IN THE NAME OF GOD

Invasive fungal Infection: diagnosis and sensitivity

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Invasive fungal infections (background)

- The global burden of invasive fungal infections has shown an upsurge in recent years due to the higher load of immunocompromised patients.
- It can also occur in immunocompetent patients admitted to intensive care unit, without classical host factors.
- Around 1.9 million patients get an acute infection each year.
- An estimated 3 million people globally suffer from chronic fungal infections.

Invasive fungal infections (background)

- The most common etiologic agents are *Candida* species, *Aspergillus* species, Mucorals, *Cryptococcus* species, and *Pneumocystis* species.
- In addition, *Blastomyces*, *Histoplasma*, *Paracoccidioides*, and *Coccidioides* are endemic fungal strains .
- Nearly 70% of all IFIs in the world are caused by invasive candidiasis, followed by cryptococcosis (20%) and aspergillosis (10%).
- Invasive aspergillosis in immunocompromised individuals, has an extremely high mortality rate ranging between 40 and 90%.

Diagnosis

- In the case of IFIs, studies show significant daily increases in mortality and hospitalization costs for each day without the proper antifungal medicines, highlighting the severity of such delays.

- All the conventional approaches, including microscopy, histopathology, and culture-based tests, rely heavily on personnel with high levels of expertise in fungal identification and detection, and this is practically not possible in every setting.

Diagnosis

- Many cryptic fungal species are unable to be isolated and grown on fungal culture media, thus escaping conventional detection.

- For example, Fontecha and team identified four *Candida* strains (*C. albicans, C. glabrata, C. parapsilosis, and C. haemulonii*) which they referred to as cryptic, as they were nonculturable on conventional fungal media

- The invasive nature of the tissue sample, i.e. biopsies from deep tissues or tissue fluids extracted is difficult especially from very old patients, or from neonates, or from patients with hematologic malignancies.

Potassium hydroxide (KOH) wet mount:

- It is a rapid presumptive test for diagnosing the fungal infection.
- A small piece of the excised samples or tissue or BAL/sputum are kept in the 20% KOH.
- We can fasten the process of dissolving by keeping the mount at 37°C.
- It is essential to differentiate the *Mucorales* hyphae from the *Aspergillus* hyphae.
- Thin, septate, regular acute angle dichotomous branched hyaline hyphae of *Aspergillus* are observed in clinical samples.
- It is essential to differentiate the fungal hyphae from artefacts, especially the cotton fibres.

Calcofluor white stain

- It is a non-specific fluorochrome dye used widely to diagnose fungal pathogens in clinical samples.
- It binds specifically to the β 1-3, β1-4 glycoside chain of the chitin, an essential fungal cell wall component.
- Once the sample is stimulated with UV light in a fluorescent microscope, the fungal pathogen appears as the apple green or bluish against the white background, depending on the filter used.
- The addition of KOH in CFW stain increases the visibility of the fungal elements in clinical samples.
- Care should be taken while examining the clinical samples, especially the cotton fibres; when present, they fluoresces as bluish as the CFW stain also bind the β 1-3, β1-4 glycoside of cellulose.

Culture

- Although conventional culture tests remain the cornerstone of diagnosing fungal infections, the challenges associated with many fold.
- These include relatively low sensitivity, slow turnaround time, laborious process and the invasive nature of the specimens required for the testing.
- Blood culture sensitivity for yeasts ranges between 50 and 95 percent, while molds have even lower sensitivity values ranging from 1 to 5 percent
- In case of invasive candidiasis, blood culturing is considered a gold standard, but the long turnaround time (in the case of yeasts, up to five days; and moulds, up to four weeks) may put the patient at increased risk due to delay in delineating the necessary treatment plan

Culture

- Culture is an essential diagnostic tool to determine the causative fungal pathogens with their antifungal susceptibility.
- Sabouraud's Dextrose agar (SDA) is most widely used to cultivate the fungal pathogen.
- The received sample is cut into multiple small pieces, directly inoculated over SDA and Potato dextrose agar (PDA) and incubated at 25°C.
- The causative fungal pathogen is identified by standard mycological procedures including colony characteristics, morphological features on lactophenol cotton blue mount.

Candidiasis

- *Candida* infections can be described as mucocutaneous candidiasis, locally invasive candidiasis (e.g., oesophageal candidiasis, vaginal candidiasis) and invasive candidiasis.
- Invasive candidiasis among non-neutropenic adults mainly manifests as candidemia and deep-seated candidiasis with or without candidemia.
- According to CDC's surveillance data, the in-hospital all-cause (crude) mortality for patients suffering from candidemia is above 25%

Candidiasis

- Although *Candida albicans* account for 40 to 50% of cases across the globe, *Candida* non-*albicans* are a frequent cause of concern due to their tendency to show azole resistance.

- *Candida glabrata* is one of the most commonly isolated non- *albicans Candida* species and is notorious for reduced antifungal drug susceptibility.

- *Candida auris* is another difficult-to-treat isolate, an emerging drug-resistant pathogen responsible for several outbreaks in the past decade. It is known to survive on human skin and tough environmental conditions, facilitating rapid transmission in intensive care units.

