



Cryptococcus neoformans population diversity is not associated with clinical outcomes of HIV-associated cryptococcal meningitis patients in Zimbabwe



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Introduction

HIV and cryptococcal meningitis coinfection are a major public health problem in most developing countries. *Cryptococcus neoformans sensu stricto* is responsible for the majority of HIV-associated cryptococcosis cases in sub-Saharan Africa.

The HIV epidemic has raised the profile of *Cryptococcus neoformans sensu stricto* from a rare yeast to one of the most important fungal cause of morbidity and mortality worldwide. *Cryptococcus neoformans sensu stricto* is a major cause of HIV-associated cryptococcal meningitis (CM) globally.

Despite the available information, little is known about cryptococcal population diversity and its association with clinical outcomes in patients with HIV-associated cryptococcal meningitis in sub-Saharan Africa.

Objectives

1. To determine the prevalence of *Cryptococcus neoformans sensu stricto* molecular types AFLP1/VNI, AFLP1A/VNB/VNII and AFLP1B/VNII in the cohort ($n=55$) of Zimbabwean patients.
2. To determine the genetic diversity of *Cryptococcus neoformans* isolates and clinical outcomes of Zimbabwean patients with HIV associated cryptococcal meningitis.
3. To determine genetic diversity of *C. neoformans* from the current cohort when compared by microsatellite typing with those of isolates collected from other countries within sub-Saharan Africa.

Methodology

This was a cohort study which investigated *Cryptococcus* isolated during the laboratory diagnostic process from adult HIV-CM co-infected patients. The study was conducted at a central hospital in Harare, Zimbabwe between September 2013 and September 2014.

Patients were followed up for the duration of their hospital stay to determine their clinical outcomes. Molecular typing was done using amplified fragment length polymorphism (AFLP) genotyping and microsatellite typing.

Results

Demographics of the cohort

The majority of patients with HIV-associated *C. neoformans sensu stricto* meningitis in this cohort were males ($n=33/55$; 60.0%). The age of the patients ranged from 18–58 years with a median age of 36 years.

All but one of the patients ($n=54/55$; 98.2%) was admitted with headache and were treated empirically with 2 g ceftriaxone ($n=51/54$; 94.4%) before final diagnosis of cryptococcal meningitis was made.

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Table 1. Patient demographic characteristics

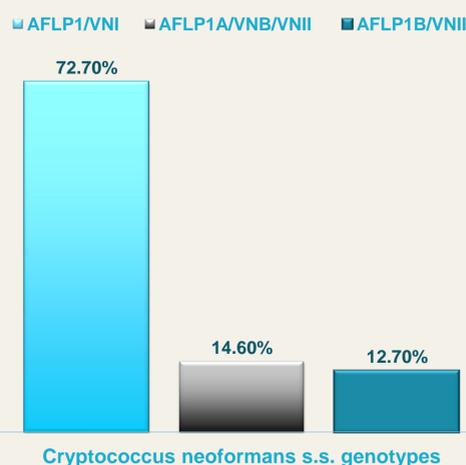
Characteristic	Patients' n= 55 (%)	Median (IQR)
Age (years)	54 (98.2)	36 (IQR 30 – 43)
Headache duration (days)	54 (98.2)	14 (IQR 7 – 21)
CD4 ⁺ cell count cells/mm ³	47 (87.5)	24 (IQR 12 – 40)
Weeks since HIV diagnosis	54 (98.2)	8 (IQR 2 – 104)
Hospital stay (days)	54 (98.2)	17.5 (IQR 10 – 22)

IQR – Interquartile range

Cryptococcus neoformans genotyping

After genotyping the majority of the isolates were mating-type α ($n=51$; 92.7%) and only 4 (7.3%) were mating-type α .

Figure 1: Prevalence of *C. neoformans sensu stricto* molecular types



The association of *C. neoformans sensu stricto* genotype and clinical outcome of patients

Overall in-hospital mortality was 55.6% ($n=30$) and no difference between infecting genotype and clinical outcome of patient ($P=0.73$) or CD4⁺ counts ($P=0.79$) was observed.

Genetic relatedness of Zimbabwean and other African *C. neoformans sensu stricto* isolates

Figure 2: Minimum spanning tree analysis representing the genotypes of 141 *C. neoformans sensu stricto* isolates from Africa based on a nine-locus microsatellite typing panel

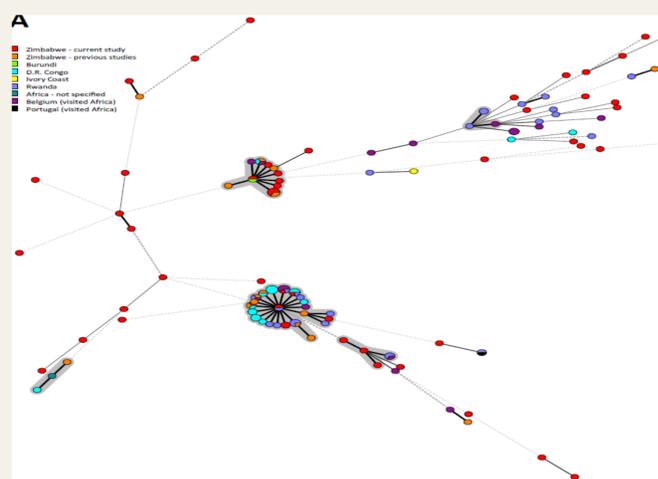


Table 2. Simpson's Diversity Index.

	$n_{ISOLATES}$	$n_{GENOTYPES}$	Simpson's D	Isolates from study
Africa	141	103	0.9930	This study
Zimbabwe (current study)	55	51	0.9953	This study
D.R. Congo	20	12	0.9211	Swinne <i>et al.</i> , 1986
Rwanda	26	22	0.9846	Bogaerts <i>et al.</i> , 1999

The Simpson's Diversity index was 0.9930 for all the African isolates ($n=141$) with 101 genotypes and the Zimbabwean *C. neoformans sensu stricto* had 51 genotypes present with a Simpson's Diversity index of 0.9953

Conclusion

- In summary, this study presents the first molecular epidemiological survey in Africa to compare the genotypic diversity of *C. neoformans sensu stricto* from clinical, environment and veterinary samples.
- Zimbabwean *C. neoformans sensu stricto* genotypes demonstrated a high level of genetic diversity by microsatellite typing and 51 genotypes within the main molecular types AFLP1/VNI, AFLP1A/VNB/VNII and AFLP1B/VNII were identified.
- This study demonstrate that *C. neoformans sensu stricto* in Zimbabwe has a high level of genetic diversity when compared to other regional isolates.

References

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