque hémoculture prélevée, nous avons pu constater que le BacT/ALERT® semble meilleur que le BD Bactec (Figure 2). Nous nous sommes également interressés au délai d'incubation des hémocultures positives à *Candida* entre 2019 et 2021 à l'IHU Méditerranée infection de Marseille, nous avons remarqué une moyenne de 34,5 heures

pour la détection de *Candida albicans*, 46,6 heures pour la détection de *Candida glabrata*, 25,2 heures pour la détection de *Candida krusei*, 40,4 heures pour la détection de *Candida parapsilosis* et 18,6 heures pour la détection de *Candida tropicalis*.

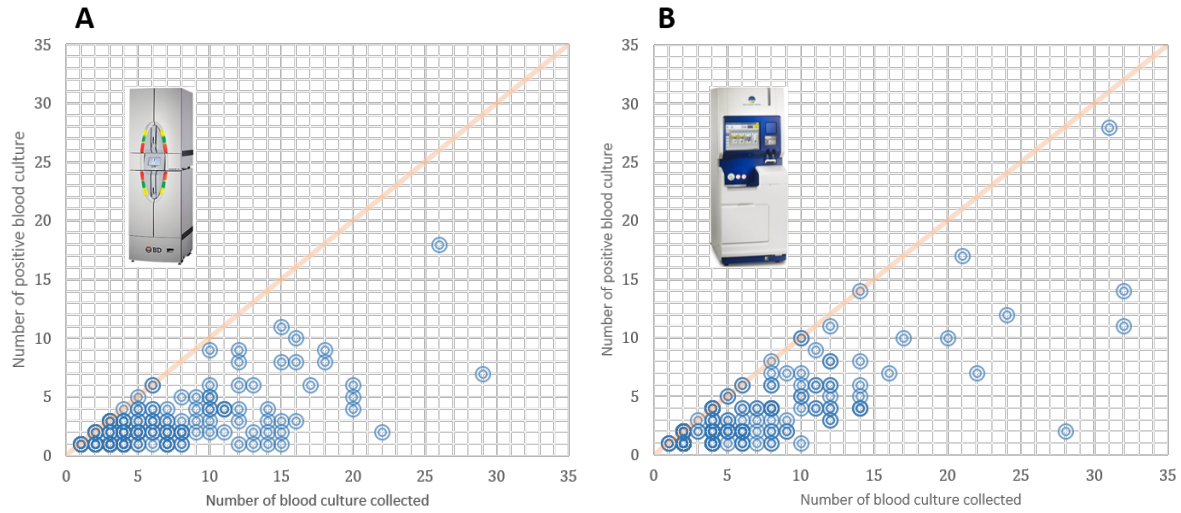

Figure 2. Nombre d'hémocultures positives à Candida en fonction du nombre d'hémoculture prélevée (A) avec l'automate BD Bactec sur la pèriode Janvier 2017-Mars 2018; (B) avec l'automate BacT/ALERT sur la pèriode Avril 2018-Octobre 2019.

Depuis une vingtaine d'années, les techniques de diagnostic moléculaire offrent la possibilité d'une détection rapide, sensible et spécifique des agents pathogènes (65). Ces outils moléculaires sont dépendants de la qualité et de la quantité d'ADN extrait et des prélèvements initiaux. A l'ère de l'approche syndromique du diagnostic des infections sanguines, la purification de l'ADN de divers organismes doit être effectuée simultanément en utilisant une seule méthode d'extraction. Dès le début de la pandémie de COVID-19, le laboratoire de microbiologie de l'Institut Hospitalo-Universitaire Méditerranée infection a eu la chance d'obtenir le prêt de nombreux automates d'extraction d'ADN et/ou d'ARN, utilisés en médecine humaine et/ou vétérinaire, afin de répondre à la quantité massive de prélèvements respiratoires (420 000 prélèvements durant l'année 2020). De ce fait, dans l'**Article 2** « *Evaluation of 11 DNA Automated Extraction Protocols for the Detection of the 5 Mains Candida Species from Artificially Spiked Blood* » nous avons évalué l'adéquation de 11 procédures automatisées récentes pour l'extraction de faibles et de fortes quantités d'ADN de *Candida* à partir d'échantillons de sang. Pour cela, nous avons artificiellement inoculé du sang-EDTA humain avec les cinq espèces de *Candida* majoritairement impliquées dans les candidémies (*Candida albicans*, *Candida glabrata*, *Candida parapsilosis*, *Candida tropicalis* and *Candida krusei*) afin d'obtenir des concentrations allant de 10 à 10^8 UFC/mL. Nous avons

identifié trois procédures d'extractions adaptées : NucliSENS™ EasyMAG™ (BioMérieux), EZ1™ DNA Blood 200 µL Kit avec pré-traitement (Qiagen) et EZ1™ DNA Tissue Kit avec pré-traitement (Qiagen). Notre étude a également mis en évidence des différences dans les performances des méthodes d'extraction en fonction des espèces de *Candida* testées.

Un des axes d'amélioration du diagnostic des candidémies reste le développement de nouveaux outils de détection. Dans cette partie, nous présentons les résultats préliminaires d'un test diagnostic élaboré à partir de la détection par capture des *Candida* en milieu liquide par l'intermédiaire de billes magnétiques recouvertes d'anticorps spécifiques. Ce projet en cours conduira à une publication à visée internationale, nous avons donc choisi de présenter les résultats préliminaires sous le format d'un **Article 3.** Cette technique de capture en solution allie des outils d'immunologie et de biologie moléculaire.

Bien que les *Candida* soient les principaux responsables de levuroses invasives, d'autres genres ont émergé ces dernières années parmi lesquels les *Trichosporon*. Ce genre compte 50 espèces dont 16 d'intérêt clinique (66). Ces basidiomycètes ubiquitaires sont largement répandus dans l'environnement et peuvent occasionnellement faire partie du microbiote de la cavité gastro-intestinale, vaginale et buccale chez l'Homme (66–70). *Trichosporon asahii* est l'espèce prédominante (71). Ce sont des pathogènes opportunistes rares qui peuvent être responsables de trichosporonoses superficielle ou invasive, difficile à diagnostiquer. L'**Article 4** « Pericardial Effusion Due to *Trichosporon japonicum*: A Case Report and Review of the Literature » est un *case report* représentant un exemple de situation clinique d'infection invasive à *Trichosporon japonicum*. A travers ce cas, nous explorons l'intérêt de réactions croisées avec des outils diagnostiques existants pour le diagnostic de ces infections. Certaines souches de *Trichosporon* ont du glucuronoxylomannane dans leur paroi cellulaire, ce qui peut entraîner une réaction croisée avec les antigènes de *Cryptococcus neoformans* et l'antigène galactomannane *d'Aspergillus* dans les sérums des patients atteints de trichosporonose invasive. Il est intéressant d'évaluer ces outils sur les infections invasives à *Trichosporon*. Dans ce *case report*, nous démontrons la présence d'une réaction croisée entre *Trichosporon japonicum* et la détection de l'antigène cryptocoque. Nous n'avons cependant pas observé de réaction avec l'antigène galactomannane *d'Aspergillus*. Le diagnostic des trichosporonoses repose uniquement sur la mise en évidence par culture du pathogène dans des échantillons de biopsies, qui peuvent être difficile à obtenir chez certains patients, ce qui présente un réel challenge. Par conséquent les méthodes de diagnostic non fondées sur la culture ont un intérêt majeur. En cas de suspicion, il est essentiel de combiner l'utilisation de la culture, de détection de l'antigène

galactomannane *d'Aspergillus* et de l'antigène cryptococcique, et l'instauration rapide d'un traitement antifongique adapté.

Article 2: Evaluation of 11 DNA automated extraction protocols for the detection of the 5 mains *candida* species from artificially spiked blood

Menu E., Landier J., Prudent E., Ranque S. et L'Ollivier C.

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RESUME

A l'ère de l'approche syndromique du diagnostic des infections sanguines, il est nécessaire d'utiliser des méthodes d'extraction polyvalentes capables de purifier simultanément l'ADN de plusieurs pathogènes (champignon, bactérie et virus).

Nous avons évalué la pertinence de 11 procédures automatisées récentes pour l'isolement de l'ADN de *Candida* à partir d'échantillons de sang artificiellement enrichis : NucliSENS™ EasyMAG™ (BioMérieux), EZ1™ DNA Blood 200 µL Kit (Qiagen), EZ1™ DNA Blood 200 µL Kit avec prétraitement (Qiagen), Kit EZ1™ DNA Tissue avec prétraitement (Qiagen), Kit QIAamp™ 96 DNA QIAcube HT (Qiagen), Macherey-Nagel™ Pathogène NucleoMag™ (Fisher Scientific), Mag-BindTM Viral DNA/RNA (Omega Biotek), MagMAX™ Viral/Pathogen Nucleic Acid Isolation Kit (Applied Biosystems), Chemagic Viral DNA/RNA 300 kit H96 (PerkinElmer), Virus DNA/RNA Extraction kit (MGI) et Bioextract™ Superball™ (Biosellal). Ces méthodes d'extraction ont été testées sur une large gamme séquentielle de concentrations de blastospores allant de 10 UFC/mL à 10^8 UFC/mL artificiellement inoculés dans du sang EDTA humain. Les faibles concentrations nous ont permis de mimer une situation clinique.

Ces techniques ont été évaluées pour les cinq espèces de *Candida* majoritairement impliquées en pathologie humaine (*Candida albicans*, *Candida glabrata*, *Candida parapsilosis*, *Candida tropicalis* and *Candida krusei*).

Cette étude nous a permis d'identifier trois procédures d'extractions adaptées à l'extraction des *Candida* à partir du sang: NucliSENS™ EasyMAG™ (BioMérieux), EZ1™ DNA Blood 200 µL Kit avec pré-traitement (Qiagen) et EZ1™ DNA Tissue Kit avec pré-traitement (Qiagen). Nous avons également observé des différences dans les performances des méthodes d'extraction en fonction des espèces de *Candida* testées.

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MDPI

Article

Evaluation of 11 DNA Automated Extraction Protocols for the Detection of the 5 Mains Candida Species from Artificially Spiked Blood

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Abstract: The molecular detection of *Candida* plays an important role in the diagnosis of candidaemia, a major cause of morbidity and mortality. The sensitivity of this diagnosis is partly related to the efficiency of yeast DNA extraction. In this monocentric study, we investigated the suitability of 11 recent automated procedures for the extraction of low and high amounts of *Candida* DNA from spiked blood. The efficacy of the DNA extraction procedures to detect *Candida* spp. in blood samples ranged from 31.4% to 80.6%. The NucliSENS^{TM} easyMAG^{TM} procedure was the most efficient, for each species and each inoculum. It significantly outperformed the other procedures at the lower *Candida* inocula mimicking the clinical setting. This study highlighted a heterogeneity in DNA extraction efficacy between the five main *Candida* species (*Candida albicans*, *Candida glabrata*, *Candida parapsilosis*, *Candida tropicalis* and *Candida krusei*). Up to five automated procedures were appropriate for *C. krusei* DNA extraction, whereas only one method yielded an appropriate detection of low amount of *C. tropicalis*. In the era of the syndromic approach to bloodstream infection diagnosis, this evaluation of 11 automated DNA extraction methods for the PCR diagnosis of candidaemia, puts the choice of an appropriate method in routine diagnosis within the reach of laboratories.

Keywords: *Candida*; candidaemia; DNA extraction

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1. Introduction

Candida species are among the top five pathogens associated with health-care bloodstream infections [1], carrying a high attributable mortality of up to 40% [2–4]. Major risk factors for developing candidaemia have well been identified and include critical illness, long-term stay in an intensive care unit, abdominal surgery, malignant haematologic diseases, intravenous catheter, parenteral nutrition, and administration of broad-spectrum antibacterial therapy [5]. Early diagnosis is critical for appropriate patient management and for improving candidaemia outcomes. Blood cultures, the current diagnostic gold standard, are limited by low sensitivity, ranging from 21% to 71% [6], and a slow turnaround, usually exceeding 48 h [6–8].

Recently, several in-house or commercially available polymerase chain reaction (PCR) assay kits have been developed. Generally, these assays target the five main *Candida* species involved in candidaemia: *Candida albicans*, *Candida glabrata*, *Candida parapsilosis*, *Candida tropicalis*, and *Candida krusei* [9,10]. In contrast to blood cultures, PCR allows for the rapid and specific detection of yeasts within whole blood, serum, or plasma samples, without requiring the sampling of large blood volumes and prior cultivation [10]. Interestingly, PCR in blood samples has shown >90% specificity, and up to 100% sensitivity. These

diagnostic indices are better than those of conventional blood culture, making PCR suited to the routine diagnosis of candidaemia [11–13]. In fact, PCR lends itself as a good tool for diagnosing candidaemia in high-risk patients. One of the cornerstones of the efficiency of PCR is DNA extraction, which is particularly dependent upon the quality and quantity of the initial material [14]. Thus, choosing an appropriate DNA extraction method is a critical step in the diagnostic laboratory workflow. DNA extraction needs to: (i) be highly efficient for DNA recovery from yeast, which are characterized by a highly complex and solid cell wall [15]; (ii) detect yeasts in low abundance, with a limit of one colony forming unit per milliliter (CFU/mL) [7]; and (iii) remove potential PCR inhibitors.

The extraction/purification of nucleic acids includes two primer steps as follows: cell lysis and separation of nucleic acids from lysate [16]. There are many automated DNA extraction methods available on the market with an efficiency which may be pathogen and/or sample matrix dependent [16,17], but no versatile method has yet been approved. The overall strategy concerning the DNA extraction of fungi is the use of an aggressive prior lysis step among cell lysis techniques including chemical lysis, mechanical lysis, ultrasonic lysis, thermal lysis and enzymatic lysis [16].

Moreover, in recent years, the diagnosis of candidaemia has been increasingly incorporated into a syndromic approach to bloodstream infection diagnosis. Thus, automated DNA extraction methods are pooled in a clinical laboratory and aim to detect a comprehensive array of microorganisms (such as viruses, bacteria and fungi) involved in blood-stream infections. It therefore appears to be essential to evaluate the efficacy of these methods. The main objective of this study was to evaluate the efficacy of eleven automated DNA extraction protocols on human blood specimens artificially spiked with *Candida* yeasts. The secondary objective was to compare the performance of these extraction protocols on the five main species implicated in candidemia (*Candida albicans*, *Candida glabrata*, *Candida parapsilosis*, *Candida tropicalis* and *Candida krusei*).

2. Materials and Methods

2.1. Preparation of Artificially Inoculated Blood

Blood samples spiked with *Candida* species were prepared as follows. *Candida albicans* ATCC 90,028, *Candida glabrata* (MH545,924), *Candida krusei* ATCC 6258, *Candida parapsilosis* ATCC 22,019 and *Candida tropicalis* (CP047,875) were cultured on Sabouraud dextrose agar plates supplemented with gentamicin and chloramphenicol (Bio-Rad, Marnes-la-Coquette, France) for 48 h at 30 °C. Yeasts cells were suspended in sterile saline solutions (API^{TM} NaCl 0.85% Medium, Biomérieux, Marcy-l'Etoile, France). A rich suspension of blastospores was prepared in a small volume of saline. Fresh EDTA-treated human blood from healthy blood donors (Convention N°7831, "Etablissement Français du Sang", Marseille, France) was inoculated with this suspension of yeasts cells to obtain eight concentrations of spiked blood samples: 0 CFU/mL, 10 CFU/mL, 50 CFU/mL, 10^2 CFU/mL, 10^3 CFU/mL, 10^4 CFU/mL, 10^6 CFU/mL and 10^8 CFU/mL. Infected blood specimens were then aliquoted in 200 µL and stored at −80 °C before DNA extraction.

2.2. DNA Extraction Methods

The DNA of each aliquot was extracted in duplicate using the following 11 extraction methods. The characteristics of each DNA extraction method are summarized in Table 1.

Method 1: DNA extraction was performed on 200 µL of whole blood inoculum by using a NucliSENS^{TM} easyMAG^{TM} system (BioMérieux, France) according to the manufacturer's instructions with a protocol optimized by pre-treatment [18]. In order to achieve maximum yield, a whole blood specimen was pre-treated in a tube containing ceramic beads (Lysing matrix D tube, MP Biomedicals Germany GmbH, Eschwege, Germany) with 500 µL of NucliSENS^{TM} easyMAG^{TM} lysis buffer (BioMérieux, Marcy-l'Etoile, France) and then disrupted in a FastPrep BIO 101 apparatus (Qbiogene, Strasbourg, France) at maximum power for 40 s. The tubes were then centrifuged at 10,000× *g* for one minute. The procedure was then performed on 200 µL of supernatant.

Table 1. Characteristics of the DNA extraction methods. The DNA extract measured by Nanodrop is expressed as a mean concentration (±standard deviation) C: chemical; M: mechanical; E: enzymatic; LB: NucliSENS™ easyMAG™ lysis buffer (BioMérieux, Marcy-l'Etoile, France); CB; ceramic beads; Ly: lyticase; PK: proteinase K; BALF: broncho-alveolar lavage fluid, DNA: deoxyribonucleic acid; RNA: ribonucleic acid.

Method	Kit	Company	Automate	Validated Sample	Validated Material	Nucleic Acids	Pretreatment			Sample Volume	Expected Elution Volume	Observed Elution Volume	Elution Appearance	DNA Quantification (ng/µL)
							C	M	E					
1	NucliSENS™ EasyMAG™	BioMérieux	NucliSENS EasyMAG	Human	Whole blood, serum, plasma, stools, respiratory samples and other body fluids.	DNA/RNA	LB	CB	-	200 µL	110 µL	110 µL	Clear	27.0 (±6.6)
2	EZ1™ DNA Blood 200 µL Kit	Qiagen	EZ1	Human	Whole blood	DNA	-	-	-	200 µL	100 µL	100 µL	Clear	30.5 (±5.5)
3	EZ1™ DNA Blood 200 µL Kit + pretreatment	Qiagen	EZ1	Human	Whole blood	DNA	LB	CB	-	200 µL	100 µL	100 µL	Clear	10.4 (±2.7)
4	EZ1™ DNA Tissue Kit + pretreatment	Qiagen	EZ1	Human	Whole dried blood, tissue, buccal cells, cultured cells, Paraffin-Embedded Tissue	DNA	-	-	Ly	200 µL	100 µL	100 µL	Clear	30.3 (±3.3)
5	QIAamp 96 DNA QIAcube HT Kit	Qiagen	QIAcube	Human	Whole blood, tissue, cells	DNA	-	-	PK	200 µL	120 µL	120 µL	Red tinted	30.2 (±6.6)
6	Macherey-Nagel™ Pathogène NucleoMag™	Fisher Scientific	KingFisher	Human	Whole blood, serum, plasma; tissue (e.g., ear notches); feces; swab wash solution	DNA/RNA	-	-	PK	150 µL	80 µL	80 µL	Clear	30.2 (±7.9)
7	Mag-Bind™ Viral DNA/RNA	Omega Bio-tek	KingFisher	Human	Whole blood, serum, plasma, saliva, and other body fluids.	DNA/RNA	-	-	PK	200 µL	100 µL	100 µL	Clear	84.5 (±29.0)
8	MagMAX™ Viral/Pathogen Nucleic Acid Isolation Kit	Applied Biosystems	MGISP-960	Human	Whole blood, swabs, urine, and viral transport media	DNA/RNA	-	-	PK	400 µL	100 µL	100 µL	Red tinted	32.2 (±35.3)
9	Chemagic Viral DNA/RNA 300 kit H96	PerkinElmer	Chemagic 360	Human	Serum, plasma, saliva, nasal or oral swab, BALF	DNA/RNA	-	-	PK	300 µL	100 µL	100 µL	Red tinted	17.8 (±19.5)
10	Virus DNA/RNA Extraction kit	MGI	MGISP-960	Human	Serum, plasma, saliva, virus culture medium, throat swabs, BALF, sputum	DNA/RNA	-	-	PK	200 µL	100 µL	25 µL	Clear	61.3 (±42.6)
11	Bioextract™ Superball™	Biosellal	KingFisher	Veterinary	Whole blood, milk, serum, organs	DNA/RNA	-	-	PK	200 µL	100 µL	100 µL	Clear	13.9 (±2.8)

Method 2: DNA extraction was performed directly on 200 µL of a whole blood specimen using the EZ1™ DNA Blood 200 µL Kit (Qiagen, Hilden, Germany) with the 10,591,402 V1.0 DNA blood card in an EZ1 Advanced XL extractor following the manufacturer's recommendations.

