Minimum inhibitory/effective concentrations (μg/ml) were as follows in increasing order: terbinafine = 0.25, amphotericin B = 1, isavuconazole = 4, micafungin > 8, itraconazole > 16, voriconazole > 16, and posaconazole > 16. To evaluate the interactions between antifungal drugs, the activity of the posaconazole in combination with terbinafine were also evaluated *M. wolfii* using agar diffusion test. A combination of posaconazole and terbinafine, significantly inhibited the mycelial growth, which indicates synergism. The patient's treatment was started on terbinafine in combination with posaconazole. On several follow-up examinations following treatment on day 30, 90 and 120, the infection had not recurred.

Conclusion: The species of *M. wolfii* a is an environmental mold belongs to the order *Mortierellales* within the subphylum *Mortierellomycotina* of Kingdom Fungi. This fungus has been mostly associated with fungal infections leading to abortion in dairy cows feeding moldy hays and ensilage.

Although posaconazole exhibited high MICs against *M. wolfii*, our *in vitro* combination study demonstrated that posaconazole and terbinafine combined are significantly more potent than either drug alone. As a suggestion, combination therapy could provide an option for the treatment of severe cases of *M. wolfii* in patients with underlying primary immunodeficiencies.

As molecular identification and sequencing techniques continue to develop and become more available, we will likely see more diverse pathogens emerge in patients with underlying primary immunodeficiencies. In this current case. Additional study is warranted to explore insight into human immunity and the efficacy of combination therapy against rare fungal species in CGD patients.

P001

Characteristics and dynamics of azole-resistant *Aspergillus fumigatus* variants emerging over a 28-year period in the Netherlands

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Poster session 1, September 21, 2022, 12:30 PM - 1:30 PM

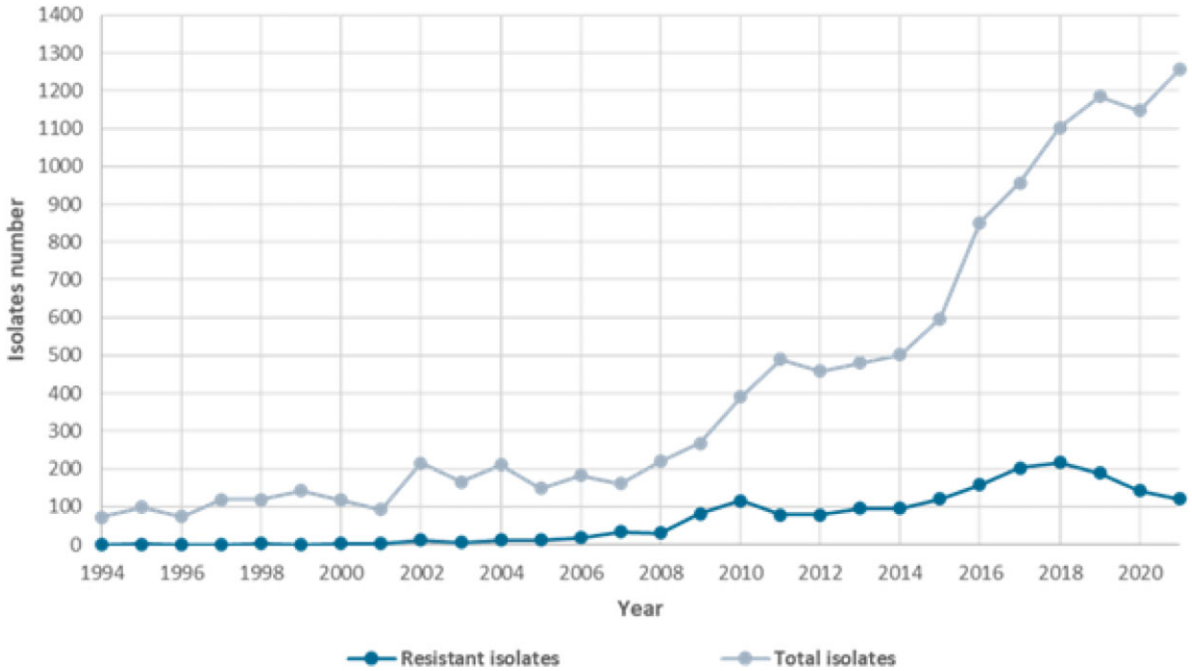
Background: *Aspergillus fumigatus*, a globally distributed opportunistic pathogen, is the main cause of invasive aspergillosis, especially in immunocompromised patients with high mortality. The emergence of azole-resistant *A. fumigatus* isolates has been a significant concern worldwide and an important clinical problem.

