Comparing variation in antifungal drug resistance in *Candida albicans* and *Candida krusei* yeast infection isolates

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Introduction

- Vulvovaginal candidiasis (VVC), or yeast infection is common in women, approximately 9% of women get 3+ infections in a year, referred to as recurrent VVC, which has no cure.1
- *Candida* species are responsible for causing VVC. *Candida albicans* is primarily responsible, but other non-albicans species are also involved.1
- Fluconazole (FLC) is a first-line treatment, but some isolates are intrinsically resistant. Recurrent infections can occur even in susceptible strains. Boric acid (BA) is an additional second-line treatment, used successfully to treat complicated VVC in Manitoba.1
- We conducted the first in-depth study on variation among vaginal and rectal yeast isolated during an active recurrent infection to examine responses to FLC and BA.

Hypothesis

Variation in antifungal resistance in VVC isolates differs based on species and body site.

Materials and Methods

Collection of Samples

Vaginal and rectal isolates were collected from two female participants with a history of recurrent VVC, one with *C. albicans* and one with *C. krusei*.

Disks Diffusion Assays

- 24 rectal and 24 vaginal isolates from each participant, were grown on solid Mueller-Hinton agar with either a 5 mg BA or 25 µg FLC disk.
- Plates were photographed at 48 h, edited with ImageJ, analyzed with DiskImageR which measured resistance and tolerance. Resistance was measured as the zone of inhibition (ZOI) at which 50% reduction in growth occurred. Tolerance was used to measure the amount of growth within the ZOI.2

Biofilm Formation

- 12 vaginal and 12 rectal isolates were grown in oxygen-restricted RPMI for 24 h to form a biofilm3
- BA or FLC was added through serial dilution and growth continued after 24 h
- XTT and menadione were then added to quantify metabolic activity
- Optical density (OD) readings of pre-drug and post-drug exposure and XTT metabolic activity were taken

Growth Curve

- 12 vaginal and 12 rectal isolates of *C. krusei* and *C. albicans* were grown in Vaginal Simulation Medium (VSM), for 48 h with OD readings taken once every hour
- Isolates were grown in low oxygen conditions3

Disk Diffusion Assays

- Only *C. albicans* had variation in resistance to BA in rectal vs. vaginal isolates.
- *C. krusei* has more variation in resistance and tolerance than *C. albicans* in disk diffusion assays
- All isolates had very low tolerance in BA in comparison to FLC.

Biofilm Formation

- Only *C. albicans* isolates were able to form biofilms in RPMI. Biomass in FLC stayed relatively consistent whereas with BA, it decreased.
- Biofilm activity stayed consistent with FLC. Activity increased with at 0.4 mg/ml and 6.4 mg/ml with BA.
- Difference in variation with rectal and vaginal isolates in FLC, possibly BA.

Conclusions

- *C. albicans* has a statistical difference between rectal and vaginal isolates in BA. Higher tolerance to FLC is seen.
- *C. krusei* has more variation in resistance and tolerance than *C. albicans* in disk diffusion assays.
- FLC has no significant inhibitory effect on *C. albicans* biofilms.
- Phenotypic difference between rectal and vaginal biofilms in FLC.
- *C. albicans* can grow better than *C. krusei* in a low-oxygen environment in VSM.

Further Directions

- This was a pilot project, more samples from females suffering from recurrent VVC will be tested.
- Whole genome sequencing to determine relatedness among isolates and compare drug resistance patterns to genotypic variation.

References


Acknowledgements

I would like to thank the U of M for the Undergraduate Research Award, for this opportunity. I acknowledge Dr. Vanessa Poliquin, and Fran Muhll, RN as clinical collaborators. Thank you to Rebeleah Kukurudz for supervising me in the lab this summer, Ola Salama for the R biofilm pipelines, and the other members of MicroStats and FunLab for feedback.