Azole resistance in *Aspergillus* species in Iran: Is there a challenge coming up?

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Management of fungal diseases in

Plants, animals and humans, and for material preservation.

Major benefits in terms of

Food security, animal health, quality of life, and survival of patients with life-threatening fungal diseases

Broad use of the same antifungal class comes with a risk

Selection of antifungal resistance

In vitro assays of azole susceptibility

Reproducible results that are predictive of therapeutic response in experimental models and patients

Azoles in human:

Superficial mycoses

Malassezia species

Cutaneous mycoses

Dermatophytes

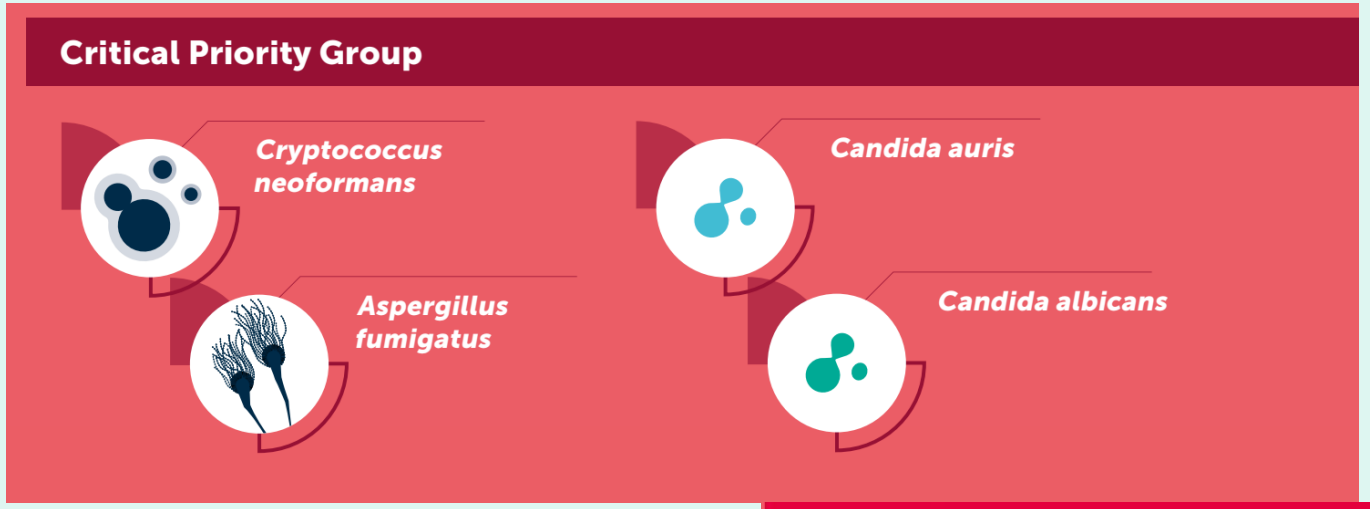
Sub-cutaneous mycoses

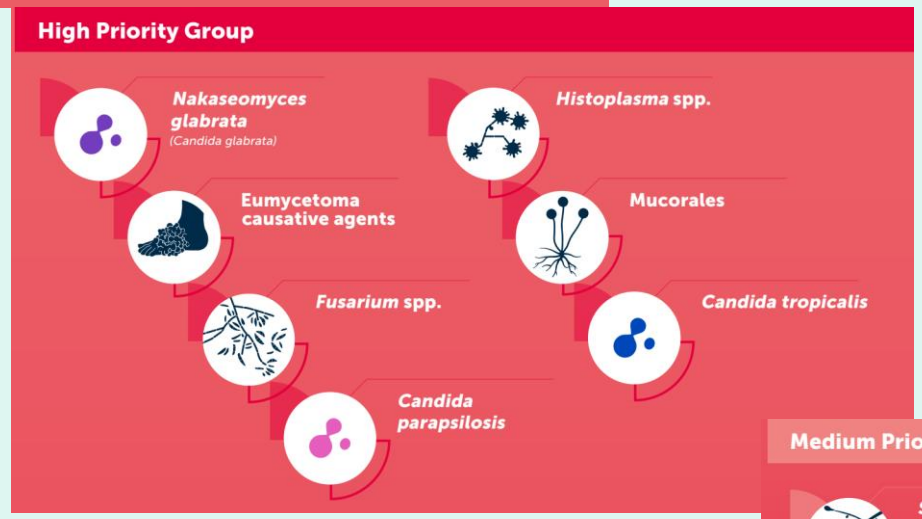
- *Sporothrix species*
- *Cladophialophora*

