Invasive Fungal Disease (IFD) and two methods of detection

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MTW

NHS
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Invasive Fungal Disease (IFD): What is it? Who is at risk?

Most pathogenic fungi can best be described as **opportunistic**, they are common in the **environment, indoors and outdoors; on and in our bodies**.

Fungi reproduce by spreading **microscopic spores,** which are often present in the **air and soil**, **or on surfaces,** where they can be **inhaled or come into contact with the surfaces of the body,** primarily the skin.

People breathe in or **come in contact with fungal spores every day**, and a **healthy immune system** will generally keep them at bay

Many treatments, disease conditions or other circumstances **weaken components of the immune system**, when fungal organisms enter the body, these fungi can grow **unchecked, spreading and invading into other tissue**.

Covid-19 or Influenza

Chronic Lung Disease or TB

Invasive medical procedures
(mechanical ventilation, lines, tubes)

Cancer

Critically ill patients in ICU

Broad-spectrum antibiotics

Cases of invasive fungal disease (IFD) are rising as the at-risk population continues to expand, and new groups at risk are constantly being identified.

Invasive Fungal Disease is the result when fungi invade and **establish themselves within the deep tissues or the bloodstream**.

IFD: Emerging, but known

2020
3 August 2005
9 October 2020
isease as neglected pathogens:
up call to public health officials
invasive fungal disease in humans.
are we aware of the real impact?

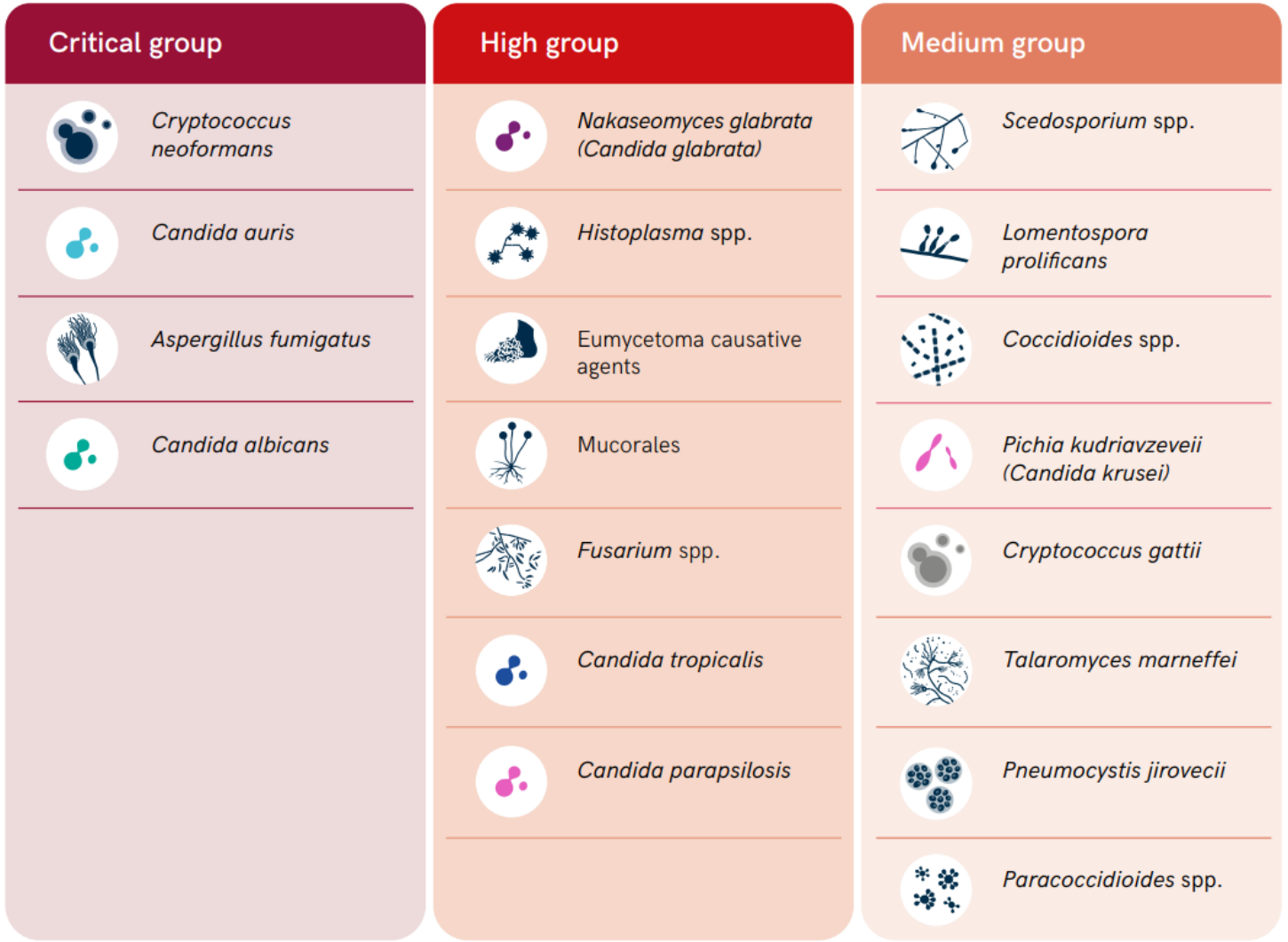
25 October 2022
20 March 2023
WHO releases first-ever list of health-threatening fungi

6 February 2023
5 August 2022
19
Humans are not prepared for a pandemic caused by fungal infections
"The Last of Us" Apocalypse Is Not Realistic, But Rising Threat of Fungal Pathogens
fungal infections: the rise of Candida auris

IFD: WHO report
Fungal priority pathogens list

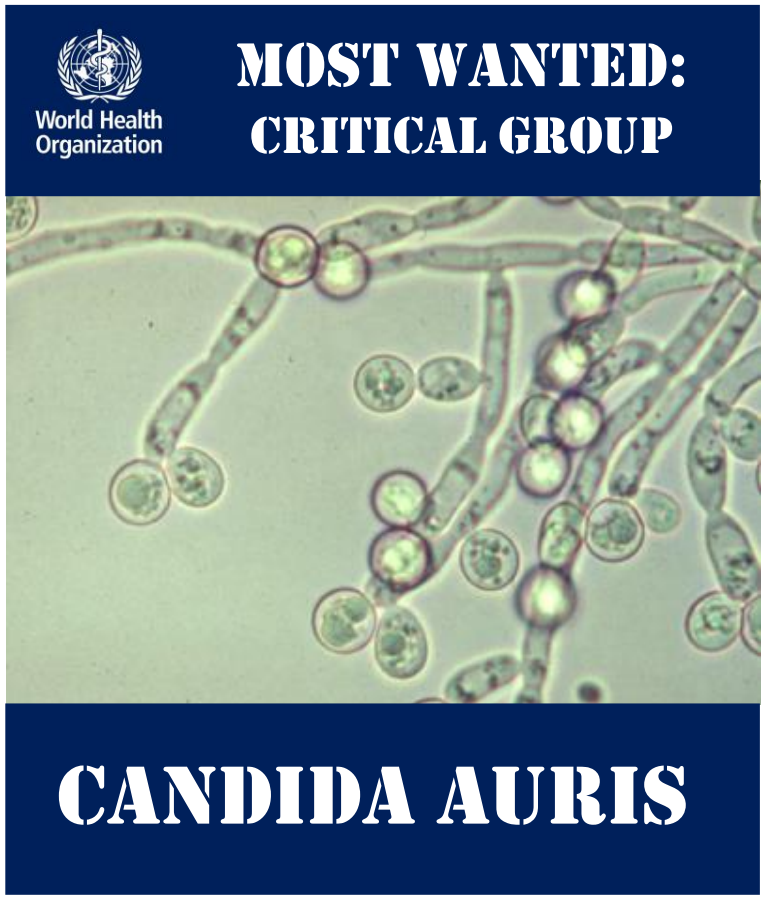
In October 2022, the World Health Organisation (WHO) developed its first fungal priority pathogens list (FPPL).

