**Objectives**:

Mucormycosis is a life-threatening disease, which is difficult to treat due to intrinsic resistance. It has been shown that the use of azole fungicides in agriculture lead to emergence of *Aspergillus fumigatus* resistance to medical antifungals. Selection pressure from azole fungicides could also be involved in the emergence of mucormycosis.

The objective of this study is to provide a detailed description of the *Mucorales* species present in an environment subjected to significant azole fungicides pressure (sawmills in Eastern France) , as well as their sensitivity profiles to various azole molecules.

**Materials & Methods:**

*Mucorales* strains, selected by culture on itraconazole or voriconazole medium at 48°C, were isolated from environnemental substrates collected during a previous study of *A. fumigatus* resistance prevalence in sawmills (Jeanvoine, J Appl Microbiol, 2017). Fungal strains identified as *Mucorales* by macro and microscopic characteristics (n= 202 ) were conserved in glycerol medium at -80°C. Data on the use of azole fungicides in the sawmills were collected to categorize the sites into “azole” and “no azole” sawmills.

For the present study, the *Mucorales* strains were thawed, and then identified at the species level by MALDI-TOF spectrometry or ITS sequencing. The MIC of 5 azole compounds has been determined, 2 medical antifungals (posaconazole and isavuconazole) and 3 agricultural fongicides (propiconazole, tebuconazole and difenoconazole). The MIC were determined according to the EUCAST protocol. The *cyp51A* gene sequencing was done for all the *Mucor circinelloides* strains (Caramalho, Sci. Rep.2017).

**Results**:

In total, 202 strains of *Mucorales*, isolated in 18 sawmills (13 “azole” and 5 “no azole”) between 2014 and 2016, could be identified at the species level. The identification revealed 6 *Mucorales* species belonging to 4 genera. Fifty three pourcent (107/202) of the strains belonged to the *Rhizomucor* genus of which 93.5% (100/107) are *Rhizomucor pusillus*. The other species are distributed as follow : 23.8% of *Rhizopus microsporus* (48/202), 11.4% of *Lichtheimia ramosa* (23/202), 4% of *Mucor circinelloides* (8/202) and 4.5% of other species.

The distribution of Mucorales species did not significantly differ between in “azole” *versus* “non azole” sawmills.

The MIC for posaconazole ranged from 0.25 to 1 µg/mL, for isavuconazole and the azoles fungicides from 4 to >16 µg/mL.

Four strains of *Mucor circinelloides* revealed mutations in proteic sequence : A256T (n=1) and E344K (n=3) but no differences in MIC were observed compared to strains without mutation.

**Conclusions**:

In conclusion, the various Mucorales species identified in the sawmill exhibit high MICs to agricultural fungicides, which can be related to the strong selection pressure in this environment. The MICs for the two azole antifungals currently used as second-line treatments were also high for these species, which are associated with severe human infections. The mutations detected in the CYP51A protein sequence of Mucor circinelloides strains do not appear to be involved in antifungal resistance. Further molecular analysis is needed to investigate the mechanisms underlying azole resistance in Mucorales