Cryptococcus neoformans/C. gattii Complex

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The epidemiology of the *Cryptococcus* species complex (SC) is well known and briefly summarized below. Among these species, most clinical isolates are *C. neoformans*; *C. gattii* has been isolated in the U.S., mostly in the Pacific Northwest area.

detection resistance | cryptococcal isolates | ECVs | mutant detection | Cryptococcus isolates

1. Introduction

Background and Epidemiology

The incidence of cryptococcal infections is difficult to calculate. Most infections occur among HIV/AIDS patients (0.4 to 1.3 cases per 100,000 population) with a mortality rate of about 12% [1]. The estimated incidence of cryptococcal meningitis occurring worldwide is 152,000/year; most of these cases are reported in sub-Saharan Africa [1][2]. www.cdc.gov/fungal/diseases/cryptococcosis-neoformans/statistics (accessed on 20 March 2023). Considering that recent research has discovered the complex genetic composition of this group, genotyping is recommended. The *C. gattii* genotype distribution is region dependent and this species is more frequently isolated from infections among AIDS patients [2][3]. By 2011, phylogenetic analysis and genotyping studies clarified the diversity among the *C. gattii*/*C. neoformans* (SC) as follows [3][4][5][6]: *C. neoformans* var. *grubii* and *C. neoformans* var. *neoformans* are two distinctive species and five species are found within the *C. gattii* SC. In a more recent report, the incidence among 233 globally collected isolates of these species was as follows: *C. neoformans*/VNI, as expected, was the most prevalent genotype followed by *C. neoformans*/VNII (34 strains, 14.6%), *C. deneoformans*/VNIV (24 strains, 10.3%), *C. bacillisporus*/VGIII (17 strains, 7.3%), *C. gattii*/VGI (6 strains, 2.6%), *C. neoformans* × *deneoformans* hybrid/VNIII (5 strains, 2.1%), and *C. deuterogattii*/VGII (1 strain, 0.4%) [7].

Some of these facts were also summarized in another study with a collection of 5686 Cryptococcal isolates from clinical, environmental, and veterinary strains as reported by the Latin American Cryptococcal Study Group [8]. As expected, *C. neoformans* VNI was the most common genotype (76%) in HIV-infected people followed by *C. gattii* VGII (12.4%) isolates mostly from otherwise healthy patients [8]. The first two molecular types are also predominant in the environment (68.6 for VNI and 20.7% for VGII). Among the smaller numbers of veterinary cases, VGII is the most prevalent molecular type (73.7%). In Latin America, due to multilocus sequence typing analysis, the *C. neoformans* population is less diverse than that of the *C. gattii*.

These species are different regarding (a) pathogenicity, (b) prevalence among patients, (c) biochemical and physiological aspects, and (d) antifungal susceptibility testing results. It is fortunate that the MALDI-TOF mass spectrometry test is able to distinguish them. In the North American clinical setting, most genotyped *C. neoformans* belong to the VNI genotype as mentioned above (>90%) [5]. While pulmonary disease incidence is higher than other infections caused by these pathogens, the central nervous system disease caused by *C. gattii* is most frequent among AIDS patients [7]. Most cryptococcal clinical isolates in the USA are *C. neoformans*.

The issue of antifungal resistance is important in the clinical setting. Both CLSI and EUCAST have developed breakpoints (BPs) and epidemiological cutoffs (ECVs/ECOFFS) for certain species/antifungal combinations as discussed below. ECVs are available for the most common *Candida* and *Aspergillus* spp. and some commercial methods, but not for the cryptococcal isolates. BPs can categorize an isolate as either susceptible or resistant, while the ECV/ECOFFs will distinguish the wild type (WT, no known resistance mechanisms) from Non-WT (NWT, harboring resistant mechanisms).

2. Antifungal Resistance Mechanisms for Treatment Agents

Antifungal resistance is common, especially among isolates infecting immunocompromised/AIDS patients and the incidence is mostly linked to prior drug exposure [9]. Triazoles and amphotericin B target the fungal cell by either direct attack and