This is the first global effort to systematically prioritise fungal pathogens, considering their unmet R&D needs and perceived public health importance.



WHO fungal priority pathogens list (FPPL) to guide research, development and public health action. Geneva: World Health Organization; 2022. Licence: CC BY-NC-SA 3.0 IGO. [https://www.who.int/publications/i/item/9789240060241]

IFD: *Candida auris*



Background: Globally distributed yeast, first identified in 2009. Originally found in the environment as a plant fungus (saprophyte), it is hypothesised that selective pressure from climate change favoured strains that had adapted to salinity and higher temperatures - similar to the conditions found in the human body.

Infection Route: Through contact with contaminated **environmental surfaces or equipment**, or from **person to person**.

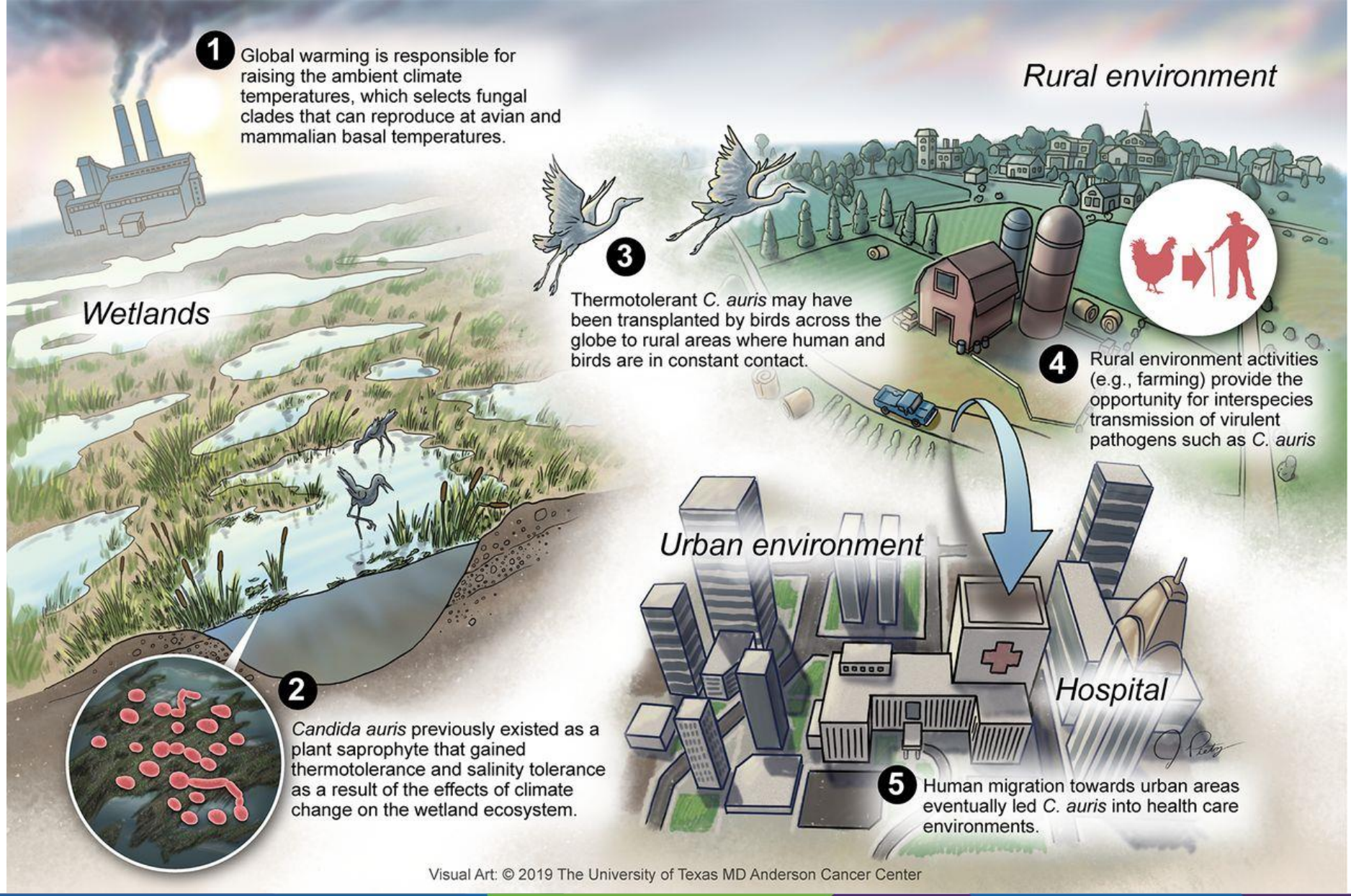
Known associates: Around 200 species of *Candida*, at least 30 are pathogenic to humans: *C. albicans, C. glabrata, C. krusei, C. parapsilosis, C. tropicalis.*

Candida Diseases: Generally, causes wound infections and ear infections; but for patients having **internal lines or tubes inserted**, patients who are **critically ill or immunosuppressed**, *candida auris* can enter internal organs or the bloodstream causing **invasive candidiasis**.

This is **life-threatening with high mortality**. Invasive candidiasis caused by *candida auris* has a mortality rate of between **29% to 53%**.

Outbreak potential: HIGH
Some strains can be **pan-resistant to antifungals**, in addition to being **thermoresistant** and partially **resistant to commonly used disinfectants**.