Method 3: DNA extraction was performed on 200 µL of a whole blood specimen by using the EZ1™ DNA Blood 200 µL Kit (Qiagen, Hilden, Germany) with the 10,591,402 V1.0 DNA blood card in an EZ1 Advanced XL extractor supplemented by the pre-treatment procedure used in Method 1.

Method 4: DNA extraction was performed on 190 µL of a whole blood inoculum using the EZ1™ DNA Tissue Kit (Qiagen, France) with the 10,677,990 V1.0 DNA bacteria card in an EZ1 Advanced XL extractor. In order to achieve maximum yield, the pre-treatment consisted of a digestion with 10 µL of Lyticase (25 units/µL, SigmaAldrich, Saint-Louis, MO, USA) at 30 °C for 30 min following the manufacturer's recommendations.

Method 5: DNA extraction was performed on 200 µL of a whole blood specimen using the QIAamp™ 96 DNA QIAcube HT kit (Qiagen, France) following the manufacturer's recommendations.

Method 6: DNA extraction was performed on 150 µL of a whole blood specimen using the Macherey-Nagel™ Pathogène NucleoMag™ kit (Fisherscientific, Waltham, MA, USA) in a KingFisher Flex (Thermofisher scientific, Waltham, MA, USA) following the manufacturer's recommendations.

Method 7: DNA extraction was performed on 200 µL of a whole blood specimen using the Mag-Bind™ Viral DNA/RNA kit (Omega Bio-tek, Norcross, GA, USA) in a KingFisher Flex (Thermofisher Scientific, France) following the manufacturer's recommendations.

Method 8: DNA extraction was performed on 400 µL of a whole blood specimen using the MagMAX™ Viral/Pathogen Nucleic Acid Isolation Kit (Applied Biosystems, Foster City, CA, USA) in a KingFisher Flex (Thermofisher scientific, France) following the manufacturer's recommendations.

Method 9: DNA extraction was performed on 200 µL of a whole blood specimen using the Chemagic Viral DNA/RNA 300 kit H96 (PerkinElmer, Waltham, MA, USA) in a Chemagic 360 instrument (PerkinElmer, Waltham, MA, USA) following the manufacturer's recommendations.

Method 10: DNA extraction was performed on 200 µL of a whole blood specimen using the Virus DNA/RNA Extraction Kit (Wuhan MGI Tech Co., Wuhan, China) in a MGISP-960 instrument (Wuhan MGI Tech Co., Wuhan, China) following the manufacturer's recommendations.

Method 11: DNA extraction was performed on 200 µL of a whole blood specimen using the Bioextract™ Superball™ kit (Biosellal, Dardilly, France) in a KingFisher Flex (Thermofisher scientific, France) following the manufacturer's recommendations.

2.3. Real Time PCR Assay

Following two extractions with each protocol, all DNA extracts (16 per method in total) were tested in duplicate by PCR. Real-time PCR was carried out using a pan-*Candida* primer set with a *Candida* spp.-specific probe targeting the internal transcribed spacer 2 (ITS2) region of nuclear ribosomal DNA [19]. Primer and probes sequences were provided by Eurogentec (Angers, France).

PCR assays were performed on a LightCycler™ 480 (Roche Diagnostics, Bâle, Switzerland) instrument. Individual real time PCR reactions were carried out in 17 µL of volume in a 96-well plate (Roche Diagnostic) containing 15 µL Master mix (Roche Diagnostics GmbH, Mannheim, Germany), 900 nM of forward primer (CCTGTTTGAGCGTCRTTT), 900 nM of reverse primer (TCCTCCGCTTATTGATAT), 250 nM of specific TaqMAn™ probe [*Candida albicans* (6FAM-TGCTTGCGGCGGTA), *Candida glabrata* (6FAM-TTTACCAACTCGGTGTTGAT), *Candida krusei* (6FAM-GCCGAGCGAACTAGACTTT), *Candida parapsilosis* (6FAM-GAAAG GCGGAGTATAAAC) or *Candida tropicalis* (6FAM-GGCCACCACAATTTATTTCA)] and 2 µL of DNA. No-template PCR controls were included in each run. The thermal cycling

conditions were 95 °C for 10 min, followed by 45 cycles of 95 °C for 10 s, 54 °C for 30 s and 72 °C for 10 s.

2.4. Detection of Inhibitors

To detect PCR inhibitors, all DNA samples were tested both pure and after a 20-fold dilution. The expected difference in Ct values between the two concentrations is 4.33 in the absence of inhibitors. Partial inhibition was defined as a less than 3.5 difference in Ct values [18].

2.5. DNA Quantification

Extracted DNA was quantified using the NanoDrop™ ND-1000 (Thermo Scientific, Waltham, MA, USA) according to the manufacturer's instructions.

2.6. Human Gene Amplification

The human albumin gene, as a DNA extraction control, was amplified in each DNA extract on a LightCycler™ 480 (Roche Diagnostics, France) instrument. Individual real time PCR reactions were carried out in 17 µL volume in a 96-well plate (Roche Diagnostic) containing 15 µL Master mix (Roche Diagnostics GmbH, Mannheim, Germany), 500 nM of forward primer (GCTGTCATCTCTTGTGGGCTGT), 500 nM of reverse primer (AAACTCATGGGAGCTGCTGGTTC), 250 nM of specific TaqMAn™ probe (6FAM-CTGTCATGCCCACACAAATCTCTCC). The thermal cycling conditions were 95 °C for 10 min, followed by 45 cycles of 95 °C for 10 s and 60 °C for 30 s.

2.7. Specimen Testing

For each *Candida* species, DNA of the eight blood inocula (i.e., 0, 10, 50, 10^2, 10^3, 10^4, 10^6 and 10^8 CFU/mL) was extracted in duplicate. Real time PCR for each of the 16 specimens was carried out in duplicate. PCR results were considered negative when the cycle threshold (Ct) value exceeded 45 or when no amplification curve was obtained.

When a single test for the four samples was positive, the tests were again, in order to eliminate cross contamination. A positive result was defined as at least two positive results among eight replicates of the same inoculum.

2.8. Determination of PCR Sensitivity

The efficiency of the *Candida* specific PCR was evaluated by plotting a standard curve with a serial 10-fold dilution (1 to 10^{10} copy number/µL) of plasmid DNA (20AD2FVC_Candida_PMA-RQ). The efficiency, slope and Y intercept were calculated with the LightCycler™ 480 Real-Time PCR System software.

2.9. Statistical Analysis

Statistical analysis was performed using STATA 14.0 and R version 4.0 (package {ggplot2}) [20] was used for additional graphical representation.

Negative inocula were annotated at 45 Ct, either the maximum number of PCR cycles performed. Mean Ct value and 95% confidence intervals (95% CI) were calculated for each DNA extraction method. Comparisons of the mean Ct values of human albumin amplification obtained with each method used the Kruskal–Wallis non-parametric test. The crude detection rate was defined as the number of positive *Candida* PCRs over the total number of reactions performed for a given method. The 95% CI were calculated, and detection rates were compared across methods using the Chi-square test.

To compare methods, the primary outcome was the detection of the pathogen by qPCR following extraction and coded as a binary variable (detected/non detected). The effects of the extraction method, the pathogen species and the concentration of the spiked sample were analyzed using multivariate multilevel logistical regression. In order to account for the correlation between the results for duplicate PCR testing of the DNA products obtained from a single extraction; a random effect was included at the extraction level. An

interaction between the pathogen and method was tested using the likelihood ratio test (model with interaction nested in model without). Sample concentrations were included as an independent covariable. For each pathogen, we ranked the methods based on their detection performance in relation to the method with the best overall detection using the adjusted odds-ratios, 95% confidence intervals (95%CI) and *p*-values adjusted for sample concentration obtained from the multivariate model. Extraction methods were considered as performing significantly worse than the reference if the upper limit of their 95% CI was below one. Apart from the reference, two methods were considered to be significantly different from one another if they had non-overlapping 95% CI.

3. Results

3.1. Determination of PCR Performances

The results of efficiency, slope and Y intercept for each species-specific PCR are as follows: *Candida albicans* (135%; −2.315; 29.51), *Candida glabrata* (102%; −3.230; 37.44), *Candida krusei* (98.7%; −3.386; 39.08), *Candida parapsilosis* (126%; −2.484; 30.84) and *Candida tropicalis* (111.7%; −2.864; 34.64).

3.2. Human Albumin Gene Amplification and DNA Quantification

The human albumin gene was amplified to constitute a complete process control for DNA extraction. The results were expressed as the mean of Ct values obtained for all DNA extracts from a given extraction method (Figure 1A). Considering an extraction method, the Ct values of albumin-PCR do not differ according to *Candida* species or the inocula tested. A significant difference was observed between the 11 DNA extraction methods ($p \leq 0.001$) (Kruskal–Wallis test). We observed three groups: Methods 1–5 with the lowest average Ct and narrow 95%CI, Methods 6–7 with the highest average Ct and narrow 95%CI, and Methods 8–11 with high Ct values and broad confidence intervals. Overall, Methods 1–5 performed significantly better than 6–7, and Methods 8–11 had intermediate performance. Using Nanodrop^{TM} to quantify the extracted DNA, a heterogeneity between the extraction methods was found. No correlation was observed between the Ct of albumin and the amount of template DNA in the sample. Thus, Method 7 gave the highest quantity of DNA yield (84.5 ± 29.0) while it is one of the methods with highest average Ct.

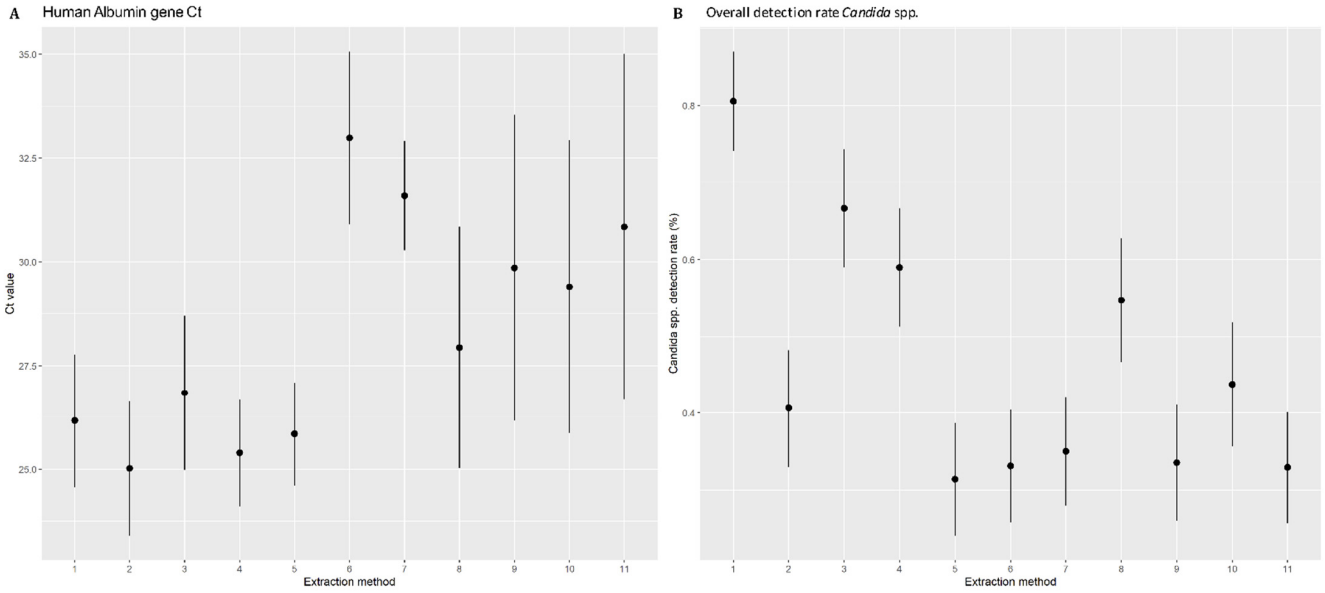

Figure 1. (**A**) Distribution of human albumin gene Ct according to the extraction methods tested. The means of the Cycle threshold and their 95% confidence interval are shown. (**B**) Distribution of the *Candida* species detection rate (in percentages) according to the extraction methods tested. The detection rates (in percentages) and their 95% confidence interval are shown.

3.3. Comparison of DNA Extraction Methods in Isolating Candida DNA from Spiked Blood

All negative control whole blood samples (n = 55) remained negative. The global performances of the automated DNA extraction of a range of yeast concentrations were expressed as an overall detection rate (Figure 1B). Only Method 1 yielded more than 80% positive results. Method 3 yielded more than 60% positive results. Methods 4 and 8 showed 59.0% and 54.7% positive results respectively. All the other methods had a detection rate below 50%, significantly lower than Methods 1, 3, 4 and 8 with positive results lower to 50%. Methods 5–7, 9 and 11 in particular presented detection rates of below 35%. Method 1 showed a homogenous distribution of Ct ranging from 15.8 to 40, rising with the gradient of the inoculum (Figure 2). Ct levels less than 20 were obtained only after DNA extraction with Methods 1, 3 and 4. Taking into account the positivity rate and the distribution of Ct, Method 1 appeared to be the most efficient, for all species and for all inocula and was therefore chosen as reference method for the next statistical analysis. Method 3, corresponding to Method 2 optimized with a mechanical pre-treatment protocol, also appears to yield acceptable results.

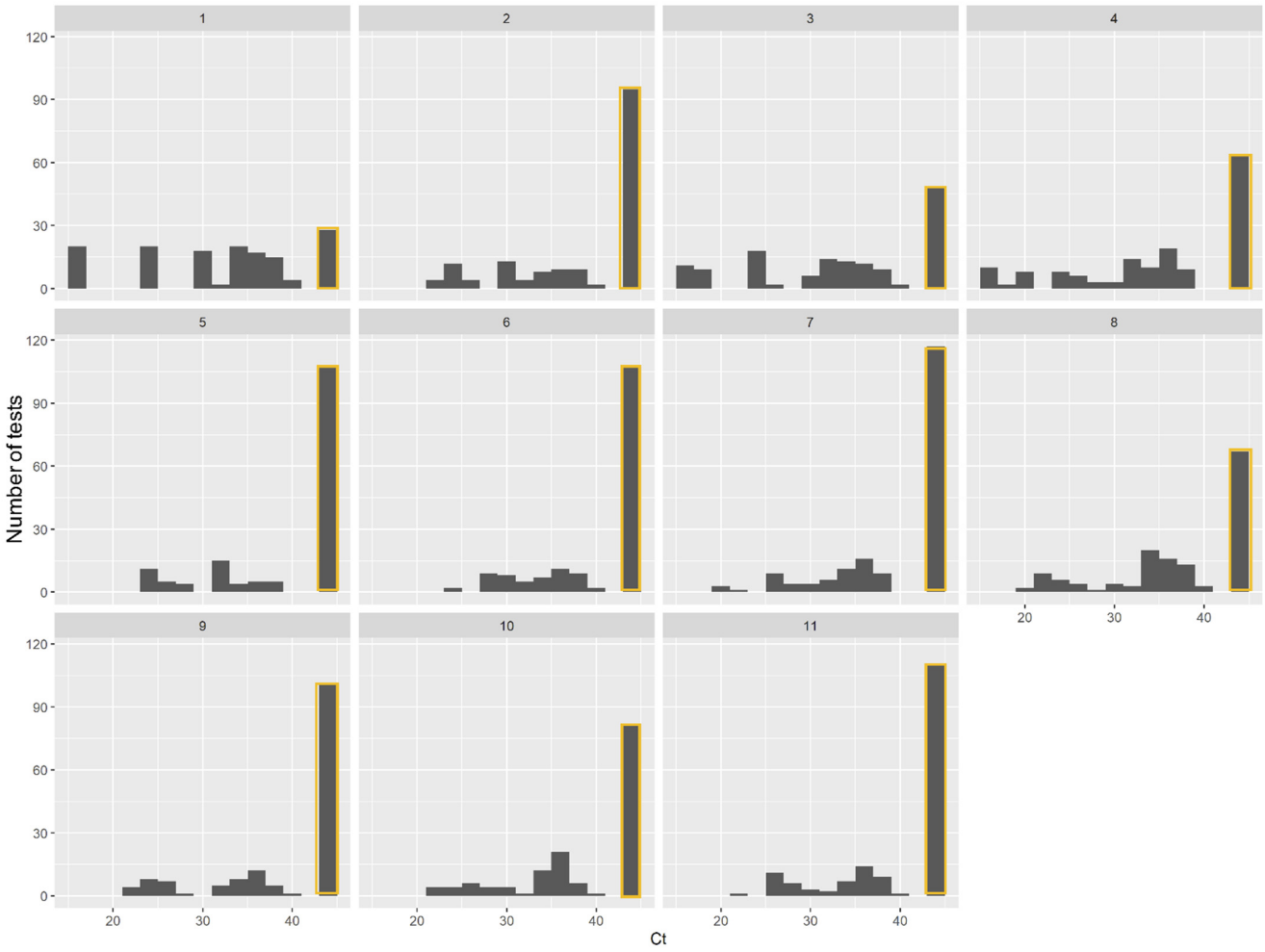

Figure 2. Number of positive *Candida* PCR test per Ct according to the 11 extraction methods. Negative results (Ct > 45 or no amplification detected) are stained orange.

3.4. Performance of DNA Extraction Methods Adjusted for Candida Species and Sample Concentration

All samples were positive for a concentration of 10^8 CFU/mL and this concentration was excluded in the subsequent analysis. We observed a significant difference (Chi-square test, $p \leq 0.001$) in DNA extraction efficiency between the five species tested: *Candida*

albicans, *Candida glabrata* complex, *Candida parapsilosis*, *Candida tropicalis* and *Candida krusei* (Figure 3).

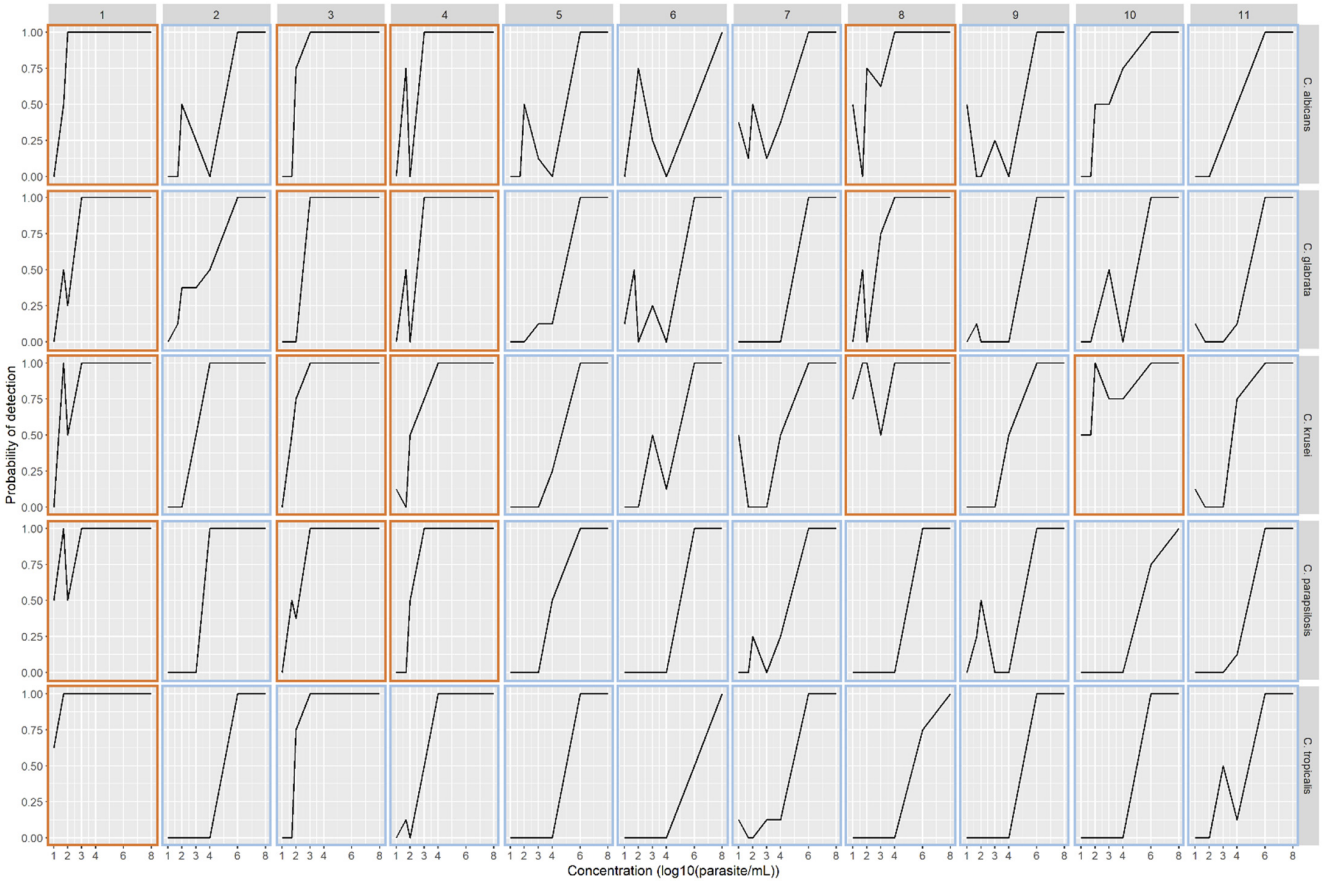

Figure 3. The probability of obtaining at least one PCR positive result (Ct < 45) on the duplicate from the two extractions for each inoculum concentration according to the *Candida* species and the automated method used.

