**Objectives**:

*Schizophyllum commune* is a saprophytic split-gill mushroom that generally grows on wooden substrates. In recent decades, it has been increasingly reported in human pathology, especially in sinusitis and allergic bronchopulmonary mycosis (ABPM). These infections have mainly been treated with itraconazole, voriconazole or amphotericin B, with or without surgery. However, only three publications have reported the antifungal susceptibility of five or more strains (up to 30 for Chowdhary & al, 2013) all using the CLSI method. Indeed, *S. commune* identification and antifungal susceptibility testing can be challenging. In culture, it appears as white cottony, wooly rapidly growing colonies of non-sporulating molds. This lack of asexual reproduction hampers the inoculum preparation and compromised standardization. We, therefore, aimed to test the susceptibilty of *S. commune* to six antifungal agents using a new standardized inoculation method based on EUCAST and CLSI references techniques.

**Materials & Methods:**

*S. commune* strains (n=113) were collected from patient specimens (n=76) mainly respiratory or sinus samples from 15 French university hospitals or harvested on decaying wood in several locations in southern France (n=37). Strains were cultured on Sabouraud medium supplemented with Bemonyl. Species identification was confirmed by MALDI-TOF mass spectrometry using the MSI-II database, and by molecular sequencing using primers NL1 and NL4. CLSI and the EUCAST techniques were adapted from the CLSI-M38-A2 and the EUCAST E.DEF 9.4 documents respectively. Inoculum was prepared by placing 1 cm2 of culture in ceramic beads tubes containing 1 ml of sterile water. The tubes were vortexed at 2,000 rotations per minutes using a MagNA Lyser automate. The inoculum was then diluted to 1/10 for EUCAST inoculums and 1/50 for CLSI inoculums. Microdilution plates were incubated at 35°C and the results were read at 96h at 100% of inhibition.

**Results**:

*S. commune* showed the lowest MIC for amphotericin B (geometric mean, GM: 0.39 and 0.096 µg/ml in EUCAST and CLSI) and voriconazole (GM: 0.24 and 0.20 µg/ml in EUCAST and CLSI) in both EUCAST and CLSI, while MICs were highest for terbinafine (GM: >8 µg/ml in EUCAST and CLSI) (Table1). MICs were discordant between EUCAST and CLSI for itraconazole (GM: 3.9 and 0.81 µg/ml in EUCAST and CLSI), posaconazole (GM: 4.22 and 0.9 µg/ml in EUCAST and CLSI) and isavuconazole (GM: 3.64 and 0.74 µg/ml in EUCAST and CLSI). Viability count was higher in EUCAST (1403 +/- 1247 colony-forming units - CFU/ml) than in CLSI (658 +/- 491 CFU/ml).

**Conclusions**:

MICs for *S. commune* were low for amphotericin B and voriconazole, intermediate for itraconazole, posaconazole and isavuconazole, and high for terbinafine. Some discrepancies appeared between EUCAST and CLSI and were probably due to differences in inoculum load. This underlines the importance of inoculum standardization of for antifungal susceptibility testing of non-sporulating molds using tubes with beads.



**Table 1.** **A**. *In vitro* profiles of susceptibility of 113 clinical and environnemental isolates of *Schizophyllum commune* in EUCAST microdilution method **B**. *In vitro* profiles of susceptibility of 113 clinical and environnemental isolates of *Schizophyllum commune* in CLSI microdilution method.