alteration of the synthesis of the enzyme Erg 11 or ergosterol depletion, respectively [9][10][11][12]. Resistance to flucytosine is generally due to the genetic mutations that impair the uptake of the agent or interfere with the target nucleic acid synthetic pathway [9]. In addition, the cell capsule formation may alter the cell wall (including melanin production) which also leads to therapy tolerance. In other cases, the formation of resistant and large titan cells has been reported (>10 µ) and was associated with stress adaptation/alteration [12]. Three efflux pumps are regulated independently by different transcription factors in response to fluconazole exposure. Deletion of *AFR1* in H99 and R265 drastically reduced the levels of resistance to the triazoles which indicated that *AFR1* is the major drug efflux pump [9][11]. However, the fluconazole susceptibility was not affected when *AFR2* or *MDR1* was deleted in both strains [11]. On the other hand, the target of the echinocandins is the glucan synthase Fks1, an important enzyme during cell wall synthesis. As mentioned above, the *Cryptococcus* spp. are inherently resistant to the echinocandins as cell changes lead to rapid or transient adaptation and resistance to these agents, including the new agent rezafungin [13]. Three publications included data from three new agents (rezafungin, manogepix, and VT-1598) versus *C. neoformans* [13][14]. As expected, MICs for the latter species were high for both the established echinocandins and rezafungin (2–≥8 µg/mL) and low for manogepix (0.03–2 µg/mL). The geometric means of VT-1598 were lower (0.016 and 0.039) than those for fluconazole versus *C. neoformans* (1.89) and *C. gattii* (2.71) [13].

3. Antifungal Susceptibility Methods for Testing Cryptoccal Isolates

Various antifungal susceptibility methods (reference and commercial) have been established for the detection of antifungal resistance which plays an important role in the clinical setting. The in vitro data could help to select the best treatment for a patient's fungal infection and could identify the local or global antifungal resistance epidemiology. These methods, developed for the antifungal evaluation of yeasts species including the Cryptococcal isolates, are well known as summarized below.

4. Reference Methods for *C. neoformans* SC and *C. gattii* SC

The CLSI published its broth dilution method for yeasts in 1997, the M27A document [15][16]. Since then, this methodology has been revised to determine minimal inhibitory concentrations (MICs), including those for the cryptococcal isolates, and minimal effective concentrations (MECs) for the echinocandins vs. the molds [15]. The EUCAST also developed a broth microdilution method for testing the susceptibilities of yeasts and molds, as well as the cryptococcal species [15][17]. The differences between both reference methods are briefly summarized below as detailed by both groups.

5. Standard Testing Conditions for *Cryptococcus* Isolates

The CLSI and EUCAST recommendations for *Cryptococcus* isolates differ as follows: (round vs. flat microdilution trays; RPMI broth with 0.2% vs. 2% glucose, an inoculum size of 0.5–2.4 × 10^3 vs. 0.5–2.4 × 10^5, MICs determined after 72 h vs. 48 h, visual and spectrometric reading, amphotericin B endpoint: 100% vs. 90% [16][17] (EUCAST E. Def 7.3.2 2022). Despite these differences, the results obtained by both methods are supposed to be comparable. However, the problem is those differences could be important, because classification endpoints (BPs or ECVs/ECOFFs) are species and method dependent.

6. Yeast Nitrogen Broth

In addition to the reference RPMI broth, the yeast nitrogen base (YNB) broth, supplemented with 0.5% glucose and buffered to pH 7, was introduced to enhance the growth of *C. neoformans* and improve the MIC clinical relevance [18]. The MIC is determined by the spectrophotometer and defined as the lowest drug concentration that reduces 50% of the growth in the control well (drug-free). The inter-laboratory agreement of MICs by this method was excellent among three sites (83 and 96% agreement within 1 and 2 log dilutions, respectively) [19]. In a third study, 149 isolates of *C. neoformans* var. *neoformans* from Ugandan AIDS patients were tested using the RPMI and the YNB broths [20]. An overall agreement of 88% between the two microdilution methods was observed, but the MIC range using the YNB could be wider. The perception was that patients infected with strains with low MICs could be detected [20]. Most data are by the RPMI CLSI broth.

7. Antifungal Resistance Detectors: BPs and ECVs/ECOFFS

Breakpoints and ECVs

The best predictors of clinical outcomes are the BPs. However, the development of BPs requires animal model pharmacokinetic/pharmacodynamic (PK/PD) data, ECVs, and, most importantly, the clinical/microbiological outcome data from clinical trials [21][22]. Although some EUCAST BPs have been developed mostly based on PK/PD data and MIC distributions, to the best of the knowledge CLSI BPs are not available for these species [21]. On the other hand, the ECV is a newer interpretive endpoint that identifies the NWT (mutants) strains [23]. The ECV development only requires in vitro data according to the guidelines in the M57 document as follows [23]: (1) defined by the iterative statistical method and (2) the modes of the distributions entering the pool must be at least one to two dilutions from the global/overall mode. This step ensures inter-laboratory agreement of MIC values by the same method [23][24][25][26]. Another requirement is that the BP or ECV should be based on the same methodology or the concept of a method-specific categorical endpoint [21][22][23][24][25]. It is interesting that Appendix B of the CLSI M59 document lists some yeasts as intrinsically resistant to the echinocandins, as follows: *C. krusei*, *C. lusitaniae*, and *Cryptococcus* spp. [24]. Furthermore, the wild-type MIC distributions, ECVs, and resistance mechanisms are needed for the establishment of BPs in addition to the correlation of in vitro vs. in vivo results from clinical trials [21]. It is not the ECV's role to categorize a fungal isolate as susceptible or resistant as BPs do. The terms WT and NWT are not the same as "susceptible" and "resistant".