The performances of automated protocols for DNA extraction for each *Candida* species were expressed as the odds ratio of PCR detection, adjusted for inoculum concentration using a multilevel logistical regression model. Method 1 was used as a reference method to make it easier to rank the other methods. A statistically significant interaction was identified between *Candida* species and method ($p < 0.0001$, likelihood ratio test), indicating that methods could perform differently for a given species, and results from the model with interaction are presented.

Only Method 1 was effective for *Candida tropicalis* DNA extraction, with a probability of detection >50%, even for the low amount of blastospores (i.e., 10 CFU/mL). Its performance for *C. parapsilosis* also appeared higher than in Methods 3 and 4, without reaching statistical significance. With regards to *C. albicans*, *C. glabrata* and *C. krusei* no significant difference between the reference Method 1 and Methods 3 and 4 in terms of DNA extraction efficiency was observed (Figure S1). *Candida krusei* appears to be less susceptible to extraction methods. After adjusting for blastospore concentration, no significant differences were observed in the detection of *C. krusei* between the reference Method 1 and Methods 3, 4, 8 and 10. Method 8 showed irregular efficiency for *C. albicans* and *C. glabrata* DNA extraction.

3.5. Detection of PCR Inhibitors

No inhibitors were detected in any DNA extract. All of the eleven extraction methods apparently performed equally well in eliminating PCR inhibitors.

4. Discussion

In this study, we evaluated the suitability of 11 recent automated procedures for the isolation of *Candida* DNA from artificially spiked blood samples: NucliSENS™ EasyMAG™ (BioMérieux) (Method 1), EZ1™ DNA Blood 200 µL Kit (Qiagen) (Method 2), EZ1™ DNA Blood 200 µL Kit with pre-treatment (Qiagen) (Method 3), EZ1™ DNA Tissue Kit with pre-treatment (Qiagen) (Method 4), QIAamp™ 96 DNA QIAcube HT Kit (Qiagen) (Method 5), Macherey-Nagel™ Pathogène NucleoMag™ (Fisher Scientific) (Method 6), Mag-Bind™ Viral DNA/RNA (Omega Biotek) (Method 7), MagMAX™ Viral/Pathogen Nucleic Acid Isolation Kit (Applied Biosystems) (Method 8), Chemagic Viral DNA/RNA 300 kit H96 (PerkinElmer) (Method 9), Virus DNA/RNA Extraction kit (MGI) (Method 10) and Bioextract™ Superball™ (Biosellal) (Method 11). These extraction methods were tested over a wide sequential range of blastospore concentrations from 10 CFU/mL to 10^8 CFU/mL. The detection of *Candida* DNA in spiked blood was performed by an *in-house* PCR targeting the ITS2 region of nuclear ribosomal DNA with an efficiency greater than 98% [19]. It should be noted that there are many in-house or commercially available real-time PCR assay kits targeting various genetic sequences (18S rDNA, 28S rDNA, 5.8S rDNA, ITS regions and mitochondrial DNA) [12,13,21,22]. All DNA extracts were tested in duplicate, which gave reproducible results in the majority of cases, for all methods and all concentrations, although there were gaps for high (>37) cycle thresholds.

As no consensus has been found concerning the best blood fraction to be tested for the diagnosis of candidaemia [23], like Metwalli et al. [24], we reasoned that inoculating fresh uninfected EDTA-treated human blood with *Candida* species, would best mimic the real conditions of candidaemia. This implies that the methods are suitable for extracting DNA from media that are rich in cells and PCR inhibitors [25]. The difference before and after 20-fold dilution demonstrates that all 11 methods were able to remove PCR inhibitors. Surprisingly, no correlation was found between the Ct values and the amount of template DNA in the sample. Concerning the Chemagic Viral DNA/RNA 300 kit H96 (PerkinElmer), probably the red tinted sample disrupted DNA quantification. Few studies comparing extraction methods quantify DNA, suggesting that house-keeping gene amplification is a better of evaluating extraction efficiency. However, we observed that the extraction methods were not equally efficient in isolating human DNA. We thus distinguished two main groups, the best results being obtained with the four Qiagen automated procedures namely: EZ1™ DNA Blood 200 µL Kit (Qiagen), EZ1™ DNA Blood 200 µL Kit with pre-treatment (Qiagen), EZ1™ DNA Tissue Kit with pre-treatment (Qiagen), QIAamp™ 96 DNA QIAcube HT Kit (Qiagen) and NucliSENS™ EasyMAG™ (BioMérieux). With the exception of the Chemagic Viral DNA/RNA 300 kit H96 (PerkinElmer) and Virus DNA/RNA Extraction Kit (MGI), all the extraction methods tested were designed for use on whole blood. The Bioextract™ Superball™ (Biosellal), is provided for veterinary laboratories and exhibits higher Ct values with respect to human albumin gene amplification.

Few studies have compared current automated nucleic acid extraction methods for the isolation of DNA of the five main *Candida* species from whole blood [24,26–28]. Here, all 11 extraction methods were equivalent at concentrations greater than 10^6 CFU/mL. At the lowest concentration (between 10 CFU/mL and 100 CFU/mL), the NucliSENS™ easyMAG™ (BioMérieux) procedure stood out significantly from the ten other methods. We specifically tested low blastospore concentration, as these concentrations are relevant in a clinical setting. The NucliSENS™ easyMAG™ system had previously been optimized to allow for the extraction of fungal DNA [16]. Interestingly, no significant difference was observed between the EZ1™ DNA Blood 200 µL Kit with pre-treatment (Qiagen) and the EZ1™ DNA Tissue Kit with pre-treatment (Qiagen) with regard to the detection rate (66.7% and 59.0% respectively). These two methods were optimized by the addition of a pre-treatment comprising chemical and mechanical lysis for one, and enzymatic lysis for the other. The extraction procedure EZ1™ DNA Blood 200 µL kit was evaluated with and without pre-treatment. Optimization of the protocol by adding a chemical and mechanical pre-treatment have be relevant with a significant improvement in the *Candida* detection rate,

especially for concentrations lower than 10^4 CFU/mL (40.6% without pre-treatment vs. 66.7% with pre-treatment). This is in line with a previous study that clearly demonstrated that introducing a bead beating step to the EZ1 procedure improved fungal DNA extraction from human specimens [28]. *Candida* species have particularities concerning their yeast cell wall, which must have an impact on their lysis susceptibility. The QIAamp™ 96 DNA QIAcube HT Kit (Qiagen), Macherey-Nagel™ Pathogène NucleoMag™ (Fisher Scientific), Mag-Bind™ Viral DNA/RNA (Omega Biotek), Chemagic Viral DNA/RNA 300 kit H96 (PerkinElmer) and Bioextract™ Superball™ (Biosellal) resulted in the worst efficiencies, with less than 35% of the sample being detected. It should be noted that some of them were validated for viral DNA. Interestingly, the performances of extraction methods when amplifying the albumin gene mirror those performance in *Candida* PCR. This paper highlights a difference in DNA extraction efficiency between the five different species mainly involved in invasive candidiasis. For *C. albicans*, *C. glabrata* and *C. krusei* DNA extraction, three methods, namely NucliSENS™ easyMAG™, EZ1™ DNA Blood 200 µL Kit with pre-treatment and EZ1™ DNA Tissue Kit with pre-treatment, are equivalent and effective. It is notable that, for the species *C. tropicalis*, only the NucliSENS™ easyMAG™ procedure has been shown to be effective with a probability of detection >50%, even for the low amount of blastospore (i.e., 10 CFU/mL). The difference in efficiency of the DNA extraction methods according to *Candida* species may be explained by the difference in matrix and composition of their wall in terms of filamentation capacity, the quantity of matrix carbohydrates, protein, and also its cell-surface hydrophobicity [29]. These characteristics, specific to each species, have been evaluated in their biofilm-forming capacity. Thus, *Candida tropicalis* has shown a higher biofilm-forming capacity than *C. krusei*, *C. parapsilosis* and *C. albicans*, characterized by high hydrophobicity, and its ability to form a very dense and intertwined biofilm [29], which may explain the need for more aggressive DNA extraction. The difference between species can also be related to a heterogeneous compatibility between certain extraction methods and a given PCR species assay. Despite these differences, NucliSENS™ easyMAG™ yielded the best results for the five *Candida* species.

Finally, in the era of the syndromic approach to bloodstream infection diagnosis, the purification of DNA from various organisms must be performed simultaneously using this type of extraction system with just a single extraction method. It is, therefore, necessary to evaluate these automated pieces of equipment for each pathogen (fungus, bacteria and virus). Of the procedures tested in this study, seven allowed for the simultaneous extraction of both DNA and RNA, including the NucliSENS™ easyMAG™ procedure. This versatile technique thus stands out from the other techniques tested here, allowing for the efficient isolation of DNA from the five species of *Candida* involved in human pathology.

5. Conclusions

The present study aimed to evaluate eleven automated DNA extraction protocols on artificially spiked blood specimens with the five main *Candida* yeasts for the suitable routine diagnosis of invasive candidaemia. This is the first study to demonstrate a difference in DNA extraction performance between *Candida* species (*Candida albicans*, *Candida glabrata* complex, *Candida parapsilosis*, *Candida tropicalis* and *Candida krusei*). Fortunately, one extraction method (i.e., NucliSENS™ easyMAG™ (BioMérieux)) displayed adequate performance for detecting the DNA of five *Candida* species in whole blood samples, which is mandatory for the current syndromic diagnosis of bloodstream infections.

Supplementary Materials: The following are available online at https://www.mdpi.com/2309-608X/7/3/228/s1, Figure S1: adjusted odds ratio for *Candida* spp. DNA detection according to 11 automated extraction protocols.

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Preliminary results - Article 3: Solution capture of *Candida* yeast

RESUME

Nous présentons sous le format d'un article les résultats préliminaires obtenus pour le développement de deux outils diagnostiques pan-*Candida*. Ces deux techniques reposent sur la détection de levures de type *Candida* par des anticorps pouvant se lier à des antigènes communs entre différentes espèces appartenant à ce genre.

D'une part, nous avons évalué l'intérêt de l'immuno-PCR en plaque, outil diagnostique inovant similaire à la méthode ELISA mais faisant intervenir un anticorps lié à un fragment d'ADN qui est amplifié par PCR. Dans un second temps, nous nous sommes orientés vers une approche fondée sur une méthode de capture immunomagnétique des levures *Candida* en solution, suivi par une detection par PCR.

INTRODUCTION

Les *Candida* sont des micromycètes opportunistes appartenant aux communautés microbiennes commensales de l'Homme (1,2). Ils peuvent être responsables de fongémies, qui demeurent la première cause d'infections fongiques systémiques (IFS) dans le monde (3). Cinq espèces de *Candida* sont responsables de 90% des fongémies: *C. albicans*, *C. glabrata*, *C. krusei*, *C. tropicalis*, et *C. parapsilosis* (4). Cependant les 10% restant ne sont pas à négliger. Dans les populations pédiatriques par exemple, plusieurs études s'intéressant à la distribution des espèces de *Candida* dans les candidémies ont rapporté une prévalence de *Candida lusitaniae*, *Candida famata*, *Candida utilis* et *Candida kefyr* supérieure à celle de *Candida glabrata* et *Candida krusei* (5,6). De même, dans des cohortes de patients d'hématologie, *Candida lusitaniae* arrive en troisième position des *Candida* responsables de candidémies (7). Ces infections sont difficiles à diagnostiquer en raison de leurs symptomatologies non spécifiques et des limites des méthodes de diagnostic. Les hémocultures, « gold standard » diagnostic des candidémies, ont une sensibilité limitée, allant de de 21% à 71%, et un délai de positivité dépassant les 48 heures (8,9). Pourtant, un retard dans l'initiation du traitement antifongique représente une perte de chance pour le patient (10). Les méthodes moléculaires « maison » et commercialisées, basées sur la détection de l'ADN par PCR sont limitées aux cinq espèces majoritairement impliquées en pathologie humaine (11–13). Le développement d'outils diagnostiques pan-*Candida* présenterait donc un intérêt majeur.

L'immuno-PCR nous a semblé être une approche prometteuse, car elle repose sur la détection par des anticorps pouvant se lier à des antigènes communs entre *Candida*. Cette technique est similaire à la méthode ELISA mais fait intervenir un anticorps lié à un fragment d'ADN, par conjugaison streptavidine-biotine ou par une liaison covalente, et qui est amplifié par PCR (14). Développée et présentée par Sano *et al*. en 1992 (15), cette technologie a déjà montré son intérêt dans le diagnostic d'infections virales (16,17), bactériennes (18–20) et parasitaires (21). Dans ce projet, nous avons donc décidé de mettre au point une technique d'immuno-PCR et de l'évaluer dans le diagnostic de candidémie.

Dans un second temps, nous nous sommes orientés vers une approche fondée sur une méthode de capture immunomagnétique des levures *Candida*, comme décrite par Apaire-Marchais *et al*. (22), suivie par une détection par PCR des levures ainsi isolées du milieu liquide initial.

APPROCHE 1 : IMMUNO-PCR

Matériel et méthodes

Les tests décrits ci-dessous détaillent les étapes de mise au point réalisées au préalable, en partant de barrettes pré-coatées avec des blastospores de *Candida albicans* afin d'ajuster les concentrations en anticorps primaire, secondaire et en ADN biotinylé.

Préparation de l'ADN biotinylé

L'ADN biotinylé à lier à l'anticorps secondaire a été préparé en amplifiant par PCR une séquence du plasmide pUC19 avec l'amorces 1 biotinylée en 5' (5'-biotin_ATTGTTGCCGGGAAGCTAGAGTAAGTAGTT-3') et l'amorce 2 (TATGCAGTGCTGCCATAACCATGA) (Eurogentec (, France) comme décrit par Niemeyer et al. (23).

Préparation des plaques coatées par Candida albicans

Un inoculum de concentration variable de *Candida albicans* (ATCC90028) a été réalisé dans une solution saline tamponnée au phosphate (PBS) (Gibco^{TM}, Thermo Fisher Scientific, Kanagawa, Japon). 100µL d'inoculum ont été déposé par puits en utilisant les barrettes TopYield (Thermo Fisher Scientific, Kanagawa, Japon) et incubées 12 heures à 37°C.

Puis les levures ont été fixées avec 100µL de paraformaldéhyde (PFA) à 3,2% (AlfaAesar, Thermo Fisher Scientific, Ward Hill, Etats-Unis) et le tout était lavé au PBS.

Saturation des barrettes

Les barrettes TopYield (Thermo Fisher Scientific, Kanagawa, Japon) ont été saturées à 4°C pendant 12 heures par différentes solutions de saturation. Condition 1 : TBS-TRITON 0.01% à pH 8.0, ADN de sperme de saumon (Salmon Sperm DNA Solution, Thermo Fisher Scientific, Carlsbad, Etats-Unis), sérum de chèvre (Goat serum, Dominique Dutscher, Bernolsheim, France). Condition 2 : TBS-TRITON 0.01% à pH 8.0, ADN de sperme de saumon (Salmon Sperm DNA Solution, Thermo Fisher Scientific, Carlsbad, Etats-Unis), lait en poudre (Régilait, Saint-Martin-Belle-Roche, France), BSA 0,5%. Condition 3 : TBS-TRITON 0.01% à pH 8.0, ADN de sperme de saumon (Salmon Sperm DNA Solution, Thermo Fisher Scientific, Carlsbad, Etats-unis), sérum de chèvre (Goat serum, Dominique Dutscher, Bernolsheim, France), lait en poudre (Régilait, Saint-Martin-Belle-Roche, France). Puis les barrettes ont été lavées 4 fois par une solution de Tween 0,05%/EDTA 5 mM/Tris-buffered saline buffer 1X (TE-TBS).

Chaque condition a été réalisée en duplicata.

Immuno-PCR en plaque

Cent microlitres d'anticorps primaire type IgM monoclonal de souris A97H anti-*Candida albicans* (Thermo Fisher Scientific, Carlsbad, Etats-unis) dilué au 1/1000e dans tampon de dilution (TETBS) ont été ajouté par puits. Les puits étaient ensuite couverts avec de la bande adhésive et incubés 1 heures 30 minutes à 37°C sous agitation. Une série de 5 lavages était ensuite réalisée en remplissant les cupules de solution de TETBS (les 2 derniers lavages durant 10 minutes). Un témoin négatif comprenant uniquement 100 µL de tampon de dilution (TETBS) a également été réalisé.

Puis, 100 µL d'anticorps secondaire type IgG biotinylé anti-IgM de souris (Biotin-SP-conjugated AffiniPure Goat Anti-Mouse IgM, µ Chain specific, Jackson Immuno Research Labs, West Grove, Etats-Unis) dilué au 1/10 000e dans du tampon de dilution (TETBS) ont été ajoutés dans chaque puits (témoin neg sans anticorps primaire inclus). Les puits étaient ensuite couverts avec de la bande adhésive et incubés 1 heures 30 minutes à 37°C sous agitation. Une série de 5 lavages était ensuite réalisée en remplissant les cupules de solution de TETBS (les 2 derniers lavages durant 10 minutes).

Liaison ADNb-Neutravidine- Anticorps secondaire extemporanée

Dans chaque puits, 60 µL de solution de neutravidine (Thermo Fisher Scientific, Carlsbad, Etats-Unis) à une concentration de 4,25 nM ont été ajoutés. Les puits étaient ensuite couverts avec de la bande adhésive et incubés 45 minutes à température ambiante sous agitation, suivi de 5 lavages au TETBS. Puis, 0,25 pmol d'ADN biotinylé était ajouté par puits pour obtenir un ratio molaire (ADNb :Nv) de 1 :1. Les puits étaient ensuite couverts avec de la bande adhésive et incubés 45 minutes à Température Ambiante sous agitation. Une série de 7 lavages au TETBS (les 2 derniers lavages doivent durer 10 minutes) a été réalisée. Puis un lavage prolongé a été réalisé avec du TETBS incubé pendant 12 heures à 4°C.

Libération de l'ADN

Une étape de digestion enzymatique avec l'enzyme BamHI (Promega, Madison, Etats-Unis) était ensuite réalisée comme suit : après élimination de la solution de lavage par puits était ajouté un mélange de 34,6µL d'eau stérile, 4µL de RE 10X Buffer et 0,4µL de BSA puis 1µL d'enzyme de restriction. Les puits étaient ensuite couverts avec de la bande adhésive et incubés 2 heures à 37°C.

Détection de l'ADN par PCR

Les analyses PCR ont été réalisées sur un instrument LightCycler^{TM} 480 (Roche Diagnostics, Bâle, Suisse). Les réactions de PCR en temps réel ont été réalisées dans un volume de 17 µL dans une plaque à 96 puits (Roche Diagnostics) contenant 15 µL de Master mix (Roche Diagnostics GmbH, Mannheim, Allemagne), 900 nM d'amorce F2 directe (CAGCAATAAACCAGCCAGCC) (24), 900 nM d'amorce 2 réverse (TATGCAGTGCTGCCATAACCATGA) (23), 20 nM de sonde TaqMAn^{TM} (5'-FAM-ACCGAAGGAGCTAACCGCTTTTTTGCAC-TAMRA-3') et 2 µL d'ADN libéré à l'étape précédente. Chaque extrait d'ADN a été déposé en duplicata.