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Susceptibility of clinical *Candida* species isolates to antifungal agents by E-test, Southern Iran: A five year study

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BMC Infectious Diseases

RESEARCH ARTICLE Open Access

Antifungal susceptibility testing of *Candida* species isolated from the immunocompromised patients admitted to ten university hospitals in Iran: comparison of colonizing and infecting isolates

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RESEARCH ARTICLE

Epidemiology and Antifungal Susceptibility of *Candida* Species Isolated from 10 Tertiary Care Hospitals in Iran

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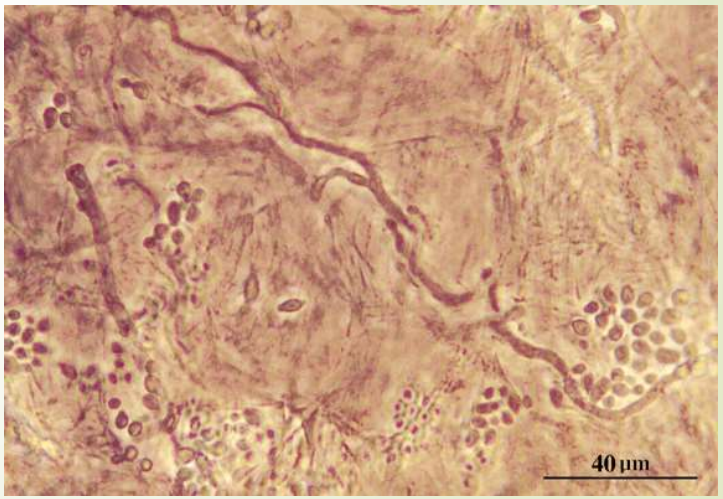
Etiologic agents

- Bassetti and co- worker reported the etiologic agents of invasive candidiasis in intensive care units in Europe were *Candida albicans* (57%) as the most common isolate, followed by *Candida glabrata* (21%) and *Candida parapsilosis* (13%).



Iran

- *2011: Candida albicans* (48%) was the most frequently isolated species, followed by *Candida kruzei* (16.1%), *Candida glabrata* (13.5%), *Candida kefyr* (7.4%), *Candida parapsilosis* (4.8%), *Candida tropicalis* (1.7%) and other species (8.5%)

- 2017: *C. albicans* 237 (56.3%), *C. tropicalis* 74 (8.8), *C.glabrata* 71 (8.3%), *Candida famata* 48 (5.7%), *C. parapsilopsis* 47 (5.6%), *Candida kefyr* 38 (4.5%), *Candida krusei* 23 (2.7%), *Candida dubliniensis* 13 (1.5%) and *C. intermedia, Candida lusitaniae and Candida guilliermondii* 22 (2.6%)

- 2022: *C. albicans* (285, 47.7%), followed by *C. glabrata* (125, 20.9%), *C. parapsilosis* (60,10%), *C. tropicalis* (23, 3.8%), *Candida krusei* (17, 2.8%), *C. orthopsilosis* (13, 2.2%), *Candida guilliermondii* (12, 2%), *Candida kefyr* (11, 1.3%), and *Candida lusitaniae* (3, 0.5%).

Proven invasive candidiasis

- A positive culture obtained from a specimen collected by a sterile procedure (including newly [<24hrs] placed drain) from a normally sterile site showing clinical or radiological features of infectious process,

- Histopathology, cytopathology or direct microscopy of a specimen collected by a sterile procedure from a normally sterile site showing budding yeast cells, which is further confirmed by culture or PCR. Note that the presence of pseudo-hyphae or true hyphae alone is not confirmatory of *Candida* as they can be seen with other fungi like *Trichosporon* spp, *Geotrichum* spp etc.
- • A positive PCR followed by DNA sequencing when yeast is detected in formalin- fixed parafin -embedded tissue.

Blood culture

- Blood culture sensitivity is 50 to 95% with a turnaround time of 3 to 7 days for candidemia and is even lower for deep-seated candidiasis without candidemia.

- Turnaround time can vary according to the species, as some may grow slower (e.g., *Candida glabrata*) than others.

Serological tests

- These tests employ the detection of antigens and/or antibodies in the patient's serum or body fluid.
- They significantly faster compared to fungal cultures.
- Antibody–based testing can be unreliable in immunocompromised patients due to the high rates of false–negative results.
- Commonly used serological tests for invasive candidiasis include
- beta–D–glucan assay (pan–fungal screening test)
- serum mannan/anti–mannan assay
- serum CAGTA (*Candida albicans* germ tube antigen)

Serological tests

- Serum BDG levels are raised in patients infected with *Candida* spp, *Aspergillus* spp, *Pneumocystis jirovecii*, *Acremonium* spp, etc., exceptions being *Cryptococcus* spp, *Blastomycets* and *Mucorales*.
- Various BDG tests are available in the market, namely, Fungitell assay, Wako – glucan test, and Goldstream Fungus (1–3)–b–D Glucan,
- Only Fungitell assay is FDA approved.
- The Fungitell assay has a sensitivity of 75 to 80% and a specificity of 60 to 80% in patients with invasive candidiasis.
- False positive results are seen in patients on haemodialysis, concurrent bacteraemia, immunoglobulin use, etc.

Molecular–based methods

- Different types of fungal PCRs are used for rapid and accurate diagnosis.
- These are especially helpful in diagnosis of drug resistance and identification of species which are difficult to culture.
- T2 *Candida* which utilizes magnetic resonance and molecular methods (PCR) for rapid diagnosis of candidemia.
- Two to four ml of whole blood from the patient is directly inserted into a fully automated T2 *Candida* instrument which causes *Candida* cell lysis using mechanical stress.

