THE BURDEN OF CANDIDA PARAPSILOSIS BLOODSTREAM INFECTIONS: FROM AZOLE RESISTANCE TO BIOFILM PRODUCTION.

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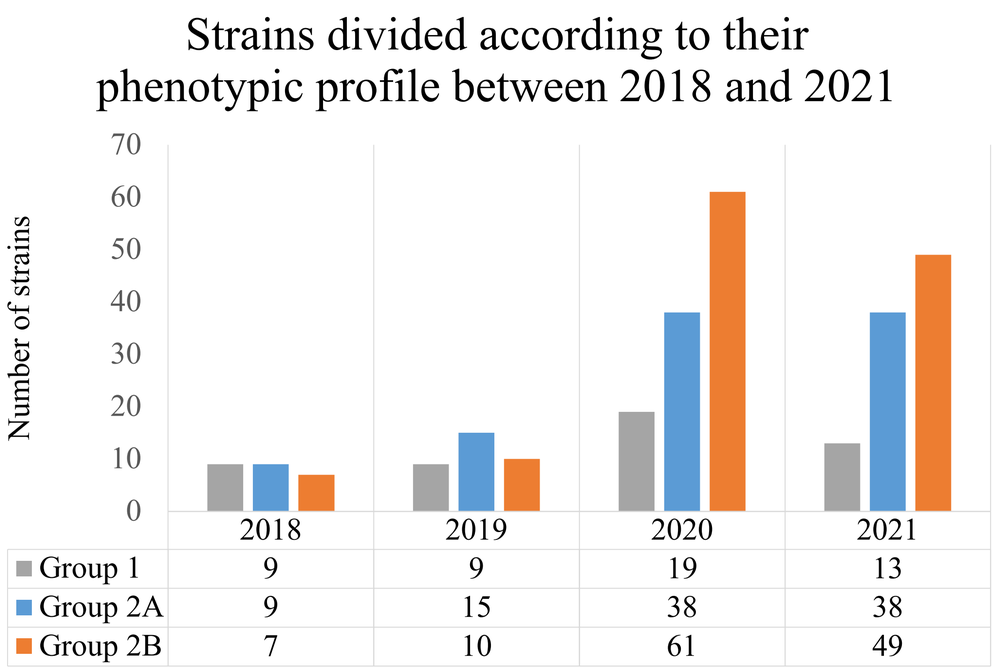
THE AIM OF OUR STUDY

Starting from 2018, a shift has occurred in the epidemiology of candidemia in our hospital setting, with particular reference to strains of *Candida parapsilosis* showing high resistance to fluconazole and reduced sensitivity to other azoles. Consequently, the aim of our work is to evaluate the correlation between phenotypic/genotypic resistance to azoles in *C. parapsilosis* strains isolated from blood cultures, with specific reference to the ERG11 gene, and their ability to produce biofilm.

MATERIALS AND METHODS

C. parapsilosis strains were isolated from blood cultures using the BACTEC system broth culture and identified phenotypically using MALDI-TOF mass spectrometry. Antifungal susceptibility was determined using the microbroth method (Sensititre YeastOne Colorimetric Broth) and interpreted according to the Clinical & Laboratory Standards Institute (CLSI) guidelines. The different isolated strains were classified into three groups (Figure 1): Group 1 comprised strains susceptible to fluconazole (MIC <= 2 mcg/ml) and voriconazole (MIC <= 0.12 mcg/ml), Group 2A included strains resistant to fluconazole (MIC >= 8 mcg/ml) and voriconazole (MIC >= 1 mcg/ml), and Group 2B consisted of isolates resistant to fluconazole (MIC >= 8 mcg/ml) and intermediate to voriconazole (MIC 0.25-0.5 mcg/ml).

The study of genotypic resistances was conducted by sequencing the ERG11 gene using Sanger sequencing. Analysis of biofilm production was assessed using the Ramage method. Evaluation was quantified by assigning a biofilm score (BS) according to the following scheme: strains with a score of 1+ or 2+ were considered low producers, 3+ or 4+ moderate producers, and 5+ or 6+ good producers of biofilm.



Graph 1: Number of strains of *C. parapsilosis* belonging to different groups of resistance and sensitivity to azoles in the period 2018-2021.