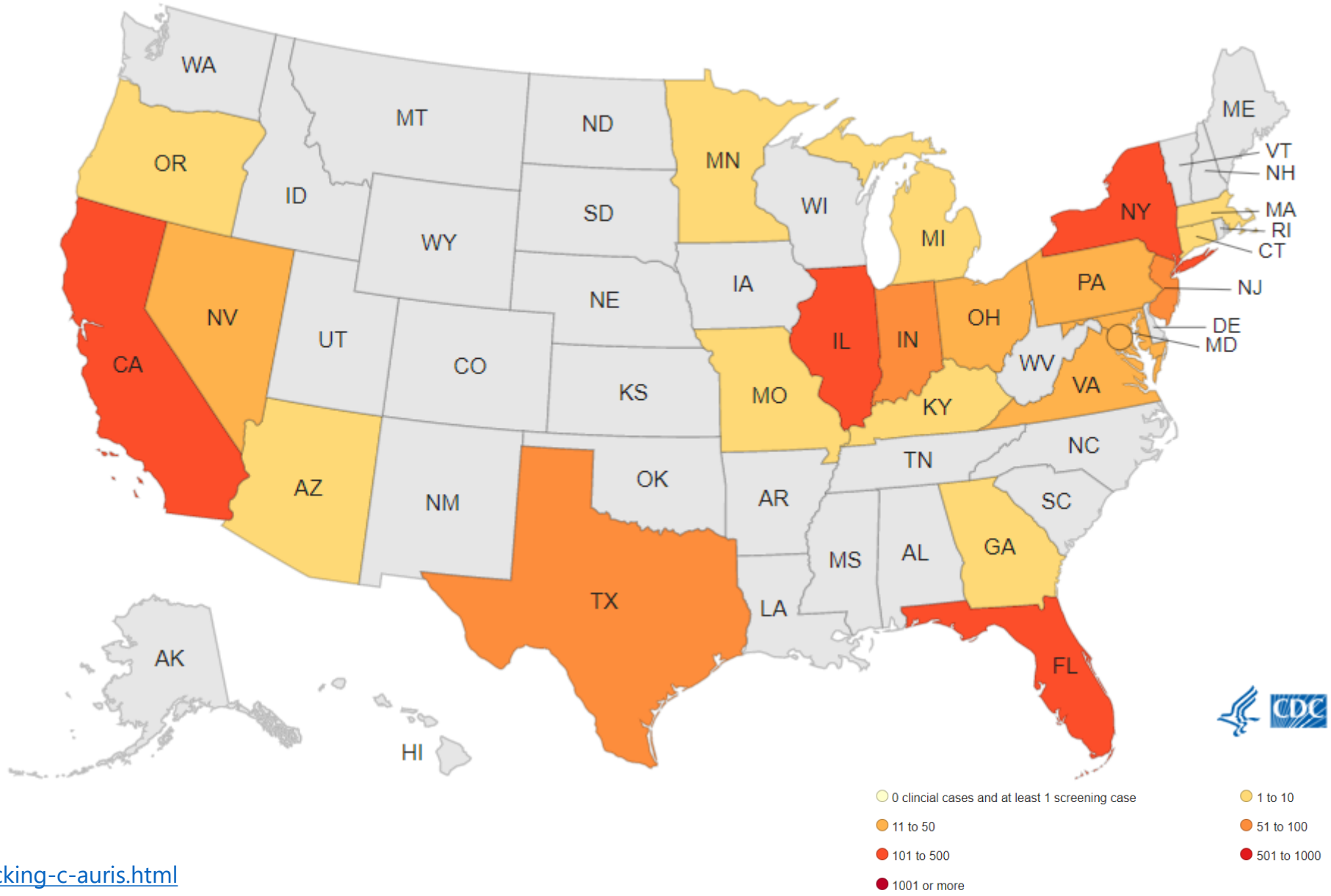
WHO fungal priority pathogens list (FPPL) to guide research, development and public health action. Geneva: World Health Organization; 2022. Licence: CC BY-NC-SA 3.0 IGO. [https://www.who.int/publications/i/item/9789240060241]
Ellwanger, J.H. and Chies, J.A. (2022) "Candida auris emergence as a consequence of climate change: Impacts on Americas and the need to contain greenhouse gas emissions," *The Lancet Regional Health - Americas*, 11, p. 100250. Available at: https://doi.org/10.1016/j.lana.2022.100250


1 Global warming is responsible for raising the ambient climate temperatures, which selects fungal clades that can reproduce at avian and mammalian basal temperatures.
Rural environment
Wetlands
3 Thermotolerant C. auris may have been transplanted by birds across the globe to rural areas where human and birds are in constant contact.
4 Rural environment activities (e.g., farming) provide the opportunity for interspecies transmission of virulent pathogens such as C. auris
Urban environment
Hospital
2 Candida auris previously existed as a plant saprophyte that gained thermotolerance and salinity tolerance as a result of the effects of climate change on the wetland ecosystem.
5 Human migration towards urban areas eventually led C. auris into health care environments.
Visual Art: © 2019 The University of Texas MD Anderson Cancer Center

Cases of *candida auris* throughout the U.S.A.
(clinical and screening cases)

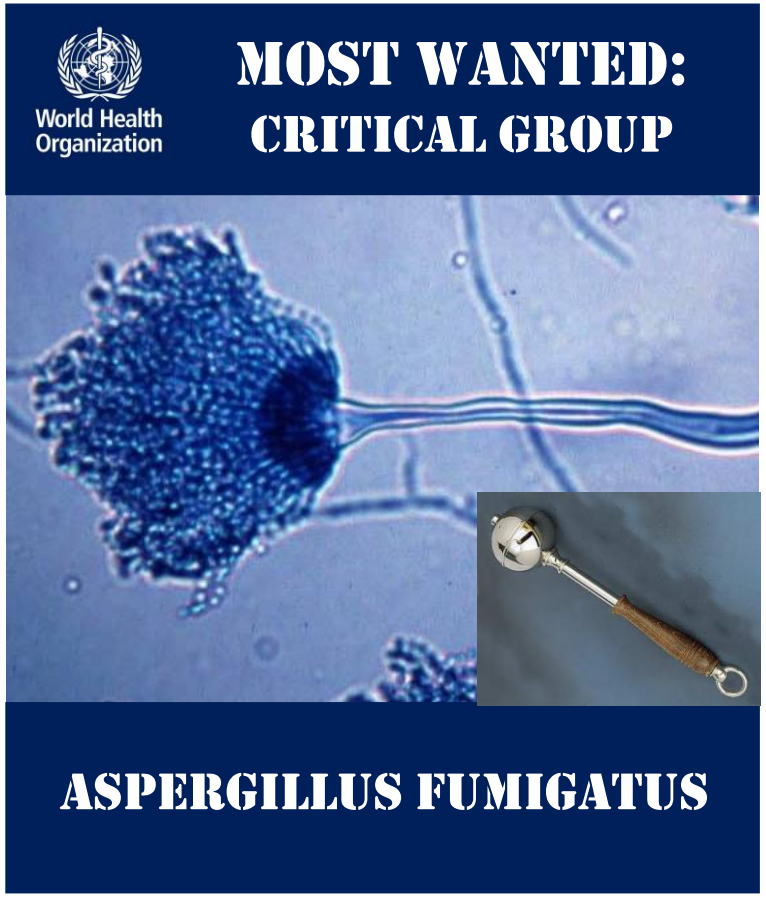
2017
2018
2019
2020
2021
2022

[illegible] clinical cases
[illegible] screening cases



https://www.cdc.gov/fungal/candida-auris/tracking-c-auris.html

IFD: *Aspergillus fumigatus*

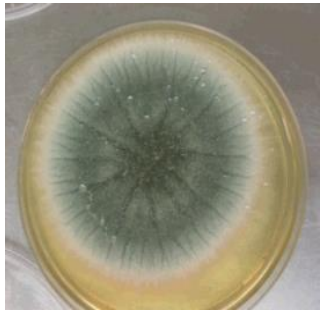


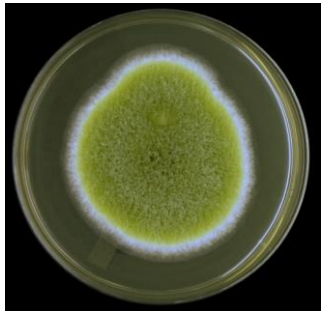
Background: Globally distributed environmental mould with pathogenic potential, commonly found in the surrounding environment but indoors and outdoors.

