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RESEARCH NOTE

A Prospective International *Aspergillus terreus* Survey: An EFISG, ISHAM and ECMM Joint Study

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Running title: TerrNet Study

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Abstract

Objectives: A prospective international multicentre surveillance study was conducted to investigate the prevalence and amphotericin B (AMB) susceptibility of *Aspergillus terreus* species complex infections.

Methods: Three hundred seventy cases from 21 countries were evaluated.

Results: The overall prevalence of *A. terreus* species complex among patients investigated and with mold positive cultures was 5.2% (370/7116). AMB MICs were ranging from 0.125 to 32 mg/L, (median 8mg/L).

Conclusions: *A. terreus* species complex infections cause a wide spectrum of aspergillosis and the majority of cryptic species display high AMB MICs.

Introduction:

Aspergillus terreus species complex holds an exceptional position within the aspergilli, as it appears to be a rare pathogen of infection and displays polyene resistance [1,2,3]. *A. terreus* is a common cause of invasive aspergillosis (IA) at the M. D. Anderson Cancer Center in Houston, USA, and the University Hospital of Innsbruck, Austria [3,4,5]. Almost no data are available on how frequently this species occurs elsewhere and whether differences within amphotericin B (AMB) susceptibility exist. Our objective was to investigate the global prevalence of *A. terreus* species complex in fungal diseases and to survey AMB susceptibility.

Methods:

An international surveillance network was established on behalf of the European Fungal Infection Study Group, the International Society for Human and Animal Mycology *Aspergillus terreus* working group, and the European Confederation of Medical Mycology. 38 centres from 21 countries participated. Each centre collected isolates and reported the number of *A. terreus* and fungal pathogens detected for 12 consecutive months (2014-2015). Patient characteristics', epidemiological data, and antifungal treatment were documented through an online questionnaire using www.clinicalsurveys.net online platform. Patients were classified according to the European Organisation for the Research and Treatment of Cancer/Mycoses Study Group consensus definitions [6] by the participating centres. Unless otherwise noted, the isolation of *A. terreus* from sputa of non-neutropenic patients was categorized as colonisation. Isolates were sent to the Division of Hygiene and Medical Microbiology for molecular species identification [7,8] and susceptibility testing according to EUCAST (European Committee on Antimicrobial Susceptibility Testing) method [2]. A. *terreus* strains were identified to the cryptic species level by sequencing partial beta-tubulin and applying a validated in-house database owned by Jos Houbraken, CBS Fungal Biodiversity Center, Utrecht, The Netherlands. An AMB epidemiological cut-off value of 4mg/L was set for *A. terreus* [2].

This study was approved by the Ethics Commission of the Medical University of Innsbruck (UN4926).

Results:

461 cases were enrolled of which 91 were excluded because of insufficient patient documentation (n=45) or lack of fungal isolates (n=46) being available. Consequently, this survey comprises 370 eligible cases with an equal number of corresponding *A. terreus* isolates. Cases derived from Europe

(n=261), followed by Middle East (n=70), India (n=19), South America (n=10), and North America (n=10) (Figure 1). *A. terreus* sensu stricto (n=315), *A. citrinoterreus* (n=36), *A. alabamensis* (n=6), *A. hortai* (n=10), *A. floccosus* (n=1), and *A. neoafricanus* (n=1) were identified. One isolate (*A. terreus 1214*) was most close to *A. alabamensis* and might represent a new species. Thus, cryptic species accounted for 14.9% (55/370) with *A. citrinoterreus* (36/55, 65.5%) being dominant. AMB MICs ranged from 0.125 to 32 mg/L for *A. terreus* sensu stricto; MICs for all cryptic species were consistently higher, ranging from 2 to 32 mg/L, see Table 1. According to the EUCAST cut-off values, 194 isolates (52.4%) were classified as non-wild types. A proportion of 6.3% (n=20) of the *A. terreus* sensu stricto isolates displayed lower MICs, ranging from 0.25 – 0.5 mg/L. Isolates were predominantly acquired from Spain (n=85) and Austria (n=49), see Figure 1.

