Infections caused by Candida species have increased worldwide substantially over the latest decades, and are a significant cause of morbidity and mortality, mostly among critically ill patients. Candida glabrata is the second most common Candida responsible for these infections in the USA and the third in Europe, and is characterized by a high antifungal resistance.

- To understand the role of mannans in C. glabrata biofilms and in biofilm cells resistance to antifungal drugs (fluconazole - Flu, amphotericin B - Amb, caspofungin - Csf and micafungin - Mcf).

1. Biofilm structure:
   - Confocal microscopy

2. Biofilm matrix analysis:
   - Biomass reduction (Crystal Violet)
   - Mannans (Quantitative Alcian Blue Binding Assay)
   - Polysaccharides (Dubois method)
   - β-1,3 glucans (Glucatell® Kit)

The biofilm matrices showed to reduce their mannans content in the presence of all drugs in C. glabrata ATCC2001 and C. glabrata HT6.

Interestingly, in C. glabrata Δmnn2, these compounds were unable to be detected in the biofilm cell walls, in all conditions. 

β-1,3 glucans increased in the biofilm matrices of the strains in contact with all drugs, when compared to the control group.

C. glabrata HT6 and the mutant, C. glabrata Δmnn2 showed to have the highest amounts in these sugars.

The KO of the MNN2 gene does not reveals any microscopic changes in the cell wall.

The lack of mannans leads to a more fragile biofilm and a higher biomass loss after a drug stress.

The polysaccharides content increase in the biofilm matrix of C. glabrata strains in contact with Flu, Amb and Mcf, but the mannans have the opposite behavior.

All the strains produce high quantities of β-1,3 glucans when in the presence of all drugs, specially the mutant, which is probably attempting to compensate the lack of mannans in the matrix;

C. glabrata Δmnn2 has a more fragile biofilm than the other strains, which can alter its drug resistance.

## Results

### A. Susceptibility of the biofilm to the antifungal agents

<table>
<thead>
<tr>
<th>Method</th>
<th>C. glabrata strains</th>
<th>ΔT=24 h</th>
<th>+ΔT=24 h</th>
</tr>
</thead>
<tbody>
<tr>
<td>C. glabrata strains:</td>
<td>C. glabrata ATCC2001 (wild-type)</td>
<td>+ΔT=24 h</td>
<td>+ΔT=24 h</td>
</tr>
<tr>
<td></td>
<td>C. glabrata HT6 (parent)</td>
<td>+ΔT=24 h</td>
<td>+ΔT=24 h</td>
</tr>
<tr>
<td></td>
<td>C. glabrata Δmnn2 (mutant)</td>
<td>+ΔT=24 h</td>
<td>+ΔT=24 h</td>
</tr>
</tbody>
</table>

Change half medium and add:
- a. RPMI-1640 (control group)
- b. Antifungal: Flu, Amb, Csf or Mcf* (*at MBECs concentrations)

Polysaccharides tended to increase in the biofilm matrices of the strains in contact with all drugs, specially with Flu, Amb for C. glabrata HT6 and Mcf for C. glabrata Δmnn2 (P<0.0001).

The lack of mannans leads to a more fragile biofilm a higher biomass loss.

C. glabrata Δmnn2 presented the top biomass reduction, specially when in contact with echinocandins.

### Conclusion

- The KO of the MNN2 gene does not reveals any microscopic changes in the cell wall;
- The lack of mannans leads to a more fragile biofilm and a higher biomass loss after a drug stress;
- The polysaccharides content increase in the biofilm matrix of C. glabrata strains in contact with Flu, Amb and Mcf, but the mannans have the opposite behavior;
- All the strains produce high quantities of β-1,3 glucans when in the presence of all drugs, specially the mutant, which is probably attempting to compensate the lack of mannans in the matrix;
- C. glabrata Δmnn2 has a more fragile biofilm than the other strains, which can alter its drug resistance.

### Acknowledgements

This study was supported by the Portuguese Foundation for Science and Technology (FCT) under the scope of the strategic funding of UID/MED/04865/2013 and COMPETE 2020 (POCI-01-0145-FEDER-026648) and BioTechnology operation (NORTE-01-0145-FEDER-000049) funded by the European Regional Development Fund under the scope of Norte2020 - Programa Operacional Regional do Norte.

The authors also would like to thank Mário Dinis and Ana Beatriz for the kind donation of caspofungin and micafungin.