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ORIGINAL ARTICLE/ARTICLE ORIGINAL

Direct identification of molds by sequence analysis in fungal chronic rhinosinusitis

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Summary Fungi are widely implicated in chronic rhinosinusitis. Direct microscopic examination (DME) is used to confirm the biological diagnosis of fungal rhinosinusitis (FRS). Diagnostic sensitivity of DME is better than culture, however DME does not allow fungal species identification. In this study, we included 54 sinus samples demonstrating hyphae on DME. Direct sequencing was compared to culture for the identification of the fungal species. Sequence analysis identified fungi in 81.5% of cases while culture was positive in only 31.5%. The most common genus was *Aspergillus* and the identified species belonged to section *Fumigati* or to section *Flavi*. Among other fungi identified by sequence analysis, *Schizophyllum commune* was present in three samples attesting to the importance of this Basidiomycetes in FRS. Our results clearly demonstrate the superiority of sequencing compared to culture when performed on specimens with hyphal elements at DME, and contributes to the epidemiological knowledge of fungi involved in FRS.

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Introduction

Chronic rhinosinusitis (CRS) is a very frequent chronic illness affecting all age groups. Current data support CRS as a predominantly multifactorial inflammatory disease with involvement of virus, bacteria or fungi [1—3]. In 5 to 10% of CRS, fungi are responsible for the disease, which encompass a wide spectrum, ranging from noninvasive disease, including fungus balls and allergic fungal disease, to invasive mycosis [4]. Each disease entity has a characteristic presentation and a specific clinical course.

Mycological diagnosis of fungal rhinosinusitis (FRS) is based on the implementation of different methods for the detection of fungi. Table 1 summarizes the different methods for mycological diagnostic procedures, and their respective advantages and drawbacks. The observation of hyphae in sinus samples by direct microscopy examination (DME) confirms the diagnosis [5,6], but does not allow identification of the fungal species involved. The culture of sinus samples can also confirm diagnosis, however, sensitivity is low and depends on the clinical forms of presentation (fungus ball, allergic fungal disease, fungal invasive sinusitis...) [4,7,8]; for example, in FRS with fungus ball, the percentage of positive culture and fungal identification ranges from 10 to 73% [7]. The development of molecular tools had improved detection of fungi in sinus samples, but the success of pathological fungi molecular identification depends on the choice of methods (Table 1). PCR may amplify commensal fungi that have no role in a pathogenic process but are part of the normal microbiota, and non-viable fungal DNA, as well as fungi implicated in infection. Whichever mycological approach is chosen, a final positive result should be interpreted according to the specificity of the method and the radiological and clinical data, in order to distinguish infection from colonization. In addition, it remains important to ensure correct identification of the species involved, especially for invasive FRS, where an appropriate antifungal treatment is promptly required depending on the fungal species.

The aim of this study was to identify fungi involved in FRS based on a positive direct microscopic examination of endoscopically guided sinus aspiration samples (50 samples) and biopsies (four samples). For this purpose, traditional culture methods were compared to direct sequencing of PCR products obtained after amplification of fungal DNA from sinus contents.

Material and methods

Samples

This study was conducted over three years (2011—2013) at the mycology laboratories of Montpellier and Nîmes University Hospitals in southwest of France. During this period, 50 endoscopically guided sinus aspirates samples and four sinus biopsies presenting hyphal elements on DME were included. A total of 54 samples were analysed and frozen at −20 °C before DNA extraction and sequencing.

Direct microscopic examination, culture and mycological identification

The presence of hyphal elements was observed by DME after Grocott's methenamine silver stain without centrifugation. Then, samples were plated on Sabouraud chloramphenicol gentamicin agar, with or without actidione, and incubated at 27 °C and 37 °C for four weeks. Cultures were examined each day during the first week and twice a week thereafter. The identification of fungi from positive cultures was based on macromorphology and micromorphology and confirmed by sequencing for the strains of *Schizophyllum commune*.

Sequencing

DNA extraction

Before DNA extraction, samples were centrifuged with MagNA Lyser Green Beads (Roche) at 6600 rpm during 90 s

Table 1 Mycological methods for fungal rhinosinusitis diagnosis.

Methods	Advantages	Drawbacks
Direct examination [Observation of hyphal elements]	Rapidity, simplicity Specificity (100%): assertion of FRS by presence of hyphal elements	Lack of sensitivity No fungal species identification
Culture [Strain isolation]	Species identification (by morphological examination, or matrix-assisted laser desorption/ionization-time of flight, or DNA sequencing) Detection of fungal mixture Evaluation of antifungal susceptibility	Lack of sensitivity Positive culture: difficult to differentiate colonization to infection Risk of contamination by conidia from the atmosphere during time of culture
Gene amplification [Detection of gender/species specific fungal DNA, detection of pan-fungal DNA]	Rapidity Fungal species identification: (i) by targeted PCR dependent on the choice of the primers or probes selected; or (ii) by pan-fungal PCR followed by sequencing	Positive PCR: difficult to differentiate colonization to infection Specific PCR: limited number of target species Pan-fungal PCR: no detection of fungal mixture Risk of contamination by amplicon
Next-generation sequencing [Sinus microbiota data]	Identification of a wide range of fungal species Improvement of eco-epidemiology data	Not suitable for routine diagnosis

FRS: fungal rhinosinusitis.

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