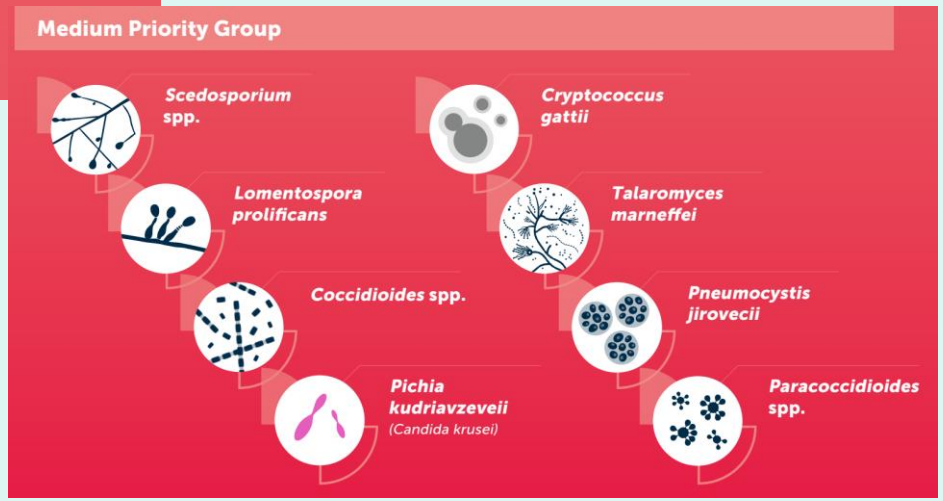
Systemic mycoses

- *Aspergillus species*
- *Mucorales*
- *Fusarium*
- *Scedosporium*

WHO fungal priority pathogens list





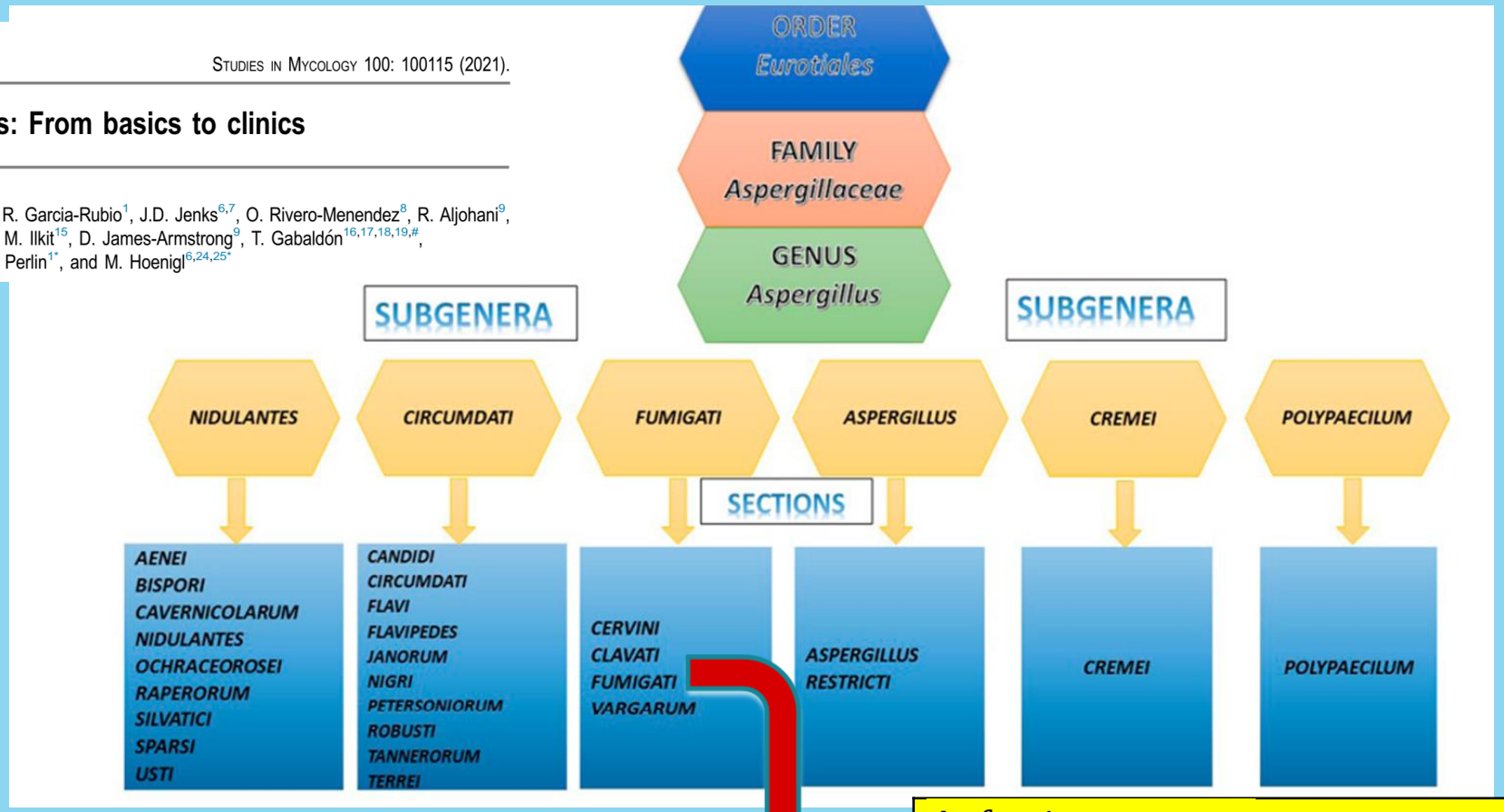


available online at www.studiesinmycology.org

STUDIES IN MYCOLOGY 100: 100115 (2021).

