**Objectives:**

The emergence of azole-resistant *Aspergillus fumigatus* strains in clinical samples represents a significant challenge to the effectiveness of antifungal therapies that has been linked with the environmental use of fungicides. The main objective of this study was to investigate the prevalence and genetic characteristics of A. fumigatus strains in both clinical and environmental settings. Specifically, we aimed to assess the presence of azole-resistant strains in both ambient air and clinical samples, examine whether identical genotypes are shared between them, and explore potential connections between environmental and clinical reservoirs.

**Materials & Methods:**

We conducted a comprehensive study over 30 months, analyzing clinical *A. fumigatus* isolates from two hospitals referred to the National Reference Laboratory, as well as environmental isolates obtained from ambient air sampling in two points located in the area of influence of these hospitals. Totally Suspended Particles (TSP) air samples were collected monthly in gelatine filters following UNE CEN/TS 16115-1:2013 Technical Specification.

To assess antifungal resistance, environmental isolates were screened using EUCAST E.Def 10.1 and confirmed by EUCAST E.Def 9.4. Clinical isolates were evaluated according to EUCAST E.Def 9.4. All clinical, azole resistant environmental and a proportion of susceptible environmental isolates were genotyped following TRESPERG method. Resistance mechanisms were further investigated by sequencing the *cyp51A* gene and its promoter.

In addition, we applied next-generation sequencing (NGS) to environmental and clinical isolates with the same TRESPERG genotype to further examine the genetic relationship among them.

**Results:**

We analyzed a total of 950 *A. fumigatus* isolates, including 442 clinical and 508 environmental samples. Azole resistance was detected in 3.6% (n=16) of clinical isolates and 15.4% (n=78) of environmental isolates. Notably, 43.8% (n=7) of the clinical isolates and 76.9% (n=60) of the environmental isolates exhibited the TR34/L98H mutation, while the remaining isolates did not show any mutations in the *cyp51A* gene or its promoter.

A total of 14 TRESPERG genotypes were common to both clinical and environmental strains. Specifically, 6 of these TRESPERG genotypesincluded isolates with differing susceptibility profiles, encompassing both resistant and susceptible strains. Although NGS results are still pending, preliminary findings suggest a promising level of genetic similarity between certain clinical and environmental isolates with the same TRESPERG genotype, reinforcing the potential connection between environmental and clinical strains.

**Conclusions:**

Our findings demonstrate that azole-resistant *A. fumigatus* strains are present in the ambient air in Madrid in a higher proportion than in the clinical isolates. TR34/L98H mutation was the most frequently found mechanism of antifungal resistance in both the clinical and the environmental strains but isolates without cyp51A mutations were also found.

The shared genotypes between clinical and environmental isolates highlight the potential for environmental transmission of resistant strains. Surveillance programs for antifungal resistance in the environment are necessary as well as further understand the drivers of resistance in order to develop preventative measures to mitigate the impact of azole resistance in the clinical setting.