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Multicentric Analysis of the Species Distribution and Antifungal Susceptibility of Clinical Isolates from *Aspergillus* Section *Circumdati*

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ABSTRACT The clinical involvement and antifungal susceptibility of *Aspergillus* section *Circumdati* are poorly known. We analyzed 52 isolates, including 48 clinical isolates, belonging to 9 species inside the section *Circumdati*. The whole section exhibited, by the EUCAST reference method, a poor susceptibility to amphotericin B, but species/series-specific patterns were observed for azole drugs. This underlines the interest in getting an accurate identification inside the section *Circumdati* to guide the choice of antifungal treatment in clinical practice.

KEYWORDS antifungal susceptibility testing, *Aspergillus* section *Circumdati*, MALDI-TOF mass spectrometry, azole resistance, *Aspergillus sclerotiorum*, *Aspergillus westerdijkiae*, *Aspergillus ochraceopetaliformis*

Aspergillus section *Circumdati*, whose the type species is *Aspergillus ochraceus*, currently includes 27 species distributed in 3 series (*Circumdati*, *Sclerotiorum*, and *Steyniorum*) (1). They share the ability to produce mycotoxins, especially the nephrotoxic, carcinogenic, and teratogenic ochratoxin A (1, 2). They are also occasionally involved in human disease (3–5), including invasive infections (6), and are known to exhibit low susceptibility to antifungal drugs (3). However, since new *Aspergillus* species have been described recently on a phylogenetic basis (1), the species distribution, clinical involvement, and antifungal susceptibility of species inside the *Aspergillus* section *Circumdati* have been poorly explored. Lack of data may be explained by the difficulty to collect these species, mainly related to issues in getting a correct identification at the species level (7). The MSI-2 application is a French independent and freely accessible online mass spectrum database (https://msi.happy-dev.fr) built in collaboration with the BCCM/IHEM (Belgian Coordinated Collections of Micro-organisms/Institute of Hygiene and Epidemiology Mycology), which includes references from 159 different *Aspergillus* species (8). The

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MSI application is of great interest to discriminate species based on current species concepts (9).

The aims of this study were to assess the antifungal susceptibility of isolates from section *Circumdati* by the reference method and to describe their clinical involvement. The network of users of the MSI application (320 laboratories from 51 countries [data obtained on 1 October 2022]) allowed collection of a consequent number of isolates in the setting of a multicentric prospective study.

(Part of this study was presented during the 8th Trends in Medical Mycology Congress, Nice, France 11 to 14 October 2019).

MSI online identification data were analyzed prospectively over a course of a 27-month period (August 2017 to October 2019). During this period, 12 regular MSI users were asked, when they identified a species belonging to the section *Circumdati*, for the shipment of the isolate to La Pitié-Salpêtrière Hospital, a tertiary care center in Paris, France. They were also asked to fill out a standardized report form. Written or verbal informed consent was not required because samples were collected through routine clinical work and patient identifiable information was anonymized prior to analysis. The participating centers were university hospitals ($n = 11$) or nonhospital laboratories ($n = 1$) located in metropolitan France (Paris area [$n = 5$], Bordeaux, Rouen, Montpellier, Nice, Saint-Etienne, and Toulouse) and French Guiana. All isolates were submitted to DNA sequence-based identification (*benA* and *cmd* genes) (10), and the resulting sequences were deposited in GenBank (Table 1). Antifungal susceptibility was assessed by EUCAST broth microdilution (BMD) reference method, with minor modifications. (The incubation temperature was lowered from 37°C to 30°C.) (https://www.eucast.org/fileadmin/src/media/PDFs/EUCAST_files/AFST/Files/EUCAST_E_Def_9_3_1_Mould_testing__definitive.pdf). One among three quality control strains (*Candida parapsilosis* ATCC 22019, *Candida krusei* ATCC 6258, or *Aspergillus fumigatus* ATCC 204305) was included in each plate alternatively.