It was first described in 1729, by an Italian priest and biologist Pier Antonio Micheli. The shape of the Aspergillus reminded him of an aspergillum (see right insert), a device used for sprinkling holy water during a liturgical service.

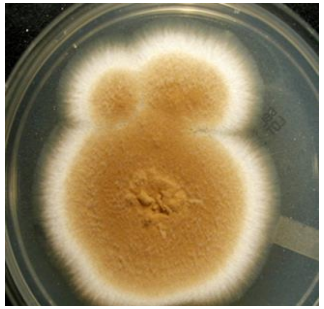
Infection route: Inhaled from the environment, cannot spread person-to-person.

Known associates: There are around 180 species of Aspergillus, but fewer than 40 are pathogenic: *A. fumigatus* (67% of cases), *A. flavus* (13%), *A. niger* (9%).


A. fumigatus


A. flavus


A. niger

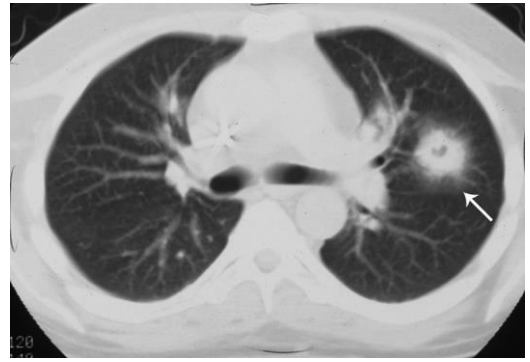

A. terreus

Outbreak potential: Mostly sporadic but has outbreak potential. Previous outbreak sources have been traced to contaminated biomedical devices, a water leak above a nurse's station, dust on ceiling tiles.

WHO fungal priority pathogens list (FPPL) to guide research, development and public health action. Geneva: World Health Organization; 2022. Licence: CC BY-NC-SA 3.0 IGO. [https://www.who.int/publications/i/item/9789240060241]

IFD: *Aspergillus* Disease

Figure 1: CT scan image of the chest in a patient with aspergillosis depicts a left upper lobe lesion with a thin ground glass halo

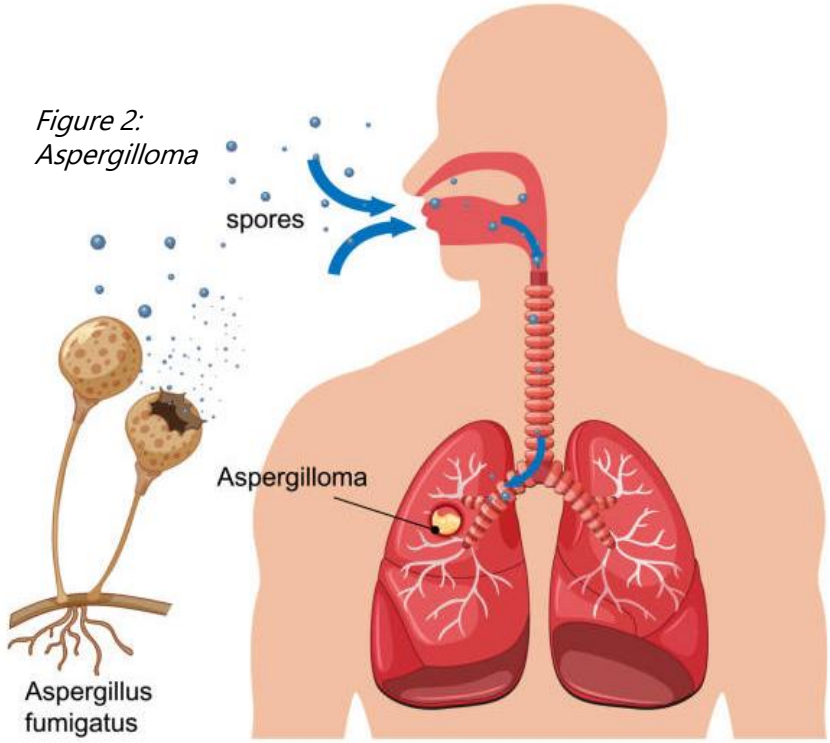


Chronic pulmonary aspergillosis (CPA)
Aspergillus infection causes **cavities in the lungs**. Can be long-term.

Aspergilloma
"Fungus ball" grows in a **pre-formed cavity**, such as from CPA and other cavitating lung diseases (see Figure 1 & 2)

Invasive Aspergillosis (IA)
Occurs mostly in **immunocompromised** hosts, when colonisation in the lungs **spreads to other sites**.
Mortality rates in those with azole-resistant *A. fumigatus* infection are high: **47–88%.**

Figure 2: Aspergilloma



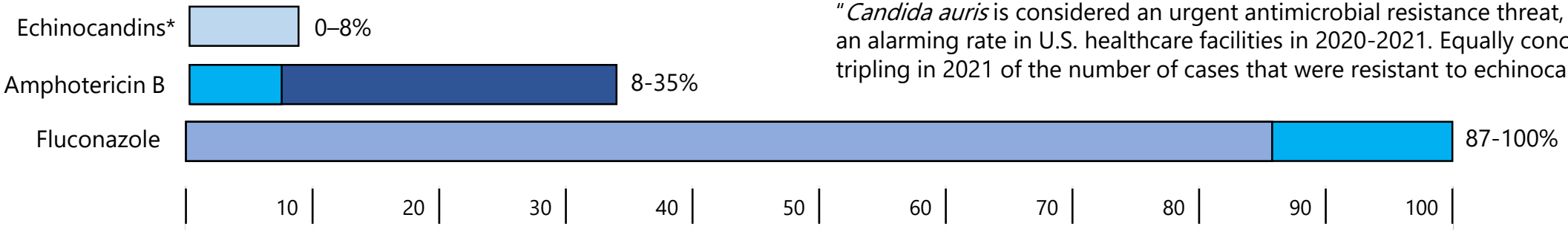
IFD: Reason for concern - Antifungal Resistances

Aspergillus fumigatus antifungal resistance

"Emerging [*A. fumigatus*] resistance to azoles is concerning." – Who FPPL Report

Candida auris antifungal resistance

"*Candida auris* is considered an urgent antimicrobial resistance threat, it spread at an alarming rate in U.S. healthcare facilities in 2020-2021. Equally concerning was a tripling in 2021 of the number of cases that were resistant to echinocandins." - CDC



*First line of treatment

Increasing threat of spread of antimicrobial-resistant fungus in healthcare facilities (2023) Centers for Disease Control and Prevention. Available at: https://www.cdc.gov/media/releases/2023/p0320-cauris.html (Accessed: March 27, 2023).
WHO fungal priority pathogens list (FPPL) to guide research, development and public health action. Geneva: World Health Organization; 2022. Licence: CC BY-NC-SA 3.0 IGO. [https://www.who.int/publications/i/item/9789240060241]

IFD: Reason for concern - An opportunity for an opportunistic pathogen

Severe COVID-19 can potentially require invasive treatments, which can include feeding tubes and mechanical ventilation; together any previous systemic therapy and a less effective innate immune system, can cause predisposition to invasive fungal infections.