Aspergillus fumigatus and aspergillosis: From basics to clinics

A. Arastehfar[1,#], A. Carvalho[2,3,#], J. Houbraken[4,#], L. Lombardi[5], R. Garcia-Rubio[1], J.D. Jenks[6,7], O. Rivero-Menendez[8], R. Aljohani[9], I.D. Jacobsen[10,11], J. Berman[12], N. Osherov[13], M.T. Hedayati[14], M. Ilkit[15], D. James-Armstrong[9], T. Gabaldón[16,17,18,19,#], J. Meletiadis[20], M. Kostrzewa[21], W. Pan[22], C. Lass-Flörl[23], D.S. Perlin[1*], and M. Hoenigl[6,24,25*]



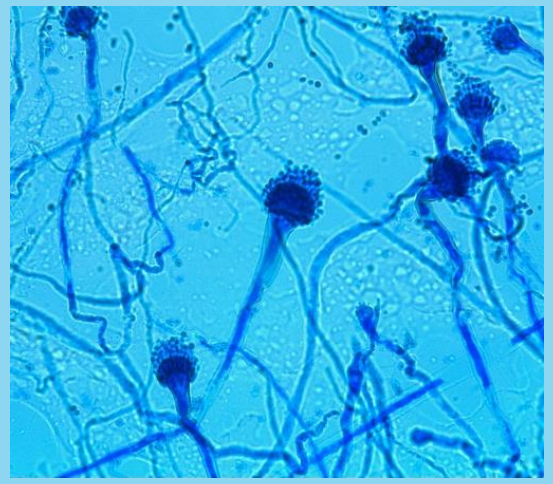
Aspergillus fumigatus
A. udagawae (Neosartorya udagawae)
A. Thermomutatus (Neosartorya pseudofischeri)
A. lentulus
A. spinosus
A. hiratsukae (Neosartorya hiratsukae)
A. Fischerianus (Neosartorya fischeri)
A. fumisynnematus

Species

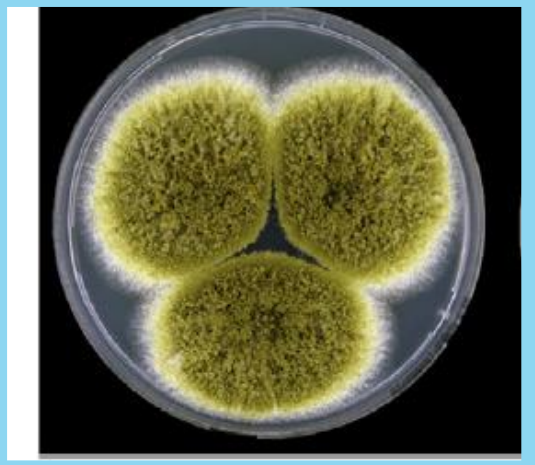
A. fumisynnematus
A. fumigatiaffinis
A. novofumigatus
A. laciniosa (Neosartorya laciniosa)
A. wyomingensis
A. pseudoviridinutans
A. felis
A. parafelis
A. pseudofelis

