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Molecular epidemiology and in vitro antifungal susceptibility testing of 108 clinical Cryptococcus isolates from Denmark

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Background: Cryptococcosis is the encompassing description of disease symptoms caused by species within the basidiomycetous yeast genus *Cryptococcus*. The majority of cryptococcal infections are caused by members of the recently taxonomically revised *C. gattii*/*C. neoformans* species complexes. Here we report the molecular characterization and *in vitro* antifungal susceptibility testing of 108 clinical cryptococcal isolates from Denmark, collected during the period 1973-2013.

Material/methods: The 108 clinical Danish *Cryptococcus* spp. isolates were subjected to qPCR to determine the species and serotype, and amplified fragment length polymorphism (AFLP) fingerprinting was performed to determine the species and genotype. *In vitro* antifungal susceptibility testing was performed for the compounds amphotericin B, 5-flucytosine, fluconazole, voriconazole and isavuconazole according to the EUCAST E.Def 7.2 reference method using incubation at 30 °C for 2 days.

Results: The qPCR-based species identification was confirmed by AFLP fingerprinting, the majority of the isolates were *C. neoformans* (formerly known as var. *grubii*; serotype A; *n*=66) and could be split into genotype AFLP1 (*n*=61) and AFLP1B (*n*=5). Twenty isolates were found to be *C. deneoformans* (formerly var. *neoformans*; serotype D; genotype AFLP2), and 13 were genotyped as being *C. neoformans* × *C. deneoformans* hybrids (serotype AD; genotype AFLP3). Seven isolates were typed as *C. gattii sensu lato* by qPCR, but AFLP fingerprinting revealed that one was a *C. deneoformans* × *C. gattii* interspecies hybrid (genotype AFLP8). Two isolates were molecularly identified as *C. curvatus*. All isolates were susceptible to amphotericin B (MICs ≤ 1 mg/L) with only discrete differences among the species. *Cryptococcus neoformans* was slightly less susceptible than *C. deneoformans* (MIC_{50} 0.125 vs. 0.06 mg/L, and GM 0.149 vs. 0.096 mg/L, respectively). Similarly, flucytosine susceptibility was uniform with an MIC_{50} of 4-8 mg/L for all species with the exception of *C. curvatus* that was less susceptible (MICs >32 mg/L) (Figure). *Cryptococcus gattii sensu lato* isolates were somewhat less susceptible to the azoles than the other species. MICs for Fluconazole (>32 mg/L), voriconazole (≥ 0.5 mg/L) and isavuconazole (0.06 and 0.25 mg/L, respectively) were elevated for 1/19 *C. deneoformans* isolate and 1/2 *C. curvatus* isolate (Fig). Similarly, flucytosine MIC was elevated for 1/61 *C. neoformans* AFLP1 isolate (>32 mg/L).

Conclusions: The epidemiology of cryptococcal isolates from Denmark reflects that of other Western European countries, with the majority of clinical isolates identified as *C. neoformans* followed by *C. deneoformans*. Antifungal susceptibility testing according to the EUCAST E.Def 7.2 reference method revealed species specific differential susceptibility but suggested that acquired resistance was an infrequent phenomenon.