COVID-19–Associated Pulmonary Aspergillosis (CAPA)

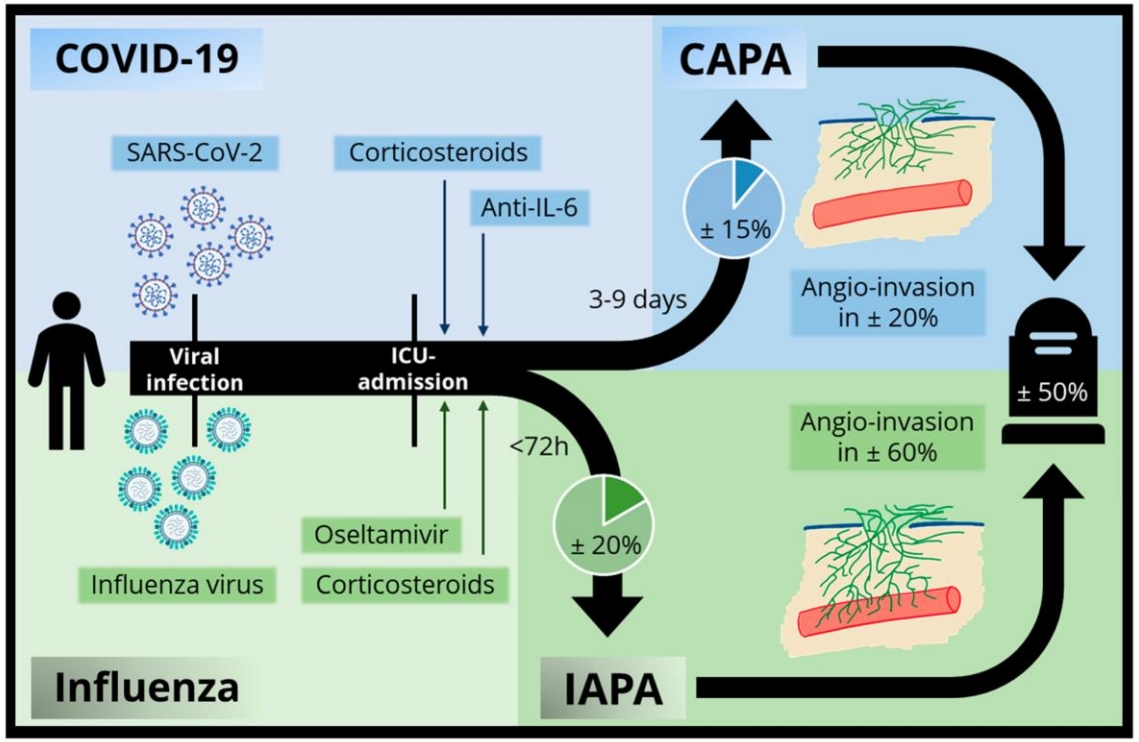
Incidence:

15% (among 2953 ICU-admitted COVID-19 patients)[2]
Against a prevalence of CPA, in general of:
<1 per 100,000 (in Europe)[1]

Mortality rate:

50% patients WITH severe influenza or COVID-19 and aspergillus co-infection

patients with severe influenza or COVID-19 WITHOUT aspergillosis **25–35%**



[1] Focus: Rare Disease: Chronic Pulmonary Aspergillosis: A Brief Review. (n.d.). PubMed Central (PMC). https://www.ncbi.nlm.nih.gov/pmc/articles/PMC8686779/
[2] Feys, S., Almyroudi, M. P., Braspenning, R., Lagrou, K., Spriet, I., Dimopoulos, G., & Wauters, J. (2021). A Visual and Comprehensive Review on COVID-19-Associated Pulmonary Aspergillosis (CAPA). Journal of Fungi, 7(12), 1067. https://doi.org/10.3390/jof7121067

IFD: Reason for concern - An opportunity for an opportunistic pathogen

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COVID-19–Associated Candidemia (CAC)

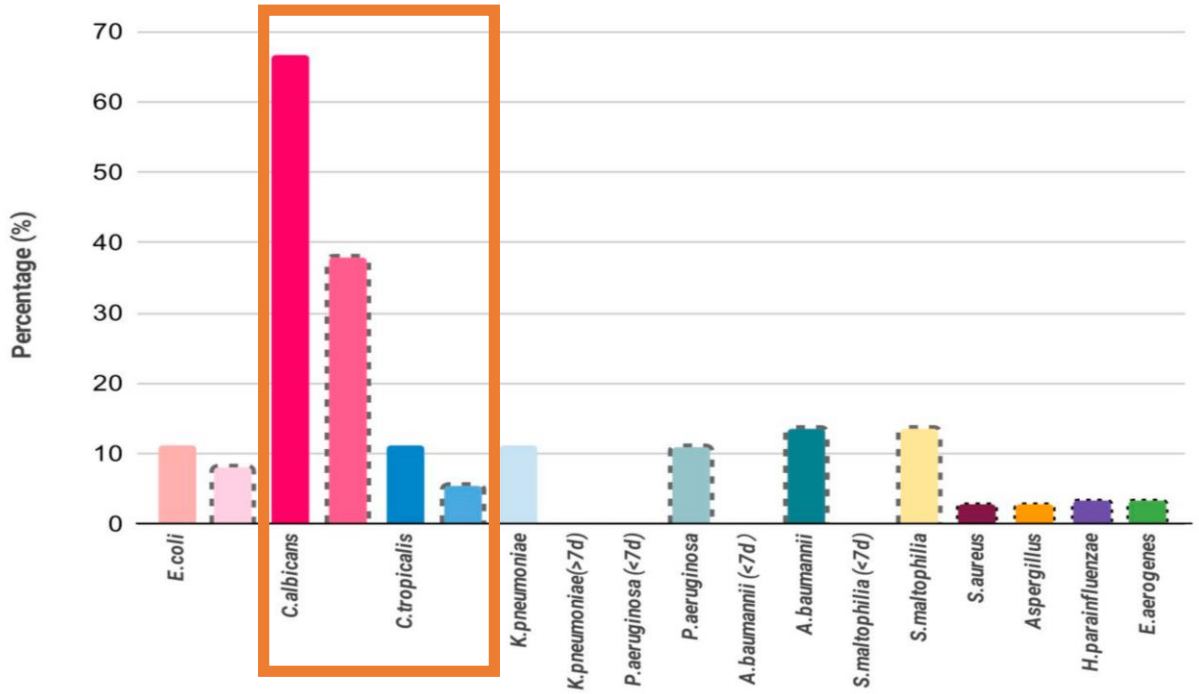
Incidence:

x2 two-fold increase in the incidence of candidemia in patients with Covid-19 compared to those who without[1]

