TITLE: Development of resistance in *Aspergillus fumigatus* by prolonged exposure to Voriconazole in an immunocompromised patient

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ABSTRACT: *A. fumigatus* are filamentous fungi that are among the most abundant in the environment and can be the causal agent of various pathologies. The clinical presentation of Aspergillosis is determined by the interaction between microorganism and host and the clinical manifestations are determined by host immune response, having Invasive Aspergillosis the worst clinical prognosis. The first therapeutic choice for Aspergillosis are azole antifungals, but their prolongued clinical use and the indiscriminate use of high concentrations of analogues in agriculture, lead these microorganisms to seek escape routes expressing resistance mechanisms to these compounds. Nine clinical sequential *A. fumigatus* isolated from 2018 to 2020 were evaluated to perform this study. All from the same patient who underwent heart transplantation. With the first isolation of *A. fumigatus*, long-term treatment with Voriconazole was started. Broth Microdilution to obtain the Minimum Inhibitory Concentration was performed according to CLSI document M38-Ed3 guidelines for Caspofungin, Micafungin, Amphotericin B, Voriconazole, Itraconazole, and Posaconazole. The search for mutations in the *CYP*51A gene was performed. Microsatellite patterns were analyzed to correlate the isolates with each other. The results obtained through microdilution in broth showed two strains with resistance to Voriconazole (>8µg/mL) and sequencing of the *CYP*51A gene showed that the same two isolates (LIF3546 and LIF3608) carried a G1413A mutation that was responsible for a G448S amino acid substitution. Microsatellite genotyping showed the same genotypic pathern for all nine isolates, suggesting the selection of resistant ones due to the pressure of prolongued use of voriconazole. The G448S mutation found has already been characterized as having an important role in the development of resistance to Voriconazole what can be confirmed as all isolates were genotypic related to the first isolate (2018). Surveillance of resistance to *Aspergillus fumigatus* has become essential within the clinical laboratory, as azole resistance is increasingly detected every day.