RESULTS

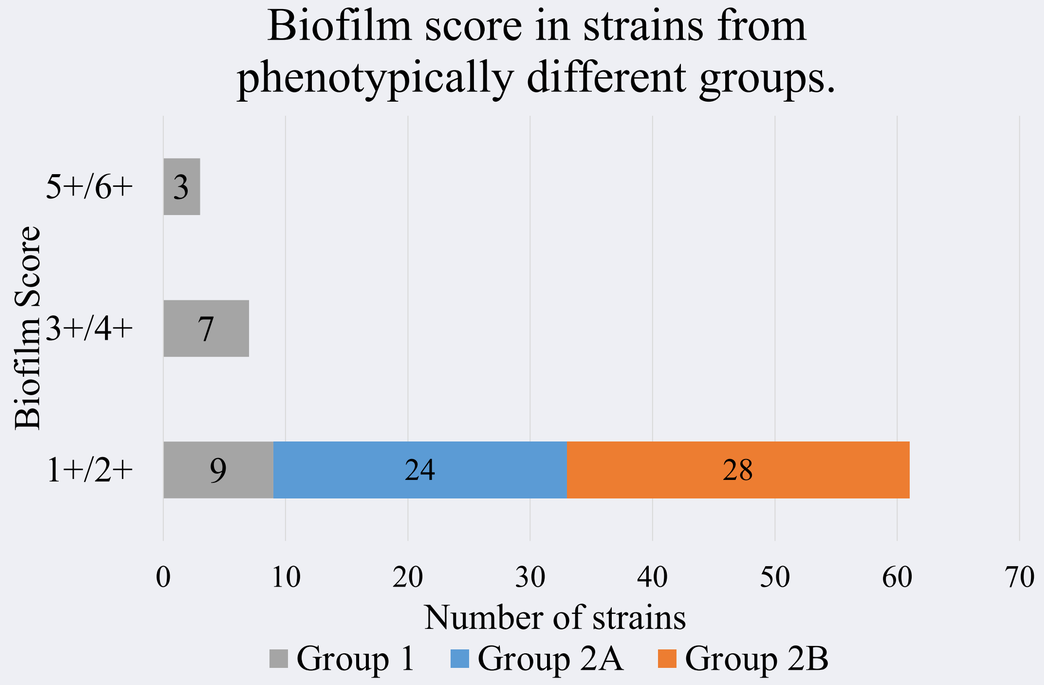
Regarding 46% of the strains isolated in 2021 (46/100), the sequences of the ERG11 gene were analysed, revealing the presence of mutations Y132F, I197I, and R398I. Of these 46, 74% belonged to groups 2A and 2B. Only one strain belonging to group 1 exhibited the R398I mutation, which did not correlate with phenotypic resistance. Furthermore, these strains showed low biofilm production (BS 1+,2+).

As for the biofilm analysis, a total of 71 strains of *C. parapsilosis* were evaluated. Strains, belonging to groups 2A and 2B, were associated with low biofilm production. Conversely, azole-sensitive strains analysed so far have shown higher biofilm production (see Figure 2).
In particular, of all tested isolates belonging to group 1, 36.84% exhibited a BS equivalent to 3+/4+ (7/19), and 15.78% showed a high BS corresponding to 5+/6+, while the remaining 47.36% showed a low BS (1+/2+). A low BS (1+/2+) was observed in all the tested strains that were phenotypically resistant to fluconazole (n=52). Overall, 14.1% of the analysed strains (10/71), all belonging to group 1 showed a BS ranging from 3+ to 6+.

CONCLUSIONS

The obtained results suggest that strains with mutations Y132F and I197I correlate with the phenotypic profile of fluconazole resistance, although the latter, being synonymous, does not result in a change in the amino acid sequence of the protein of interest and therefore does not impact the morphology of the target region. The R398I mutation, on the other hand, does not correlate with a specific resistance phenotype.

Additionally, it can be hypothesised that azoles, not being endowed with anti-biofilm activity against various *Candida* species, unlike echinocandins and liposomal amphotericin B, may have reduced efficacy "in vivo," even against the phenotypically sensitive strains isolated in our hospital setting, especially if candidemia is catheter-related.



Graph 2: Correlation between different phenotypic profiles of *C. parapsilosis* strains and biofilm production.