Mortality rate:

92.5% in Covid-19 patients

non-Covid-19 patients with Candidemia **79.4%**

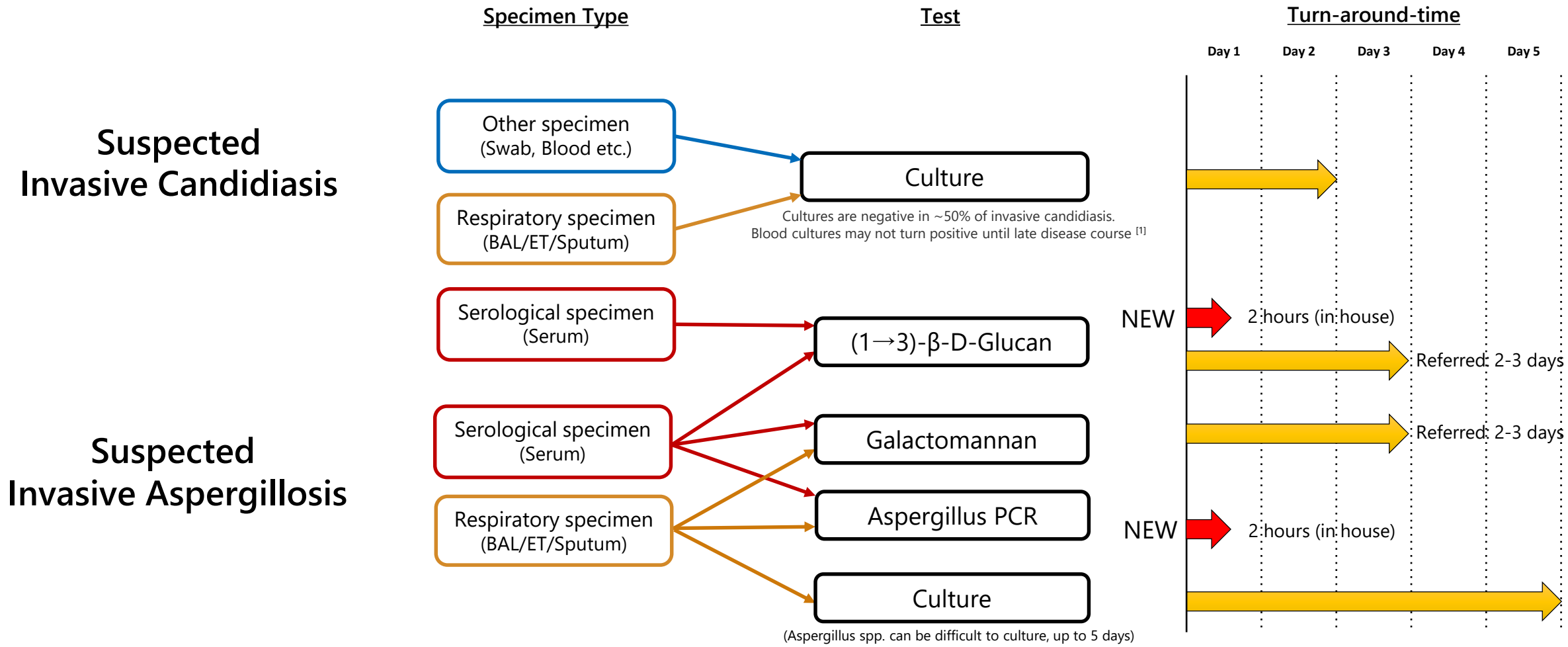


Distribution of respiratory pathogens in COVID-19 mortality cases (n=27) [2]
Solid line: <7 d length of stay (LOS); Dotted line: >7 d LOS

[1] Kayaaslan, B., Eser, F., Kaya Kalem, A., Bilgic, Z., Asilturk, D., Hasanoglu, I., Ayhan, M., Tezer Tekce, Y., Erdem, D., Turan, S., Mumcuoglu, I., & Guner, R. (2021). Characteristics of candidemia in COVID-19 patients; increased incidence, earlier occurrence and higher mortality rates compared to non-COVID-19 patients. Mycoses, 64(9), 1083–1091. https://doi.org/10.1111/myc.13332

[2] Lu, D.-E., Hung, S.-H., Su, Y.-S., & Lee, W.-S. (2022). Analysis of Fungal and Bacterial Co-Infections in Mortality Cases among Hospitalized Patients with COVID-19 in Taipei, Taiwan. Journal of Fungi, 8(1), 91. https://doi.org/10.3390/jof8010091

IFD: New, Rapid Diagnostic pathways



[1] Clancy, C. J., & Nguyen, M. H. (2018). Diagnosing Invasive Candidiasis. *Journal of Clinical Microbiology*, *56*(5), Article e01909-17. https://doi.org/10.1128/jcm.01909-17

Beta-D-Glucan: What it is?

Beta-D-Glucan (BDG) is a type of carbohydrate: a glucose polymer that is a major part of the cell walls of certain: cereals, bacteria and fungi.

Most pathogenic fungi have (1→3)-β-D-Glucan as a component of their cell walls; and small, but detectable quantities are released into the blood circulation during IFD.