Afin d'apprécier les fixations non spécifiques, chaque étape de l'immuno-PCR a été évaluée indépendamment : Témoin Ø I^{re} : Manipulation réalisée à partir de l'étape anticorps IIre ; Témoin Ø I^{re} Ø II^{re} : Manipulation réalisée à partir de l'étape neutravidine ; Témoin Ø I^{re} Ø II^{re} Ø Nv: Manipulation réalisée à partir de l'étape ADNb.

Un témoin dit « blanc » a également été testé, en réalisant l'ensemble des étapes de l'immuno-PCR dans un puits non coaté par *C. albicans*.

Les conditions de cyclage thermique étaient les suivantes : 94 ◦C pendant 30 secondes, suivi de 40 cycles de 94 ◦C pendant 30 secondes, 54 ◦C pendant 30 secondes et 72 ◦C pendant 1 minute.

Résultats

En raison du faible nombre d'échantillons, les résultats sont présentés sous la forme de moyenne et d'intervalle, les écart-type ne pouvant être calculés.

Malgré l'utilisation de différentes solutions de saturations, empêchant les fixations non spécifiques, nous n'avons pas réussi à nous affranchir du bruit de fond lié à la fixation non spécifique de l'anticorps secondaire et/ou à l'élimination insuffisante de l'ADN biotinylé (Table 1).

Table 1. **Ct obtenus avec les trois conditions de saturations testées avec deux concentrations en *Candida albicans* de 1000 et 10 000 UFC/mL**. qRT-IP : Ensemble des étapes d'immuno-PCR réalisée ; Témoin Ø Ire : Manipulation réalisée à partir de l'étape anticorps IIre ; Témoin Ø Ire Ø IIre : Manipulation réalisée à partir de l'étape neutravidine ; Témoin Ø Ire Ø IIre Ø Nv: Manipulation réalisée à partir de l'étape ADNb; m : moyenne des Ct ; NR : Non réalisé.

		Condition 1		**Condition 2**		**Condition 3**	
		m	Intervalle [min-max]	m	Intervalle [min-max]	m	Intevalle [min-max]
1 000 UFC/mL	qRT-IP	18,95	[18,65-19,21]	18,54	[18,44-18,69]	19,08	[18,87-19,44]
	Témoin Ø I^{re}	20,41	[20,01-20,87]	19,78	[19,35-20,27]	20,24	[20,05-20,41]
	Témoin Ø I^{re} Ø II^{re}	NR	NR	21,22	[20,31-22,11]	NR	NR
	Témoin Ø I^{re} Ø II^{re} Ø Nv	NR	NR	24,59	[22,95-26,25]	NR	NR
10 000 UFC/mL	qRT-IP	16,84	[16,36-17,26]	16,15	[16,00-16,33]	16,04	[15,99-16,10]
	Témoin Ø I^{re}	19,17	[19,12-19,25]	19,13	[19,09-19,20]	19,04	[18,83-19,26]
	Témoin Ø I^{re} Ø II^{re}	NR	NR	19,41	[19,40-19,42]	NR	NR
	Témoin Ø I^{re} Ø II^{re} Ø Nv	NR	NR	26,16	[26,09-26,23]	NR	NR
0 UFC/mL	Blanc	NR	NR	33,29	[29,81-35,00]	NR	NR

APPROCHE 2 : CAPTURE EN SOLUTION

Pour cette deuxième approche nous avons testé trois combinaisons de billes magnétiques – anticorps spécifiques (Table 2).

Table 2. Combinaisons de billes magnétiques/Anticorps testées.

	Billes magnétiques	
Anticorps spécifiques	CELLection™ Pan Mouse IgG Dynabeads (Thermo Fisher Scientific, Kanagawa, Japon)	MagnaLink™ 4FBMagnetic Beads (Solulink, Inc, Le Perray en Yveline, France)
IgG monoclonal anti-bêta-(1-3)-D-glucane de souris (anticorps recombinant Creative Biolabs, New-York, Etats-Unis)	•BiAc1	
IgG monoclonal anti-*Candida albicans* de souris (Meridian Lifescience, Memphis, Etats-Unis)	•BiAc2	
IgM monoclonal MAb 5B2 (Lille, France)		•BiAc3[1]

[1]Le couplage BiAc3 a été réalisé par le laboratoire Tebu-Bio (Le Perray en Yvelines, France).

Matériel et méthodes

Préparation de la suspension de levures

Une solution saline tamponnée au phosphate (PBS) (Gibco™, Thermo Fisher Scientific, Kanagawa, Japon) inoculée avec des espèces de *Candida* a été préparée extemporanément comme suit. *Candida albicans* (ATCC 90028), et *Candida tropicalis* (CP047,875) ont été cultivés sur des plaques de gélose Sabouraud dextrose supplémentées en gentamicine et en chloramphénicol (Bio-Rad, Marnes-la-Coquette, France) pendant 48 heures à 30 °C. Les levures ont été mises en suspension dans du PBS (GibcoTM, Thermo Fisher Scientific, Kanagawa, Japon) et comptées sur des cellules de Kova (KOVA Glasstic®, Kova international, Swatar, Malte) pour obtenir quatre concentrations d'inocula : 250 UFC/mL, 500 UFC/mL, 1000 UFC/mL et 5000 UFC/mL.

Couplage billes – anticorps spécifiques

L'anticorps type IgM monoclonal 5B2 nous a été aimablement donné par le laboratoire de recherche « Glycobiology in Fungal Pathogenesis & Clinical Applications » (Inserm U1285 CNRS UMR 8576) dirigée par le Professeur Boualem Sendid (Lille, France). Les billes

immunomagnétiques (MagnaLink^{TM} 4FB Magnetic Beads, Solulink, Inc, Le Perray en Yveline, France) recouvertes de MAb 5B2 (1 mg/ml) (BiAc3) ont été obtenues auprès de Tebu-Bio (Le Perray en Yvelines, France).

Pour les autres anticorps, la procédure suivie est détaillée ci-dessous.

Les billes magnétiques (CELLection^{TM} Pan Mouse IgG Dynabeads, Thermo Fisher Scientific, Kanagawa, Japon) ont été lavées avec 1 mL de solution PBS-BSA à 1% pendant 1 heure à température ambiante avec agitation rotative, lavées 3 fois avec du PBS et remises en suspension dans le Tampon 1 (PBS supplémenté avec 0,1% de BSA à pH 7,4). Ensuite, 25µL ont été préincubés avec 0,3 µg d'anticorps monoclonal anti-bêta-(1-3)-D-glucane de souris (anticorps recombinant Creative Biolabs, New-York, Etats-Unis) (BiAc1) ou d'un anticorps monoclonal anti-*Candida albicans* de souris (Meridian Lifescience, Memphis, Etats-Unis) à (BiAc2) pendant 1 heure à température ambiante avec agitation rotative.

Validation de l'étape de fixation des anticorps aux billes magnétiques par immunofluorescence (BiAc2)

Les billes magnétiques (CELLection^{TM} Pan Mouse IgG Dynabeads, Thermo Fisher Scientific, Kanagawa, Japon) ont été lavées avec 1 mL de solution PBS-BSA à 1% pendant 1 heure à température ambiante avec agitation rotative, lavées 3 fois avec du PBS et remises en suspension dans le Tampon 1. 25µL ont été pré-incubés avec anticorps monoclonal anti-*Candida albicans* de souris (Meridian Lifescience, Memphis, Etats-Unis) à 0,3 µg pendant 1 heure à température ambiante avec agitation rotative. Après lavage avec le tampon 1 (3 lavages), les billes ont été remises en suspension dans 20µL d'anticorps secondaire anti-IgG de souris couplés à la l'isothiocyanate de fluorescéine (Goat anti-Mouse IgG (H+L) Secondary antidbody FITC, Thermo Fisher Scientific, Kanagawa, Japon) dilués au 1/50^{e} dans du PBS puis la solution a été incubée pendant 1 heure à 37°C avec agitation rotative. Une nouvelle étape de lavage avec le tampon 1 (3 lavages) a été réalisée. Les billes couplées ont été déposées entre lame et lamelle avec de la glycérine tamponnée. Le montage a ensuite été observé au microscope à fluorescence avec un filtre Fs09 (Zeiss, Oberkochen, Allemagne) présentant une longueur d'onde d'excitation entre 450 et 490 nm et une longueur d'onde d'émission supérieure à 515 nm.

Un témoin négatif a été réalisé en parallèle avec ajout uniquement de l'anticorps secondaire couplé à la fluorescéine aux billes magnétiques.

Capture immunomagnétique en solution des levures

Après lavage avec le tampon 1, 100 µl d'une suspension de levures aux 3 concentrations différentes ont été ajoutés aux couples BiAc retenus par l'aimant. Le tout a été incubé pendant 3 heures à 37°C dans un incubateur rotatif à 600 rpm (ThermoMixer F1.5, Eppendorf, Montesson, France). Le surnageant a été jeté après que la suspension cellulaire eut été placée près de l'aimant (DynaMag™-2, Invitrogen, Thermo Fisher Scientific, Kanagawa, Japon) pendant 2 minutes afin de retenir les billes magnétiques ayant fixées les levures. L'extraction de l'ADN à partir des levures piégées a ensuite été réalisée directement sur les billes.

Chaque couple BiAc a été testé en duplicata pour chaque concentration de levure.

Extraction de l'ADN

Après l'étape de capture, les BiAc ont été placées dans un tube contenant des billes de céramique (tube Lysing matrix D, MP Biomedicals Germany GmbH, Eschwege, Allemagne) avec 500 µL de tampon de lyse NucliSENS^{TM} easyMAG^{TM} (BioMérieux, Marcy-l'Etoile, France), puis elles ont été désagrégées dans un appareil FastPrep BIO 101 (Qbiogene, Strasbourg, France) à puissance maximale pendant 40 secondes. L'extraction d'ADN a été réalisée sur 200µL du surnageant en utilisant les kits de sang ADN EZ1&2^{TM} (Qiagen, Hilden, Allemagne) avec la carte de sang ADN 10591402V1.0 dans un extracteur EZ1 Advanced XL.

Pour comparer la sensibilité de notre test à la PCR actuellement réalisée en routine sur sang total, l'ADN a été extrait à partir de 100 µL (même volume que l'inoculum utilisé pour la capture en solution) et 200 µL (même volume que celui utilisé actuellement pour l'extraction d'ADN à partir du sang en routine) d'inoculum de levures, constituant nos deux Témoin inoculum, Témoin I^{100} et Témoin I^{200} respectivement.

Un témoin négatif dit « Témoin billes » a également été réalisé. Il correspond aux billes magnétiques non couplées aux anticorps spécifiques. Nous l'avons également testé pour un inoculum de 0 UFC/mL composé de PBS pur, afin d'éliminer une détection non spécifique par PCR.

Détection par PCR en temps réel

Tous les extraits d'ADN ont été testés en quadruple. La PCR en temps réel a été réalisée à l'aide d'un ensemble d'amorces pan-*Candida* avec une sonde spécifique de *Candida albicans* ou *Candida tropicalis* ciblant la région de l'internal transcribed spacer 2 (ITS2) de l'ADN ribosomal nucléaire (25). La production des amorces et des sondes a été réalisée par Eurogentec

(Angers, France). Les tests PCR ont été effectués sur un instrument LightCycler^{TM} 480 (Roche Diagnostics, Bâle, Suisse) comme décrit précédemment (26). Chaque extrait d'ADN a été déposé quatre fois.

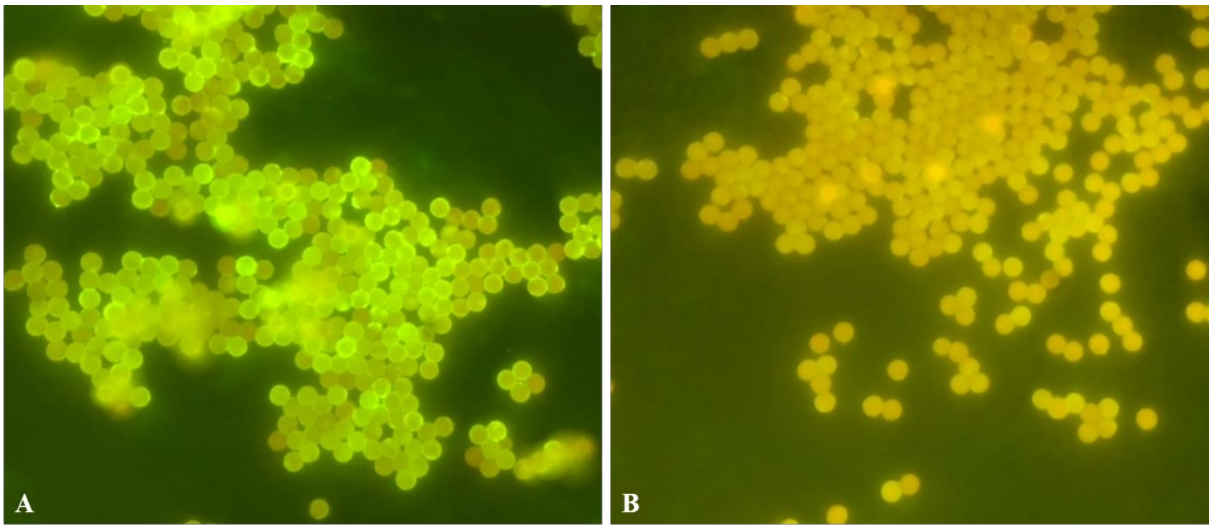
Les résultats de la PCR ont été considérés comme négatifs lorsqu'aucune courbe d'amplification n'était obtenue. Les inocula négatifs ont été annotés à 45 Ct, soit le nombre maximum de cycles PCR effectués.

Résultats

Fixation des anticorps aux billes magnétiques

Nous avons pu constater que dans les conditions recommandées par le constructeur, il y avait bien une fixation des anticorps monoclonaux anti-*Candida albicans* aux billes magnétiques et ainsi valider la première étape (Figure 1). Mais cette fixation semble hétérogène.

Figure 1. Observation au microscope à fluorescence de la fixation des anticorps monoclonaux anti-*Candida albicans* aux billes magnétiques (BiAc2) . (A) Présence d'anticorps monoclonaux anti-*Candida albicans*. (B) Absence d'anticorps monoclonaux anti-*Candida albicans* (témoin négatif).



Détection des levures par capture en solution puis PCR en temps réel

Au total trois couples BiAc ont été testés : BiAc1, BiAc2 et BiAc 3 (Table 2) La détection des levures par capture en solution a été évaluée sur 4 concentrations : 5000 UFC/mL, 1000 UFC/mL, 500 UFC/mL et 250 UFC/mL (Table 3).

En raison du faible nombre d'échantillons, les résultats sont présentés sous la forme de moyenne et d'intervalle, les écarts-types ne pouvant être calculés.

Table 3. Résultats exprimés en Ct obtenus avec les billes magnétiques recouvertes des trois différents anticorps primaires spécifiques pour différentes concentrations de *Candida albicans*. Témoin I^{100} : Extraction de 100µL d'inoculum ; Témoin I^{200} : Extraction de 200µL d'inoculum ; Témoin billes : billes magnétiques non couplées aux anticorps spécifiques ; m : Moyenne ; NA : Non applicable.

		BiAc3		BiAc2		BiAc1	
		m	Intervalle [min-max]	**m**	Intervalle [min-max]	**m**	Intervalle [min-max]
250 UFC/mL	Témoin I^{100}	**40,66**	[35,81-45]	**37,14**	[37,07-37,20]	**35,77**	[35,48-36,06]
	Témoin I^{200}	**39,43**	[35,96-45]	**37,37**	[37,08-37,66]	**37,21**	[35,17-39,24]
	Test	**43,39**	[38,37-45]	**41,86**	[37,87-45]	**41,56**	[35,51-45]
	Témoin billes	**NA**	NA	**44,19**	[38,48-45]	**39,02**	[35,41-45]
500 UFC/mL	Témoin I^{100}	**35,57**	[35,11-35,77]	**40,97**	[36,93-45]	**36,10**	[35,62-36,58]
	Témoin I^{200}	**36,50**	[36,04-36,88]	**37,56**	[36,46-38,66]	**35,96**	[35,28-36,63]
	Test	**40,17**	[36,43-45]	**43,14**	[37,36-45]	**42,82**	[35,77-45]
	Témoin billes	**NA**	NA	**43,33**	[37,78-45]	**42,32**	[37,64-45]
1000 UFC/mL	Témoin I^{100}	**37,04**	[35,61-38,33]	**36,30**	[35,97-36,62]	**34,83**	[34,62-35,04]
	Témoin I^{200}	**35,04**	[34,74-35,22]	**34,96**	[34,36-35,56]	**35,66**	[34,22-37,09]
	Test	**39,64**	[37,16-45]	**43,42**	[38,41-45]	**39,86**	[36,25-45]
	Témoin billes	**NA**	NA	**41,16**	[37,20-45]	**43,21**	[37,80-45]
5000 UFC/mL	Témoin I^{100}	**33,77**	[33,30-34,06]	**NA**	NA	**NA**	NA
	Témoin I^{200}	**32,97**	[32,70-33,52]	**NA**	NA	**NA**	NA
	Test	**37,17**	[35,21-38,89]	**NA**	NA	**NA**	NA
	Témoin billes	**NA**	NA	**NA**	NA	**NA**	NA
0 UFC/mL	Témoin billes	**NA**	NA	**45**	[45-45]	**45**	[45-45]

Tests avec Candida tropicalis

Le couple biAc1 a également été évalué sur une souche de *Candida tropicalis* (CP047,875). En raison du faible nombre d'échantillons, les résultats sont présentés sous la forme de moyenne et d'intervalle, les écarts-types ne pouvant être calculés.

Table 4. Résultats exprimés en Ct obtenus avec les différentes billes magnétiques recouvertes d'anticorps spécifiques pour différentes concentrations de *Candida tropicalis*.

Témoin I^{100} : Extraction de 100µL d'inoculum ; Témoin I^{200} : Extraction de 200µL d'inoculum ; Témoin billes : billes magnétiques non couplées aux anticorps spécifiques ; m : Moyenne ; NR : Non réalisé.

Anticorps anti BDG

		m	Intervalle
250 UFC/mL	Témoin I^{100}	40,00	[40,00-40,00]
	Témoin I^{200}	37,25	[38,50-36,44]
	Test	45	[45-45]
	Témoin billes	45	[45-45]
500 UFC/mL	Témoin I^{100}	36,28	[36,07-36,48]
	Témoin I^{200}	36,15	[36,05-36,25]
	Test	45	[45-45]
	Témoin billes	45	[45-45]
1000 UFC/mL	Témoin I^{100}	35,51	[35,50-35,52]
	Témoin I^{200}	35,51	[35,13-35,88]
	Test	45	[45-45]
	Témoin billes	45	[45-45]
5000 UFC/mL	Témoin I^{100}	30,93	[30,86-30,99]
	Témoin I^{200}	29,85	[29,84-29,85]
	Test	32,89	[32,85-32,93]
	Témoin billes	34,68	[34,34-35,02]

Nous avons évalué l'intérêt de la capture en solution de *Candida tropicalis* à l'aide du couple BiAc1. Pour des concentrations élevées de *C. tropicalis*, nous retrouvons des résultats similaires à ceux obtenus par PCR directement sur l'inoculum. Les levures se fixent également de façon non spécifique sur les billes magnétiques. Pour de plus faible inocula (i.e. ≤ 1000 UFC/mL), ce phénomène n'est plus observé.