References

1. Rajasingham, R.; Govender, N.P.; Jordan, A.; Loyse, A.; Shroufi, A.; Denning, D.W.; Meya, D.B.; Chiller, T.M.; Boulware, D.R. The global burden of HIV-associated cryptococcal infection in adults in 2020: A modelling analysis. Lancet Infect. Dis. 2022, 22, 1748–1755.
2. Harris, J.R.; Lockhart, S.R.; Debess, E.; Marsden-Haug, N.; Goldoft, M.; Wohrle, R.; Lee, S.; Smelser, C.; Park, B.; Chiller, T. Cryptococcus gattii in the United States: Clinical aspects of infection with an emerging pathogen. Clin. Infect. Dis. 2011, 53, 1188–1195.
3. Kidd, S.E.; Hagen, F.; Tscharke, R.L.; Huynh, M.; Bartlett, K.H.; Fyfe, M.; Macdougall, L.; Boekhout, T.; Kwon-Chung, K.J.; Meyer, W. A rare genotype of Cryptococcus gattii caused the cryptococcosis outbreak on Vancouver Island (British Columbia, Canada). Proc. Natl. Acad. Sci. USA 2004, 101, 17258–17263.
4. Espinel-Ingroff, A.; Kidd, S.E. Current trends in the prevalence of Cryptococcus gattii in the United States and Canada. Infect. Drug Resist. 2015, 8, 89–97.
5. Hagen, F.; Khayhan, F.; Theelen, B.; Anna Kolecka, A.; Polacheck, I.; Sionov, E.; Rama Falk, R.; Sittiporn Parnmen, S.; Lumbsch, H.T.; Boekhout, T. Recognition of seven species in the Cryptococcus gattii/Cryptococcus neoformans species complex. Fungal Genet. Biol. 2015, 78, 16–48.
6. Hagen, F.; Lumbsch, H.T.; Arsenijevic, V.A.; Badali, H.; Bertout, S.; Billmyre, R.B.; Bragulat, M.R.; Cabañes, F.J.; Carbia, M.; Chakrabarti, A.; et al. Importance of resolving fungal nomenclature: The case of multiple pathogenic species in the Cryptococcus genus. mSphere 2017, 2, e00238-17.
7. Pharkjaksu, S.; Chongtrakool, P.; Chayakulkeeree, M.; Mitrpant, C.; Angkasekwinai, P.; Bennett, J.E.; Kwon-Chung, K.J.; Ngamskulrungroj, P. Cryptococcus neoformans/gattii Species Complexes from Pre-HIV Pandemic Era Contain Unusually High Rate of Non-Wild-Type Isolates for Amphotericin B. Infect. Drug Resist. 2020, 13, 673–681.
8. Firacative, C.; Meyer, W.; Castañeda, E. Cryptococcus neoformans and Cryptococcus gattii Species Complexes in Latin America: A Map of Molecular Types, Genotypic Diversity, and Antifungal Susceptibility as Reported by the Latin American Cryptococcal Study Group. J. Fungi 2021, 7, 282.
9. Rogers, T.R.; Verweij, P.E.; Castanheira, M.; Dannaoui, E.; White, P.L.; Arendrup, M.C. Molecular mechanisms of acquired antifungal drug resistance in principal fungal pathogens and EUCAST guidance for their laboratory detection and clinical implications. J. Antimicrob. Chemother. 2022, 77, 2053–2073.
10. Kim, S.J.; Kwon-Chung, J.; Milne, G.W.; Hill, W.B.; Patterson, G. Relationship between polyene resistance and sterol compositions in Cryptococcus neoformans. Antimicrob. Agents Chemother. 1975, 7, 99–106.
11. Sanguinetti, M.; Posteraro, B.; La Sorda, M.; Torelli, R.; Fiori, B.; Santangelo, R.; Delogu, G.; Fadda, G. Role of AFR1, an ABC transporter-encoding gene, in the in vivo response to fluconazole and virulence of

Cryptococcus neoformans. Infect. Immun. 2006, 74, 1352–1359.

