TerrNet

A Global Aspergillus terreus Surveillance Study

An initiative of the ISHAM Aspergillus terreus working group and ECMM

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Content:

1. Background

Invasive aspergillosis (IA) has emerged worldwide as an important cause of infections among patients undergoing cancer chemotherapy, haematopoetic stem-cell transplantation, or solid organ transplantation (1, 2). Aspergilli are ubiquitous fungi and the major pathogens are *Aspergillus fumigatus*, *Aspergillus flavus* and *Aspergillus terreus* (2). Among the various species *A. terreus* takes an exceptional position: most isolates are in vitro and in vivo resistant to Amphotericin B (AMB) (3). *A. terreus* is regarded intrinsically resistant to AMB, which is one of the broadest antifungal drugs and widely used for life threatening fungal infections. AMB MICs (48 h / 37°C) are significantly higher (≥4 µg/ml broth dilution, >8 µg/ml Etest) than for *A. fumigatus* (0.5 - 1 µg/ml) (4, 5). Drug resistant fungal infections are becoming more prevalent and are major health issues facing us today.

A. terreus is a common cause of IA in some geographically disparate institutions, such as The University of Texas M. D. Anderson Cancer Center (MDACC) in Houston, TX, and The University Hospital of Innsbruck (UHI), Austria (6, 7). At the UHI, a tertiary-care hospital with 2000 beds, infections due to *A. terreus* have been noted since 1994. *A. terreus* isolates collected from patients of MDACC and UHI were analyzed using random amplification of polymorphic DNA-PCR with three different primers. No genetic relationship between strains was detected, indicating great genetic diversity of A. *terreus* (6, 7). All strains tested, showed an MIC > 2 µg/ml AMB (Etest) but the underlying mechanism behind the reduced susceptibility remains to be fully understood (8). Also, no data are available on how frequent this species occurs outside Innsbruck and Houston and if the low MIC range of isolates include isolates that are truly susceptible or represents low MIC isolates due to inherent test variation. In general, AMB resistance has been reported in some *Candida* species, as well as *Cryptococcus, Trichosporon, A. terreus, Scedosporium*, and *Fusarium* species (4). Mechanisms of resistance to polyenes include several mechanisms (6) and much of the knowledge came from studies on mutant isolates of *S. cerevisiae*, and *Candida* species. Tortorano and coworkers (9) collected an AMB susceptible *A. terreus* with an AMB MIC of 0.19 µg/ml; such finding suggests that there might exist AMB susceptible *A. terreus* isolates. Whether such isolates represent AMB susceptible variants or represent a new species (10, 11, 12) needs to be further investigated.

Recently, acquired azole resistance in an *A. terreus* isolates was identified and itraconazole resistance was linked to an M217I *cyp51a* alteration. Genotyping suggests an endogenous origin (13). The TerrNet study will help to collect various A. terreus from all over the world and allows detailed molecular studies on genetic relationship and resistance.

2. Aim

The aim of TerrNet is to determine the global prevalence of *A. terreus* in mould infections, and to broaden the knowledge on epidemiology, on clinical courses of infections and to investigate mechanism behind differences in amphotericin B and azole susceptibility.

Main objectives

- to determine worldwide distribution of *A. terreus*
- to identify global prevalence
- to evaluate global epidemiology and strain diversity
- to identify new species within the section terrei
- to collect amphotericin B susceptible strains (if indicated also azole resistance)
- to identify patient population at risk
- to determine the clinical pattern of disease
- to characterize in vitro susceptibility
- to check in vitro susceptibility and antifungal-drug combinations
- to check experimental antifungal drugs in vitro and in vivo in animal models.