Interactive quiz

Aspergillus fumigatus



Aspergillus lentulus



Aspergillus flavus

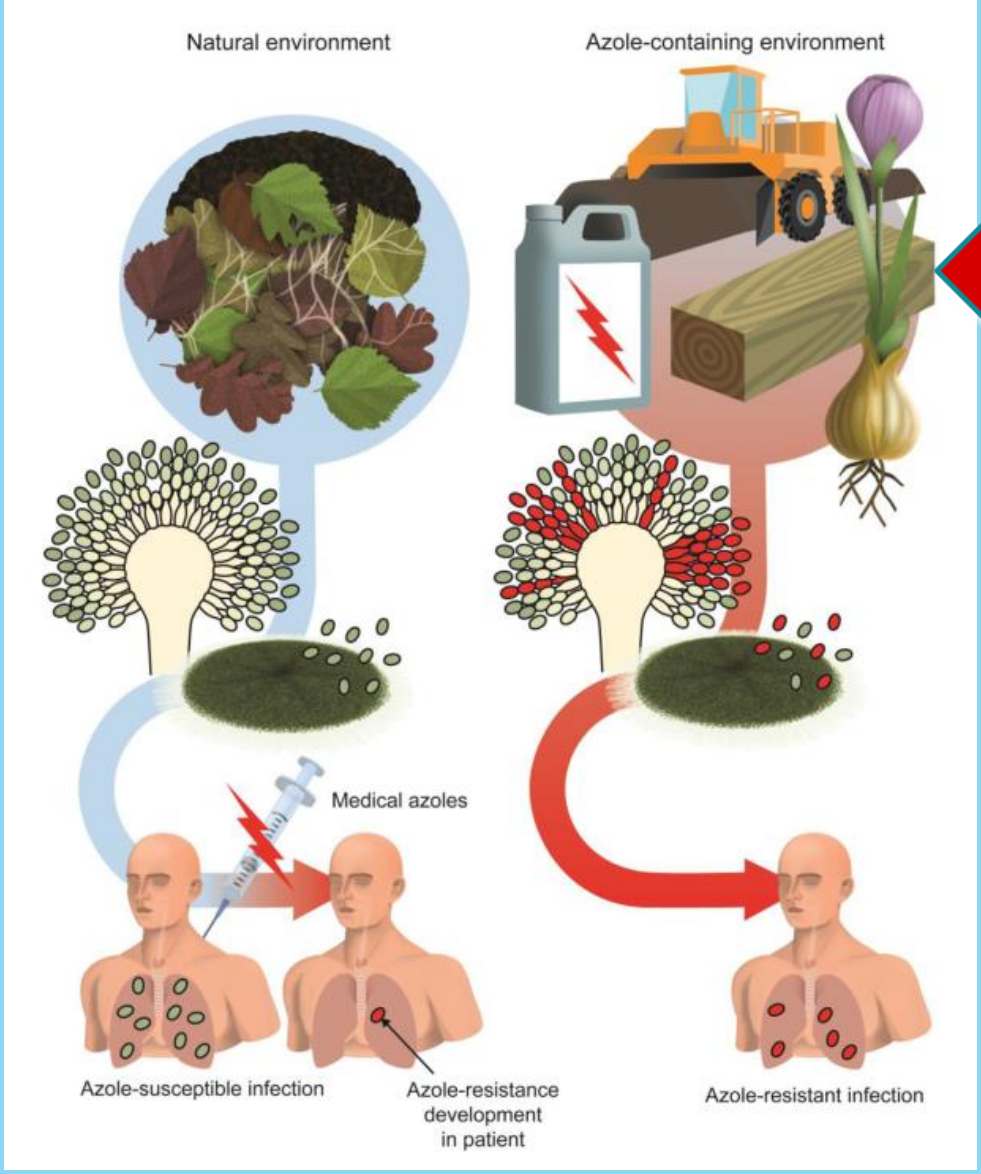
Aspergillus caelatus

Mode of transmission and Portal of exit

- The portal of exit is closely related to the mode of transmission.
- Aspergillosis is not transmitted form person to person
- the emphasis on focusing control measures on preventing the exit of that organism from the infected patient does not appear to a major component.

Reservoir

- **Two main environmental sources:**
  - Outside air entering hospital through gaps in filters, windows, backflow of contaminated air
  - Moist environments (e.g., plumbing, leaks, rainwater, air conditioning condensate


Natural environment
Azole-containing environment
Medical azoles
Azole-susceptible infection