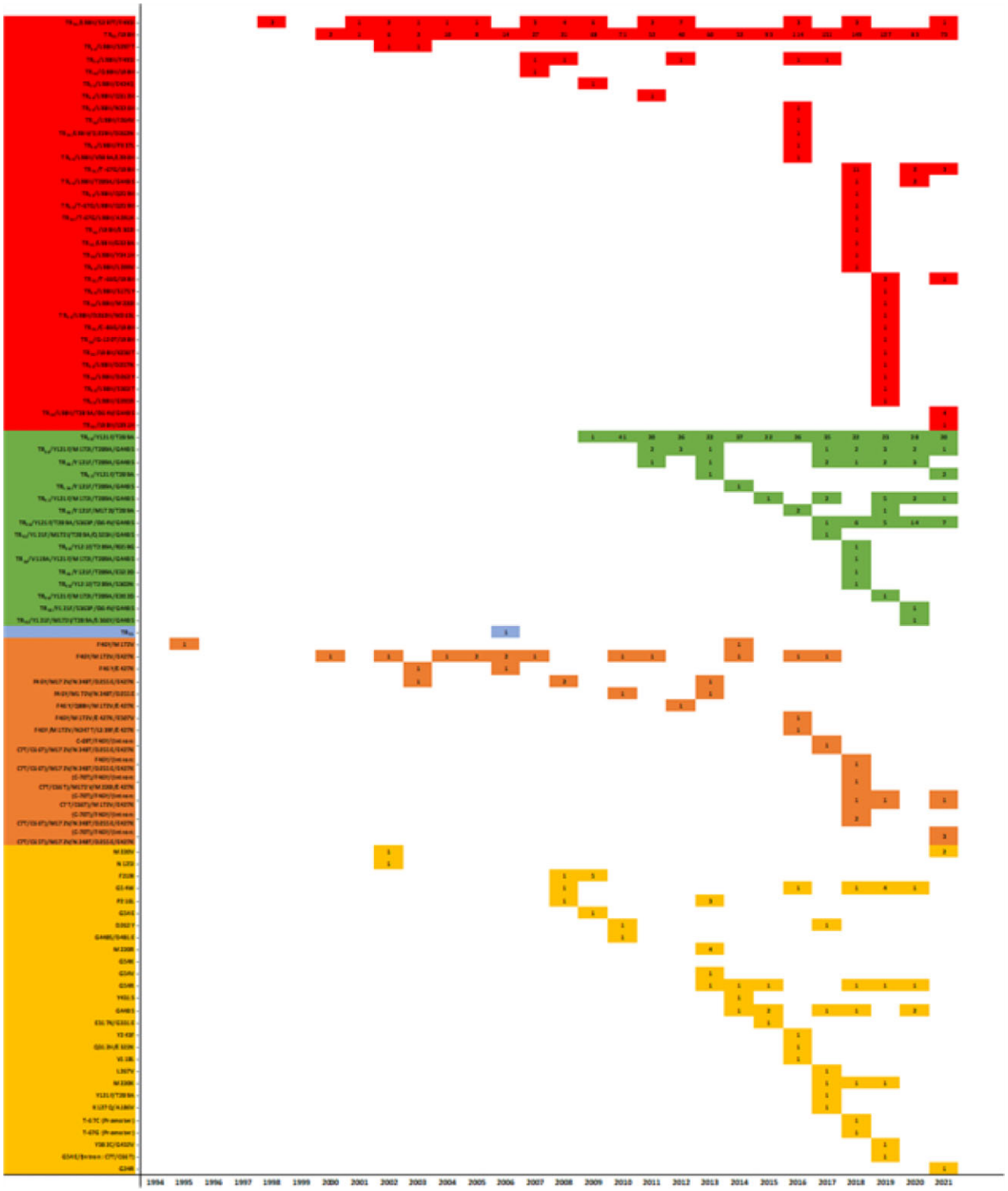
Objectives: We aim to determine the presence of variants in a large collection of clinical *A. fumigatus* isolates from the Netherlands, if the number of variants increased over time and if the presence of additional short nucleotide polymorphisms (SNPs) or tandem repeats (TR) variations impacted on the triazole phenotype.