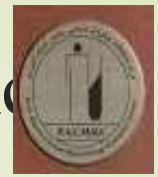
Molecular–based methods

- Amplified DNA product is detected using super magnetic nanoparticles.
- It detects the five most commonly pathogenic *Candida* species (*C. albicans*, *C. tropicalis*, *C. parapsilosis*, *C. krusei,C. glabrata*) within a turnaround time of fewer than 5 hours.
- T2 Candida has a sensitivity of 91% and specificity of 94% for candidemia but has poor sensitivity for detecting deep–seated candidiasis without candidemia.

Molecular–based methods

- PCR–based tests for the direct detection of *Candida* DNA in whole blood represent excellent technology for rapid diagnosis.
- Multiplex PCR enables the detection of multiple pathogens in a single test by employing distinct pairs of primers for each target.
- Real–time multiplex PCR (m–PCR) like Septifast (Roche Diagnostics) is now widely used as an advance molecular assay.
- The technology helps in the quick detection of multiple organisms.
- The sensitivity and specificity of m–PCR have been reported to vary between 60% to 100% and 90 to 96%, respectively, in various studies.

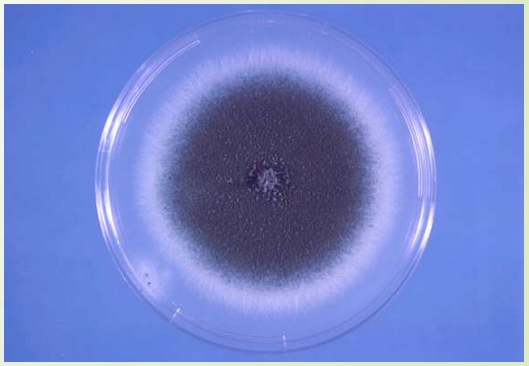
Combined approaches using artificial intelligenc



- Artificial intelligence systems are typically designed to mimic human cognitive functions, and they often involve the use of algorithms, data, and self–correction.
- Machine learning is a branch of artificial intelligence which uses historical data to learn, recognize patterns, and make predictions or decisions.
- Currently artificial intelligence is being used to assist in interpretation of microscopic images of fungal structures and automated histopathological analysis.

Invasive aspergillosis

- Invasive aspergillosis mainly manifests with pulmonary involvement.
- Risk factors include previous lung conditions or comorbidities like Chronic obstructive airway disease, bronchiectasis, decompensated liver disease, chronic heart failure, adult respiratory distress syndrome, etc.
- Morality due to invasive pulmonary aspergillosis varies between 65 to 90%.
- Invasive pulmonary aspergillosis is one of the most common “missed diagnoses” found in autopsy studies of ICU patients.

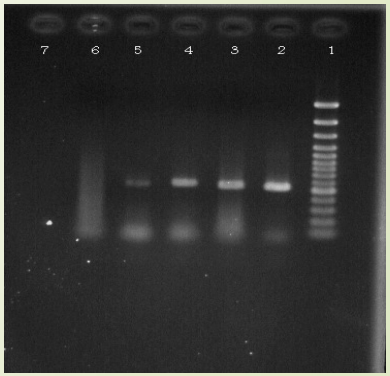


Diagnosis of invasive aspergillosis

- Diagnosing invasive aspergillosis is even more challenging than invasive candidiasis due to multiple factors like poor sensitivity (1–5%) of blood cultures
- Difficulty in performing lung biopsy in mechanically ventilated patients
- Difficulty in differentiating colonization from infection in the respiratory specimen
- Non–specific clinical and radiological features
- etc .

Diagnosis of invasive aspergillosis

- **Proven IPA** requires evidence of filamentous growth with the background of tissue damage.
- A positive culture obtained from a specimen collected via a sterile procedure from a normally sterile site, along with clinical/radiological abnormalities suggestive of infectious disease process (excluding bronchoalveolar lavage fluid, nasal sinuses and urine sample)
- Histopathology, cytopathology or direct microscopy of a specimen collected via a sterile procedure showing hyphae compatible with *Aspergillus* spp. along with tissue damage, **which is further confirmed by culture or PCR.**
- A positive PCR followed by DNA sequencing when mould is detected
- in formalin– fixed parafin –embedded tissue.



Diagnosis of invasive aspergillosis

- **Probable IPA** requires the patient to qualify for all the following criteria:
- Cytology, direct microscopy or culture showing the presence of *Aspergillus* hyphea in lower respiratory tract specimen
- Galactomannan assay is the most widely used and studied assay among the various non–culture–based techniques to diagnose invasive pulmonary aspergillosis.
- A positive galactomannan assay (>0.5 in plasma/serum or >0.8 in bronchoalveolar lavage)

Diagnosis of invasive aspergillosis

- It is an antigen–based assay (polysaccharide cell wall component) used for testing *Aspergillus* spp infection in serum, bronchoalveolar lavage fluid and cerebrospinal fluid.
- The only FDA–approved galactomannan test is Platelia *Aspergillus* enzyme immune assay (EIA) by Bio–Rad.
- Its overall sensitivity and specificity range between 67 to 100% and 86 to 100%, respectively.