The prevalence of *A. terreus* will be investigated by prospectively species identifying all *Aspergillus* isolates from routine specimens that are received at clinical microbiological laboratories in various countries in the 1–year study period from January 2014 to January 2015. Consecutive isolates will be enrolled in order to have isolates uniformly spread throughout the seasons. All strains are included irrespective of the clinical relevance of the isolate. For every isolate the surveillance questionnaire is to be completes on the TerrNet web-site. All isolates will be stored and send to the Department of Hygiene and Medical Microbiology of the Innsbruck Medical University for storage, susceptibility testing and further characterization.

Segal et al. (14, 15, 16) have reported on the development of an intralipid formulation of Nystatin (NYT-IL) that had increased activity against A. *terreus*. Combinations of that preparation with antifungals of different modes of activity (e.g. echinocandins) exhibited a synergistic effect. We plan further investigations in this direction (in vitro combination studies and experimental systemic A. *terreus* infection in animal models).

3. Data collection

In order to be able to calculate the prevalence of *A. terreus* the following data will be recorded:

- centre
- date of isolation
- strain identification number
- strain identification
- origin of the sample (BAL, sputum etc)
- gender
- year of birth
- underlying disease of the patient
- antifungal drug use at time of strain isolation
- outcome of antifungal treatment
- diseases related to *A. terreus* infection.

If possible, these parameters should be recorded; if not available leaves the box blanc. A web-based system will be available for real-time inclusion of data in a central database, by means of completing an online questionnaire. The access to the web-site will be restricted to contributing centers and the web-site will provide updates on the inclusion rate of *Aspergillus* isolates that are screened.

4. Molecular based studies

1. Investigate amphotericin B resistance in *A. terreus* (genomic approach)
2. Study immune response and virulence potential of *A. terreus* (complement)
3. To set up animal models to establish in vivo and in vitro correlation
4. To create an *A. terreus* proteome map

In case we are able to collect azole-resistant isolates, we will also study the molecular mechanisms of these isolates.

5. Storage of isolates and ongoing studies

A culture collection will be established at the Department of Hygiene and Medical Microbiology of the MUI in Innsbruck. The isolates will be available to any group member. After receiving the strain, the identification will be confirmed and the phenotypic susceptibility profile determined using EUCAST reference method. All isolates will be characterized by molecular methods in order to rule out other closely related species of the *Aspergillus* section terrei. Molecular-based studies on potential non-wt strains ($\geq$ 3 µg/ml or $\leq$ 0.125 µg/ml AMB MICs, Etest) will be performed.

6. Collaborators

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7. Ethical Considerations and Data Privacy Protection

In the current study 2 aspects have to be considered separately:

1 Documentation of clinical data
2 Work with isolates of *Aspergillus terreus*

There is no interventional aspect to this study. Therefore, there are neither associated risks nor benefits for the patient when participating in the study. The digital documentation of the clinical data will take place in an anonymised fashion. No identifiable data, e.g. name or date of birth will be entered into the database. There will also be no pseudonyms which would make a retrospective re-identification of the patient possible. Clinical data collected refers to common conditions and treatment modalities in medical care, such that no re-identification of the individual case on the basis of these data will be possible. Under these circumstances, we consider an informed consent of the patient not necessary. Regular data backup, hierarchized management of rights and authentication protocols ensure the protection of data from unauthorized access and loss. Contributors can only view the cases submitted by themselves. All clinical data fall under medical confidentiality. All data and results will be stored for at least 10 years after publication of results.

To ensure anonymity, the results of microbiological examinations will only be communicated to the treating physician and entered into the database along with the clinical data in one session by the treating physician.

8. Authorship Policy

Authorship will be restricted to those centers contributing clinical/microbiological data or translational work. For each contributing center, there will be authorship positions available. This will extend to a maximum of two: one clinician, and one microbiologist/medical mycologist, if applicable.

9. Contact Information

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10. References

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11. Costs

Web-site	10.000 Euro

Antifungal susceptibility testing	10.000 Euro
Molecular analysis	20.000 Euro
Strains: mailing	5.000 Euro
Meeting	5.000 Euro
Publication costs	2.000 Euro
Student	35.000 Euro