Methods: The Radboud University Medical Center has collected 11 813 clinical *A. fumigatus* isolates since 1994. The collection includes isolates cultured from patients admitted to our own center, isolates sent from other hospitals for identification and *in vitro* susceptibility testing, and isolates sent from five university medical centers and five teaching hospitals that contribute to the national *Aspergillus* resistance surveillance. The genotypes were detected by Cyp51A Sanger sequencing. All isolates were subjected to *in vitro* susceptibility testing using the EUCAST microdilution reference method. Minimal inhibitory concentrations (MICs) were determined for itraconazole, voriconazole, posaconazole, in all isolates and for isavuconazole in isolates cultured in 2015 and thereafter.

Results: In total, 1826 *A. fumigatus* isolates harbored azole-resistant mutations in the Cyp51A-gene with 92 genotypes. Tandem Repeat-associated resistance genotypes accounted for 55.43% of the variants and were involved in 1728 isolates (94.63%). TR34/L98H and TR46/Y121F/T289A resistance mutations remained dominant, and increasingly additional SNPs in the Cyp51A-gene or changes to the gene promoter were observed. The G448S mutation was relatively common and present in various genetic backgrounds. This SNP was most often found in isolates harboring the TR46 resistance mechanism (8 variants) and was also observed in two variants in the TR34 genetic background. TR34 and TR46 resistance mutations are associated with 1170 (64.07%) isolates that exhibited a pan-azole resistance phenotype, 547 (29.96%) a multi-azole resistance phenotype, and 75 (4.11%) resistance to a single azole. TR34/L98H confers high itraconazole resistance, while T289A confers high voriconazole resistance in the TR46 background. Isolates with a G448S point mutation show high MICs for both voriconazole and itraconazole. The TR34/L98H/T289A/G448S isolate showed low itraconazole MICs but high voriconazole resistance, and mutations in the promoter region, TR34/C-86 G/L98H, and (T-66 G)/TR34/L98H variants, showed increased voriconazole and isavuconazole MIC compared with the parent phenotype. TR46/Y121F/M172I/T289A/G448S variant was observed with an increased itraconazole (GM MIC 16 mg/L, 1→16 mg/l) and decreased voriconazole (GM MIC 18.664 mg/l, 4→16 mg/l) compared with the parent MIC of TR46/Y121F/T289A, while TR92/Y121F/M172I/T289A/G448S and TR46/Y121F/T289A/G448S variants showed the consistent MIC distribution with parent genotype. The variants with more combination mutations showed pan-azole resistance with increased MIC distribution.

Conclusion: Our survey showed a significant increase in resistance genotypes in clinical *A. fumigatus* over a period of 28 years. Azoles resistance phenotypes vary from resistant variants in clinical isolates; it is an implication for clinical *A. fumigatus* infection treatment options and antifungal stewardship.





P002

Antifungal activity of antimicrobial synthetic peptides against *Candida* species of public health importance

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Background: Candidiasis is one of the most frequent opportunistic infections in immunosuppressed and/or hospitalized patients. In countries like Colombia, candidiasis is associated with a mortality rate of ~ 46%. Growing pharmacological resistance of *Candida* spp., and the appearance of the emerging pathogen *Candida auris*, have turned candidiasis into a major public health problem. Different types of antimicrobial peptides have been investigated as a therapeutic alternative to control candidiasis effectively and safely.

Objective: This work aimed at evaluating the *in vitro* antifungal activity of three synthetic antimicrobial peptides (35 409, 1609, and 29 009) obtained from *Plasmodium falciparum* Rif1 protein against *C. auris*, *C. albicans*, *C. glabrata*, *C. parapsilosis*, *C. krusei*, and *C. tropicalis*, species with worldwide clinical importance.

Methods: The minimum inhibitory concentrations (MIC) of the three peptides against *Candida* species were determined by the plate microdilution method; the peptides' effect on biofilm formation in *C. auris* and *C. albicans* species was also evaluated through the XTT metabolic activity assay. Additionally, the structural damages in *C. auris* and *C. albicans* caused by the action of the peptides were observed by transmission electron microscopy (TEM) and finally, the *in vitro* peptides' cytotoxicity against L929 murine fibroblasts was verified.

Results: Our findings showed that the three peptides herein evaluated, displayed antifungal activity in both planktonic and sessile *Candida* cells. Likewise, the TEM evidenced morphological alterations induced by the peptides, both in the membrane and at the intracellular level of the yeasts. As well, total safety against the murine cell line L929 with 24 h of treatment was observed.

Conclusions: From these results, we conclude that the antimicrobial peptides 35 409, 1609, and 29 009 are potential therapeutic alternatives against the most important *Candida* species in Colombia and the world.