Discussion

La première approche fondée sur l'immuno-PCR universelle a déjà montré son intérêt dans plusieurs domaines de l'infectiologie (27). Cependant, l'interférence dans les immuno-essais est un problème souvent limitant à l'utilisation de ces techniques (15,28). Le bruit de fond lié à la fixation passive des anticorps ou des fragments d'ADN biotinylés sur la surface en polycarbonate des tubes est une des limites reconnues de l'immuno PCR (29). Les témoins négatifs sont souvent détectés aux alentours de 23 Ct (30), et parfois plus de 30 Ct (20). Certaines publications semblent s'être affranchies du bruit de fond après plus de 25 lavages (21). Dans notre expérience, nous n'avons en effet pas pu nous affranchir d'un bruit de fond de 19,3 Ct en moyenne. Ceci constitue une limite moindre dans le cadre de pathogènes bactériens, où la charge bactérienne est généralement élevée. L'article de Fischer *et al*., compare l'immuno-PCR suivi par une détection par PCR en temps réel au dosage immunologique enzymatique dans la détection d'enterotoxines A et B staphylococciques (30). Il rapporte une sensibilité de l'immuno-PCR évaluée à 0,6 pg d'enterotoxine B staphylococcique total par puits, correspondant à 21 Ct pour un bruit de fond à environ 22 Ct. Bien que cette approche soit considérée comme fiable dans cet article, il semble utopiste d'imaginer sa mise en place en laboratoire de routine diagnostique, notamment pour la détection de faibles concentrations en toxine basée sur une différence de 1 Ct avec le témoin négatif. De plus, lors d'infections fongiques les levures vont être présentent en faible quantités et pourront être détectées à des Ct élevés. Le bruit de fond que nous retrouverons ne permet pas d'utiliser un seuil en dessous duquel la détection est validée. Enfin, cette approche a été évaluée à partir d'inoculum de levures dans du PBS. Son application pour la détection de levure à partir de prélèvements sanguins, rajoutera des interférences potentielles qu'il est nécessaire d'évaluer. Enfin, l'une des solutions afin d'éliminer le bruit de fond est l'utilisation d'anticorps polyclonaux (20,31,32) ou de mélange d'anticorps monoclonaux afin d'améliorer la sensibilité de détection (30).

Notre seconde approche a consisté à évaluer une technique de capture en solution de *Candida* suivi par une détection moléculaire. Cette approche a déjà montré son intérêt dans la capture puis la remise en culture de quatre espèces de *Candida* : *Candida albicans*, *Candida glabrata*, *Candida tropicalis* et *Candida krusei* à partir de prélèvements sanguins artificiellement inoculés (22). Asghar *et al*. rapporte la capture en solution de *Candida albicans* suivie par une détection par fluorescence à l'aide d'anticorps spécifiques marqués au FITC (33). Etant donné que la biologie moléculaire permet la détection rapide et spécifique de levures à partir de liquides biologiques (12), le couplage de la capture solution et la détection par PCR semble donc

prometteur. Plusieurs facteurs peuvent influencer l'efficacité de la capture en solution. Dans le commerce, on retrouve des billes magnétiques avec différentes caractéristiques de taille, des revêtements et des chimies et de système de conjugaison aux anticorps. Ces billes ne sont pas toutes équivalentes en terme de performance de capture et d'isolement (34), il est donc nécessaire de les évaluer. Il faut tenir compte des exigences en termes de choix d'anticorps ; dans notre cas nous devions sélectionner des anticorps produits à partir de souris. Or, le panel d'anticorps disponibles sur le marché spécifiques du genre *Candida* est très limité. Nous avons donc sélectionné deux anticorps type IgG monoclonal et un anticorps type IgM monoclonal. Nous avons sélectionné deux types de billes magnétiques. Les billes CELLection^{TM} Pan Mouse IgG Dynabeads (Thermo Fisher Scientific, Kanagawa, Japon) ont été couplées aux deux anticorps différents type IgG. Ces billes magnétiques ont déjà démontré leur intérêt dans la capture de cellules tumorales circulantes à partir de prélèvements sanguins (35,36), mais n'ont pas encore été évaluées dans la détection de pathogènes. Nous avons mis en évidence une fixation non spécifique de certaines espèces de *Candida* sur les billes magnétiques. Malheureusement, pour des concentrations faibles de blastospores, la détection devient aléatoire. Il est donc nécessaire de poursuivre la mise au point de cette technologie.

L'anticorps type IgM monoclonal 5B2 nous a été aimablement donné par le laboratoire de recherche « Glycobiology in Fungal Pathogenesis & Clinical Applications » (Inserm U1285 CNRS UMR 8576) dirigé par le Professeur Sendid (Lille, France) (37,38). Il réagit avec les beta-1,2-oligomannosides partagé par les mannoprotéines de *C. albicans*, *C. tropicalis*, *C. glabrata* et *C. parapsilosis* et fortement exprimé sur le phospholipomannane, un glycolipide synthétisé par *C. albicans*, *C. dubliniensis* et *C. tropicalis* (37,39). Cet anticorps a déjà été évalué dans la capture en solution de quatre espèces de *Candida* : *Candida albicans*, *Candida glabrata*, *Candida tropicalis* et *Candida krusei* à partir de prélèvements sanguins artificiellement inoculés (22). En combinaison avec un autre anticorps monoclonal type IgM (Mab 6B3), il a montré des résultats prometteurs dans la capture des *Candida* pour des concentration de 6 à 40 UFC/mL (22). Malheureusement, cet anticorps étant de type IgM, nous avons été confrontés à des problèmes de compatibilité avec les billes. En effet, dans la majorité des couplages billes-anticorps, la protéine A, la protéine G et la protéine A/G recombinante sont utilisées comme ligand d'affinité. Ces protéines ayant une très faible affinité de liaison avec les anticorps type IgM, cette technique de couplage ne peut être utilisée dans ce cas. Nous avons donc évalué les billes magnétiques MagnaLink^{TM} 4FB Magnetic Beads (Solulink, Inc, Le Perray en Yveline, France) qui sont utilisées pour immobiliser les biomolécules aromatiques

modifiées par l'hydrazine (HyNic). Le principe du couplage repose alors sur la présence d'aldéhydes aromatiques extrêmement stables (4FB) à la surface des billes qui ne réagissent spécifiquement qu'avec les fragments d'hydrazine aromatique (HyNic) fixés à la surface de certaines biomolécules. Nous avons fait appel au laboratoire Tebu-Bio (Le Perray en Yvelines, France) afin de réaliser le couplage des billes magnétiques MagnaLink^{TM} 4FB Magnetic Beads (Solulink, Inc, Le Perray en Yveline, France) à l'anticorps type IgM 5B2. Ces billes ont l'avantage de pouvoir être couplées à diverses biomolécules. Elles ont ainsi montré leur intérêt dans la détection de l'ARN du virus Ebola, sans amplification, par couplage avec une sonde de capture complémentaire du génome de ce virus (40). Dans cette application, l'anticorps est remplacé par une sonde spécifique ce qui, dans notre cas, ferait perdre le caractère pan-*Candida* de la détection, que nous recherchons. De plus une détection post-extraction d'ADN/ARN ne permet pas un enrichissement de l'échantillon à extraire et présente donc un intérêt limité dans la détection de faibles concentrations en *Candida* à partir de prélèvements sanguins. Dans notre étude, l'efficacité du couplage bille magnétique /Anticorps obtenu a malheureusement été très faible 5 µg pour 1.2 mg de billes, lié à la faible pureté de l'échantillon d'anticorps de départ. Le manque de rentabilité de ce couplage peut expliquer les résultats obtenus qui ne sont pas assez satisfaisants. Cela souligne l'intérêt de réaliser de nouveaux essais de couplage avec ces IgM monoclonaux 5B2 purifiés et d'autres billes magnétiques. L'objectif est de pouvoir identifier un couple billes magnétiques/anticorps spécifique idéal.

Que ce soit pour les couples billes magnétiques-anticorps type IgG ou billes magnétiques-anticorps type IgM, il serait également intéressant de faire varier la taille des billes, afin de voir si cela a un impact sur la fixation non spécifique des levures. Une autre alternative serait l'utilisation d'anticorps polyclonaux, ou le mélange de plusieurs couples billes magnétique – anticorps spécifiques.

Enfin, pour l'étape de détection, une des alternatives plus sensibles que la PCR peut être le dosage du bêta-(1-3)-D-glucane. Ce composant de la paroi cellulaire de *Candida* est utilisé comme biomarqueur dans le diagnostic des candidoses invasives avec une sensibilité de 92 % et une spécificité de 81 % (41–47). Une capture en solution grâce aux billes magnétiques couplées à des anticorps spécifiques suivie par une détection du bêta-(1-3)-D-glucane est donc une alternative à envisager et évaluer.

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Article 4: Pericardial effusion due to *Trichosporon japonicum*: a case report and review of the literature

Menu E., Kabtani J., Roubin J., Ranque S. et L'Ollivier C.

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RESUME

A travers ce case report, nous avons étudié une situation clinique à laquelle nos cliniciens ont été confrontés à l'hôpital de La Timone, Marseille ; un cas rare d'infection invasive à *Trichosporon japonicum*. Les *Trichosporon* sont des organismes opportunistes émergents de type levure basidiomycète. Ubiquitaires dans l'environnement, ils sont parfois impliqués dans des infections fongiques invasives (66). Ce cas illustre les difficultés diagnostic auxquelles les cliniciens sont confrontés face à ces infections.

Dans cette publication, nous discutons la valeur des différents outils diagnostiques disponibles et réalisons une revue de la littérature des infections à *Trichosporon japonicum*. La réaction croisée entre *Trichosporon japonicum* et la détection de l'antigène cryptocoque a été constatée pour la première fois avec cette espèce et non observée avec l'antigène galactomannane *d'Aspergillus*. Bien que cette réaction soit déjà connue pour d'autres espèces de *Trichosporon* sp., il est nécessaire d'évaluer ces outils sur les différentes espèces de *Trichosporon* impliquées en pathologie humaine.

En cas de suspicion d'infection fongique invasive à *Trichosporon*, en raison de la difficulté diagnostic, il est essentiel d'associer la culture, gold standard diagnostic, à l'utilisation détournée d'outils diagnostiques en raison des réactions croisées : la détermination de l'antigène galactomannane *d'Aspergillus* et de l'antigène cryptococcique, et l'instauration rapide d'un traitement antifongique.

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Case Report

Pericardial Effusion Due to *Trichosporon japonicum*: A Case Report and Review of the Literature

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Abstract: Invasive infections due to *Trichosporon* spp. are life-threatening opportunistic fungal infections that may affect a wide array of organs. Here, we described a case of pericardial effusion due to *Trichosporon japonicum* in a 42-year-old female after a heart transplantation. *T. japonicum* was isolated from the pericardial fluid, pericardial drain hole and the swab of the sternal surgery scar wound. The late mycological diagnosis due to blood culture negative, the ineffective control of pulmonary bacterial infection and the late start antifungal therapy were the contributing factors in the patient's death.

Keywords: *Trichosporon japonicum*; yeast; pericarditis

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1. Introduction

Trichosporon are emerging opportunistic basidiomycetous yeast-like organisms. Ubiquitous in the environment, they are occasionally involved in invasive fungal diseases [1]. Patients with hematological malignancies, persistent neutropenia, intravenous and urinary catheters, those who have had thoracic or abdominal surgery, and those who are immunosuppressed or pre-exposed to antifungal therapy, especially to echinocandins, are at risk of invasive trichosporonosis [2]. The most common species involved in clinical infections are *Trichosporon asahii*, followed by *T. inkin*, *T. faecale*, and *T. asteroides* [3].

In this report, we present a rare case of a *Trichosporon japonicum* infection in a 42 years old female following heart transplantation. We discussed the value of the different diagnostic tools available and performed a review of the literature on *T. japonicum* infections.

2. Case Presentation

2.1. Patient History

A 42-year-old female was admitted at La Timone hospital (Marseille, France) to undergo a heart transplantation. She had a history of congenital cardiopathy (single ventricle) with multiple cardiac decompensation episodes, severe left ventricular dysfunction (LVEF 25%) and New York Heart Association class III dyspnea. The patient was on mechanical ventilation throughout the duration of the hospitalization, and did not develop any fever. At day 6 post transplantation, a routine trans-thoracic echography showed a 1 cm pericardial effusion (Figure 1). The pericardial effusion progressively increased to 1.2 cm at day 10, and 1.5 cm at day 17. At day 13, the patient developed an acute respiratory distress syndrome, and HSV-1 PCR was positive (in blood and bronchoalveolar lavage fluid); the patient was treated with acyclovir. The following bacteria were repeatedly cultured from respiratory samples from day 6 post transplant: *Pseudomonas aeruginosa* (one bronchial aspirate and

one bronchoalveolar lavage fluid), *Stenotrophomonas maltophilia* (three bronchial aspirate, one bronchoalveolar lavage fluid and one sputum) and *Citrobacter freundii* (one bronchial aspirate and one bronchoalveolar lavage fluid). Following that, the patient was treated with an adapted antibiotic therapy, which unfortunately does not enable the control of the infection. From the first day post transplant, the patient had a supranormal white blood cell count with an average of 28 $\times$ 10^9/L (range: 18 $\times$ 10^9–43 $\times$ 10^9).

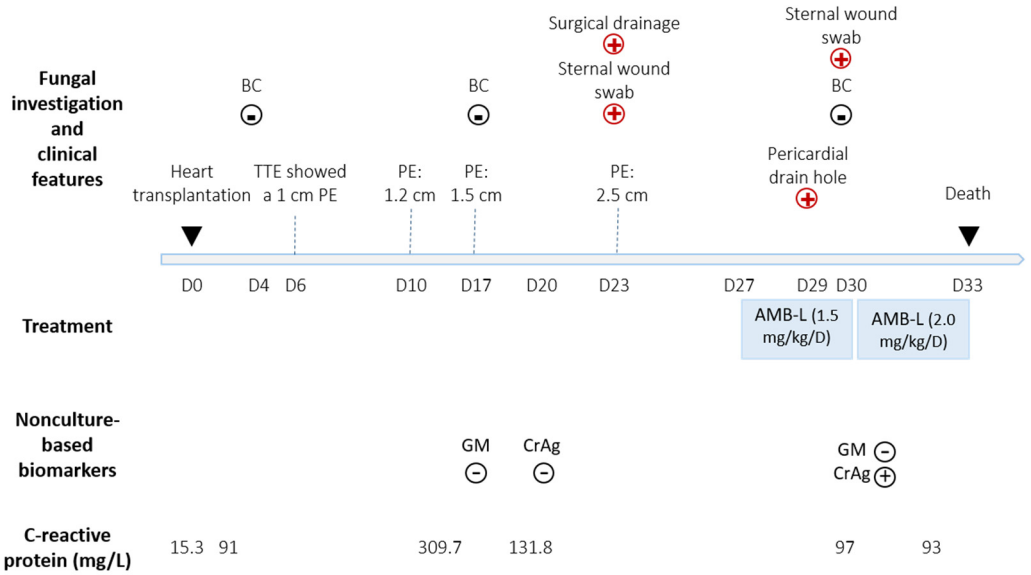

Figure 1. Timeline of *Trichosporon japonicum* pericarditis course. TTE: trans-thoracic echography; AMB-L: Amphotericin B liposomal; BC: Blood culture; PE: Pericardial effusion; CrAg: Cryptococcal antigen; GM: *Aspergillus* galactomannan; +: positive; −: negative; ✚: positive *Trichosporon japonicum* culture; ▪: negative *Trichosporon japonicum* culture.

2.2. Diagnostic Assessment and Therapeutic Intervention

At day 23, the pericardial effusion became circumferential and measured 2.4 cm. The patient underwent a surgical drainage. The pericardial aspirate was purulent and grew cream-colored dry wrinkled colonies with irregular margins after 3 days (Figure 2A). Bacterial culture was negative. Fresh microscopic examination of the colonies revealed round yeast cells with blastoconidia and arthroconidia (Figure 2B,C). *Trichosporon japonicum* was identified by MALDI-TOF mass spectrometry (MicroflexLT, Bruker Daltonics GmbH, Bremen, Germany) with a Logscore value at 2.02 obtained from the standard manufacturer library, and it was deposited (strain number: IHEM 28563). DNA sequence-based identification was performed as previously described [4]. The 100% identity with the rRNA intergenic spacer IGS1 sequence (GenBank AB066426.1) of JCM8357, the type strain of *T. japonicum*, confirmed this identification. The other genomic regions rRNA Internal Transcribed Spacer 1 and 2, the D1/D2 domains of the rRNA large-subunit were less informative but in agreement with this identification. All nucleotide sequences of the present isolate were submitted to GenBank (Acc. No OM865139, OM865141 and OM897590). The NCBI BLASTn results are detailed in Supplementary Table S1. The same yeast was isolated from the swab of sternal surgery scar wound (day 23 and day 30) and pericardial drain hole (day 29). Blood culture (BacT/ALERT system, bioMerieux, Craponne, France) remain negative for the duration of the stay. *Aspergillus* galactomannan antigenaemia (Bio-Rad Laboratories, Marnes-La-Coquette, France) was negative, at day 17 and day 30. Cryptococcal serum antigen (CrAg® LFA kit, IMMY, Norman, OK, USA) was negative at day 20 but positive (1:80 titer) at day 30.

The patient was treated with liposomal amphotericin B (1.5 mg/kg/D) starting from day 27. The patient developed a multiple organ failure and died at day 33.

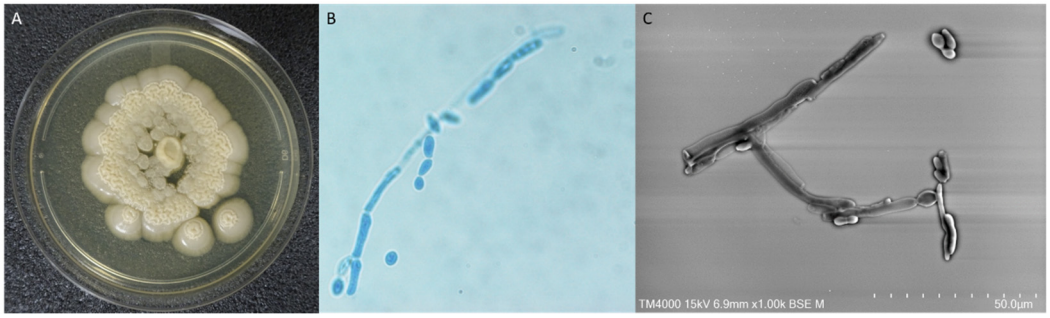

Figure 2. *Trichosporon japonicum* morphological features. (**A**) Colony of *Trichosporon japonicum* on Sabouraud dextrose agar media. (**B**) Fresh microscopic examination of the colonies stained with Mycetblue® (Biosynex, Graffenstaden, France) ×1000 original magnification. (**C**) Scanning Electron Microscopy examination (15 KeV, lens mode 3, Scale bar 50 µm) of the colonies using the TM4000 PlusTM (Hitachi, Japan) instrument.

Antifungal susceptibility testing was performed on the strain cultured from the pericardial fluid by using the colorimetric broth microdilution Sensititre Yeast-OneTM YO10 (Thermo Fisher Diagnostics, Dardilly, France) and/or the agar diffusion EtestTM (BioMérieux, Craponne, France). The following minimum inhibitory concentration (MIC) values of *T. japonicum* isolate were found: amphotericin B (YO10: 0.5 mg/L; Etest: 0.5 mg/L), micafungin (YO10: >8 mg/L), caspofungin (YO10: >8 mg/L), anidulafungin (YO10: >8 mg/L), 5-fluorocytosine (YO10: 4 mg/L), voriconazole (YO10: 0.12 mg/L; Etest: 0.125 mg/L), posaconazole (YO10: 0.25 mg/L; Etest: 0.75 mg/L), itraconazole (YO10: 0.12 mg/L), fluconazole (YO10: 4 mg/L) and isavuconazole (Etest: 0.38 mg/L).

3. Discussion

Trichosporon japonicum was first isolated in 1998 from air collected in the house of a patient with summer-type hypersensitivity pneumonitis in Japan [5]. So far, *T. japonicum* infections have been scarcely reported in the literature. A review of the English language literature in Medline by using the following keywords: "*Trichosporon japonicum*" AND "Human" (Table 1) found only five references reporting a total of six human cases of *T. japonicum* infections. These infections were documented in blood (n = 2) [6,7], urinary tract (n = 2) [8] and respiratory tract (n = 2) from respiratory samples [9,10]. The mean age of reported cases was 24 years (range: 8–50), the sex ratio was 1.5 and three patients died. Regarding the patients' risk factors, two patients were kidney transplant recipients, two patients had a hematologic malignancy (AML, ALL), and similarly to our patient, one underwent cardiac surgery. No data was provided about the remaining case in whom *Trichosporon japonicum* DNA was detected in lower respiratory specimens [10]. In the present case, our patient developed a pericardial effusion following heart transplantation. The heart preservation fluid culture remained sterile, which should rule out transmission by the graft. Finally, our patient is the second case described following heart surgery and the third after solid organ transplantation.