Mucormycosis

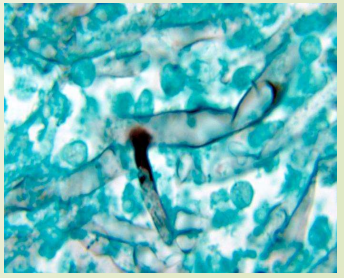
- Mucormycosis is a rare opportunistic infection caused by fungi that belong to the family Mucorales and usually live in soil and decaying organic material such as vegetable matter.
- Fungi that commonly cause mucormycosis include *Rhizopus* spp., *Mucor* spp., *Rhizomucor* spp., *Cunninghamella* spp, *Apophysomyces* spp., and *Lichthemia* (formerly known as Absidia) spp.
- The hyphae of the causative fungus are non-pigmented.
- The older colonies become grey to brown in colour due to the development of pigmented spores
- Nevertheless, the name "black fungus disease" comes from the black discolouration of the necrotic tissue that the fungus causes by invading blood vessels and destroying surrounding tissue.

Mucormycosis

- Mucormycosis rapidly involves neighbouring organs and can be fatal.
- The first wave of COVID-19 in India from July to December 2020 came with an increased number of cases of mucormycosis.
- During the second wave, a huge surge of cases including a staggering 47,508 cases and 4,425 deaths due to mucormycosis have been reported from India between 5 May and 3 August 2021.
- Several other countries have reported small numbers of cases during the same period. The first few cases, in the Arabian Peninsula, were reported in Oman, along with few cases in Iran, Egypt and Iraq. Many cases have also been reported in the Latin American nations of Chile and Uruguay.

Diagnosis

- The tissue necrosis resulting from angio-invasion is a classical feature of mucormycosis but, the other fungi, like *Aspergillus*, *Lomentospora* (*Scedosporium*), and *Fusarium* spp, also result in tissue necrosis.
- A high index of suspicion is needed for early diagnosis of mucormycosis in patients having multiple predisposing risk factors.
- Endoscopy mediated nasal cavity examination, especially the middle turbinate and sinuses may provide the initial evidence for mucormycosis.
- MRI of the paranasal sinuses and orbit help to diagnose mucormycosis where the hyperinflamed densed sinus mucosa with bone erosion, including the periosteum, observed.



Culture

- The primary concern about the culture is its low sensitivity.
- This low culture positivity may be attributed to different factors ranging from sample collection, storage to sample processing.
- The storage of samples at 4°C affects the viability of *Mucorales*.
- The tissue sample processing, including grinding or homogenization, also affects the viability of these fungal pathogens.
- However, good communication between the clinician and diagnostic laboratories can easily overcome these issues.

Histopathological examination

- Histopathological examination of the excised tissue is essential to determine the tissue reactions and the causative fungal pathogens.
- On microscopic examination, tissues from the suspected case of mucormycosis show necrosis, inflammatory infiltrate rich in neutrophils and fungal hyphae.
- H& E staining is most widely used for histopathological examination.
- PAS staining detects the carbohydrate component of the fungal cell wall, rich in β 1-3, β 1-4 D-glucan.
- Grocott-Gomori's Methenamine Silver staining (GMS) is a unique stain used to detect the fungal element in the clinical samples.

Molecular methods:

- Different molecular methods, semi-nested PCR, nested PCR with RFLP, real Time PCR targeting the ITS region or specific primers targeting a restricted number of mucoralean genera/species, are being used to diagnose mucormycosis.
- Among these, **the 18S ribosomal RNA gene is the most common target**, but others; 28S rDNA, the mitochondrial gene *rnl*, the cytochrome b gene or the Mucorales-specific CotH gene are also being targeted.
- Once the targeted region is amplified, it is sequenced to determine the causative fungal pathogen.
- Care should be taken while interpreting the results of molecular-based diagnostic tools as they don't differentiate between the actual pathogen/colonization or contamination.

