Invasive fungal infections due to Aspergillus species have become a major cause of morbidity and mortality among immunocompromised patients. Aspergillus infections species are acquired via inhalation of conidia present in the environment. The major pathogens associated with invasive aspergillosis are members of the Aspergillus section Fumigati, followed by members of the Aspergillus section Flavi, Nigri, and Terrei. Infections due to A. terreus species occur worldwide, and the global prevalence of A. terreus species in fungal disease revealed an overall occurrence of 5.2% [1]. This is of serious concern due to the *in vivo* and *in vitro* resistance of A. terreus against the commonly used antifungal amphotericin B [2]. At the Institute of Hygiene and Medical Microbiology at the Medical University of Innsbruck, A. terreus infections are recognized as a frequent agent of invasive aspergillosis, noted since 1994 [3]. The reason for this epidemiological situation is unclear. To investigate the ecological niche and epidemiology of A. terreus complex species, a large number of environmental samples are collected and analyzed for the frequency of A. terreus in Tyrol, Austria. Our data describe the environmental distribution of A. terreus species complex in Tyrol, a wide geographical area in Austria, to gain a better understanding of the elevated density of A. terreus infections.

**BACKGROUND**

The reason for the frequency of A. terreus found in patients samples at the Medical University of Innsbruck is unclear. A comparative study of the environmental distribution of Aspergillus species in Austria, Denmark, and Spain showed that A. terreus was exclusively present in samples from Austria [4]. The reason for this elevated A. terreus density is unknown.

**OBJECTIVES**

This study aims to investigate the ecological niche and epidemiology of A. terreus complex species in Tyrol, Austria. Therefore, a large number of environmental samples, including soil, air, water, living and dead plant material, decaying material, dust, and excreta are collected at different locations in Tyrol and screened for the frequency of A. terreus.

**METHODS**

**RESULTS**

![Map showing environmental distribution of A. terreus in Tyrol, Austria.](image)

Fig. 1. Environmental distribution of A. terreus in Tyrol, Austria. A total of 1740 environmental samples were collected in Tyrol, a wide geographical area (12,640 km²) located in Austria, from June to October 2019. Sampling locations covered urban and rural environments and mountains with altitudes ranging from 540 m to 3100 m. On average, A. terreus was present in 4.0% (n=70) out of 1740 samples. However, the frequency of positive A. terreus samples varies between regions. In the eastern parts of Tyrol ("Tyrolean Oberland"), A. terreus was present on average in 9% of collected samples. In one particular area (Ziller Valley), the frequency was even as high as 25%. In contrast, in the western parts of Tyrol ("Tyrolean Lowlands"), A. terreus was present on average, only in 2.6 % of collected samples.

![Graph showing number and percentage of A. terreus isolates.](image)

Tab. 1. Frequency of pathogenic Aspergillus spp. in collected environmental samples. Most frequently, A. fumigatus was present in 64.4% of environmental samples followed by A. niger (28%), A. flavus (20.6%), and A. terreus (4%).

<table>
<thead>
<tr>
<th>Aspergillus Species</th>
<th>Frequency (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A. fumigatus</td>
<td>1138 (64.4)</td>
</tr>
<tr>
<td>A. niger</td>
<td>453 (28.0)</td>
</tr>
<tr>
<td>A. flavus</td>
<td>358 (20.6)</td>
</tr>
<tr>
<td>A. terreus</td>
<td>70 (4.0)</td>
</tr>
</tbody>
</table>

![Graph showing MIC and MFC values for A. terreus isolates.](image)

Tab. 2. *In vitro* Amphotericin B (AMB) susceptibilities of A. terreus environmental isolates. Antifungal susceptibility testing was performed according to EUCAST guidelines. Minimum inhibitory concentrations (MIC, in µg/mL) of all isolates were determined after growth for 48 h at 37°C. MIC 50 and MIC 60 values were defined as the lowest AMB concentration, at which 50 and 90% of the isolates were inhibited, respectively. Minimal fungicidal concentrations (MFCs) were defined as the lowest drug concentration resulting in 99.9% killing.

<table>
<thead>
<tr>
<th>MIC (µg/mL)</th>
<th>A. terreus isolates (n=70)</th>
</tr>
</thead>
<tbody>
<tr>
<td>range</td>
<td>MIC50</td>
</tr>
<tr>
<td>A. terreus</td>
<td>1-4</td>
</tr>
</tbody>
</table>

**CONCLUSION**

- In Tyrol, a wide geographical area (12,640 km²) located in Austria, A. terreus species complex displays an overall environmental distribution of 4.0%.
- The frequency of A. terreus scientifically varies between western and eastern regions.
- Most frequently, A. terreus was isolated from soil, followed by living and dead plant material, outdoor air, and decaying material.
- *In vitro* susceptibility testing revealed AMB resistance of the majority of environmental A. terreus isolates.
- The ecological niche of A. terreus needs to be further investigated.

**FUTURE PERSPECTIVES**

- Increase the sample collection during winter months.
- Molecular phylogenetic studies of collected A. terreus isolates for genotype identification within the section Terrei.
- Comparison of collected environmental A. terreus isolates with clinical isolates.
- Characterization of (i) sample types, e.g., pH value and nutrient composition, and (ii) environmental features, e.g., humidity, temperature ranges, altitudes, wind regime or exposition site, to determine a common ecological factor and consequently identify the environmental niche of A. terreus.

**REFERENCES**

[1] Rissegger et al.; Microbiol Infect. ; 2017