Underlying diseases e described in Table 2. Species distribution did not differ per underlying disease and specimen investigated (Table 2). Diseases comprised IA (25.1%), allergic broncho-pulmonary aspergillosis (12.4%), chronic aspergillosis (11.4%), COPD exacerbation (5.5%), aspergilloma (3.7%), otitis externa (2.5%), and wound infections (0.7%). 25.1% and 27.3% of the patients suffered from proven and probable IA, 28.6% were colonized, 10.1% had onychomycosis, and 8.9% had mycological documented diseases such as otitis externa, aspergilloma and others.

Using a random effects model the pooled estimated proportion was 5.6% (95% CI 3.8 to 7.7) with I^2 = 92% ($p<0.0001$) and the proportions ranged from 0.0% to 58.3%. These calculations were done with MedCalc 16.8.4. Four reference centres and one centre dealing with onychomycosis only were excluded from the analysis.

A total of 68 patients received antifungal treatment at the time of fungal diagnosis, 12 were treated with AMB or liposomal - AMB. The remaining 56 received combinations of azoles and echinocandins and improved. Only one patient died due to the *A. terreus* infection. No information on outcome was available in 13 patients.

Discussion:

Infections due to *A. terreus* species complex were detected in 21 countries and 38 centres with an overall prevalence of 5.2% among mold infections. High AMB MICs were frequently observed and crossed all cryptic species. Infections were reported from all over the world with three main specific findings. Firstly, Spain and Austria were the countries with the highest density of *A. terreus* isolates collected. Secondly, the number of *A. terreus* cases enrolled varied from centre to centre, and displayed a broad range from zero to several cases per country. Thirdly, it seems that few susceptible AMB variants exist within *A. terreus* sensu stricto.

Taking into account the differences on the environmental conditions, host related characteristics, and the use of antifungal agents, it is not possible to conclude on the particular biogeography of *A. terreus* species complex. In addition, one has to be aware that data collected may depend on the quality of care, patient demographics, infection control practices, frequency of specimen collection, and laboratory methodology. Hence, further studies are needed to determine whether specific risk and/or environmental factors are associated with infections by *A. terreus.*

Notable was the fact that *Aspergillus* section *Terrei* was most commonly isolated from patients suffering from chronic lung diseases (39.2%). No similar data have been reported [10] and it remains to be seen whether *A. terreus* reflects an emerging pathogen of this disease entity.

A. terreus is a poor target for AMB and hence is reported as resistant [2]. The role of isolates with MICs <0.5 mg/L needs further evaluation. The pharmacodynamic target may be attained with the standard AMB dose for isolates with MICs ≤0.25 mg/l [10] and infections were successfully treated with high dose liposomal-AMB [11].

Cryptic species accounted for 14.8%, with *A. citrinoterreus* being the most prevalent. Although the clinical implications of sibling species of *A. terreus* are less well understood, our study confirms that these species are generally resistant to AMB and are causing a wide spectrum of invasive and non-invasive aspergillosis. Guinea et al. [12] observed *A. citrinoterreus* acting mainly as a co-pathogen with *A. fumigatus*.

Our study has some limitations. We do not have a comprehensive worldwide *A. terreus* survey network and some countries are missing for a variety of reasons. Also, generally, the diagnosis of fungal infections is difficult to obtain and may often be based on detection of biomarkers rather than on isolation of the infecting organism. Hence, some cases may have been missed and chronic lung diseases were not specified in more detail. Further, we have no data available on co-infections which may complicate diseases. The centres included represent a convenience sample. However, this is the largest and geographically most diverse study on the contemporary epidemiology of *A. terreus* species complex infections worldwide.

Our study shows that *A. terreus* sensu stricto is widely distributed in climatically divergent countries, and that cryptic species display high AMB MICs. *A. terreus* species complex was most commonly isolated from patients suffering from chronic lung diseases (39.2%).

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Transparency Declaration

We declare that we have no conflicts of interest related to this study.