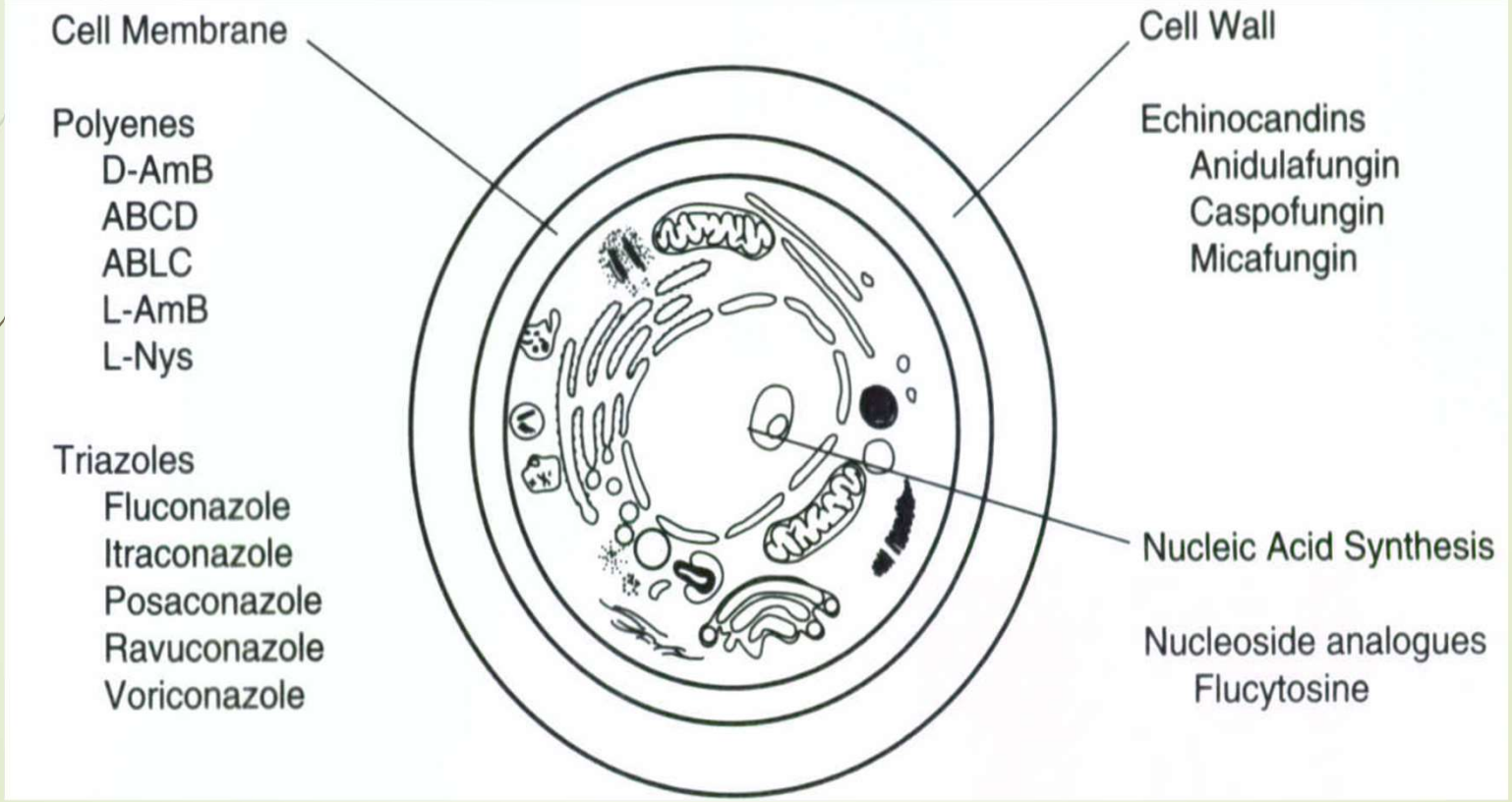
Serological test:

- Presently, ELISA testing targeting the serum *R. arrhhizus, Rhizomucor pusillus* antibody, western immunoblotting targeting *R. arrhhizus* antigen, Cytoplasm, hyphae walls and septate, are available for diagnosis of mucormycosis.
- In 2017, Kanako Sato *et al*. have reported the unique protein antigen, protein RSA of 23 kDa, in the serum and lung homogenates of *R.arrhizus* infected mice compared to uninfected mice.
- **Blood cultures can be obtained; however, they usually are negative despite the angioinvasive nature of the organism**.

Antifungal agents

- One of the major reasons for early and timely diagnosis of fungal pathogen is the increased mortality rates occur-ring due to emergence of fungal pathogens not responding to commonly deployed antifungal class of drugs.
- Fungal pathogens belonging to *Candida*, *Aspergillus*, *Cryptococcus*, and *Pneumocystis* spp. have been showing notable rates of antifungal resistance worldwide making antifungal drug resistance.
- Of these, *Candida auris* and *Aspergillus fumigatus* are now officially listed on the urgent antimicrobial resistance threat list published by the US CDC. As per CDC data, there has been a significant upsurge in clinical cases of C. auris (an emerg-ing multidrug resistant yeast) from 329 in 2018 to 1012 in 2021.

Antifungal agents



Antifungal agents

- Azoles act by inhibiting the synthesis of ergosterol, an essential component of the fungal cell membrane. They generally exhibit **fungistatic properties**, except for voriconazole, which demonstrates **fungicidal activity against *A. fumigatus***.
- Additionally, some of the species of *Candida* (e.g. *C. glabrata, C. krusei*) are intrinsically "less susceptible" to this class of antifungals.
- The newer azole isavuconazole has been found to have good in–vitro activity against *C. glabrata and C. krusei.*

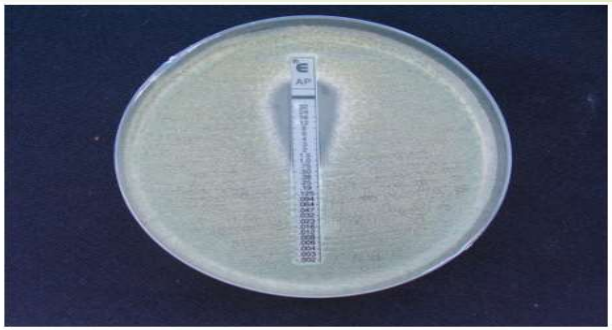
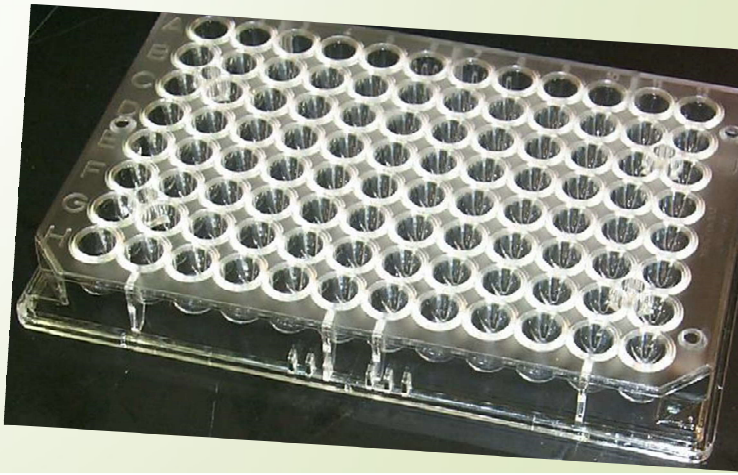


Figure 2 Amphotericin B E-test strip on RPMI medium showed a MIC of 1 µg/mL (susceptible to amphotericin B).