Azole-resistance development in patient
Azole-resistant infection


FUNGICIDE
AZOLES

Azole-resistant *Aspergillus*

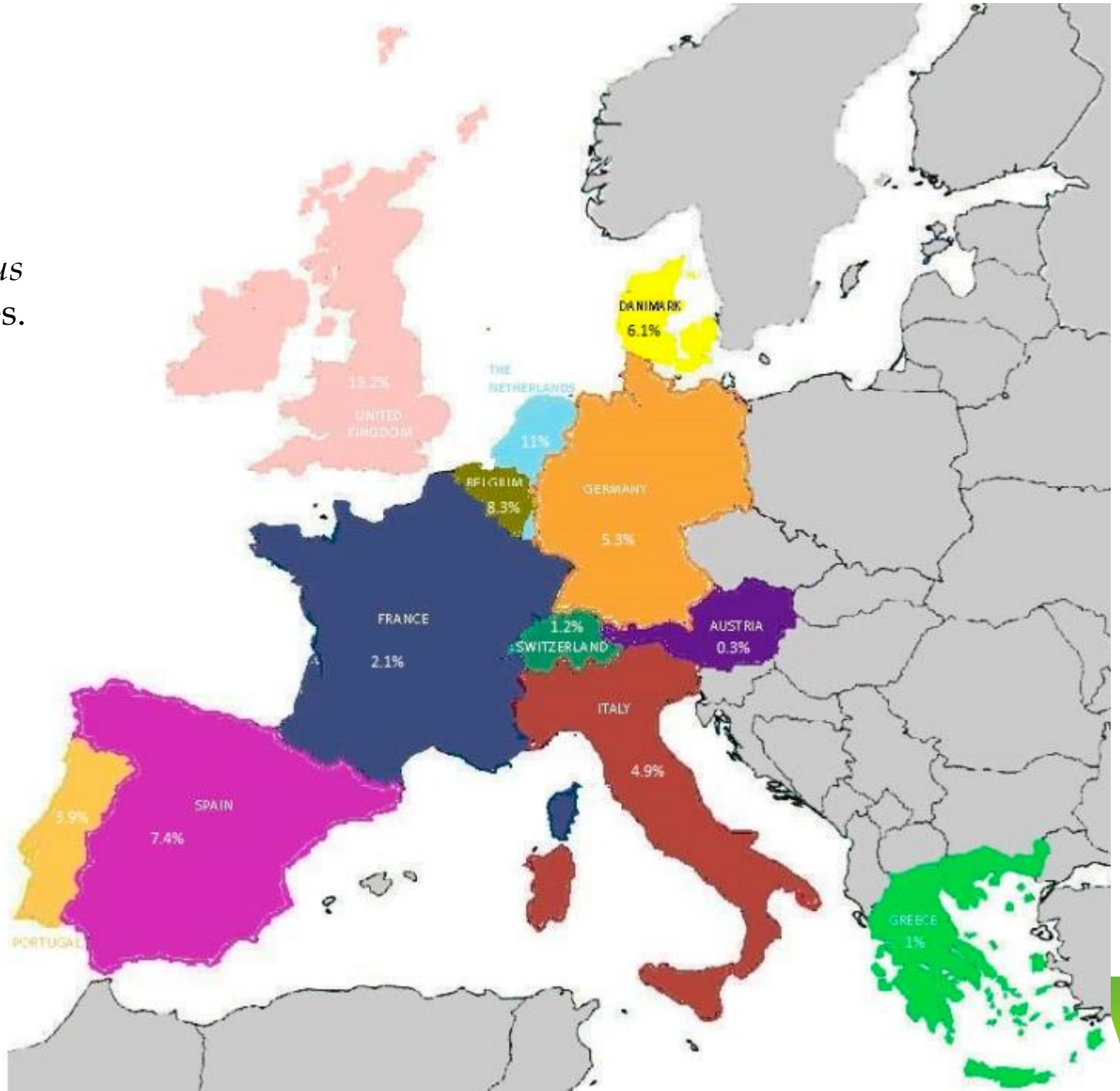
* The Netherlands was the European country using the greatest amount of azole fungicide, followed by Germany and France.
* In fact, the first environmental pan-azole-resistant *Aspergillus fumigatus* isolate was detected in the Netherlands [Lass-Florl, C.; et al. A nationwide passive surveillance on fungal infections shows a low burden of azole resistance in molds and yeasts in Tyrol, Austria. *Infection* **2018**, *46*, 701–704.].
* For clinical isolates, a study performed in a cardiothoracic center in London detected a higher prevalence of azole-resistant *Aspergillus fumigatus* (13.2%) principally associated with the environmentally driven TR34/L98H mutation.