CLF received research grants, travel grants or honorarium as a speaker or advisor from Gilead Sciences, Pfizer, Schering Plough, MSD and Basilea, VAA received research grants or honorarium as a speaker or advisor from Astellas, Pfizer and Schering Plough, MSD. WB received honorarium as a speaker from Pfizer, Schering Plough and MSD. CF received research grants or honorarium as a speaker or advisor from Astellas, Gilead Sciences, MSD and Basilea. AH received travel grants from Astellas and served on the advisory board of Gilead. EJ received travel grants or honorarium as a speaker or advisor from Bio-Rad, Gilead Sciences, Pfizer, Schering Plough, and MSD. DPK has received research support from Pfizer, Astellas Pharma, honoraria from Astellas, Merck, Pfizer and he serves on the advisory boards of Merck, Amplyx, Cidara and F2G. JFM received grants from Astellas, Basilea and Merck. He has been a consultant to Astellas, Basilea and Merck and received speaker’s fees from Merck, United Medical and Gilead. ALC received educational grants from Pfizer, Gilead and United Medical and research grant from Pfizer, Astellas. MCA received research grants/contract work (Paid to SSI) from Amplyx, Basilea, Cidara, F2G, Gilead, Pfizer & T2Candida, speaker honoraria from Astellas, Basilea, Gilead, MSD, Novartis, Pfizer & T2Candida. NK has received research support from Pfizer, honoraria from Astellas, Merck and Pfizer, and he serves on the advisory boards of Merck and Pfizer. OAC is supported by the German Federal Ministry of Research and Education, has received research grants from Actelion, Aranis, Astellas, AstraZeneca, Basilea, Bayer, Cidara, Duke University (NIH UM1AI104681), F2G, Gilead, GSK, Leeds University, MedPace, Melinta Therapeutics, Merck/MSD, Miltenyi, Pfizer, Rempex, Roche, Sanofi Pasteur, Scynexis, Seres Therapeutics, The Medicine Company, is a consultant to Anacor, Amplyx, Actelion, Astellas, Basilea, Cidara, Da Volterra, F2G, Gilead, Inositec AG, Janssen Pharmaceuticals, Matinas, Menarini, Merck/MSD, Paratek Pharmaceuticals, Scynexis, Seres, Summit, Vical, Vifor, and received lecture honoraria from Astellas, Basilea, Gilead and Merck/MSD. BW received research support from Pfizer, travel grants or honorarium as a speaker or advisor from Gilead Sciences, Pfizer, Astellas, MSD and Basilea. LK grant from Gilead and has been an adviser to Astellas, Gilead, Schering-Plough, has received research grants from Gilead, Merck, Sharpe&Dohme, Schering-Plough and has received honoraria for educational lectures from Gilead, Pfizer, Merck, Sharpe & Dohme, Schering-Plough and Janssen. AMT received research support from Gilead Sciences, travel grants or honorarium as speaker from Gilead Sciences, Pfizer, Astellas. KL has received research grants from Gilead, MSD and Pfizer, received travel support from MSD, Pfizer and Gilead and received lecture honoraria from Gilead, MSD, and Pfizer. SAA received Investigator Initiated Research grant support from Pfizer and speaker honoraria or travel grants from Astellas, Gilead, Merck and Pfizer. SO received research grants from Pfizer and Astellas, honorarium as advisor from MSD, Astellas Pharma and Gilead Sciences. ER reports grants, and non-financial support from Astellas, Pfizer, Gilead, and Merck, outside the submitted work. AAI received research and/or travel grants from Gilead Sciences, F2G and Pfizer. OU was consultant for and has received travel grants from Merck, Astellas, Pfizer, and Gilead. S.M.H. has received research and travel grants from Astellas, Gilead, and MSD, research grants from Basilea, and travel grants from Pfizer; was consultant to

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ACCEPTED MANUSCRIPT

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Table 1. Distribution of amphotericin B MICs against *Aspergillus terreus* species complex isolates collected during the study period and tested according to EUCAST methodology