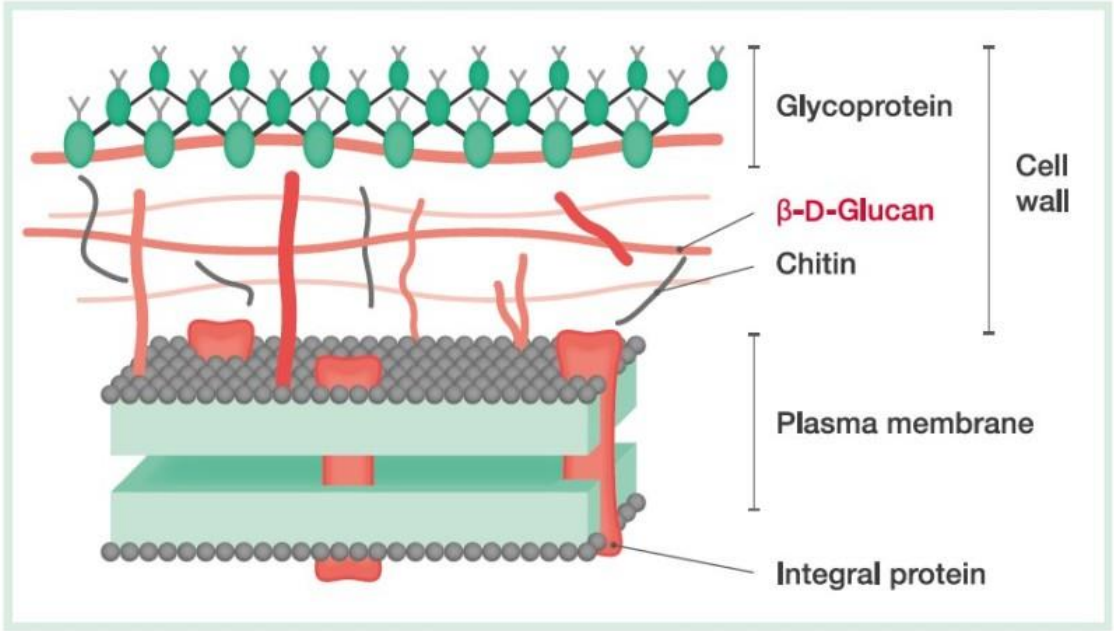
(1,3)-β-D-Glucan

(1, 3): Type of *O-glycosidic* bond (a covalent bond that joins a carbohydrate molecule to another group) i.e., Cellulose is a (1, 4)-β -glucan.

Beta (β): two types (isomers) of dextrose-(D) glucose: alpha (α) and beta (β) glucans

- *Alpha-glucans:* building block of starch, also used to form lactose, maltose, and galactose.
- *Beta-glucans:* building block of cellulose, also used to form lactose, maltose, and galactose.

D-Glucan: dextrose-(D) glucose (isomer of glucose)



Cross-section of fungal cell wall

Fungitell STAT Assay: Overview vs Plate Assay

MTW Microbiology is currently in the process of verifying a scaled-down version of the plate-based assay currently used by UKSHA Regional Mycology reference laboratory in Bristol.

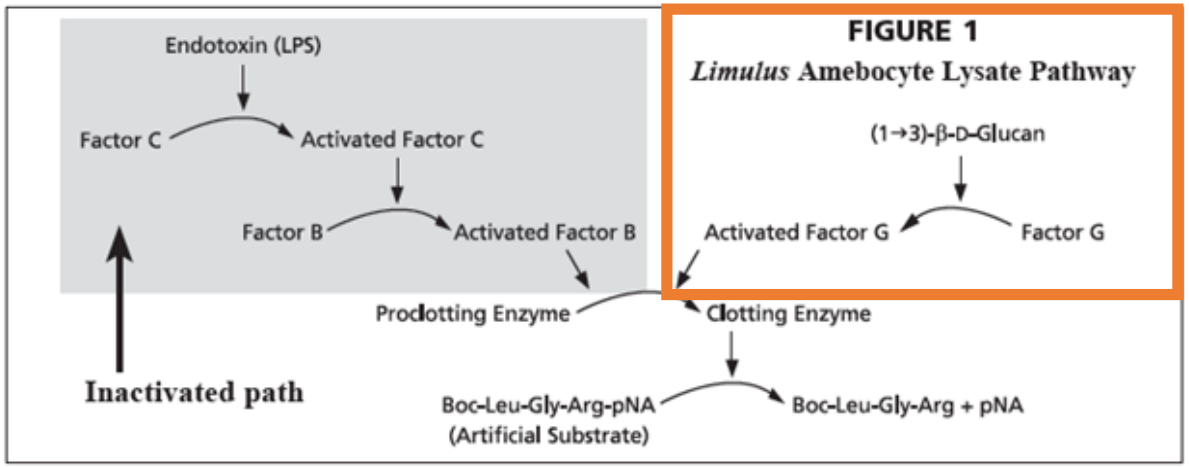
	Fungitell® Assay (Bristol)	Fungitell STAT® Assay (MTW)
Test Type	Plate-based	Tube-based
Max. tests per run	96	6 (inc. IQC)
Process Footprint	Large	Small (17x11cm)
Time for Test	Around 60 mins	Around 60 mins
Overhead Times	1 day for delivery for DX 1-2 days for testing & releasing	Same-day (60 mins)

Fungitell STAT Assay: Test Principle

The assay is a chromogenic, quantitative EIA, based on the clotting cascade of the **Limulus Amoebocyte Lysate** (LAL) pathway of *Limulus polyphemus* (the Atlantic horseshoe crab)

Horseshoe crabs are special, their blood contains important immune cells called amoebocytes that are exceptionally sensitive to both bacterial endotoxin lipopolysaccharide (membrane component of some gram-negative bacteria: such as *Neisseria* and *Haemophilus*). When encountered, this sets off a clotting cascade, which clots around the bacteria to encase them. Since the 1970's this pathway has been used by pharmaceutical companies to check batches of vaccines for bacterial contamination.

The LAL cascade is also triggered against Beta-D-Glucan via a different factor (G), the assay modifies the pathway so that only activated factor G will convert the inactive pro-clotting enzyme to the active clotting enzyme. This in turn cleaves a substrate, leaving a product (pNA) which absorbs light at a certain wavelength.



NATURAL HISTORY MUSEUM

"Horseshoe crab blood: the miracle vaccine ingredient that's saved millions of lives"

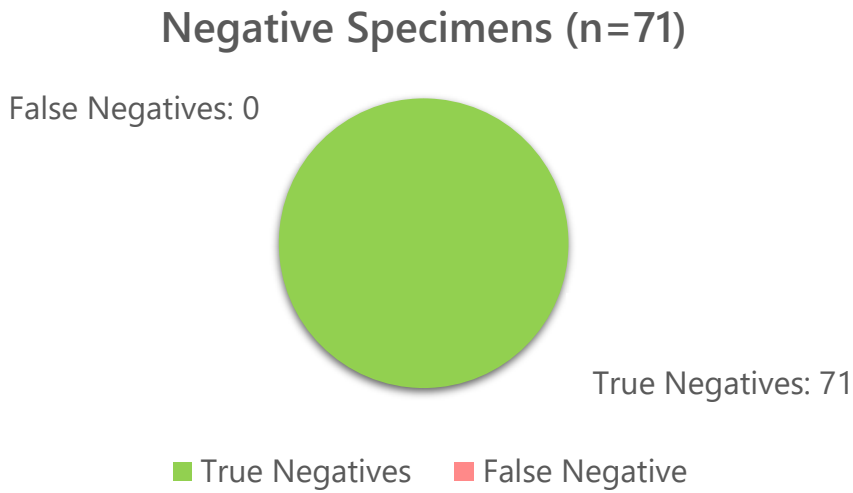
Fungitell STAT Assay: Verification Results