Epidemiology of Azole-resistant *Aspergillus*

* Many studies have principally focused on azole-resistant *A. fumigatus* isolates because they represent the predominant pathogen of aspergillosis.
* The first case of ARAF was reported in the late 1980s in the Netherlands.
* The overall azole resistance rate of *A. fumigatus* was reported as ranging from 0.6 to 27.8%, depending on the isolation country, the type of disease, and the emergence of the environmental resistance mechanism.
* Most of the environmental azole-resistant isolates were found in Europe (56.7%) than in other countries due to the higher azole fungicide application per hectare of agricultural land (Burks, C.; Darby, A.; Gomez Londoño Momany, M.; Brewer, M.T. Azole-resistant *Aspergillus fumigatus* in the environment: Identifying key reservoirs and hotspots of antifungal resistance. *PLoS Pathog.* **2021**, *17*, e1009711.).

Prevalence of azole-resistant *Aspergillus fumigatus* strains in European countries.

Pathogens **2023**, *12*, 1305.



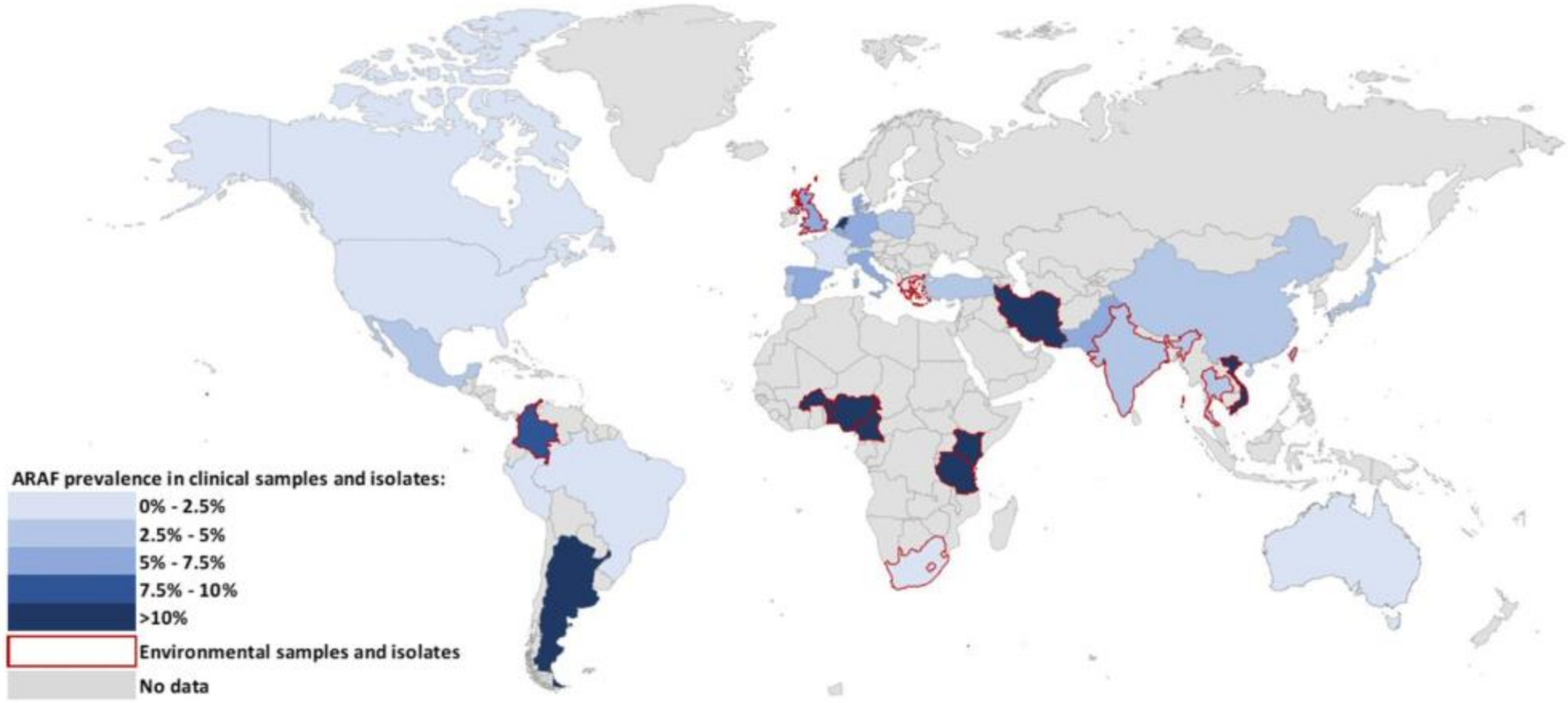


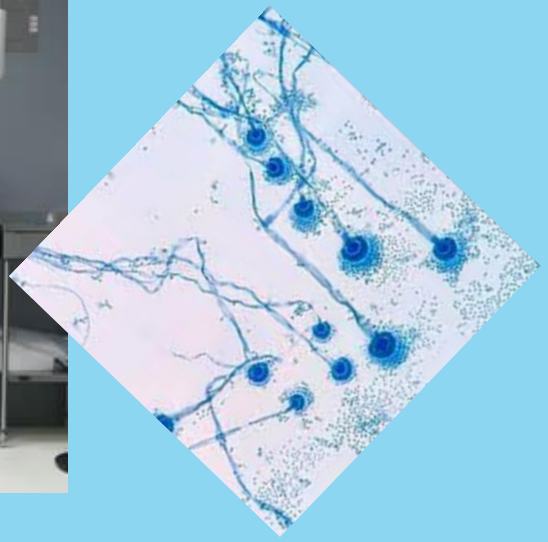
Fig. 1 Worldwide prevalence of azole resistant *Aspergillus fumigatus* (ARAF) clinical and environmental samples and isolates

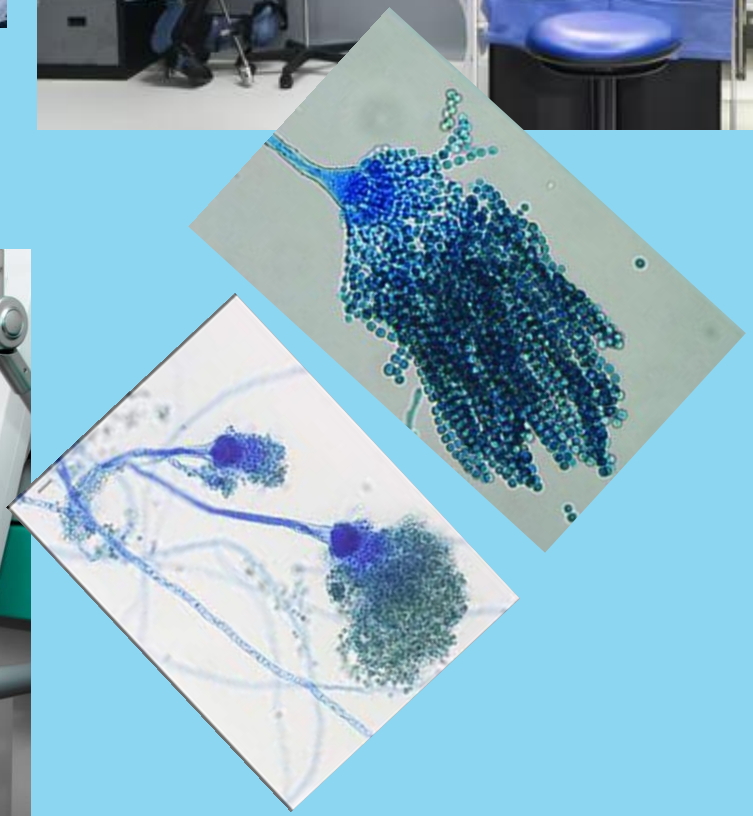
A note!

- In a recent study [Fakhim, H.; et al. Trends in the prevalence of amphotericin B resistance (AmBR) among clinical isolates of Aspergillus species. J. Mycol. Med. 2022, 32, 101310], among 26,909 *Aspergillus* isolates analyzed, resistance to amphotericin B was detected in 36.8% of *A. terreus*, 14.9% of *A. flavus*, 5.2% of *A. niger*, and 2.01% of *A. fumigatus* isolates.
- Some *A. lentulus* and *A. ustus* isolates have been reported to show amphotericin B resistance.
- Additionally, an increasing trend in amphotericin B resistance was observed in *A. fumigatus* between 2016 and 2020, together with a decreasing trend in amphotericin B resistance in *A. terreus* and *A. flavus*.