Prompt diagnosis and timely management of trichosporonosis are essential. The gold standard diagnosis is the culture with growth between 48 and 72 h on Sabouraud medium [11] and their ability to grow on non-specific media. In the present case, the repetitive blood cultures remained negative, but the samples grew rapidly (within 72 h). *T. japonicum* was identified by the MALDI TOF MS and DNA sequencing confirmed the identification. Whereas the ITS and D1/D2 domains of rDNA are considered as the gold standard for medically important yeasts identification [12], in this present case, they could not differentiate *T. japonicum*, *T. asahii* and *T. asteroides*. The IGS region is more discriminating and has confirmed the species (Table S1). Our results confirm previous reports that the rRNA IGS1 region nucleotide sequence is the most discriminating and relevant for the precise species identification within the *Trichosporon* genus [1,11,13–15].

Table 1. Cases report of *Trichosporon japonicum* infections, review of literature (including the present case).

Age	Gender	Comorbidity Conditions	Clinical Presentation	Site of Positive Culture	Treatment		Outcome	Reference
					Molecule	Duration		
8	F	AML	Respiratory distress	Sputum	AMB-L (5 mg/kg/D) + ITRA (100 mg/D)	NS	Death	[9]
-	-	-	Hypersensitivity pneumonitis	BALF	-	-	-	[10]
18	F	Transcutaneous biventricular assist device	Fungemia	Blood, aortic cannula, removed left ventricular apex cuff	AMB-L + 5FC switch VORI	11 days / 6 weeks	Survival at 2 months	[6]
36	M	Kidney transplant recipient	Urinary tract infection	Urine	VORI + CASPO	NS	Survival	[8]
50	M	Kidney transplant recipient	Urinary tract infection	Urine	VORI + CASPO	15 days	Death	[8]
8	M	ALL	Fungemia	Blood	AMB (3 mg/kg/D) + VORI (8 mg/kg twice a day)	11 days	Death	[7]
42	F	Heart transplant recipient	Pericardial effusion	Pericardial fluid	AMB-L (1.5 mg/kg/D)	6 days	Death	Present case

AML: Acute Myeloid Leukemia; ALL: acute B cell lymphoblastic leukemia; F: Female; M: Male; D: day; BALF: Broncho alveolar lavage fluid; AMB-L: Liposomal amphotericin B; ITRA: itraconazole; 5FC: 5-fluorocytosine; CASPO: caspofungin; VORI: voriconazole; NS: Not Specified.

Like *Cryptococcus* spp., *Trichosporon* spp. are Basidiomycota, and cross-reactions between cryptococcal polysaccharide antigen detection and *Trichosporon* species are known [16,17]. Until now, the reported cases of cross-reactions concerned the following species: *Trichosporon cutaneum* [18,19], *Trichosporon beigelii* [16,20–23], *Trichosporon asahii* [24,25] and *Trichosporon dermatis* [26]. We present the first case of a positive cryptococcal antigen detection during a *Trichosporon japonicum* infection. Interestingly, in the literature, only Bogomin et al. [6] performed a cryptococcal antigen detection in a *T. japonicum* fungemia in a patient with a transcutaneous biventricular assist device. The latex agglutination cryptococcal capsular polysaccharide antigen test returned negative, although performed concomitantly with positive blood cultures. In our patient, the serum cryptococcal antigen using CrAg® LFA kit (IMMY, Norman, OK, USA) was negative 3 days before the pericardial fluid puncture and positive 7 days after. Three situations have been reported regarding the time to serum cryptococcal antigen positivity in patients infected with the other *Trichosporon* species. Karigane et al. reported *Trichosporon asahii* fungemia in an AML patient with positive cryptococcal antigenemia 5 days before the isolation of the fungus [25], while others reported a positive cryptococcal antigen concomitantly [16] or after the yeast isolation [20,26]. Interestingly, in our patient, the cryptococcal antigen was positive in several biological fluids, such as urine or cerebrospinal fluid, all confirmed by a subsequent *Trichosporon* positive culture [19,22].

Cross-reaction between *Aspergillus* galactomannan detection and *Trichosporon* spp. have been reported [27,28]. A dual positivity of cryptococcal antigen and *Aspergillus* galactomannan has been described in case of disseminated *Trichosporon dermatis* infection [26]. In our patient, *Aspergillus* galactomannan assay in the serum at day 17 and day 30 was negative. We tested cryptococcal antigen (IMMY, Norman, OK, USA) and Platelia *Aspergillus*

galactomanan (Bio-Rad Laboratories, Marnes-La-Coquette, France) in the supernatant from a *T. japonicum* culture. Both returned positive, suggesting that *T. japonicum* share common epitopes with *Cryptococcus* and *Aspergillus* cell wall components. As recommended in the ESCMID and ECMM joint clinical guidelines [28], in case of suspected invasive *Trichosporon* infection, the diagnosis should include the combined use of culture, *Aspergillus* galactomannan and cryptococcal antigen determination. It would be interesting to extend the use of these tools to the first-line diagnostic approach of invasive fungal infections.

There is no consensus on the treatment of trichosporonosis and data concerning MIC interpretation for all antifungal drugs are scarce [3]. Like in most basidiomycetes, the use of echinocandins is not recommended due to natural resistance [29]. Consistently, in our case, the isolates of *T. japonicum* showed high MIC for echinocandins and low MIC for azoles except for fluconazole. Despite data suggesting a discrepancy between in vitro and in vivo activity, susceptibility testing is still recommended for epidemiological knowledge [3,8]. Recent guidelines moderately recommend voriconazole for initial antifungal therapy, exhibiting excellent in vitro and in vivo activity against *Trichosporon* spp. [3,8]. In the present case, treatment with amphotericin B was initiated following sensitivity testing and late in the course of the disease. Therapeutic failure is thus difficult to assess.

4. Conclusions

Trichosporon japonicum infection is rare. In case of suspected invasive *Trichosporon* infection, prompt diagnosis, including the combined use of culture, *Aspergillus* galactomannan and cryptococcal antigen determination and the rapid initiation of antifungal treatment are essential.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/pathogens11050598/s1, **Table S1**: Molecular identification of *Trichosporon japonicum* sequencing ITS, D1/D2 and IGS genetic regions.

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Institutional Review Board Statement: The study was conducted according to the guidelines of the Declaration of Helsinki, and approved by the Ethics Committee of the Assistance Publique des Hôpitaux de Marseille (APHM) (protocol code 2019-73 on 29 May 2019).

Informed Consent Statement: Patient consent was waived due to her inability to sign a written consent (hospitalization in intensive care unit then death). This case, completely anonymized, reports the history of the patient with her management in accordance with ethical respect. No further analysis was performed.

Data Availability Statement: Not applicable.

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Conflicts of Interest: The authors declare no conflict of interest.

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Table S1. Molecular identification of *Trichosporon japonicum* sequencing ITS, D1/D2 and IGS genetic regions.

	ITS				D1/D2				IGS1			
Samples	ID	Type strain ID collection	Nucleotide alignement (identity %)	Genbank accession no.	ID	Type strain ID collection	Nucleotide alignement (identity %)	Genbank accession no.	ID	Type strain ID collection	Nucleotide alignement (identity %)	Genbank accession no.
Pericardial fluid	*Trichosporon japonicum*	CBS 8641	472/473 (99.79%)	NR_073263.1	*Trichosporon japonicum*	CBS 8641	492/493 (99.80%)	KY109955.1	*Trichosporon japonicum*	JCM8357	458/458 (100%)	AB066426.1
	Trichosporon asteroides	CBS 2481	471/473 (99.58%)	KY105727.1	*Trichosporon asahii*	CBS 2479	491/492 (99.80%)	KY109922.1	*Trichosporon asteroides*	CBS 2481	462/477 (96.86%)	EU934802.1
	Trichosporon insectorum/ faecale	CBS10422/ CBS 4828	470/473 (99.37%)	KY105746.1 / KY105736.1	*Trichosporon asteroides*	CBS 2481	491/493 (99.59%)	KY109937.1	*Trichosporon faecale*	CBS 4828	407/509 (79.96%)	KM488293.1
	Trichosporon asahii	CBS 2479	469/473 (99.15%)	KY105709.1	*Trichosporon insectorum*	CBS 10422	489/491 (99.59%)	KY109953.1	*Trichosporon asahii*	PUMCHBY15	403/505 (79.80%)	JF303013.1
Swab no. 1 (sternal surgery scar wound)	*Trichosporon insectorum*	CBS 10422	425/425 (100%)	KY105746.1	*Trichosporon japonicum*	CBS 8641	547/547 (100%)	KY109955.1	*Trichosporon japonicum*	JCM8357	448/448 (100%)	AB066426.1
	Trichosporon faecale	CBS 4828	425/425 (100%)	KY105736.1	*Trichosporon asteroides*	CBS 2481	546/547 (99.82%)	KY109937.1	*Trichosporon asteroides*	CBS 2481	453/468 (96.79%)	EU934802.1
	Trichosporon japonicum	CBS 8641	425/425 (100%)	NR_073263.1	*Trichosporon asahii*	CBS 2479	545/547 (99.63%)	KY109922.1	*Trichosporon faecale*	CBS 4828	395/479 (79.48%)	KM488293.1
	Trichosporon asahii	CBS 2479	423/423 (100%)	AB018013.1	*Trichosporon insectorum*	ATCC MYA-4361	542/547 (99.09%)	NG_042467.1	*Trichosporon asahii*	PUMCHBY15	391/493 (79.31%)	JF303013.1
Swab no. 2 (sternal surgery scar wound)	*Trichosporon insectorum*	CBS 10422	427/427 (100%)	KY105746.1	*Trichosporon japonicum*	CBS 8641	486/486 (100%)	KY109955.1	*Trichosporon japonicum*	JCM8357	470/470 (100%)	AB066426.1
	Trichosporon faecale	CBS 4828	427/427 (100%)	KY105736.1	*Trichosporon asahii*	CBS 2479	486/486 (100%)	KY109922.1	*Trichosporon asteroides*	CBS 2481	490/506 (96.84%)	EU934802.1
	Trichosporon japonicum	CBS 8641	427/427 (100%)	NR_073263.1	*Trichosporon insectorum*	CBS 10422	485/486 (99.79%)	KY109953.1	*Trichosporon faecale*	CBS 4828	432/534 (80.90%)	KM488293.1
	Trichosporon asahii/asteroides	CBS2479 / CBS 2481	426/427 (99.77%)	KY105709.1 / KY105727.1	*Trichosporon asteroides*	CBS 2481	485/486 (99.79%)	KY109937.1	*Trichosporon asahii*	PUMCHBY15	427/529 (80.72%)	JF303013.1
Swab no. 3 (Subcutaneous mediastinal sample)	*Trichosporon japonicum*	CBS 8641	483/483 (100%)	NR_073263.1	*Trichosporon japonicum*	CBS 8641	496/496 (100%)	KY109955.1	*Trichosporon japonicum*	JCM8357	455/455 (100%)	AB066426.1
	Trichosporon asteroides	CBS 2481	482/483 (99.79%)	KY105727.1	*Trichosporon asahii*	CBS 2479	496/496 (100%)	KY109922.1	*Trichosporon asteroides*	CBS 2481	461/476 (96.85%)	EU934802.1
	Trichosporon insectorum/faecale	CBS10422/ CBS 4828	481/483 (99.59%)	KY105746.1 / KY105736.1	*Trichosporon insectorum*	CBS 10422	495/496 (99.80%)	KY109953.1	*Trichosporon faecale*	CBS 4828	403/505 (79.80%)	KM488293.1
	Trichosporon asahii	CBS 2479	480/483 (99.38%)	KY105709.1	*Trichosporon asteroides*	CBS 2481	495/496 (99.80%)	KY109937.1	*Trichosporon asahii*	PUMCHBY15	399/501 (79.64%)	JF303013.1

ID: identification; ITS: internal transcribed spacer; D1/D2: domains of the ribosomal DNA large-subunit; IGS: intergenic spacer; no.: number

Partie III : Prévention des infections fongiques systémiques - Identification de la source

AVANT PROPOS

La détection et l'investigation des épidémies ont pour finalité une action de santé publique et la prévention de futures épidémies. L'identification des sources potentielles des infections fongiques systémiques fait partie de la prise en charge multidisciplinaire de ces infections.

Les micromycètes peuvent être à l'origine d'épidémies nosocomiales lors de travaux de construction et de démolition (i.e. *Aspergillus* spp.) (48–50), lors l'utilisation de médicaments contaminés (i.e. *Exserohilum rostratum*) (52) ou de dispositifs médicaux contaminés (i.e. *Rhizopus arrhizus*, *Rhizopus rhizopodiformis* and *Rhizopus microsporus*) (72–74). Ces épidémies concernent majoritairement les patients immunodéprimés et ont pour origine une inhalation de spores dans la majorité des cas. Bien qu'elles concernent généralement un faible nombre de patients, leur issue peut être fatale. Une investigation environnementale doit être systématiquement réalisée afin d'identifier la source et de prévenir les épidémies futures (49,75).

En Europe, plusieurs épidémies nosocomiales de fongémies à *Saprochaete clavata* ont été décrites (76–80), sans réussir à identifier les sources potentielles de ces infections. La troisième partie de cette thèse repose sur la description d'une épidémie à *Saprochaete clavata* dans un centre anti-cancéreux de Marseille, faisant l'objet de l'**Article 5**. Nous avons en collaboration avec le Centre national de référence des Mycoses invasives et des antifongiques (CNRMA, Institut Pasteur) investigué cette épidémie afin d'identifier la source.

Article 5: *Saprochaete clavata* outbreak infecting cancer center through dishwasher

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RESUME

Ces dernières décennies, nous avons assisté à l'émergence de champignons responsables d'infections fongiques invasives opportunistes tel que *Saprochaete* spp., en raison du nombre croissant de patients immunodéprimés ou présentant des facteurs de risques de ces infections (21,81). Le management de ces infections inclut la prévention et donc l'identification de sources potentielles.

Dans cette publication est rapportée une épidémie d'infections invasives à *Saprochaete clavata* dans un centre anticancéreux Marseillais entre Janvier 2017 et Décembre 2017. L'investigation sur le terrain a reposé sur une approche multidisciplinaire comprenant l'étude clinique, épidémiologique, environnementale et microbiologique.

Au total, l'épidémie a concerné 9 patients hospitalisés dans 3 services différents, suggérant une source commune de contamination. L'enquête environnementale a permis d'identifier *S. clavata* dans différents échantillons environnementaux, notamment dans un lave-vaisselle de la cuisine centrale et dans un autre, à la disposition des patients et de leurs familles, dans le service de transplantation de cellules souches. Le séquençage du génome entier a confirmé que les isolats environnementaux et cliniques des patients appartenaient au même clade phylogénétique.

Les résultats suggèrent que les appareils ménagers liés à l'alimentation, tels que les lave-vaisselles, peuvent être des niches écologiques anthropophiles pour *S. clavata* et d'autres champignons opportunistes.

Saprochaete clavata Outbreak Infecting Cancer Center through Dishwasher

Estelle Menu, Alexis Criscuolo, Marie Desnos-Ollivier, Carole Cassagne, Evelyne D'Incan, Sabine Furst, Stéphane Ranque, Pierre Berger, Françoise Dromer

Saprochaete clavata is a pathogenic yeast responsible for rare outbreaks involving immunocompromised patients, especially those with hematologic malignancies. During February 2016–December 2017, we diagnosed *S. clavata* infections in 9 patients (8 with fungemia), including 3 within 1 month, at a cancer center in Marseille, France. The patients (median age 58 years), 4 of 9 of whom had acute myeloid leukemia, were hospitalized in 3 different wards. Ten environmental samples, including from 2 dishwashers and 4 pitchers, grew *S. clavata*, but no contaminated food was discovered. The outbreak ended after contaminated utensils and appliances were discarded. Whole-genome sequencing analysis demonstrated that all clinical and environmental isolates belonged to the same phylogenetic clade, which was unrelated to clades from previous *S. clavata* outbreaks in France. We identified a dishwasher with a deficient heating system as the vector of contamination.

Saprochaete clavata (previously *Geotrichum clavatum*) is a rare emerging pathogen, an ascomycetous yeast-producing arthroconidia that causes invasive fungal infections in immunocompromised patients. The species has mainly been reported in Europe, often associated with sporadic cases or small outbreaks (*1*,*2*). Unlike *Magnusiomyces capitatus* (*3*,*4*), which has been associated with dairy products, *S. clavata* has rarely been isolated from environmental samples (*5*,*6*). Patients most at risk for infections from *Geotrichum* spp. have hematologic diseases with severe neutropenia (*7*) and are undergoing chemotherapy, mainly with cytarabine (*1*) or caspofungin (*8*). They often have central venous catheters (*9*).

In recent years, *S. clavata* fungemia outbreaks associated with high mortality rates in vulnerable patients with malignancies have been described throughout Europe, mainly in France (*1*), Italy (*2*,*10*), Czechia (*11*), and Spain (*12*). No source of contamination was identified in any of these outbreaks despite thorough investigation.

During February 2016–December 2017, the Paoli-Calmettes Institute, a cancer center in Marseille, France, was faced with an outbreak of *S. clavata* infections involving 9 patients hospitalized in 3 different wards, suggesting a common source of contamination. We describe the findings of an outbreak investigation that recovered *S. clavata* in different environmental samples, including from a dishwasher in the central kitchen and another, available to patients and their families, in the stem-cell transplant ward. Whole-genome sequencing (WGS) confirmed that the environmental and clinical isolates from patients belonged to the same phylogenetic clade. Handwashing, avoiding direct skin contact, checking air quality, and sterilizing food are routine practice to prevent contamination in hematology wards; however, examining dishwashers for contamination and operability may not be done routinely. Our findings should prompt adding dishwasher inspections to guidelines for preventing infection.

Materials and Methods

Case Definition Criteria

We defined *S. clavata* infection by obtaining ≥1 positive results for *S. clavata* blood culture from a usually sterile body site or from a bronchoalveolar lavage or tracheal aspirate of the respiratory tract. Infection was also confirmed by observing pleural fluid in a patient with pleural effusion or lung infection.

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Mycologic Investigation

We collected a rectal swab specimen from all patients hospitalized in the stem-cell transplant unit during December 20–30, 2017. In addition, during December 22, 2017–January 19, 2018, we collected 95 environmental samples from food (powdered milk, a pea-sized amount from each package of cheese); tap water in 2 patients' room and water used for the coffee machine in 1 kitchen (500 μL); air filters; food-contact surfaces; non–food-contact surfaces in the rooms of infected patients; various kitchenware (vacuum flasks, cutlery); tables and chairs in the ward's kitchen; and microwaves, refrigerators, and dishwashers, including the dishwasher in the ward's central kitchen. For the dishwashers, we sampled inner surfaces, door seals, and the water outlet.

We used Sigma Transwab MW176S MWE medical wire sterile dry cotton swabs (Sigma Transwab, https://www.mwe.co.uk) for sampling as wide an area as possible. We discharged swabs in liquid Amies medium, then streak-plated the samples on Sabouraud dextrose agar plates supplemented with gentamicin and chloramphenicol (Bio-Rad, https://www.bio-rad.com) and BBL CHROMagar Candida plate (BD, https://www.bd.com). We identified species using Bruker Biotyper version MBT 3.1 matrix-assisted laser desorption/ionization time-of-flight (MALDI-TOF) mass spectrometry (Bruker, https://www.bruker.com) and nucleotide sequence analysis of the internal transcribed spacer (ITS) regions of the rRNA gene, as described elsewhere (*13*). The ITS sequences of the isolates were compared to those of the *S. clavata* type strain CBS425.71 (GenBank accession no. KF984489) isolated in Baltimore, Maryland, USA, in 1971.

All the strains we recovered from environmental and clinical samples and identified as *S. clavata* were stored at –20°C in cryotubes with bead tune Cryosystème Protect (Dutscher, https://www.dutscher.com). After subculturing all of the samples on Sabouraud agar slant (Bio-Rad), we sent them to the French National Reference Center for Invasive Mycoses and Antifungals (Institut Pasteur, Paris, France) for further characterization and comparison with selected clinical isolates collected through the nationwide surveillance program (Appendix Table, https://wwwnc.cdc.gov/EID/article/26/9/20-0341-App1.pdf).