Antifungal agents

- Polyenes, act by binding to fungal ergosterol and destabilisation the fungal cell membrane. Amphotericin B and Nystatin are the currently available polyenes.
- **Amphotericin B** is one of the most commonly used antifungals among ICU patients and has the broadest spectrum of activity.
- Pyrimidine analogue, Five–flucytosine is the antifungal pyrimidine analogue which acts by destabilising the fungal DNA and RNA.



Antifungal agents

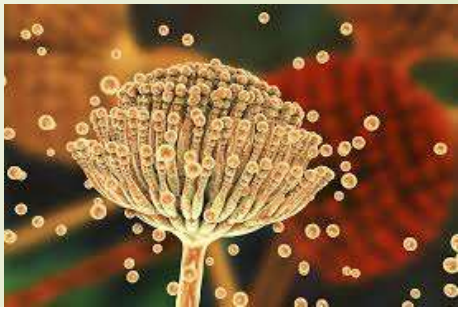
- Echinocandins are the unique class of antifungals with the least systemic toxicity due to their action on fungal cell walls, which is not present in human cells.
- Caspofungin, anidulafungin and micafungin are the three agents in this group with equal efficacy.
- Echinocandins block beta 1–3 glucan synthase leading to decreased beta–1–3 glucan production, which is an essential component of fungal cell walls.
- **Echinocandins are generally well tolerated and have a favourable safety profile.**

Azole Resistance Develops in the Body and in the Environment

- Azole-resistant *A. fumigatus* infections are difficult to treat, and infected patients are up to 33% more likely to die than patients with infections that can be treated with azoles.
- We know of two ways that *A. fumigatus* can develop resistance to azoles.
- **Inside the body**: Strains of *A. fumigatus* in people who take azole antifungals for a long period of time can become resistant, survive treatment, and continue to cause infection.
- **Outside the body**: Strains of *A. fumigatus* on decaying plants in the environment can be exposed to azole compounds used as fungicides that are chemically similar to azole antifungal medications.

Emergence Globally and Within the United States

- Aspergillosis is one of the leading missed diagnoses in intensive care units.
- At medical centers in some parts of the world, an estimated 19% of *A. fumigatus* infections are resistant to azole antifungals.
- In a large U.S. study, antimicrobial resistance was identified in up to 7% of *Aspergillus* specimens from patients with stem cell and organ transplants.
- The true burden of azole-resistant aspergillosis in the United States is unknown.



Etiologic agents

- A multicentre study which included 30 ICUs from 8 countries, found *Aspergillus fumigatus* the most common (92%) species causing invasive aspergillosis.
- Other species reported in the literature are *Aspergillus flavus*, *Aspergillus terreus*, *Aspergillus niger* etc .

Infection and Drug Resistance

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ORIGINAL RESEARCH

A 9-Year Experience of *Aspergillus* Infections from Isfahan, Iran

This article was published in the following Dove Press journal:
Infection and Drug Resistance

prevelence of Aspergillis in Iran

- Aspergillus flavus (n=54),
- A. fumigatus (n=24),
- A. niger (n=15),
- A. terreus (n=12)

Table 2 MIC Range, MIC_{50}, MIC_{90}, Geometric Mean (GM) and MIC Values Distribution of the Three Antifungals

Aspergillus Species (No.)	MIC Range (μg/mL)	MIC_{50} (μg/mL)	MIC_{90} (μg/mL)	GM	MIC Values (μg/mL)						
					0.25	0.5	1	2	4	8	16
A. flavus/oryzae (32)	AMB (0.5–4)	1	4	0.94	0	15	9	4	4	0	0
	ITR (0.5–16)	2	4	1.57	0	7	7	12	3	2	1
	VOR (0.25–8)	0.5	2	0.82	5	12	6	6	2	1	0
A. terreus (2)	AMB (4–4)	N/A	N/A	4	0	0	0	0	2	0	0
	ITR (0.5–1)			0.7	0	1	1	0	0	0	0
	VOR (0.5–1)			0.7	0	1	1	0	0	0	0
A. niger (1)	AMB (1)	N/A	N/A	N/A	0	0	1	0	0	0	0
	ITR (1)				0	0	1	0	0	0	0
	VOR (1)				0	0	1	0	0	0	0