Range 6.9%- 55.2%		Range 0-11.9%
14.3% *A. fumigatus* isolate resistant to voriconazole	2023	4.1% and 6.1% of *A. fumigatus* isolates resistant to VOR and NWT for ITR. 2% and 11.9% *A. flavus* isolates resistant to VOR and NWT for ITR.
55.2% and 6.9% *A. fumigatus* isolates were NWT and resistant for ITR and VOR respectively. 6.9%) had cross resistant to ITR and VOR.	2022	No Azole resistance in 55 *A. fumigatus* clinical isolates were found
12.9% of *A. fumigatus* isolates were NWT and resistant for ITR and VOR. 12.9% isolates had cross resistant to ITR and VOR.	2018	*8% of A. fumigatus* isolates NWT for itraconazole.
8.9% *A. fumigatus* isolates were NWT for ITR and 5.1% isolates were resistant to VOR.	2016	3.5% *A. fumigatus* isolates cross resistant to ITR and VOR.
12.2% *A. fumigatus* isolates cross resistant to ITR and VOR	2013	3.2% *A. fumigatus* isolates cross resistant to ITR and VOR.
Environment isolates		Clinical isolates


126
98
60
100
36






A.D.A.M.

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Electronic equipment and appliances in special wards of hospitals as a source of azole-resistant *Aspergillus fumigatus*: a multi-centre study from Iran

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Results

- A total of 693 samples were collected from electronic medical equipment and appliances in 23 educational hospitals from 18 provinces of Iran.

Table I
Distribution of isolated *Aspergillus* species (*N*=89) on azole-containing agar plates

Subgenus	Section	Species	No. of isolates
Fumigati	*Fumigati*	*A. fumigatus*	37 (41.6%)
Circumdati	*Flavi*	*A. flavus*	5 (5.6%)
		A. oryzae	4 (4.5%)
	Nigri	*A. tubingensis*	21 (23.6%)
		A. niger	14 (15.7%)
		A. welwitschiae	2 (2.2%)
		A. luchuensis	2 (2.2%)
		A. japonicus	2 (2.2%)
		A. awamori	1 (1.1%)
	Circumdati	*A. terreus*	1 (1.1%)

In Vitro Antifungal Susceptibility Test

- Among different species of *Aspergillus*, *A. fumigatus* showed the highest MIC_{50}/MIC_{90} to all azoles tested (VCZ: 2/>16 µg/mL; ICZ: 1/16 µg/mL; PCZ: 0.25/1 µg/mL).
- VCZ showed the highest *in vitro* antifungal activity against *A. flavus*
- Whereas *A. niger* had the highest *in vitro* susceptibility to PCZ and ICZ.
- MICs 4, 2, and 1 (µg/mL) were observed as the highest MICs of *A. fumigatus* to VCZ, ICZ, and PCZ, respectively.
- A proportion of 12.8% (89/693) azole resistance *Aspergillus* species were recovered.
- VCZ resistance *A. fumigatus* were recovered as 5.3% (37/693).

Cont. *In Vitro* Antifungal Susceptibility Test

- In terms of the defined MIC breakpoint and ECV for azoles against *Aspergillus* spp. isolates, among the 37 *A. fumigatus* isolates obtained from ACAP, 8.1% were classified as intermediate, and 51.3% were classified as resistant to VCZ.
- The non-wild type of *A. fumigatus* against ICZ was identified in 56.8% of isolates.
- The non-wild type of *A. niger* to VCZ was observed in 7.1% of isolates.
- One isolate of *A. flavus* (1/5, 20%) was reported as non-wild type to PCZ.
- Of the 37 isolates of *A. fumigatus*, 48.6% showed cross-resistance to VCZ and ITZ.