WGS

After checking purity on chromogenic medium, we extracted DNA using a NucleoMag Plant kit (Macherey-Nagel, https://www.mn-net.com) in a KingFisher Flex system (Thermo Fisher Scientific, https://www.thermofisher.com). We sequenced whole genomes from each selected isolate (17 clinical and 10 environmental isolates) at the Mutualized Platform for Microbiology (Institut Pasteur, Paris, France) using a NextSeq 500 sequencer (Illumina, https://www.illumina.com). We constructed libraries using Nextera XT technology (Illumina) and sequenced genomes using a 2 × 150 nt paired-end run strategy. We preprocessed all reads with AlienTrimmer version 0.4.0 (https://bioweb.pasteur.fr/packages/pack@AlienTrimmer@0.4.0) to remove exogenous or low-quality bases, leading to a mean of 8.47M paired-end reads per sample (≈140 × sequencing depth, mean). We deposited FASTQ files for all isolates from Marseille at the European Nucleotide Archive BioProject (accession no. PRJEB36345).

Phylogenetic Analysis

For phylogenetic comparison, we used WGS data from 10 isolates studied during an outbreak described by Vaux et al. (BioProject accession no. ERP003645) (*1*); all reads from the BioProject ERP003645 isolates were preprocessed as described in previous sections. (The patients from whom the cultures were isolated correspond to patients 11–20 in the Appendix Table.) These reads included 5 isolates from epidemic clade A (CNRMA12.494, CNRMA12.559, CNRMA12.637, CNRMA12.667, CNRMA12.647) and 5 from epidemic clade B (CNRMA8.1167, CNRMA11.1183, CNRMA12.304, CNRMA12.615, CNRMA12.634). Overall, we studied a total of 38 isolates: 10 from BioProject ERP003645; 26 clinical and environmental isolates recovered in Marseille during the outbreak or its investigation, plus 1 clinical isolate, CNRMA15.181, recovered in 2015 in the same hospital in Marseille; and the *S. clavata* strain (CBS425.71).

For each preprocessed read sample, we performed short read mapping against the genome sequence of *S. clavata* clade A isolate CNRMA12.647 (GenBank accession no. CBXB000000000.1) using minimap2 version 2.17-r941 (*14*). We then inferred a pseudogenome following 4 rules: 1) we considered only aligned reads and sequenced bases associated with a Phred score >20; 2) we replaced each position with the character states observed in >80% of the aligned residues at that position; 3) we replaced every position covered by <10 aligned reads with the unknown character state "?"; and 4) we replaced all polymorphic positions located within strand-biased (set as <5 aligned reads on ≥1 strand) or over-covered regions (set as >200×) with the character state "X." Finally, after pooling all pseudogenome sequences into a unique matrix of aligned nucleotide

characters, we discarded each position containing >10% undefined character states (?, –, X, or N), resulting in 12,053,164 characters (including 261 variable characters), which we used to infer a maximum likelihood phylogenetic tree using IQ-TREE (http://www.iqtree.org) (*15*). To approximate the number of single-nucleotide polymorphisms (SNPs) shared by each branch of the phylogenetic tree, each branch length was multiplied by the total number of analyzed characters (i.e., 12,053,164) and the result was rounded to the closest integer.

Growth Temperature Testing

We analyzed the ability of 3 isolates of *S. clavata* (CBS425.71 type strain, CNRMA15.100, CNRMA14.292) and 3 isolates of *M. capitatus* (CBS162.80 type strain, CNRMA17.803, CNRMA17.775) to grow at high temperatures after 48 and 72 h of incubation. We subcultured isolates on Sabouraud agar medium at 30°C for 48h, then plated suspensions containing 10^3, 10^2, 10, and 1 colony-forming units in 5 μL of sterile distilled water on Sabouraud agar plates and incubated samples of each concentration at 30°C, 35°C, 37°C, 40°C, 45°C, and 48°C.

Results

Characteristics of the Patients

In December 2017, *S. clavata* infections were diagnosed in 3 patients (numbers 7–9 in the Table) within 3 weeks of admission to the Paoli-Calmettes Institute. This timing suggested a common source of contamination, even though the patients were hospitalized in 2 different wards, the stem-cell transplant

Table. Characteristics of patients with a culture positive for *Saprochaete clavata* in Marseille, France, February 2016–December 2017*

Characteristic	Patient no. 1	2	3	4	5	6	7	8	9
Age, y	58	38	45	66	57	68	65	56	68
Sex	M	F	M	F	M	M	F	M	M
Hospitalization ward	H	H	T	ICU	T	H	T	T	H
Immune status									
Underlying disease	Lymphoma	AML	MDS	Lymphoma	CLL	AML	ALL	AML	AML
Lymphocyte count, G/L	<0.1	0.1	5.6	0.2	0.1	0.1	0.8	0.1	0.1
Severe neutropenia, <500 /mm^3	Yes	Yes	No	Yes	Yes	Yes	Yes	Yes	Yes
Duration of neutropenia at time of positive culture, d	6	51	0	4	36	27	0	21	21
BMT	Yes	No BMT	Yes	No BMT	Yes	No BMT	Yes	Yes	Yes
Days from BMT to first positive culture	9		90		75		61	3	>90
Clinical signs at the time of positive culture									
Fever, temperature >38°C	Yes	Yes	Yes	Yes	NA	Yes	NA	Yes	Yes
Digestive symptoms	Yes	Yes	NA	NA	NA	NA	Yes	Yes	Yes
Diarrhea	Yes		NA	NA	NA	NA	Yes	Yes	
Constipation	NA	Yes	NA	NA	NA	NA		NA	Yes
Pulmonary symptoms	NA	Yes	Yes	NA	NA	NA	Yes	NA	Yes
Skin lesions	NA	NA	NA	Yes	Yes	NA	Yes	NA	NA
Positive culture results									
Date of first positive culture	2016 Feb 3	2017 Jan 16	2017 Jan 18	2017 Feb 26	2017 Apr 17	2017 Jun 29	2017 Dec 5	2017 Dec 10	2017 Dec 29
Days after admission	16	51	6	14	80	27	68	20	21
No. positive samples	1	1	2	7	5	9	1	10	5
Blood	1	1	None	5	4	9	1	9	5
Respiratory tract	None	None	2	2	1	None	None	None	None
Stool, rectal swab	None	None	None	None	None	None	None	1	None
Outcome									
Death within 90 d	No	Yes	Yes	Yes	No	Yes	Yes	No	No
Days after first positive culture	DNA	12	57	7	DNA	4	6	DNA	DNA
Treatment									
Venous access	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Echinocandins	Micafungin	NP	NP	NP	NP	NP	Caspo	NP	NP
Azoles	NP	PCZ	NP	NP	VCZ	PCZ	VCZ	VCZ	VCZ, PCZ
Cytarabine	Yes	Yes	NP	NP		Yes	Yes	Yes	Yes
Ibrutinib	NP		NP	NP	Yes				
Apheresis platelet concentrates	NP	Yes	NP	NP	Yes	Yes	Yes	Yes	Yes

*ALL, acute lymphoblastic leukemia; ANL, acute myeloid leukemia; BMT, bone marrow transplant; Caspo, caspofungin; CLL, chronic lymphocytic leukemia; DNA, does not apply; H, hematology; ICU, intensive care unit; MDS, myelodysplastic syndromes; NA, not available; NP, not prescribed; PCZ, posaconazole; T, stem-cell transplant; VCZ, voriconazole.
†Bronchoalveolar lavage, tracheal aspirate.

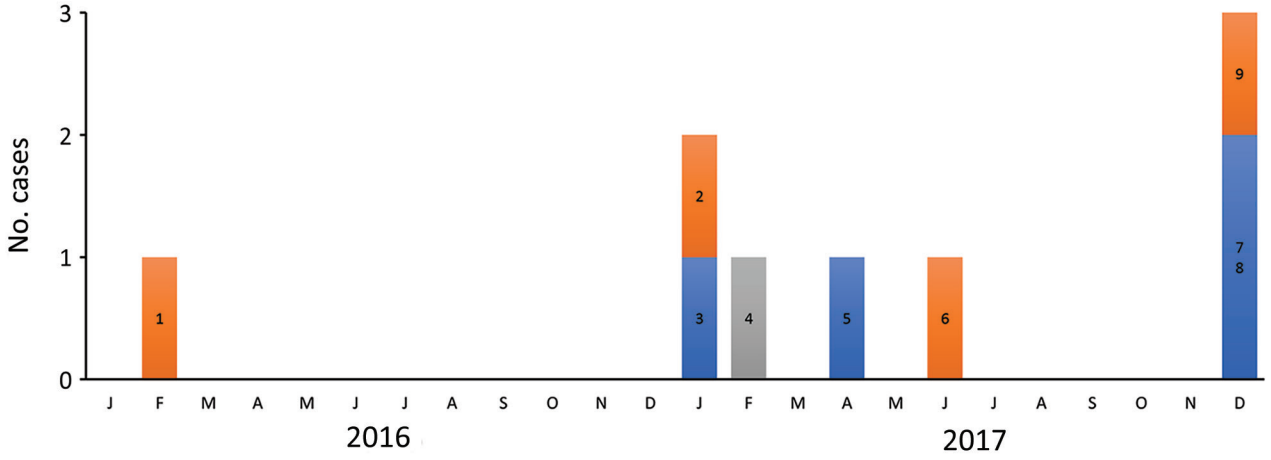

Figure 1. Timeline of outbreak for 9 cases of infection with *Saprochaete clavata* identified in a single center at the Institut Paoli-Calmettes, Marseille, France, February 2016–December 2017. The patients were hospitalized in 3 wards: the hematology unit (orange bar sections), the stem cell transplant unit (blue bar sections), and the intensive care unit (gray bar sections). Numbers 1–9 correspond to patient numbers in the Table.

and hematology units. A retrospective review of laboratory files revealed that *S. clavata* infection had been diagnosed in 6 additional patients during February 2016–July 2017 (Table; Figure 1), bringing the total identified to 9 patients. The 6 patients found retrospectively had been hospitalized in 3 different wards, the stem-cell transplant, hematology, and intensive care units. The median age of the 9 patients was 58 years (range 38–68 years); 6 (67%) were male. All of the patients had central venous catheters; 4 (44%) were treated for acute myeloid leukemia and 6 (67%) had cytarabine chemotherapy. Of the 41 samples testing positive for *S. clavata*, 35 (85%) were blood cultures; fungemia was detected in 8 (89%) of 9 patients on the basis of a mean of 4 (range 1–9) blood samples positive for *S. clavata*. In 5 patients, results were positive only for the blood samples. Results from rectal swab cultures were positive only for patient 8 (Table). Of note, 5 patients had digestive symptoms. The 90-day case fatality rate was 55% (5/9); median survival time for those 5 patients was 7 days after the first positive culture.

Mycological and Environmental Investigation

Among the 95 environmental samples, 75 were sterile, and 10 tested positive for fungi other than *S. clavata* (*Penicillium rubens*, *Lecytophora* sp., *Aspergillus creber*, *Alternaria citri*, *Trichoderma viride*, *Exophiala dermatidis*, *Alternaria alternata*, *Candida lusitaniae*, *Candida parapsilosis*, *Scopulariopsis cinerea*, and *Geotrichum capitatum*). Of the 10 *S. clavata*–positive samples, we collected 6 from the kitchen in the stem-cell transplant ward: 4 samples from the dishwasher (water outlet, interior surfaces, and door seal) and 2 samples from vacuum flasks, 1 each used for coffee and milk. Two of those samples had additional fungi species: milk recovered in 2015 in a patient pitcher lid in the hematologic ward contaminated with *C. lusitaniae* and a coffee pitcher lid from the stem cell transplant ward contaminated with *C. lusitaniae* and *C. parapsilosis*. In the stem cell transplant ward, only a sample from a table surface in patient 8's room tested positive for *S. clavata*. In the hematology ward, we collected *S. clavata*–positive samples from the coffee and milk pitcher lids but found no contamination of the dishwasher. In the central kitchen dishwasher, samples from the prewash area (Figure 2), where water is sprayed to loosen food particles on the dishes, tested positive for *S. clavata*. Finally, samples from 2 different cheeses, proposed as possible vectors at the time of the outbreak, tested negative.

Study of Growth Temperature

The isolates of *S. clavata* and *M. capitatus* tested exhibited similar growth at various temperatures. No growth was detected at ≥48°C.

WGS

Bioinformatic analysis of the WGS data yielded a robust phylogenetic classification for 38 isolates (Figure 3). The 5 isolates belonging to clade A and the 5 isolates from clade B (isolation years 2008–2012) clustered in 2 distinct clades, as described elsewhere (*1*). All of the isolates collected in Marseille after February 2016 clustered into a third new monophyletic clade, referred to as clade C, and had an estimated <10 SNP difference. Multiple isolates recovered from patients

2, 5, 8, and 9 exhibited ≤1 SNP mean difference. Isolates from both environmental and clinical samples clustered in clade C, suggesting a clonal outbreak with a probable common source. The CNRMA15.181 isolate, which was recovered at the same center in January 2015, clustered in neither clade C nor in any other previously identified clade.

Interventions and Control Measures

We discarded and replaced all *S. clavata*–contaminated fomites and the ward's dishwasher as soon as contamination was determined. Even if the water temperature could have achieved >60°C, the dishwasher was discarded because of incomplete drain cycles, seals in poor condition, and overall aging. We discarded the old vacuum flasks and replaced them with simpler models in which the entire device is accessible to washing (Figure 4). In addition, we instituted mandatory guidelines for thorough cleaning and washing after each use.

Discussion

Small outbreaks and sporadic cases of invasive infections due to *Geotrichum* spp. have been reported mostly, but not exclusively, in Europe. As in this outbreak, patients infected by *M. capitatus* and *S. clavata* often share a common clinical background of severe hematologic malignancy and neutropenia. *M. capitatus* (previously known as *G. capitatum*) is the most common reported involving patients in hematology wards (*4*,*16*); *S. clavata* infections are less often reported but occur as sporadic cases or small outbreaks that are usually (*2*,*10*,*11*), but not always (*1*), monocentric.

No study of *S. clavata* outbreaks has so far succeeded in identifying the contamination source (*10*). Contaminated milk jugs have been identified as the source of outbreaks from *M. capitatus* (*17*), and several reports have noted the role of food as a potential source of outbreaks of *Geotrichum* spp. (*17*,*18*). However, because of the lack of accurate databases, earlier reports relied on the association of arthroconidia with lack of urease activity to identify *Geotrichum* spp., and others misidentified *S. clavata* as *M. capitatus* (*19*). Therefore, it is possible that cheese and milk that were reported in the literature (*5*,*17*) to be positive with *Geotrichum* spp. could actually have been contaminated by *S. clavata*. However, to our knowledge, no report has associated *S. clavata* with cheese production (*20*).

Previously, we discovered that some yeast strains recovered from dishwashers were *S. clavata* and not *M. capitatus* as initially reported (*1*,*6*), which might reinforce ingestion as a possible route of *S. clavata* infection. This finding influenced our decision to sample dishwashers and the jugs and vacuum flasks used to deliver food to patients in the hematology and stem cell transplant wards at the cancer center. Recovering *S. clavata* from the dishwashers and jugs was the first step in explaining this monocentric outbreak, because the contaminated utensils from the hematology ward had been washed in those dishwashers. Another possible factor in the dishwasher's involvement in spreading infection might have been the nonremovable lids on the jugs, which could have prevented the dishwasher from completely removing food residues. In a laboratory setting,

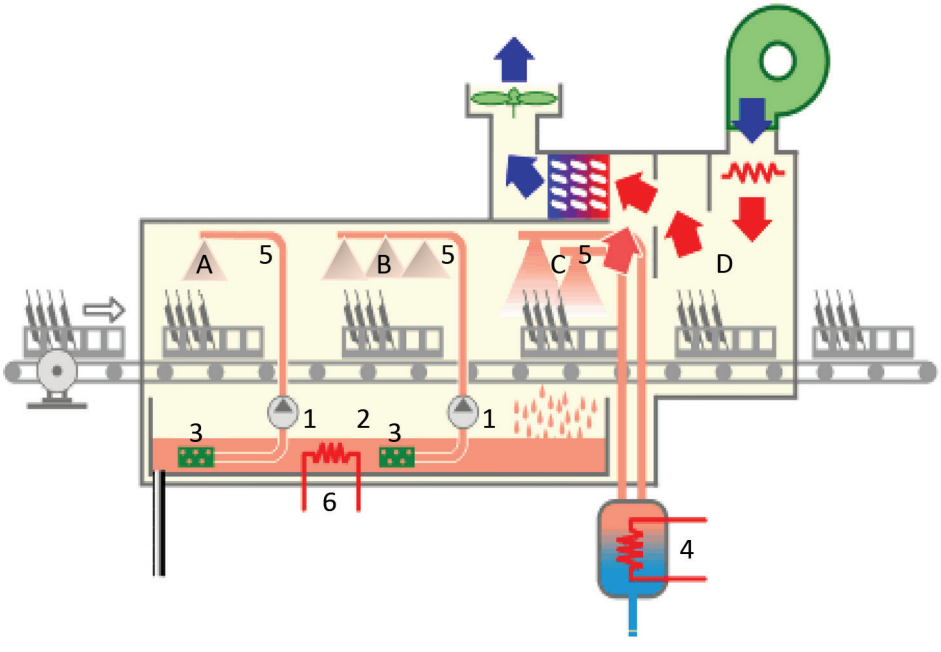

Figure 2. Schematic of dishwasher implicated in outbreak of *Saprochaete clavata* at the Institut Paoli-Calmettes, Marseille, France, February 2016–December 2017. A) Prewash area; B) wash area; C) rinse area; D) drying area. 1, pump; 2, prewash and wash trays; 3, filters; 4, rinse water heater; 5, wash arm; 6, wash heat resistor. Blue arrows indicate cool air flow; red arrows indicate hot air flow. (Figure modified from https://energieplus-lesite.be/techniques/cuisine-collective6/laverie-vaisselle/lave-vaisselle-description [cited 2020 May 20].)

S. clavata has been shown to not survive temperatures >48°C or contact with fungicidal sprays (M. Desnos-Ollivier, unpub. data). Therefore, it is possible that the temperature cycle of the dishwasher, normally capable of reaching temperatures >60°C, may have been dysregulated or the procedure or the detergent used to decontaminate dishes and utensils may have been insufficient. We did not assess these possibilities, but discarding the contaminated fomites and the old dishwasher seemed to control the outbreak. The dishwasher in the central kitchen was also contaminated, but only in the prewash area, ruling out its involvement in the spread of the fungus. Nevertheless, it was decontaminated as a precaution. Finally, we did not uncover any food source for the *S. clavata* infection, possibly because the initial contamination had occurred almost 2 years earlier or because we did not test the correct food samples.

