**Objectives**: *Candida albicans* is an opportunistic fungal pathogen and the most common cause of vulvovaginal candidiasis (VVC) in women of child-bearing age. With 70 - 75% of women experiencing at least one VVC episode in their lifetime and 8% experiencing a recurrence of infection, it along with other fungal infections produce a large economic burden on the U.S. healthcare system. With resistance to fluconazole increasing especially in women with recurrent vulvovaginal candidiasis (RVVC), it is important to study the factors associated with resistance mechanisms in *C. albicans* VVC clinical isolates. Although some fluconazole resistance mechanisms are known in *C. albicans*, there are some clinical isolates with unknown fluconazole resistance mechanisms.

**Materials & Methods:** A total of 32 *C. albicans* clinical isolates from patients with treatment refractory VVC described in (PMID: 37093005) were included in this study. Each isolate was evaluated on CHROMagar Candida Plus to confirm the *Candida* species. For each clinical isolate, the minimum inhibitory concentration (MIC) was determined using a modified CLSI broth microdilution assay. The genomes of all fluconazole resistant VVC clinical isolates were sequenced from genomic DNA, isolated with the Zymo Research Quick-DNA Fungal/Bacterial Miniprep kit, using Illumina NovaSeq with paired-end 150 bp reads and 100x coverage. The genome sequences were compared to sequences of the reference genome SC5314 and of fluconazole susceptible control isolates which include 5 VVC isolates and 5 non-VVC clinical isolates.

**Results**: MIC analysis revealed that 28 of the 32 clinical VVC isolates had an MIC value between 4 and 16 μg/mL and the remaining 4 clinical VVC isolates had an MIC value between 32 and 64 μg/mL. All control VVC and non-VVC isolates were susceptible to fluconazole with MIC values less than 0.25 μg/mL. Although only 3 of the clinical VVC isolates were considered resistant with a fluconazole MIC values equal to the CLSI breakpoint (≥ 64 μg/mL), all 32 of the clinical isolates tested had MIC values greater than 4-fold above the susceptible-control strains. While the gDNA sequencing results revealed known mechanisms of resistance including amino acid substitutions in *ERG11* (Y257H, A114S, and Y132H) and *TAC1b* (A736V, G980E, and N972D), two novel amino acid substitutions in *UPC2* (G377S) and in *MRR1* (G409E) were observed in the clinical isolates with the highest MIC values (32 or 64 μg/mL).

**Conclusions**: We identified 1 novel mutation in *UPC2* (G377S) and 1 novel mutation in *MRR1* (G409E). Interestingly, this *MRR1* mutation was observed in four unrelated isolates with the highest fluconazole MIC suggesting it may impart a selective advantage in this setting. These findings likely describe fluconazole resistance mechanisms in *Candida albicans* VVC isolates.