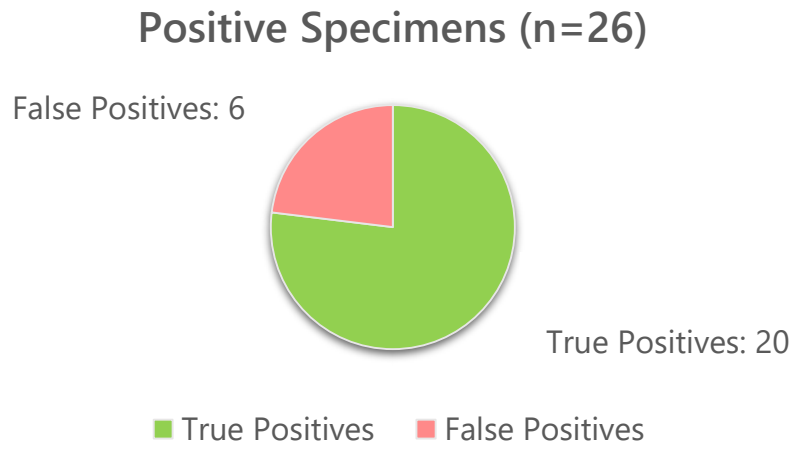
Testing of the Fungitell STAT assay against the Fungitell Assay (FA)		
	Mycology Regional Reference Unit (Public Health Wales)*[1]	**Microbiology, Maidstone & Tunbridge Wells NHS Trust**
Location of FA comparison (plate assay)	In-house	UKHSA Mycology Reference Laboratory, Bristol
Number of Specimens	107	97
STAT Test Sensitivity	67.9%	100%**
STAT Test Specificity	93.9%	92.2%
Positive Percent Agreement	99%	76.9%***
Negative Percent Agreement	98%	100%

*Case-control study of 107 serum samples from critical-care COVID-19 patients with proven/probable IFD defined according to international guidelines (107 samples)[1]

** High clinical confidence that no true cases of invasive fungal infection have been missed – Every positive that Bristol reported was matched by the STAT assay.

***Low PPA is likely due to false positives created due to environmental contamination (at MTW) – equipment was moved into safety cabinet mid-trial.





[1] (2021) *Evaluation of the Performance of the Associates of Cape Cod STAT Assay for the Diagnosis of Invasive Fungal Disease in Critical-Care Patients with COVID-19.* ASM. Available at: https://journals.asm.org/doi/10.1128/JCM.00869-21 (Accessed: March 21, 2023).

Aspergillus PCR: Fungiplex® Aspergillus IVD PCR (Bruker)

- A real-time PCR assay to detect the main species associated with Invasive Aspergillus (IA) including:
- *A. fumigatus, A. flavus, A. niger,* and differentiates *A. terreus*
- The assay targets the 28S ribosomal RNA (rRNA)

Benefits:

- The assay provides results in under 2 hours from extracted DNA, helping the laboratory support clinical decision-making when time matters.
- This reduction in the time to diagnosis, reduce the use of empiric therapy and ultimately, reduce the cost of care.

Aspergillus PCR: Fungiplex® Aspergillus IVD PCR (Bruker)

- The assay is validated for whole blood, serum, plasma and bronchoalveolar lavage (BAL) fluid
- Compatible with wide range of extraction and amplification
- Verification was carried out on GenoXtract® (Extraction) and the Hain FluoroCycler® XT

Targets (differentiated)	Performance characteristics[a]	Thermocyclers (validated)	Time to result
Aspergillus spp.[b] *A. terreus*	Sensitivity = 99.3 % Specificity = 98.5 %	√ ABI 7500 √ ABI Quantstudio 5 √ Bio-Rad CFX √ Hain FluoroCycler XT √ Mic qPCR Cycler √ Roche Lightcycler 480 II √ Qiagen RotorGene	< 2 h from extraction (1 - 100 samples)

Aspergillus PCR: Fungiplex® Aspergillus IVD PCR (Bruker)

Verification data analysis:

- Combination of both clinical and EQA samples were used
- PCR confirmation sent to Bristol Mycology for comparison (True positive, True negative)
- Re-verification at 6 months with additional data including EQA samples

	Serum	Bronchial alveolar lavage (BAL)
Sensitivity	77.8%	100%
Specificity	91.7%	90.5%

Aspergillus PCR: Fungiplex® Aspergillus IVD PCR (Bruker)

Future improvements:

The use of plasma samples over serum samples?

- The use of plasma as a specimen avoids the potential loss of free DNA due to trapping during clot formation and may improve clinical performance
- The analytical evaluation showed that the amount of cell-free DNA is greater in plasma than that in serum, which is likely the result of DNA becoming trapped during clot formation (White et al., 2015) https://doi.org/10.1128/JCM.00905-15

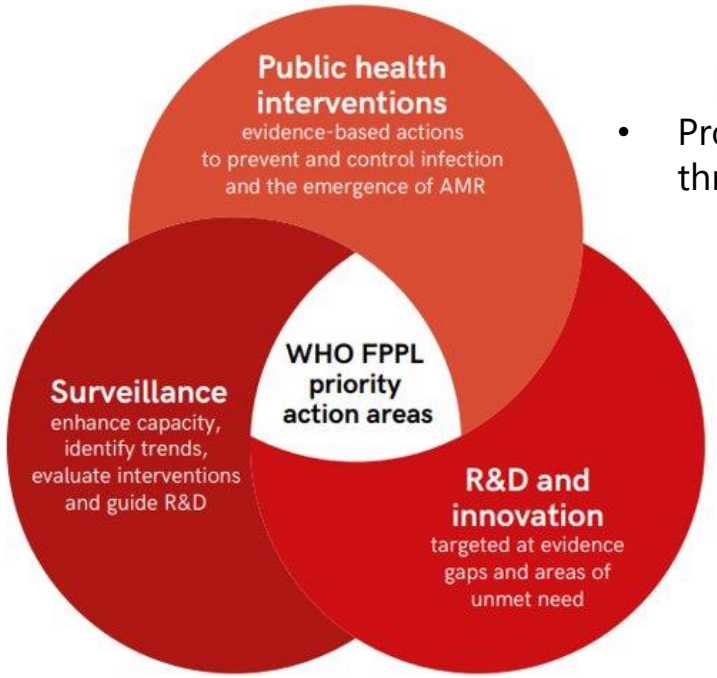
Fungiplex® *Aspergillus* Azole-R IVD PCR

- Assay is a real-time PCR designed to detect the presence of these TR 34 and TR46 mutations in the Cyp51 gene of *A. fumigatus*.
- Resistance to azoles in *Aspergillus fumigatus* is emerging as a global health problem
- Limited treatment options for azole-resistant invasive aspergillosis results in poor patient outcomes, with mortality reported as high as 88%

IFD: WHO FPPL priority action areas

Surveillance

- Build mycology diagnostic capacity to manage fungal infections and to perform surveillance
- Integrate fungal diagnostics into routine care or specialised laboratories
- Limit the inappropriate use of antifungals as well as antibiotics



Public Health Interventions

- Promote rational use of antifungal agents through antifungal stewardship intervention

R&D and Innovation

- Support research into the development of novel, accurate rapid diagnostics for priority pathogens
- Promote research to improve efficacy, efficiency and quality of fungal identification and susceptibility testing

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