Species	Amphotericin B MICs, mg/L								
	0.125	0.25	0.5	1	2	4	8	16	32
A. terreus sensu stricto	3	7	10	14	36	81	86	55	23
A. citrinoterreus					3	13	8	7	5
A. hortai					1	2	5	2	
A. alabamensis					2	3	1		
A. floccosus						1			
A. neoafricanus									1
Potential new species							1		

Table 2. Species distribution of *Aspergillus terreus* species complex isolated from the various human specimens

Species	Specimens, total numbers						
	Sputa	Bronchoalveolar lavages and tracheal secretions	Body-fluids	Biopsies	Swabs	Others	Total
A. terreus sensu stricto	126	65	53	33	17	21	315
A. citrinoterreus	14	7	3	5	3	4	36
A. hortai	4	2			1	3	10
A. alabamensis	3	2			1		6
A. floccosus					1		1
A. neoafricanus						1	1
Potential new species				1			1
Total	147	76	56	39	23	29	370

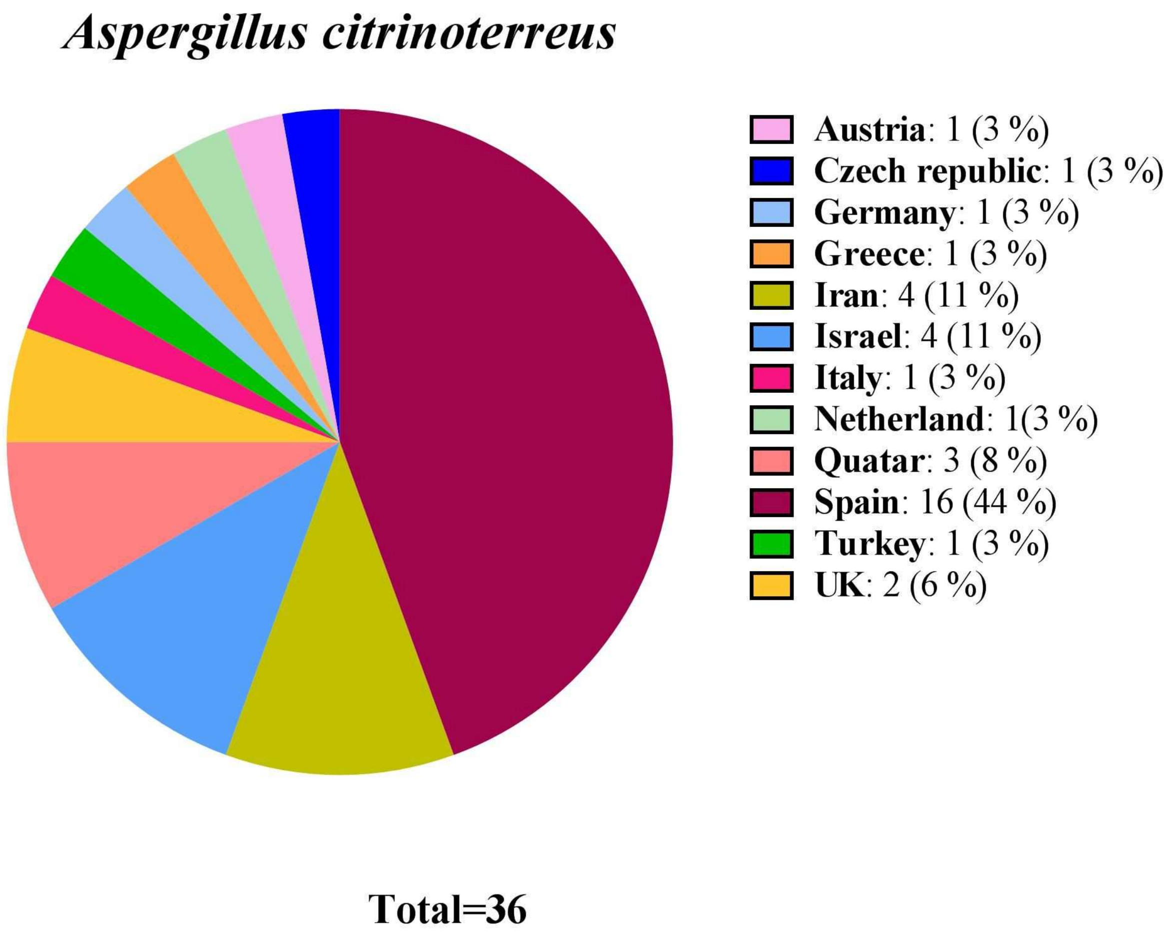
* aspirates, wound secretions, nails

Fig. 1a - c. Overview of countries and *Aspergillus terreus* species complex isolated numbers collected during the study period:

a) *Aspergillus citrinoterreus*

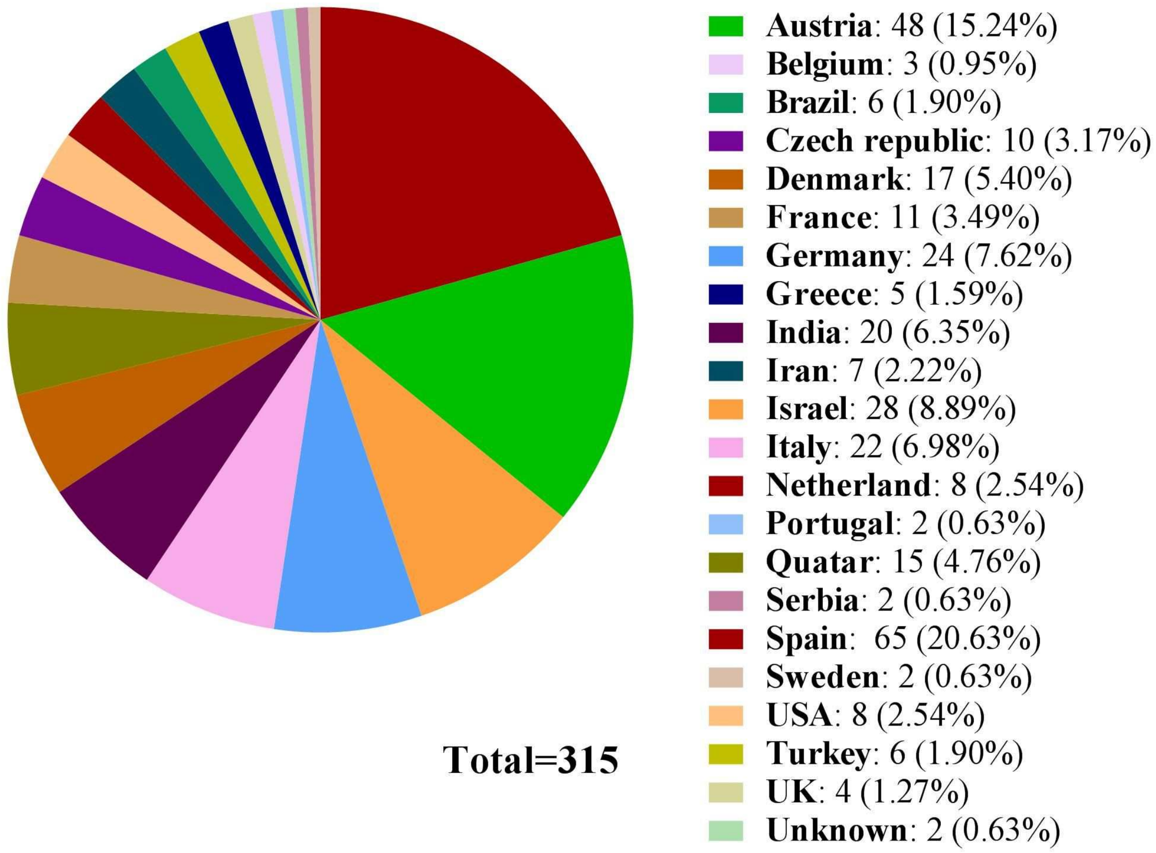
b) *Aspergillus terreus* sensu stricto

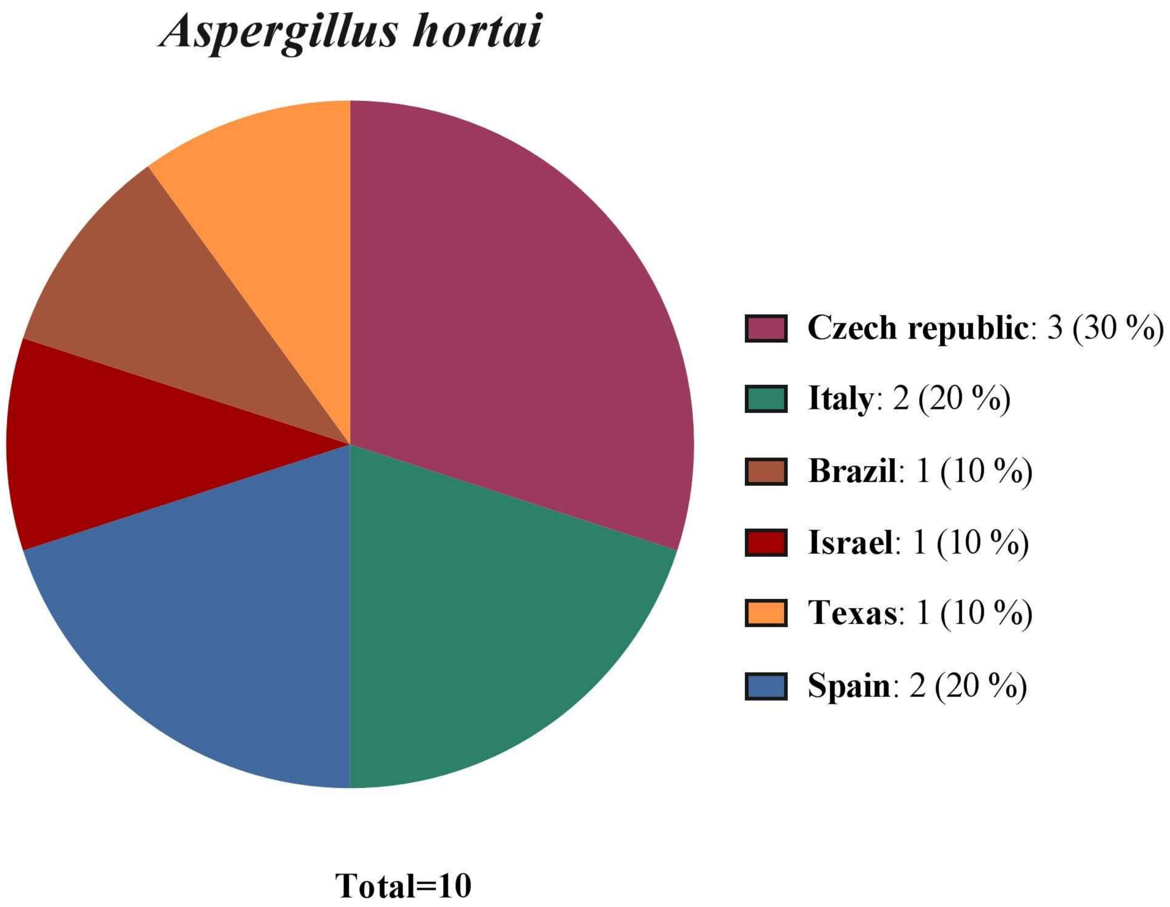
c) *Aspergillus hortai*



Aspergillus citrinoterreus
Austria: 1 (3 %)
Czech republic: 1 (3 %)
Germany: 1 (3 %)
Greece: 1 (3 %)
Iran: 4 (11 %)
Israel: 4 (11 %)
Italy: 1 (3 %)
Netherland: 1(3 %)
Quatar: 3 (8 %)
Spain: 16 (44 %)
Turkey: 1 (3 %)
UK: 2 (6 %)
Total=36

Aspergillus terreus sensu stricto




Aspergillus hortai
Czech republic: 3 (30 %)
Italy: 2 (20 %)
Brazil: 1 (10 %)
Israel: 1 (10 %)
Texas: 1 (10 %)
Spain: 2 (20 %)
Total=10