Over the study period, species belonging to the section *Circumdati* represented 288 isolates out of the 15,305 *Aspergillus* total isolates identified by the MSI application (1.9%). These included 36 *A. ochraceus* isolates (12.5%) and 252 non-*A. ochraceus* isolates (87.5%) distributed into 10 species. Among them, we collected 52 isolates, whose species distribution reflected the overall distribution obtained for all users. Based on DNA sequencing identification, isolates were distributed into 9 species: *A. sclerotiorum* ($n = 17$), *A. persii* ($n = 5$), and *A. subramanianii* ($n = 1$) for series *Sclerotiorum*, *A. westerdijkiae* ($n = 13$), *A. ochraceus* ($n = 5$), *A. affinis* ($n = 1$), and *A. ostianus* ($n = 1$) for series *Circumdati*, and *A. insulicola* ($n = 5$) and *A. ochraceopetaliformis* ($n = 4$) for series *Steyniorum* (Table 1). The accuracy of the MSI database identification was 100% at the section and series levels, but some discrepancies appeared at the species level between close species inside a given series (accurate identification, 34/52 [65%]). In this context, the MSI application appears to be a very interesting tool to give more insight into the study of rare *Aspergillus* species.

Collected isolates included 48 clinical and 4 environmental isolates, which represent to the best of our knowledge the largest collection of section *Circumdati* clinical isolates. They were mainly recovered from the respiratory tract ($n = 33$) and were responsible for chronic pulmonary aspergillosis ($n = 2$) or pulmonary colonization in patients suffering from chronic respiratory diseases ($n = 29$). Interestingly, 10 isolates (5 species) were involved in toenail onychomycosis, suggesting an important part of this section in nondermatophytes' onychomycosis as previously reported (4, 5, 11). Finally, one isolate was responsible for otomycosis, while 6 were considered environmental contamination (including 2 respiratory samples and 1 nail sample). Most of these species grow slightly at 37°C and thus are more prone to bronchial colonization or superficial infection by toxin production (1, 2, 12). Species belonging to the series *Sclerotiorum* have the ability to grow at 37°C and might be responsible for invasive infection (1), although this was not the case in this study. These findings highlight the occurrence of the section *Circumdati* as a human pathogen and the interest in an accurate *Aspergillus* identification at the species/series level.

In vitro susceptibility to azole drugs (itraconazole, voriconazole, posaconazole, and isavuconazole) and amphotericin B was assessed for the 52 isolates by EUCAST BMD method.

TABLE 1 List and characteristics of *Aspergillus* section *Circumdati* strains analyzed in this study, GenBank accession numbers generated and sequence similarity with type strains