12. Gerstein, A.C.; Fu, M.S.; Mukaremera, L.; Li, Z.; Ormerod, K.L.; Fraser, J.A.; Berman, J.; Nielsen, K. Polyploid titan cells produce haploid and aneuploid progeny to promote stress adaptation. mBio 2015, 6, e01340-15.
13. Garcia-Effron, G. Rezafungin: Mechanisms of action, susceptibility and resistance. Similarities and differences with the other four echinocandins. J. Fungi 2020, 6, 262.
14. Jacobs, S.E.; Zagaliotis, P.; Walsh, T.J. Novel antifungal agents in clinical trials. F1000Research 2022, 10, 507.
15. Alastruey-izquierdo, A.; Melhem, M.S.C.; Bonfietti, I.; Rodriguez-Tudela, J.L. Susceptibility test for fungi: Clinical and laboratorial correlations in medical mycology. Rev. Inst. Med. Trop. Sao Paulo. 2015, 57 (Suppl. S19), 57–64.
16. CLSI M27-A; Reference Method for Broth Dilution Antifungal Susceptibility Testing of Yeasts. Clinical and Laboratory Standards Institute: Wayne, PA, USA, 2017.
17. European Committee on Antimicrobial Susceptibility Testing. Overview of Antifungal ECOFFs and Clinical Breakpoints for Yeasts, Moulds and Dermatophytes Using the EUCAST E.Def 7.3, E.Def 9.3 and E.Def 11.0 Procedures. Version 3.0, Valid from 18 January 2022. 2022. Available online: https://www.eucast.org/fileadmin/src/media/PDFs/EUCAST_files/AFST/Clinical_breakpoints/AFST_EUCAST_BP_ECOFF_v3.0_ (accessed on 20 March 2023).
18. Ghannoum, M.A.; Ibrahim, A.S.; Fu, Y.; Sus Shafiq, M.C.; Edwards, J.E.; Criddle, R.S. Susceptibility testing of Cryptococcus neoformans: A microdilution technique. J. Clin. Microbiol. 1992, 30, 2881–2886.
19. Sanati, H.; Messer, S.A.; Pfaller, M.; Witt, M.; Larsen, R.; Espinel-Ingroff, A.; Ghannoum, M. Multicenter evaluation of broth microdilution method for susceptibility testing of Cryptococcus neoformans against fluconazole. J. Clin. Microbiol. 1996, 34, 1280–1282.
20. Jessup, C.J.; Pfaller, M.A.; Messer, S.A.; Zhang, J.; Tumberland, M.; Mbidde, E.K.; Ghannoum, M.A. Fluconazole Susceptibility Testing of Cryptococcus neoformans: Comparison of Two Broth Microdilution Methods and Clinical Correlates among Isolates from Ugandan AIDS Patients. J. Clin. Microbiol. 1998, 36, 2874–2876.
21. CLSI. Performance Standards for Antifungal Susceptibility Testing of Yeasts, 2nd ed.; CLSI Supplement M60; Clinical and Laboratory Standards Institute: Wayne, PA, USA, 2020.
22. European Committee on Antimicrobial Susceptibility Testing. Overview of Antifungal ECOFFs and Clinical Breakpoints for Yeasts, Moulds and Dermatophytes Using the EUCAST E.Def 7.3, E.Def 9.3 and E.Def 11.0 Procedures. Version 2.0, Valid from 24 September 2020. 2020. Available online: https://www.eucast.org/fileadmin/src/media/PDFs/EUCAST_files/AFST/Clinical_breakpoints/EUCAST_BP_ECOFF_v2.0_20-09-24.pdf (accessed on 20 March 2023).
23. CLSI M57S; Principles and Procedures for the Development of Epidemiological Cutoff Values for Antifungal Susceptibility Testing. Clinical and Laboratory Standards Institute: Wayne, PA, USA, 2022.
24. CLSI M59S; Epidemiological Cutoff Values for Antifungal Susceptibility Testing. Clinical and Laboratory Standards Institute: Wayne, PA, USA, 2022.
25. Turnidge, J.; Kahmeter, G.; Kronvall, G. Statistical characterization ofbacterial wild-type MIC value distributions and the determination of epidemiological cut-off values. Clin. Microbiol. Infect. 2006, 12, 418–425.
26. Espinel-Ingroff, A.; Turnidge, J. The role of epidemiological cutoff values (ECVs/ECOFFs) in antifungal susceptibility testing and interpretation for uncommon yeasts and moulds. Rev. Iberoam. Micol. 2016, 33, 63–75.