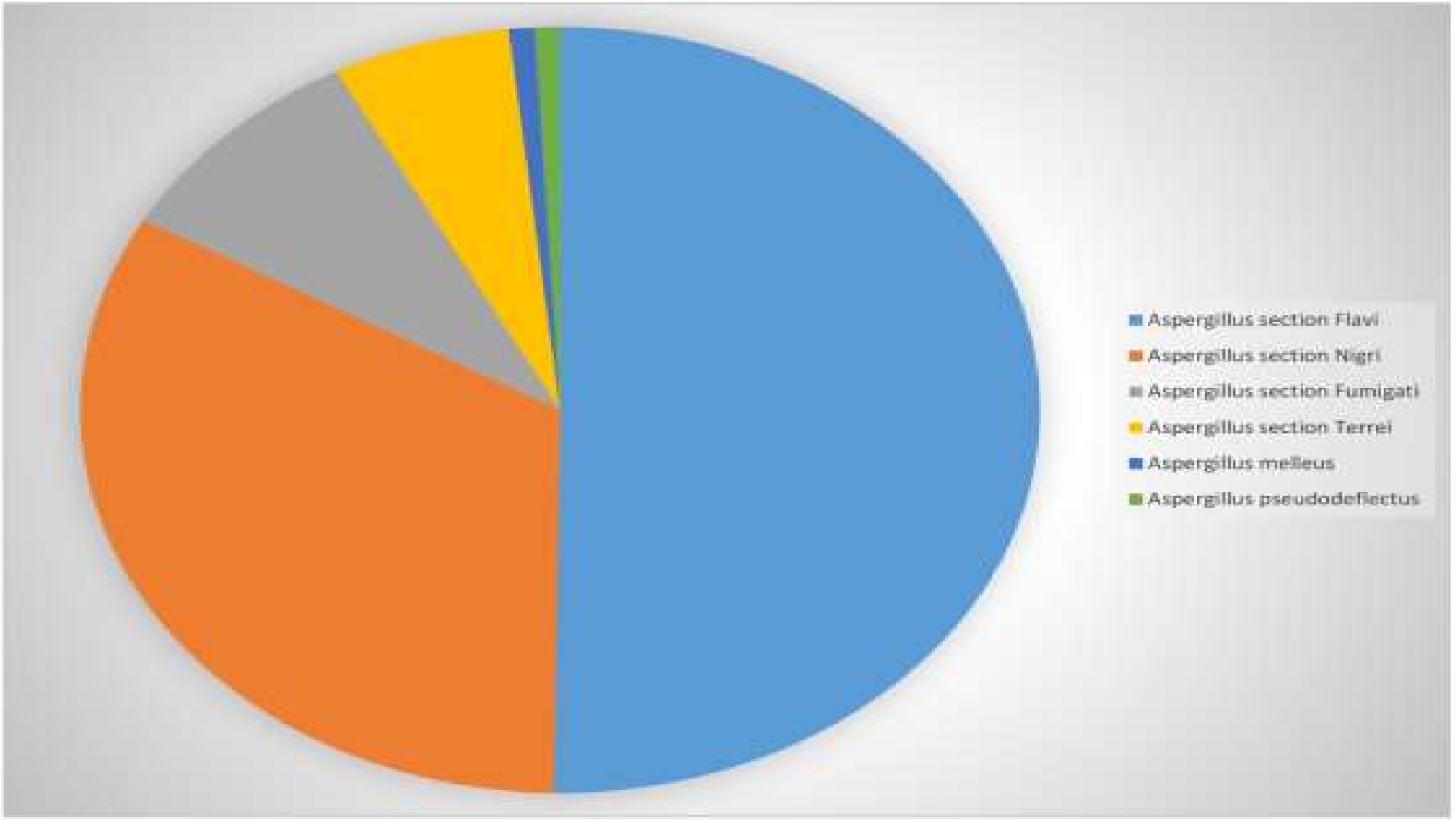

FIG 1 *Aspergillus* species isolated from clinical samples in 11 university hospitals in Iran.

TABLE 1 MIC/MEC range, MIC_{50}, MIC_{90}, MIC_{GM}, and epidemiological cutoff value distributions of *Aspergillus* species for seven antifungals, according to CLSI protocol (Table view)

Aspergillus species (no. of isolates, %)	Antifungal agent	MIC/MEC range (μg/mL)	MIC/MEC_{50} (μg/mL)	MIC/MEC_{90} (μg/mL)	MIC_{GM} (μg/mL)	ECV (μg/mL)	Wild type (%)	Non-wild type (%)
Aspergillus section *Flavi* (117, 50.2%)	Amphotericin B	0.064–8	0.5	2	0.818	8	100	0.00
	Caspofungin	0.016–8	0.016	0.064	0.033	1	97.5	2.5
	Voriconazole	0.032–8	0.25	0.5	0.334	1	96.6	3.4
	Itraconazole	0.016–8	0.25	2	0.310	8	100	0.00
	Posaconazole	0.016–8	0.25	2	0.412	4	99	1
	Luliconazole	0.008–0.5	0.008	0.016	0.012	0.064	95	5
	Isavuconazole	0.008–2	0.032	0.064	0.032	0.25	96.6	3.4
Aspergillus section *Nigri* (77, 33.1%)	Amphotericin B	0.032–4	0.25	0.5	0.281	ND[b]	ND	ND
	Caspofungin	0.016–4	0.032	0.064	0.034	ND	ND	ND
	Voriconazole	0.032–2	0.5	1	0.425	ND	ND	ND
	Itraconazole	0.016–8	4	8	2.693	ND	ND	ND
	Posaconazole	0.064–8	0.5	4	0.799	ND	ND	ND
	Luliconazole	0.008–0.016	0.008	0.016	0.009	ND	ND	ND
	Isavuconazole	0.008–4	0.064	1	0.111	ND	ND	ND
Aspergillus section *Fumigati* (21, 9%)	Amphotericin B	0.032–2	0.5	1	0.327	ND	ND	ND
	Caspofungin	0.016–0.064	0.016	0.064	0.025	ND	ND	ND
	Voriconazole	0.064–2	0.5	1	0.469	ND	ND	ND
	Itraconazole	0.032–8	4	8	0.879	ND	ND	ND
	Posaconazole	0.064–8	1	4	1.941	ND	ND	ND
	Luliconazole	0.008–4	0.008	0.032	0.019	ND	ND	ND
	Isavuconazole	0.008–0.5	0.064	0.25	0.072	ND	ND	ND