Species	Strain no.	Source (sample type)	Yr and country of isolation	GenBank accession no. (% similarity to type strain)	
				Calmodulin	β-Tubulin
A. affinis	MTP110	Clinical (respiratory tract)	2017, France	OQ208776 (99.6)	OQ197965 (99.6)
A. insulicola	BDX157	Clinical (respiratory tract)	2018, France	OQ208777 (97.7)	OQ197966 (97.1)
	BPO103	Clinical (nail)	2018, France	OQ208778 (99.3)	OQ197967 (98.7)
	BPO104	Clinical (nail)	2018, France	OQ208779 (99.3)	OQ197968 (97.6)
	PSL50	Clinical (nail)	2019, France	OQ208780 (99.2)	OQ197969 (98.9)
	PSL58	Clinical (nail)	2019, France	OQ208781 (99.5)	OQ197970 (98.5)
A. ochraceopetaliformis	MTP108	Clinical (unknown)	2017, France	OQ208782 (99.8)	OQ197971 (100)
	CAY03	Clinical (respiratory tract)	2018, French Guiana	OQ208783 (100)	OQ197972 (100)
	CAY04	Clinical (cutaneous)	2018, French Guiana	OQ208784 (100)	OQ197973 (100)
	NCE14	Clinical (nail)	2019, France	OQ208785 (99.8)	OQ197974 (100)
A. ochraceus	ROU25	Clinical (respiratory tract)	2018, France	OQ208786 (99.8)	OQ197975 (99.8)
	MTP130	Clinical (respiratory tract)	2018, France	OQ208787 (99.7)	OQ197976 (99.8)
	BDX107	Clinical (respiratory tract)	2019, France	OQ208788 (100)	OQ197977 (100)
	MTP407	Clinical (respiratory tract)	2019, France	OQ208789 (99.8)	OQ197978 (99.8)
	MTP412	Clinical (respiratory tract)	2019, France	OQ208790 (99.8)	OQ197979 (99.8)
A. ostianus	BCH54	Clinical (respiratory tract)	2018, France	OQ208791 (99.6)	OQ197980 (100)
A. persii	ROU91	Clinical (nail)	2018, France	OQ208792 (99.8)	OQ197981 (99.6)
	DWG03	Environmental (water)	2018, Belgium	OQ208793 (99.8)	OQ197982 (99.6)
	DWG04	Environmental (water)	2018, Belgium	OQ208794 (99.8)	OQ197983 (99.6)
	MTP410	Clinical (respiratory tract)	2019, France	OQ208795 (99.8)	OQ197984 (99.6)
	PSL61	Clinical (nail)	2019, France	OQ208796 (99.8)	OQ197985 (99.6)
A. sclerotiorum	NCE15	Clinical (nail)	2019, France	OQ208797 (99.7)	OQ197986 (100)
	ROU237	Clinical (respiratory tract)	2018, France	OQ208798 (100)	OQ197987 (100)
	BDX336	Clinical (respiratory tract)	2017, France	OQ208799 (100)	OQ197988 (100)
	MTP115	Clinical (respiratory tract)	2017, France	OQ208800 (100)	OQ197989 (100)
	PSL103	Clinical (respiratory tract)	2017, France	OQ208801 (100)	OQ197990 (100)
	ROU217	Clinical (respiratory tract)	2017, France	OQ208802 (99.6)	OQ197991 (100)
	ROU226	Clinical (respiratory tract)	2018, France	OQ208803 (99.7)	OQ197992 (100)
	AVC104	Clinical (respiratory tract)	2018, France	OQ208804 (100)	OQ197993 (100)
	PSL24	Clinical (cerebrospinal fluid)	2018, France	OQ208805 (100)	OQ197994 (100)
	ROU149	Clinical (respiratory tract)	2018, France	OQ208806 (100)	OQ197995 (100)
	TLS168	Clinical (respiratory tract)	2018, France	OQ208807 (99.7)	OQ197996 (100)
	TLS165	Clinical (respiratory tract)	2018, France	OQ208808 (100)	OQ197997 (100)
	BPO111	Clinical (nail)	2018, France	OQ208809 (99.7)	OQ197998 (100)
	BDX158	Clinical (respiratory tract)	2018, France	OQ208810 (99.7)	OQ197999 (100)
	BDX86	Clinical (respiratory tract)	2018, France	OQ208811 (100)	OQ198000 (100)
	BDX90	Clinical (respiratory tract)	2018, France	OQ208812 (100)	OQ198001 (100)
	STE12	Clinical (respiratory tract)	2019, France	OQ208813 (99.7)	OQ198002 (100)
A. subramanianii	PSL67	Clinical (ear)	2019, France	OQ208814 (98.7)	OQ198003 (97.1)
A. westerdijkiae	BDX321	Clinical (respiratory tract)	2017, France	OQ208815 (99.1)	OQ198004 (98.8)
	ROU221	Clinical (respiratory tract)	2018, France	OQ208816 (99.9)	OQ198005 (99.4)
	STE108	Clinical (nail)	2017, France	OQ208817 (99.6)	OQ198006 (99.8)
	ROU231	Clinical (respiratory tract)	2018, France	OQ208818 (100)	OQ198007 (100)
	ROU63	Clinical (respiratory tract)	2018, France	OQ208819 (99.3)	OQ198008 (99.6)
	ROU70	Clinical (respiratory tract)	2018, France	OQ208820 (99.3)	OQ198009 (99.6)
	DWG01	Environmental (water)	2018, Belgium	OQ208821 (99.5)	OQ198010 (100)
	DWG02	Environmental (water)	2018, Belgium	OQ208822 (99.5)	OQ198011 (100)
	BDX105	Clinical (respiratory tract)	2019, France	OQ208823 (99.8)	OQ198012 (100)
	SAT08	Clinical (nail)	2019, France	OQ208824 (99.5)	OQ198013 (99.8)
	ROU100	Clinical (respiratory tract)	2019, France	OQ208825 (100)	OQ198014 (100)
	ROU103	Clinical (respiratory tract)	2019, France	OQ208826 (98.4)	OQ198015 (99.4)
	ROU101	Clinical (respiratory tract)	2019, France	OQ208827 (99.5)	OQ198016 (99.8)