Table III

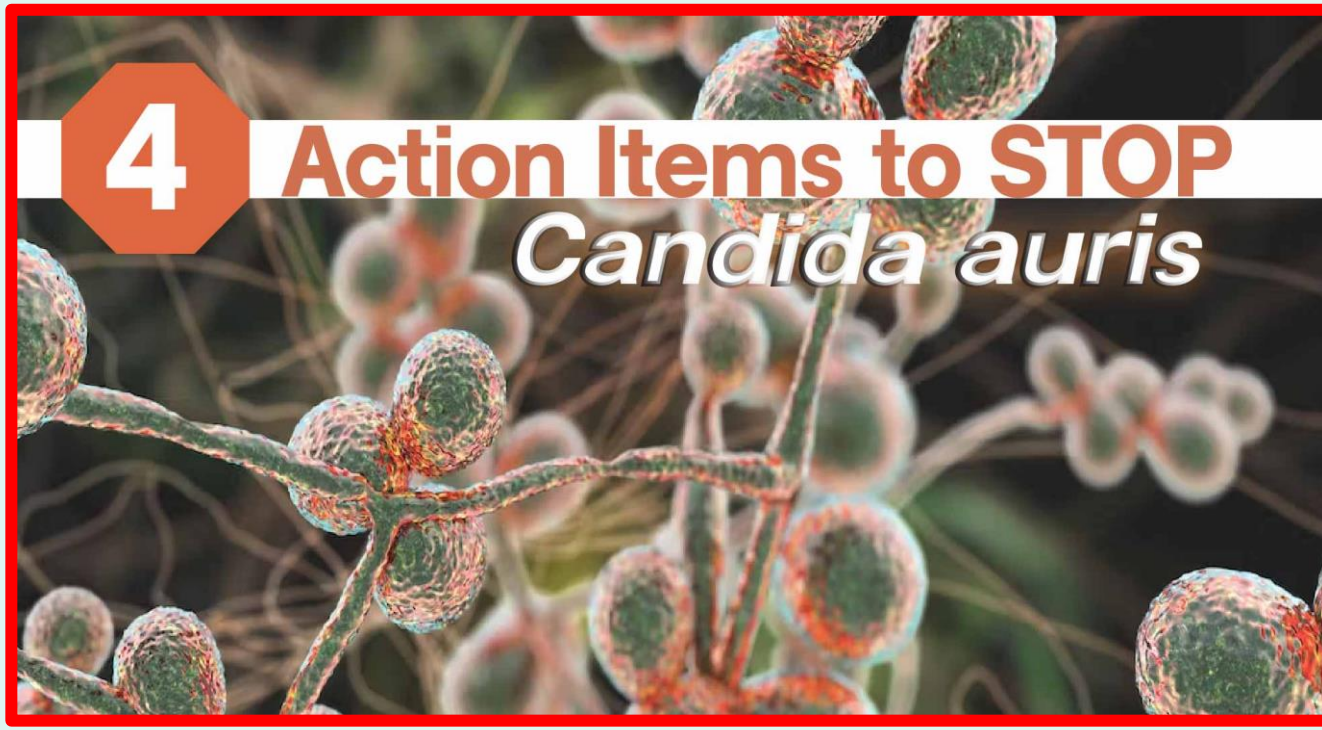
Characteristics of azole-resistant and non-wild-type *A. fumigatus* isolates and detected mutations

Isolate number	Isolated from ACAP			MIC (μg/mL)			*CYP51A* mutations											
	VCZ	ICZ	PCZ	VCZ	ICZ	PCZ	F46Y	G54E	G138C	Y121F	M172V	M220I	D255E	T289F	G432C	G448S	L98H	TR34
22s		+		4	8	0.063	+	+	+	–	+	+	+	+	+	+	–	–
3	+			4	4	1	+	+	+	–	+	+	+	+	+	+	+	+
4	+			2	4	0.063	+	+	+	–	+	+	+	+	+	+	+	+
14	+			>16	2	0.5	+	+	+	+	+	+	+	–	+	+	–	–
15	+			4	16	1	+	+	+	–	+	+	+	+	+	+	+	+
18	+			4	16	0.5	+	+	+	–	+	+	+	+	+	+	+	+
19	+			4	>16	1	+	+	+	–	+	+	+	+	+	+	+	+
21	+	+		4	16	1	+	+	+	–	+	+	+	+	+	+	+	+
29	+			>16	2	0.125	+	+	+	+	+	+	+	–	+	+	–	–
35	+			4	8	2	+	+	+	–	+	+	+	+	+	+	+	+
46	+	+		8	>16	1	+	+	+	–	+	+	+	–	+	+	+	+
47	+	+		4	>16	0.5	+	+	+	–	+	+	+	+	+	+	+	+
56		+		16	2	0.25	+	+	+	+	+	+	+	–	+	+	–	–
63	+	+		>16	2	1	+	+	+	+	+	+	+	–	+	+	–	–
72		+		8	>16	1	+	+	+	+	+	+	+	+	+	+	+	+
97	+			>16	1	0.063	+	+	+	+	+	+	+	–	+	+	–	–
23	+			4	16	1	–	–	–	–	+	+	+	+	+	+	–	–
34	+			2	2	1	+	+	+	–	–	–	–	–	+	+	–	–
65	+			>16	2	0.5	+	+	+	+	+	+	+	–	+	+	–	–

ACAP, azole-containing agar plate; MIC, minimum inhibitory concentration; ICZ, itraconazole; VCZ, voriconazole; PCZ, posaconazole.

High prevalence of Terbinafine resistance among *T. mentagrophytes/T. interdigitale* complex isolated from Iran

Iman Haghani[1,2], Maryam Babaie[1], Akbar Hoseinnejad[3,4], Ali Rezaei-Matehkolaei[4], Ramin Mofarah[5], Zahra Yahyazadeh[1], Firoozeh Kermani[6], Javad Javidnia[1,2], Tahereh Shokohi[1,2], Maryam Azish[7], Kambiz Kamyab Hesari[8], Majid Saeedi[9], Zeynab Ghasemi[8], Shaghayegh Khojasteh[10], Zohreh Hajheydari[11], Elham Mosayebi[1,2], Reza Valadan[12], Seyedmojtaba Seyedmousavi[13], Mahdi Abastabar[1,2]*, M.T. Hedayati[1,2]*



Recommendation

- Active surveillance for potential sources of fungal contamination
- *In vitro* antifungal susceptibility testing for all clinical fungal isolates routinely
- *A. flavus* should be considered as the main cause of IA in Iran
- Testing for identification of mutations in clinical fungal isolates routinely
- Presence of a real infection control committee in all hospitals

Thanks!

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