The temporal association of *S. clavata* in the environment with the outbreak offered only a potential link; genetic relatedness needed to be demonstrated. WGS is being used increasingly to investigate outbreaks, especially when genotyping methods are not readily available, such as for rare species. In 2012, following the discovery of a clade, A, as the source of a multicenter outbreak of *S. clavata* infections in France, we designed a real-time PCR so we could rapidly distinguish isolates belonging to clade A or to another clade, B (NRCMA, unpub. data). Since the isolates recovered in Marseille belonged to neither of those clades, we used WGS to study strain relatedness. All of the isolates recovered in Marseille after early 2016,

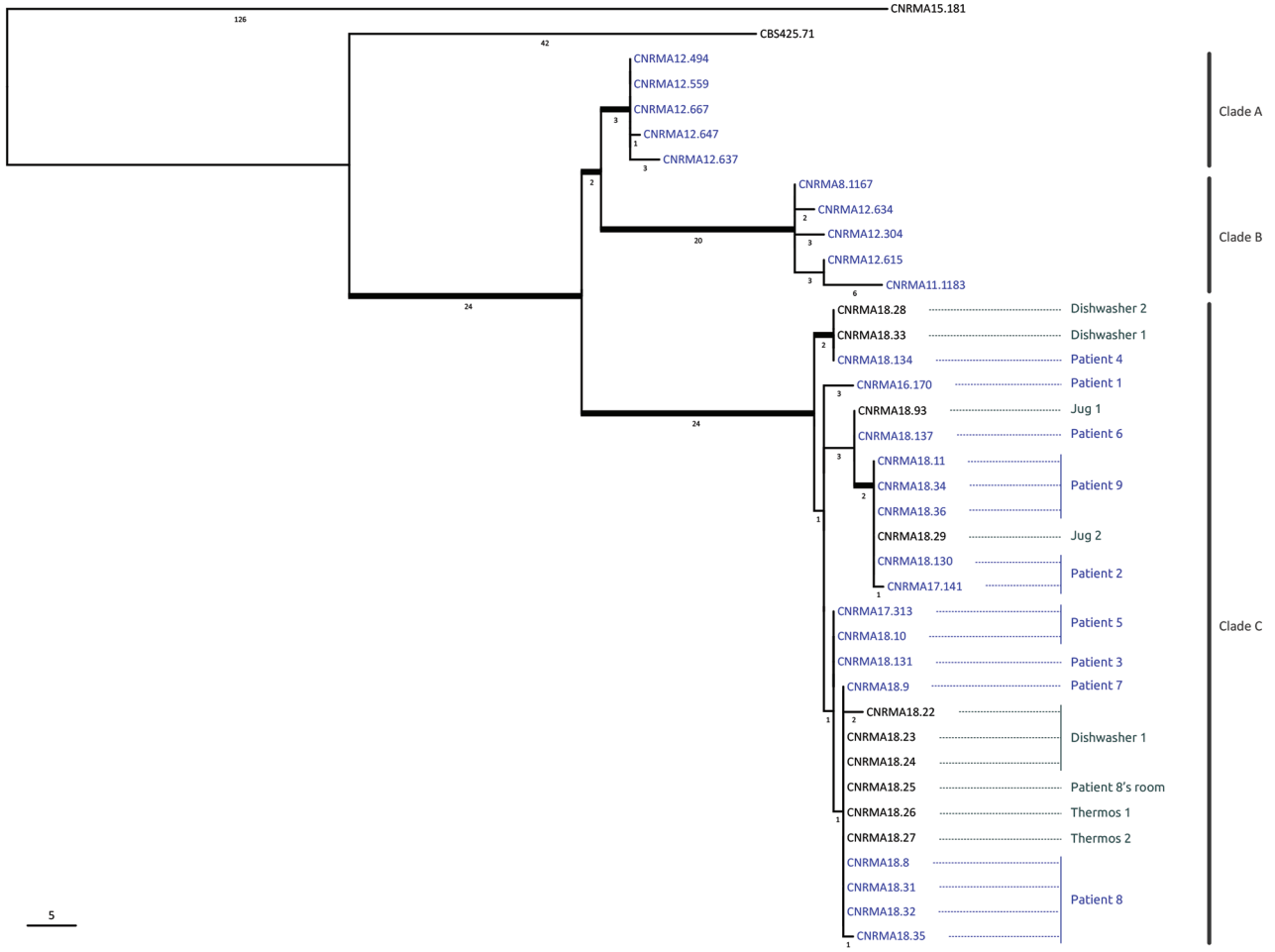

Figure 3. Phylogenetic tree of 38 *Saprochaete clavata* isolates, including isolates from outbreak of *Saprochaete clavata* at the Institut Paoli-Calmettes, Marseille, France, February 2016–December 2017. The unrooted maximum-likelihood tree was inferred from 12,053,164 nt characters with evolutionary model HKY (Hasegawa, Kishino, and Yano, 1985) + FO (base frequencies optimized by ML) + I (proportion of invariable sites optimized by ML). Thick branches are supported by >70% bootstrap supports (500 replicates). The approximated number of single-nucleotide polymorphisms is indicated below each branch. Blue indicates clinical isolates; gray indicates nonclinical isolates. Patient numbers correspond to those in the Table; clades A, B, and C are indicated at the right. Scale bar indicates single-nucleotide polymorphisms.

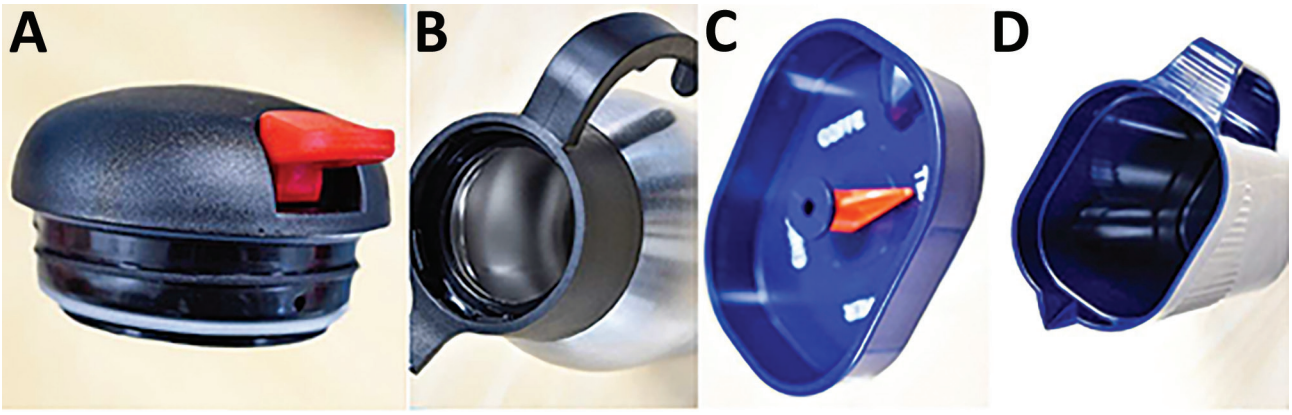

Figure 4. Vacuum flask styles used before and after outbreak of *Saprochaete clavata* at the Institut Paoli-Calmettes, Marseille, France, February 2016–December 2017. A) Old seal; B) old vacuum flask; C) new seal; D) new vacuum flask. The newer model is fully accessible to washing.

including environmental isolates, clustered together into a novel clade, C, different from the previously identified clades. Identifying a unique clade suggested a common source for the contamination, which was restricted to this cancer center in Marseille. Of note, a single case diagnosed in the cancer center in 2015 did not belong to clades A, B, or C and was thus considered a sporadic case.

Our investigation found that a dishwasher made available to patients in the kitchen of the stem cell transplant ward had been the vector of contamination. The fact that patient 4 had been hospitalized in neither the stem cell transplant ward nor the hematology ward before being infected leaves open the hypothesis that contaminated food, of an unknown source, could have contaminated utensils and then the dishwashers, which became vectors of *S. clavata* for other patients. This transmission scheme is supportable using our findings: the contaminated milk or coffee pitchers were used in both hematology and stem cell transplant units; environmental and clinical isolates clustered within the same clade; and the outbreak ended after we removed the pitchers, replaced the contaminated and potentially dysfunctional dishwasher in the stem cell transplant ward, and disinfected the dishwasher in the central kitchen.

Our findings suggest that food-related household appliances, such as dishwashers, can be anthropophilic ecologic niches for *S. clavata* and other life-threatening fungi. Combined with the trend toward providing patients a low-bacterial diet rather than a sterile diet (*21*), this possibility increases the potential for contaminated food. Therefore, routine procedures to protect severely ill patients from airborne or contact contamination should include regular microbiologic sampling, dishwasher testing and maintenance, and controlling the supply and distribution of food. In general, these findings stress the need for continuous extensive vigilance in hospital settings.

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Discussion et perspectives

Les infections fongiques systémiques (IFS) sont un problème majeur de santé publique. Elles sont responsables d'une morbidité et d'une mortalité élevées (1,5 million de personnes/an) chez les patients à haut risque à travers le monde (1) et leur incidence est en constante augmentation. Le 25 octobre 2022, l'OMS a publié une liste de 19 agents fongiques impliqués en pathologie humaine, prioritaires en recherche et développement (5). Les agents inclus ont été divisés en trois groupes de priorité selon des critères définis (résistance aux antifongiques, mortalité, accès au diagnostic, incidence annuelle, complications et séquelles …) : critique élevée et moyenne. A travers notre travail sur l'amélioration du diagnostic des IFS, nous nous sommes intéressés à 10 agents fongiques appartenant à cette liste, répartis dans les trois groupes de priorité. Parmi les agents critiques, nous avons étudié *Candida albicans* et *Aspergillus fumigatus*. Parmi les agents de priorité élevée nous nous sommes intéressés à *Nakaseomyces glabrata* (*Candida glabrata*), *Candida parapsilosis*, *Candida tropicalis*, *Fusarium* sp. et les mucorales. Dans le groupe de priorité moyenne, nous nous sommes intéressés à *Scedosporium* spp., *Lomentospora prolificans* et *Pichia kudriavzeveii* (*Candida krusei*). Le diagnostic des IFS est un défi en raison de présentations cliniques et radiologiques non spécifiques et d'outils diagnostiques souvent insuffisamment performant (82). De plus, il est parfois compliqué de pouvoir évaluer le caractère invasif de certains champignons, quand ils sont isolés à partir de prélèvements biologiques, ce qui nécessite des connaissances concernant les organes où il a déjà été isolé.

Dans un premier temps nous avons réalisé une revue la plus exhaustive possible de la littérature répertoriant 565 champignons filamenteux identifiés chez l'Homme. L'objectif étant d'offrir un catalogue des moisissures identifiées chez l'Homme par culture et/ou biologie moléculaire associée ou non à l'histopathologie. Pour chaque champignon identifié chez l'Hommes, des précisions concernant les organes où il a été identifié ainsi que la sémiologie des infections ont été apportées. Des répertoires exhaustifs sont disponibles dans la littérature mais se limitent à l'identification et la nomenclature de certains genres précis tels que *Penicillium* spp. ou *Aspergillus* spp. (83–85). D'autres plus généraux mais moins exhaustifs, rapportent les principales espèces isolées en pathologie humaine (44,86,87). Mais ces revues ne s'intéressent pas aux champignons plus rares, comme par exemple *Paecilomyces variotii* ou *Purpureocillium lilacinum* pourtant rapportés comme émergent dans les infections sino-

pulmonaire (88). Les revues sont souvent limitées à un site anatomique pour un taxon donné (89–92), à une pathologie (93,94), où à une population à risque (95). Cette revue peut ainsi aider les cliniciens à voir que certains champignons isolés au niveau de sites non stériles, peuvent être responsables d'infections invasives. Ce répertoire peut également avoir un intérêt avec les progrès de la génomique et l'intérêt grandissant porté au mycobiome (96,97). En effet, on peut être amené avec ces nouveaux outils à détecter des champignons rares au niveau de certains sites anatomiques. Savoir si ces champignons ont déjà été identifiés chez l'Homme peut aider à l'interprétation et la compréhension de leur potentiel pathogène (98,99). Une des limites de notre répertoire est l'absence de distinction entre colonisation et infection, et ce majoritairement lors d'identification à partir de sites non stériles. Or, l'utilisation des nouveaux outils moléculaires dans le diagnostic des infections fongiques peut conduire à des difficultés d'interprétation des résultats (100,101). Ce problème se retrouve lors d'identification de micromycètes à partir de sites non stériles, ne permettant alors pas de trancher entre colonisation, infection ou contamination environnementale (27). Ainsi, les recommandations récentes de l'EORTC/MSG, limitent même l'amplification de l'ADN fongique par PCR combinée au séquençage aux prélèvements de tissus uniquement lorsque des éléments fongiques sont observés à l'histopathologie (102). Il serait donc nécessaire de s'intéresser de façon indépendante à chaque site anatomique, afin d'approfondir les facteurs de risque associés à ces infections et de réaliser la distinction entre infection et colonisation. Enfin, notre catalogue permet de présenter de façon succincte l'état des connaissances au 16 juin 2020 sur les champignons filamenteux identifiés chez l'Homme, les organes où ils ont été identifiés et la sémiologie des infections. Ce travail doit être étendu aux levures, dermatophytes et dimorphiques.

Le deuxième axe d'amélioration du diagnostic des infections fongiques systémiques : l'amélioration du diagnostic des candidémies. Dans un premier temps nous avons évalué 11 techniques automatisées d'extraction et de purification des acides nucléiques, mises à notre disposition au cours de la pandémie du COVID-19. Nous avons ainsi pu identifier trois méthodes d'extraction valides, NucliSENS^{TM} EasyMAG^{TM} (BioMérieux), EZ1^{TM} DNA Blood 200 µL Kit avec prétraitement (Qiagen), et EZ1^{TM} DNA Tissue Kit avec prétraitement (Qiagen), permettant la détection optimale des cinq espèces de *Candida* majoritairement impliquées en pathologie humaine (i.e. *Candida albicans*, *Candida glabrata*, *Candida tropicalis*, *Candida parapsilosis* et *Candida krusei*). L'objectif étant de proposer une technique standardisée d'extraction d'acides nucléiques et pouvant s'inclure dans un diagnostic

syndromique des infections systémiques. Cependant, les infections systémiques désignent l'invasion de divers micro-organismes pathogènes dans la circulation, incluant plus de 600 pathogènes principalement bactériens et fongiques, parfois sous la forme d'infections polymicrobiennes (103–105). De rares publications ont évalué des méthodes d'extraction à la fois sur les bactéries et les champignons mais elles présentaient une majorité de techniques manuelles (106,107). Or les techniques d'extraction d'ADN manuelles ont le désavantage d'être chronophages et opérateur dépendant (108). Il faut désormais évaluer ces équipements automatisés d'extraction d'ADN, parmi lesquelles NucliSENS™ EasyMAG™ (BioMérieux), sur l'ensemble des pathogènes responsables d'infections systémiques afin d'identifier une technique d'extraction d'ADN universelle pour les infections systémiques. Dans une continuité, il serait également intéressant d'identifier un système de détection moléculaire standardisé permettant de dépister l'ensemble des pathogènes responsables et de les détecter (109,110). Une autre problématique rencontrée dans le diagnostic des infections systémiques est la nécessité d'optimiser la détection des agents pathogènes (111). Nous avons cherché à mettre au point une technique de capture en solution de *Candida* grâce à des billes magnétiques recouvertes d'anticorps couplée à une détection par PCR. L'objectif est double : améliorer la sensibilité de détection des *Candida* au cours des fongémies par rapport aux hémocultures et de permettre une détection pan-*Candida*. En effet, les outils actuels se limitent à la détection des cinq espèces de *Candida* majoritairement impliquées en pathologie humaine (65). Cependant, d'autres espèces de *Candida* responsables de candidémies ne sont pas à négliger. Par exemple, dans les populations pédiatriques, les espèces *Candida lusitaniae*, *Candida famata*, *Candida utilis* et *Candida kefyr* peuvent être retrouvées à une prévalence supérieure à celle de *Candida glabrata* et *Candida krusei* (112,113). Les résultats préliminaires obtenus sont présentés dans cette thèse. Nous avons évalué trois couples de billes magnétiques-anticorps spécifiques pour la capture en solution de *Candida*. Parmi ces couples, les billes magnétiques couplées à l'anticorps monoclonal type IgM 5B2, l'un des plus spécifiques et des plus affins pour la détection de *Candida*. Pour cet anticorps, un mauvais rendement a été obtenu lors du couplage aux billes magnétiques MagnaLink™ 4FBMagnetic Beads (Solulink, Inc, Le Perray en Yveline, France). Cependant, ce couple a quand même permis une détection de faibles concentrations de levures, bien qu'aléatoire, ouvrant la perspective d'un couplage avec de nouvelles billes magnétiques. Nous avons également évalué deux anticorps type IgG monoclonal (i.e. l'anti-bêta-(1-3)-D-glucane (anticorps recombinant Creative Biolabs, New-York, Etats-Unis) et l'anticorps anti-*Candida albicans* (Meridian Lifescience, Memphis, Etats-Unis)) couplés aux billes magnétiques CELLection™ Pan Mouse IgG Dynabeads (Thermo

Fisher Scientific, Kanagawa, Japon). Nous avons pu constater une fixation aspécifique des levures sur les billes magnétiques ainsi qu'une détection aléatoire pour de faibles concentrations de levures. Il est donc désormais nécessaire, pour la mise au point de cette technologie, de trouver une association billes-anticorps spécifiques idéale. Dans ce but, il serait intéressant de tester des billes de différentes tailles et des mélanges de couples billes-anticorps.

Nous avons également évalué l'intérêt d'outils existants dans le diagnostic d'infections invasives à levure rares émergentes, les trichosporonoses, en rapportant un cas. Ainsi, bien que connue pour d'autres espèces de *Trichosporon* (41,42), nous avons décrit une réaction croisée entre la détection de l'antigène spécifique de *Cryptococcus neoformans* et *Trichosporon japonicum*. La réaction croisée entre *Histoplamsa capsulatum* et le test Platelia *Aspergillus* a démontré son utilité à la fois pour le diagnostic et le suivi des patients atteints d'histoplasmose (40). La détection de l'antigène cryptocoque étant inclue dans les recommandations cliniques conjointes de l'ESCMID et de l'ECMM (61), en cas de suspicion d'infection invasive à *Trichosporon*, il est désormais nécessaire d'évaluer son utilité dans le suivi des patients atteints de trichosporonoses.

Enfin, les micromycètes pouvant être à l'origine d'épidémies nosocomiales, la prise en charge diagnostique passe par la maitrise des sources d'infections fongiques invasives. Lors de l'investigation d'une épidémie de *Saprochaete calavata* dans un centre anti—cancéreux, nous avons identifié pour la première fois un lave-vaisselle mis à la disposition de patients et de leurs familles ainsi que le lave-vaisselle de la cuisine centrale de l'hôpital comme source de contamination. Cela souligne que les patients vulnérables sont constamment exposés aux risques fongiques, y compris dans leur quotidien. Des épidémies nosocomiales d'infections fongiques systémiques liées à la contamination de produits laitiers et autres denrées alimentaires ont déjà été rapportées (114,115). Les patients neutropéniques étant particulièrement à risque de développer des IFS, des recommandations pour la prévention des infections opportunistes chez ces patients ont été élaborées par les Centres de control et de prévention des maladies (CDC, États-Unis) pour les adultes et les enfants (116). Ces recommandations impliquent une prophylaxie antimicrobienne, un environnement protecteur, des soins bucco-dentaires, des soins aux cathéters veineux centraux, le lavage des mains, des pratiques d'hygiène personnelle et des restrictions alimentaires. Ces recommandations peuvent entraîner des frustrations et donc une non–adhérence chez les patients qui sont déjà soumis à de multiples obligations de par leurs pathologies souvent lourdes et à un isolement. Ainsi, la tendance est de proposer aux patients à risque un régime pauvre en bactéries à base d'aliments cuits plutôt qu'un régime stérile

(117,118). Ces régimes sont plus acceptables pour les patients bien que toujours contraignants (119), mais augmentent la possibilité de contamination des aliments. Cette problématique souligne l'intérêt de l'hygiène hospitalière dans la maîtrise et l'élimination des sources d'infection. Récemment, *Saprochaete clavata* a à nouveau été isolé au niveau de la cuisine centrale de ce centre anti-cancéreux, mais cette source a été rapidement maitrisée et aucune infection n'a été recensée. Il est donc nécessaire de réaliser une veille environnementale et une désinfection régulière des sources potentielles de contamination. Enfin, les épidémies n'étant pas prévisibles, et les sources d'infections souvent méconnues, les systèmes de surveillance sont essentiels (120).

Conclusion générale

A travers ce travail de thèse nous nous sommes intéressés à trois axes d'amélioration de la prise en charge diagnostique des infections fongiques systémiques. Dans un premier temps, nous avons répertorié de façon exhaustive l'ensemble des champignons filamenteux impliqués en pathologie humaine et les organes où ils ont été isolés. Ce travail a pour objectif d'aider les cliniciens à l'interprétation de prélèvements positifs à moisissures. Dans un second temps, nous avons évalué des outils diagnostiques pour le diagnostic des candidémies et élargit l'application d'outils existants au diagnostic des trichosporonoses, infections invasives à levures rares émergentes. Nous avons également tenté de développer des outils diagnostiques innovants, dont les résultats préliminaires ont été présentés. Le dernier axe a consisté en l'étude des sources potentielles d'infections fongiques systémiques à levures responsables d'épidémies associées aux soins chez les populations à risque. Nous avons ainsi identifié de nouvelles sources hospitalières. Les résultats obtenus dans ce travail de thèse ouvrent la voie à de nombreux travaux de recherche.

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