Antimicrobial Potential of *Lactobacilli* against Planktonic and Sessile *Candida* Strains

Thesis

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by

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Summary

Vulvovaginal Candidiasis (VVC) is a female genital system infection that occurs due to *Candida* species. There are many risk factors for development of vulvovaginal candidiasis like advanced reproductive age, pregnancy, diabetes, hormonal contraception, recent antibiotic use**.** Elevated serum glucose may lead to impaired neutrophil and monocyte adherence, chemotaxis, phagocytosis, pathogen killing, and respiratory burst. Also elevated glucose level in infected tissue increase *candidia* adherence and invasion.

Several characteristics of *Candida* have been shown to be important for its pathogenicity, especially adherence to host surfaces and medical devices, Reversible morphogenetic transitions between yeast and filamentous growth, secreted hydrolytic enzymes as aspartyl proteinases, phospholipases. Our study involved 60 *Candida* strains isolated during our previous study. These strains were isolated from vagina of female with vulvovaginal candidiasis.

Our previous study reported a high prevalence of vaginal candidiasis between diabetic women. The results in our previous study improve the relevance of diabetics as a reservoir of higher phospholipase producer *C. glabrata.*

This observation highlights the need to identification of other virulence factors such as secreted aspartyl protinase and biofilm formation. *Candida* biofilms are more resistant than their planktonic counterparts to various antifungal agents so the antifungal sensitivity of *Candida* planktonic and sessile cells was examined. By phenotypic

screening of extracellular aspartyl proteinase enzymes a high significant strong proteinase activities exhibited by *Candida* isolated from vaginal samples of diabetic females were detected (82.5%). we found high significant proteinase production by *C. albicans* and *C. glabrata* in diabetic group.

By studying the frequency of *SAP1-8* genes among *Candida* isolates, non-significant differences were detected between tested groups, *SAP1* and *SAP2* were the most detected genes in both groups (100% and 100% respectively for non-diabetics versus 95% and 95% respectively for diabetics) followed by *SAP5* (75% for both groups) and *SAP3* (80% for non-diabetics versus 67% for diabetics).

Our study revealed that out of 60 strains 48 (80.0%) was biofilm producers. biofilm production was most frequently observed in *C. albicans* 27 (45%) followed by *C. glabrata* 18 (30%) and *non albicans non glabrata* 3 (5%). Biofilm production was significantly frequent among isolated *Candida* species from diabetic than non-diabetic females. Out of 40 strains among diabetic group 37 (92.5%) were biofilm producers. 11out of 20 (55%) in non diabetic group were biofilm producers. Among biofilm-positive strains, the highest biofilm production intensity was observed in diabetic group 7 (17.5%) versus 0(0%) in non diabetic group.

Well diffusion method was used to determine the inhibition activity of *L. acidophilus* against *Candida* strains. High inhibitory effect was obtained during using supernatant of *L. acidophilus*. The result revealed

that concentrations 12.5% and, 25% had no effect on *Candida* strains when clear growth was noticed, while concentration 50% minimized *Candida* growth. 100% concentration led to sharp decrease in growth of *Candida* isolates.

Secondary screening of the growth inhibitory activities of the probiotic *Lactobacilli* strains was evaluated using a plate-based microtitre assay. *L. acidophilus* had a broad antifungal inhibitory spectrum, with activity against 53 (88.3%) out of 60 *Candida* planktonic cells.
L. acidophilus supernatant had also broad antifungal eradication spectrum against *Candida* biofilm. 45 (91.8%) biofilm mass out 48 were reduced up to 50% by *L. acidophilus* supernatant. The biofilm mass of 36 (73.5%) out 48 biofilm producers were reduced up to 90%.

By studying the relation between antifungal effect of *Lactobacilli* and diabetes our study couldn't reveal significant difference in between non diabetic and diabetic group. Our study revealed that *Lactobacillus* MIC_{50} was 50% concentration. *Lactobacillus* MIC_{90} for *Candida* planktonic cells was 100% concentration. *Lactobacillus* Sessile BEC_{50} was 50% concentration while Sessile BEC_{90} was 100% concentration.

Minimal inhibitory concentration of Voriconazole, was evaluated using a plate-based microtitre assay.

Our study found significant higher resistant of *Candida* planktonic cells to Voriconazole alone than its combination with *Lactobacillus* supernatant or with Kombucha tea supernatant. There were 57 (95.0%) strains resistant to Voriconazole alone which became 41 (68.3%) by addition of *Lactobacillus* supernatant. When we added Kombucha tea

supernatant to Voriconazole we found that 56 (93.3%) strain were resistant to this combination.

By studying the association between *Candida* species and antifungal resistance among planktonic cells we couldn't find significant association between them.

We revealed no association between diabetes and *Candida* planktonic cells resistance to Voriconazole. 40 (100%) strains among diabetic group were Voriconazole resistant. These resistant strains decreased to 31 (77.5%) by addition of *Lactobacillus* supernatant. Kombucha supernatant had little effect, as 38 (90%) strain stay resistant to Voriconazole.

Voriconazole MIC_{50} and MIC_{90} for *Candida* planktonic cells were significantly decreased by combination with *Lactobacillus* supernatant.

The mean value of Voriconazole MIC was 8501.6 ± 7438.03 µg/ml which significantly decreased to 515.9 ± 898.5µg/ml by addition of *Lactobacillus* supernatant. Kombucha tea and Voriconazole combination had MIC mean value equal to 2647.2 ± 5224.7µg/ml.

By studying the association between *Candida* species and antifungal MIC among *Candida* planktonic cells we found that the mean value of Voriconazole MIC was significantly higher among *C. glabrata* than other species**.**

We found that addition of either *Lactobacillus* or Kombucha tea supernatant to Voriconazole result into significant decrease of non

eradicated sessile cells. 35 (72.9%) biofilm mass weren't reduced up to 90% by Voriconazole. Voriconazole combination with *Lactobacillus* supernatant couldn't reduce 90% of optical density of only 19 (39.5%) biofilms.

Candida sessile cells were highly resistant to Voriconazole. The mean Voriconazole BEC_{50} was 6045.08 ± 8365.2 µg/ml. Addition of *Lactobacillus* supernatant to Voriconazole lead to highly significant decrease of mean BEC_{50} to 1472.6 ± 4274.3 µg/ml. The mean Voriconazole BEC_{90} was 11718.9 ± 8357.8 µg/ml which highly significant decreased by addition of *Lactobacillus* supernatant to 4550.9 ± 6083.9µg/ml.