Advanced International Journal for Research (AIJFR)
E-ISSN: 3048-7641 ● Website: www.aijfr.com ● Email: editor@aijfr.com

Genomic Determinants of Antifungal Resistance Among Non-albicans Candida Isolates From HIV-Positive Patients in Southwestern Uganda: A Study Protocol

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Abstract

Background

Non-albicans Candida (NAC) species have emerged as significant opportunistic pathogens among people living with HIV (PLHIV), attributable in part to increasing antifungal resistance and evolving epidemiological trends. Although phenotypic resistance has been documented in Uganda, the genomic mechanisms underlying resistance among NAC isolates remain inadequately characterized. This study aims to investigate the genomic determinants associated with antifungal resistance among NAC isolates recovered from HIV-positive patients in Southwestern Uganda.

Methods

This protocol outlines a laboratory-based cross-sectional genomic study involving archived and prospectively collected NAC isolates obtained from HIV-positive patients attending Mbarara, Kabale, and Fort Portal Regional Referral Hospitals. Clinical isolates will undergo phenotypic identification and antifungal susceptibility testing using the Clinical and Laboratory Standards Institute (CLSI) broth microdilution method. Resistant isolates, together with a representative subset of susceptible isolates, will be subjected to whole genome sequencing (WGS). Bioinformatics analyses will be conducted to identify mutations in resistance-associated genes, including ERG11, FKS1, FKS2, CDR1, CDR2, MDR1, as well as regulatory pathways implicated in antifungal resistance. Genotype–phenotype correlations will subsequently be evaluated.

Discussion

This study will generate the first comprehensive genomic dataset describing antifungal resistance mechanisms among NAC isolates in HIV-positive populations in Uganda. The findings are anticipated to inform antifungal stewardship programmes, strengthen fungal surveillance systems, and support evidence-based management of candidiasis in resource-limited settings.

Keywords: Non-albicans Candida; HIV; whole genome sequencing; antifungal resistance; Uganda; genomic epidemiology.

1. Introduction

Candida infections remain among the most prevalent opportunistic fungal infections affecting PLHIV. While Candida albicans has traditionally been regarded as the predominant pathogen, NAC species such as Candida glabrata, Candida tropicalis, Candida parapsilosis, and Candida krusei are increasingly implicated in clinical disease. These organisms frequently exhibit reduced susceptibility to commonly used antifungal agents, particularly azoles.

Current diagnostic approaches in Uganda rely predominantly on phenotypic identification methods, which are unable to detect cryptic species and provide limited insight into the mechanisms of resistance. Molecular characterization through WGS offers high-resolution identification of genetic determinants associated with resistance and transmission.

Despite increasing reports of NAC infections in Uganda, the genomic basis of antifungal resistance in this setting remains poorly understood. This study therefore seeks to address this knowledge gap by characterizing resistance-associated mutations among NAC isolates obtained from HIV-positive patients in Southwestern Uganda.

Objectives

General Objective

To identify genomic determinants associated with antifungal resistance among clinical NAC isolates from HIV-positive patients in Southwestern Uganda.

Specific Objectives

1. To determine antifungal susceptibility profiles of NAC isolates.
2. To identify mutations associated with antifungal resistance using WGS.
3. To compare genomic characteristics between resistant and susceptible isolates.
4. To assess genotype–phenotype associations between identified mutations and antifungal susceptibility patterns.

Methods

Study Design

A laboratory-based cross-sectional genomic study will be conducted using archived and prospectively collected NAC isolates.

Study Sites

The study will be conducted at:

- Mbarara Regional Referral Hospital;
- Kabale Regional Referral Hospital; and
- Fort Portal Regional Referral Hospital.

Study Population

NAC isolates obtained from HIV-positive patients presenting with candidiasis during the study period.

Inclusion Criteria

- Laboratory-confirmed NAC isolates from HIV-positive patients.
- Archived or prospectively collected viable isolates.
- Availability of essential laboratory metadata.

Exclusion Criteria

- Candida albicans isolates.
- Mixed cultures or contaminated isolates.
- Non-viable isolates.
- Isolates lacking key laboratory information.

Antifungal Susceptibility Testing

Minimum inhibitory concentrations (MICs) will be determined using CLSI broth microdilution methods. Antifungal agents tested will include:

- Fluconazole;
- Voriconazole;
- Amphotericin B;
- Caspofungin or Micafungin.

Candida parapsilosis ATCC 22019 and Candida krusei ATCC 6258 will be used as quality control strains.

Whole Genome Sequencing

DNA extraction will be performed using validated fungal DNA extraction kits. Sequencing libraries will be prepared using Illumina DNA library preparation kits. Sequencing will be undertaken on Illumina platforms targeting a minimum genome coverage of 30×.

Bioinformatics Analysis

Raw sequencing reads will undergo:

- Quality assessment using FastQC;
- Trimming using Trimmomatic;
- Alignment to reference genomes;
- Variant calling using established pipelines;
- Functional annotation of variants.

Resistance-associated genes of interest include:

- ERG11;
- FKS1;
- FKS2;
- CDR1;
- CDR2;
- MDR1;
- PDR1.

Comparative analyses will identify shared and unique variants among resistant and susceptible isolates.

Statistical Analysis

Descriptive statistics will summarize resistance patterns and mutation frequencies.

Associations between genotypes and phenotypes will be assessed using:

- Chi-square tests;
- Fisher's exact tests;
- Logistic regression analyses.

Adjusted odds ratios with 95% confidence intervals will be reported.

Statistical significance will be set at $p < 0.05$.

Ethical Considerations

Ethical approval will be sought from the Mbarara University of Science and Technology Research Ethics Committee and the Uganda National Council for Science and Technology. Written informed consent will be obtained from participants for prospectively collected samples. Archived isolates will be analysed using de-identified data.

All procedures will adhere to Biosafety Level 2 standards.

Dissemination Plan

Findings will be disseminated through:

- Peer-reviewed publications;
- Scientific conferences;
- Stakeholder dissemination meetings;
- Reports to participating hospitals;
- Policy briefs to relevant public health authorities.

Discussion

The increasing burden of antifungal-resistant NAC infections among PLHIV necessitates a more comprehensive understanding of the underlying resistance mechanisms. By integrating phenotypic and genomic approaches, this study will provide foundational evidence to support antifungal stewardship initiatives and fungal disease surveillance efforts in Uganda.

Trial Status

At the time of manuscript submission, ethical approvals are being sought and laboratory preparations are underway.

Authors' Contributions

MN conceived the study and developed the protocol. JB and HI contributed to the study design, methodological refinement, and critical revision of the manuscript. All authors approved the final version.

Funding

Funding will be sought from institutional, national, and international agencies that support research on fungal diseases.

Conflicts of Interest

The authors declare that they have no competing interests.