The geometric means (GMs), MIC ranges, MIC_{50}s, and MIC_{90}s obtained are shown in Table 2. Regarding the whole *Circumdati* section, MICs were overall high for all drugs. According to the antifungal drug, from 28.9% to 94.2% of isolates exhibited MICs higher than *A. fumigatus* EUCAST clinical breakpoints (>1 mg/L for itraconazole, voriconazole, and amphotericin B, >2 mg/L for isavuconazole and > 0.125 mg/L for posaconazole) (https://www.eucast.org/fileadmin/src/media/PDFs/EUCAST_files/AFST/Clinical_breakpoints/AFST_BP_v10.0_200204_updatd_links_200924.pdf). Amphotericin B showed less *in vitro* efficacy (MIC_{50} of 4 mg/L

TABLE 2 *In vitro* antifungal susceptibility for the 52 *Aspergillus* section *Circumdati* isolates determined by EUCAST reference method

Series or section	Parameter[a]	Result for: Itraconazole	Voriconazole	Posaconazole	Isavuconazole	Amphotericin B
Series *Sclerotiorum*						
A. sclerotiorum (*n* = 17)	Range (mg/L)	>8	2 to 8	0.5 to >8	1 to 8	1 to >16
	MIC_{50} (mg/L)	>8	4	2	4	4
	MIC_{90} (mg/L)	>8	8	>8	4	8
	GM (mg/L)	>8	4.71	2.52	3.54	2.77
	n > CBP (%)	17 (100)	17 (100)	17 (100)	16 (94.12)	14 (82.35)
A. persii (*n* = 5)	Range (mg/L)	0.5 to >8	1 to 2	0.5 to 1	0.5 to 4	2 to >16
A. subramanianii (*n* = 1)	MIC (mg/L)	>8	4	1	4	2
Total (*n* = 23)	Range (mg/L)	0.5 to >8	1 to 8	0.5 to >8	0.5 to 8	1 to >16
	MIC_{50} (mg/L)	>8	4	1	4	2
	MIC_{90} (mg/L)	>8	8	> 8	4	8
	GM (mg/L)	>8	3.55	2.19	3.05	3.14
	n > CBP (%)	22 (95.65)	20 (86.96)	23 (100)	15 (65.22)	20 (86.96)
Series *Circumdati*						
A. westerdijkiae (*n* = 13)	Range (mg/L)	0.25 to 1	0.5 to 2	0.25 to 1	0.25 to 2	4 to >16
	MIC_{50} (mg/L)	0.5	0.5	0.5	0.5	>16
	MIC_{90} (mg/L)	1	2	1	2	>16
	GM (mg/L)	0.45	0.69	0.40	0.77	>16
	n > CBP (%)	0 (0)	2 (15.38)	7 (53.85)	0 (0)	13 (100)
A. ochraceus (*n* = 5)	Range (mg/L)	0.5 to 1	1	0.5	1	2 to 4
A. affinis (*n* = 1)	MIC (mg/L)	0.25	0.25	0.5	0.25	2
A. ostianus (*n* = 1)	MIC (mg/L)	>8	2	0.5	0.5	2
Total (*n* = 20)	Range (mg/L)	0.25 to 16	0.25 to 2	0.25 to 1	0.25 to 2	2 to >16
	MIC_{50} (mg/L)	0.5	0.75	0.5	0.75	>16
	MIC_{90} (mg/L)	1	2	0.55	2	>16
	GM (mg/L)	0.62	0.76	0.44	0.76	>16
	n > CBP (%)	1 (5)	3 (15)	14 (70)	0 (0)	20 (100)
Series *Steyniorum*						
A. insulicola (*n* = 5)	Range (mg/L)	1 to 4	0.5 to 1	0.5 to 1	1 to 2	>16
A. ochraceopetaliformis (*n* = 4)	Range (mg/L)	1	1	0.5 to 1	2	16 to >16
Total (*n* = 9)	Range (mg/L)	1 to 4	0.5 to 1	0.5 to 1	1 to 2	16 to >16
	MIC_{50} (mg/L)	1	1	0.5	2	>16
	MIC_{90} (mg/L)	2	1	1	2	>16
	GM (mg/L)	1.19	0.92	0.60	1.83	>16
	n > CBP (%)	1 (11.11)	0 (0)	9 (100)	0 (0)	9 (100)
Total section *Circumdati* (*n* = 52)	Range (mg/L)	0.25 to >8	0.25 to 8	0.25 to >8	0.25 to 8	1 to >16
	MIC_{50} (mg/L)	1	1	0.5	2	4
	MIC_{90} (mg/L)	>8	8	>8	4	>16
	GM (mg/L)	2.79	1.55	0.95	1.62	7.48
	n > CBP (%)	24 (46.15)	23 (44.23)	46 (88.46)	15 (28.85)	49 (94.23)

