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

Devin Habon[1*], Aleeza Gerstein[1,2]
[1]Departments of Microbiology and [2]Statistics, University of Manitoba, Winnipeg, MB
*habond@myumanitoba.ca

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 innately resistant. Recurrent infections can occur even in susceptible strains. Boric acid (BA) is an 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 by 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*.

Disk Diffusion Assays

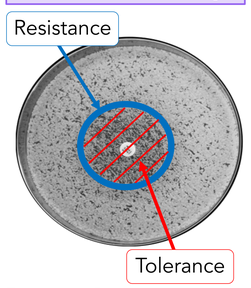

Figure 1. Resistance and tolerance on a plate.

- 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 biofilm[3]
- 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 ever hour
- Isolates were grown in low oxygen conditions[3]

Results

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.

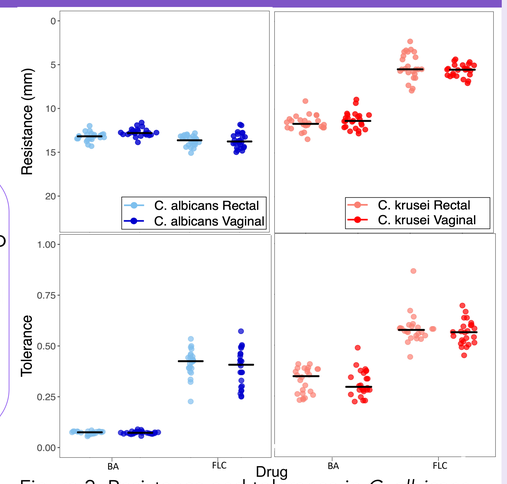

Figure 2. Resistance and tolerance in *C. albicans* and *C. krusei*. Black line is median value.

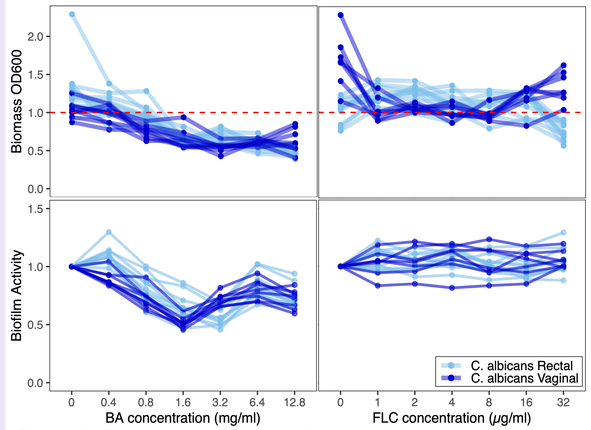

Figure 3. Biofilm formation in *C. albicans* rectal and vaginal isolates

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.

Growth Curve

- Lines represent a logistic equation fit to the curve.[3]
- After 10 h, cells reached the stationary phase.
- *C. krusei* isolates did not grow as well in VSM as the *C. albicans* isolates.
- No difference in growth for rectal or vaginal isolates, in both species.

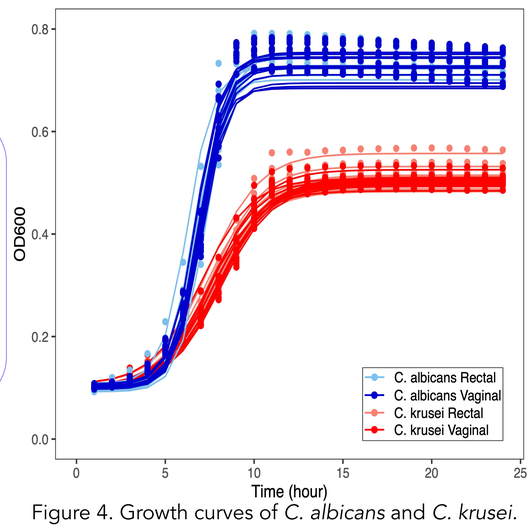

Figure 4. Growth curves of *C. albicans* and *C. krusei*.

Statistical Analysis – Disk Diffusion Assays

Table 1. Wilcoxon Signed-rank test to test rectal vs. vaginal differences in resistance.[4]

Species	Drug	V_{23}	Z	Effect Size	Test Results
C. albicans	BA	259	-3.27	0.67	0.001
C. albicans	FLC	153	-0.07	0.01	0.94
C. krusei	BA	200	-1.41	0.29	0.16
C. krusei	FLC	96	-1.52	0.31	0.13

Table 2. Wilcoxon Signed-rank test to test rectal vs. vaginal differences in tolerance.[4]

Species	Drug	V_{23}	Z	Effect Size	Test Results
C. albicans	BA	155	-0.13	0.03	0.90
C. albicans	FLC	184.5	-0.97	0.20	0.33
C. krusei	BA	161	-0.68	0.14	0.49
C. krusei	FLC	167.5	-0.88	0.18	0.38

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

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