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Potentially voriconazole resistant environmental *Aspergillus* strains in the Basque Country

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INTRODUCTION

Aspergillus constitutes one of the most prevalent fungal genera in the environment, including some opportunistic pathogen species e.g. *Aspergillus fumigatus*. These fungi are capable of causing serious infections, the most lethal invasive aspergillosis, in immunosuppressed patients which could reach a mortality rate of up to 95%. The treatment of those fungal infections consists mainly of antifungal agents such as azoles. However, in recent years, antifungal resistance has become an issue of major concern. One of the possible origins of these resistances is clinical, due to prolonged treatments in chronic patients. However, another possible origin is environmental, due to the use of azoles in agriculture^{2,3}. Therefore, it is of paramount importance to assess the prevalence of antifungal resistance in the environment. Hence, the aim of this study is to detect and analyze potentially resistant *Aspergillus* strains in the environment of the Basque Country.

MATERIAL & METHODS

1. Air sampling and growth conditions

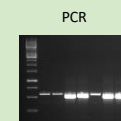
Air samples were collected in November 2021 using the MAS-100 Eco Air Sampler in 3 different environments (hospital, urban and rural areas) of each province of the Basque Country (Bizkaia, Araba and Gipuzkoa).



Growth conditions:

- Temperature: 28°C or 37°C
- Growth media: SAB or SAB-VCZ (1 mg/L)

2. Strains identification



Sequencing



The identification of the *Aspergillus*-like colonies was performed by the amplification and subsequent sequencing of the region between the Internal Transcribed Spacer 1 (ITS1) and ITS4.

3. EUCAST microdilution method



EUCAST microdilution method⁴ was carried out to assess the resistance to voriconazole of the isolated *Aspergillus* species. All experiments were performed in triplicate.

As expected, both the temperature and the antifungal acted as restriction factors. Therefore, the plates supplemented with voriconazole incubated at 37°C were the ones that showed less growth regardless of the sampling point (Figure 1). Concerning provinces, less fungal load was detected in Araba compared to Gipuzkoa and Bizkaia which could be explained by its cold and less wet weather. Moreover, Bizkaia as well as Araba did not show any variation of CFU/m³ in each experimental environment at 28°C.

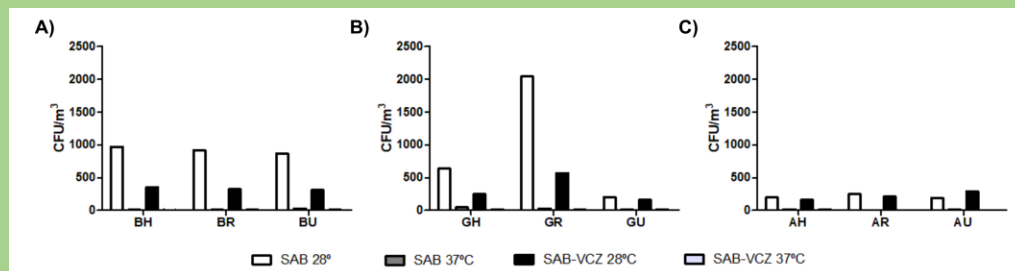


Figure 1. Representation of the colonies forming units per volume of filtered air (CFU/m³). The values of each province are shown: (A) Bizkaia, (B) Gipuzkoa, and (C) Araba. The tested conditions were: SAB 28°C (white), SAB 37°C (dark grey), SAB-VCZ 28°C (black), and SAB-VCZ 37°C (light grey). SAB: Sabouraud Agar. SAB-VCZ: Sabouraud Agar supplemented with voriconazole (1 mg/L). BH: Bizkaia Hospital area. BR: Bizkaia Rural area. BU: Bizkaia Urban area. GH: Gipuzkoa Hospital area. GR: Gipuzkoa Rural area. GU: Gipuzkoa Urban area. AH: Araba Hospital area. AR: Araba Rural area. AU: Araba Urban area.

Seven *Aspergillus*-like strains were isolated from samples corresponding to hospital and urban areas of the three provinces of the Basque Country. These strains were capable of growing in both SAB and SAB-VCZ plates (Figure 2); thus, they were studied as potentially voriconazole resistant strains. All of them were identified as *Aspergillus fumigatus* species after performing a PCR and sequencing the region between ITS1 and ITS4.

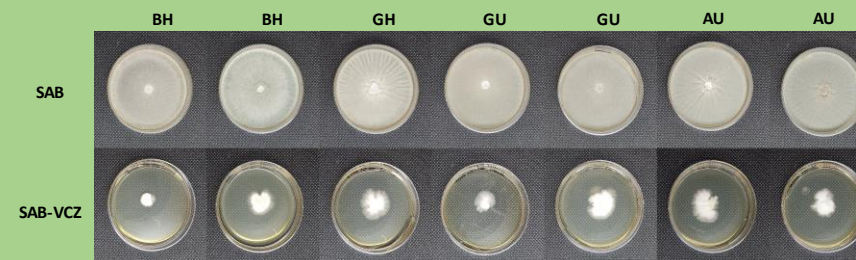


Figure 2. Growth of the *Aspergillus fumigatus* strains. The isolated strains were inoculated in plates containing Sabouraud Agar (SAB) or Sabouraud Agar supplemented with 1 mg/L of voriconazole (SAB-VCZ). All plates were incubated 7 days at 37°C. The origin of the strains is indicated above. BH: Bizkaia Hospital area. GH: Gipuzkoa Hospital area. GU: Gipuzkoa Urban area. AU: Araba Urban area.

In order to assess the possible resistance of the isolated *Aspergillus fumigatus* strains an EUCAST microdilution method was performed. After performing the experiment in triplicate, the results for the determination of the Minimum Inhibitory Concentrations showed that those strains were not resistant to voriconazole (0.125 mg/L). Therefore, although these fungi were able of growing in presence of voriconazole in agar plates, they were sensible to the mechanism of action of the antifungal.

CONCLUSION

This work shows the presence of potentially voriconazole resistant *Aspergillus fumigatus* strains in the hospital and urban environment of the Basque Country. Moreover, Araba was the province with less CFU/m³, where no differences were found between sampling points at 28°C. However, yet much work remains to be done to study the prevalence of the resistant strains in the environment of the Basque Country.

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