[a]GM, geometric mean; CBP, *Aspergillus fumigatus* Clinical Breakpoint defined by EUCAST. Note that values above the dilution range were adjusted to the next upper 2-fold dilution for the geometric mean calculation for each of the geometric means shown.

and MIC_{90} of >16 mg/L), but the MIC distribution was related to series and/or species identification. Indeed, amphotericin B MICs were significantly lower for the series *Sclerotiorum* (GM of 3.14 mg/L) than the series *Circumdati* and *Steyniorum* (GM of >16 mg/L; $P = 0.0023$). Moreover, inside the series *Circumdati*, *A. westerdijkiae* was the species exhibiting the highest amphotericin B MICs. Regarding azole drugs, susceptibility patterns are also related to series identification, with the series *Sclerotiorum* exhibiting significantly higher MICs than the series *Circumdati* and *Steyniorum* ($P < 0.0001$ for each azole drug by Mann-Whitney test [Prism v.9]). For these two latter series, azole MICs were quasi-systematically under the *A. fumigatus* EUCAST clinical breakpoints (except for posaconazole), suggesting susceptibility to these drugs.

These series/species-specific MICs patterns are in accordance with previous findings inside the section *Circumdati* (3). This indicates that an accurate identification at the series/species level could suggest the antifungal susceptibility profile and drive the therapeutic

option. However, the use of the *A. fumigatus* EUCAST clinical breakpoints to categorize isolates may be nonsuitable for the section *Circumdati* and could explain the discrepancies observed between azoles drugs. This strengthens the need to determine clinical breakpoints, or at least epidemiological cutoff values in this section to interpret MIC values. Assessment of new fungal drugs such as olorofim, may also be interesting in this slightly susceptible *Aspergillus* section (13).

In conclusion, *Aspergillus* section *Circumdati* encompasses a great number of clinically relevant species exhibiting a poor *in vitro* susceptibility to conventional antifungal drugs. The existence of species/series-specific susceptibility patterns underlines the need for prompt and accurate identification in clinical practice.

Data availability. The sequences determined for all isolates submitted to DNA sequence-based identification (*benA* and *cmd* genes) were deposited in GenBank under the accession numbers listed in Table 1.

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