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Environmental surveillance of fungi and susceptibility to antifungal agents in tertiary care hospitals

Parisa Badiee,[1] Abdolkarim Ghadimi-Moghadam,[2] Habibeh Bayatmanesh,[2] Jafar Soltani,[3] Ali Reza Salimi-Khorashad,[4] Fatemeh Ghasemi,[1] Maneli Amin Shahidi,[1] Hadis Jafarian[1]

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Mucoral Sensitivity in Iran

Mucor (*n* = 17)	Amphotericin B	0.016–4	0.25	2	0.410
	Caspofungin	0.016–16	4	8	1.451
	Voriconazole	0.5–16	4	8	4.340
	Itraconazole	0.25–16	8	8	5.543
	Posaconazole	0.125–16	2	8	1.920
	Luliconazole	1–16	4	8	3.539
	Isavuconazole	0.008–0.5	0.25	2	0.241

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Comparison of in vitro activities of newer triazoles and classic antifungal agents against dermatophyte species isolated from Iranian University Hospitals: a multi-central study

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Dermatophytosis

- The most commonly isolated were *Trichophyton mentagrophytes* (76/198), followed by
- *Trichophyton interdigitale* (57/198)
- *Trichophyton rubrum* (34/198)
- *Microsporum canis* (10/198)
- *Epidermophyton floccosum* (3/198)
- *Trichophyton benhamiae* (1/198).

Trichophyton tonsurans (12/198)

Trichophyton simii (3/198)

Trichophyton violaceum (2/198)

Table 1 Comparison of in-vitro activities of seven antifungal agents tested against *dermatophytes* species isolated from patients by CLSI method

Dermatophyte species No. (%)	Antifungal agents	Range (µg/mL)	MIC/MEC$_{50}$[a] (µg/mL)	MIC/MEC$_{90}$ (µg/mL)	GM MIC[a] (µg/mL)
*Trichophyton mentagrophytes*76 (38.4%)	Terbinafine	0.016–8	0.25	2	0.209
	Griseofulvin	0.25–8	2	4	2.356
	Caspofungin	0.016–8	0.016	4	0.077
	Fluconazole	0.5–32	32	32	24.788
	Itraconazole	0.016–8	2	8	1.610
	Luliconazole	0.008–2	0.016	0.125	0.022
	Isavuconazole	0.008–2	0.016	0.125	0.177
Trichophyton interdigitale 57 (28.8%)	Terbinafine	0.016–8	0.032	1	0.080
	Griseofulvin	0.016–8	1	4	0.915
	Caspofungin	0.016–4	0.016	4	0.060
	Fluconazole	4–32	32	32	24.438
	Itraconazole	0.016–8	1	4	1.0142
	Luliconazole	0.08–0.5	0.008	0.25	0.019
	Isavuconazole	0.008–2	0.032	0.5	0.049
Trichophyton rubrum 34 (17.2%)	Terbinafine	0.016–8	0.032	0.25	0.038
	Griseofulvin	0.016–4	0.5	4	0.534
	Caspofungin	0.016–8	0.016	1	0.041
	Fluconazole	0.064–32	16	32	7.379
	Itraconazole	0.016–8	0.5	4	0.411
	Luliconazole	0.08–0.016	0.008	0.008	0.008
	Isavuconazole	0.08–1	0.032	0.5	0.044

Dermatophytes sensitivity

- The GM MIC and MIC_{90} values for all the isolates were as follows:
- terbinafine (0.091 and 1 μg/ml)
- griseofulvin (1.01 and 4 μg/ml)
- caspofungin (0.06 and 4 μg/ml)
- fluconazole (16.52 and 32 μg/ml)
- itraconazole (0.861 and 8 μg/ml)
- isavuconazole (0.074 and 2 μg/ml)
- luliconazole (0.018 and 0.25 μg/ml).

Therapiutic drug monitoring

- Recently, therapeutic drug monitoring for antifungals is increasingly being used for treatment optimization and prevention of both sub and supratherapeutic drug concentrations.
- Routine therapeutic drug monitoring is recommended for **voriconazole** in critically ill patients. However, it is neither recommended nor discouraged for other azoles and echinocandins.
- Routine therapeutic drug monitoring should be used for flucytosine as it can cause dose–related hepatotoxicity, bone marrow suppression and gastrointestinal disturbances

Conclusion

- Timely diagnosis of IFI is necessary to prevent the high morbidity and mortality associated with systemic fungal infections, as late diagnosis always equates with a pmorbidity and mortality associated with systemic fungal infections.
- With the increased use of antifungal agents, there is a rise in antifungal resistanc.
- Isavuconazole, a newer member of the azole class, has an improved safety compared to other azoles, with fewer concerns related to hepatotoxicity and drug interactions.

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Traditionally diagnosis

- Traditionally, identification of the fungal pathogen is made by using macroscopic and microscopic techniques, which are labour–intensive, time–consuming and largely dependent upon the expertise of the clinical mycologist.
- MALDI–TOF MS is a promising alternative for the rapid identification of Candida species and in–vitro antifungal susceptibility testing.
- Using MALDI–TOF MS, allows for improved species identification, as has been done for C. auris.