**Objectives**:

This study is to determine the frequency of fluconazole-resistant *Candida* species isolated from high vaginal swabs (HVS) and to emphasize the need for antifungal susceptibility testing (AST), which is not a part of a routine laboratory protocol for vaginal cultures.

**Materials & Methods:**

**Study design and setting:**

We conducted a prospective observational study at the clinical microbiological Laboratory of Aga Khan University Hospital, Karachi, Pakistan.

**Sample Size:**

The study included 187 yeast isolates from high vaginal swabs from women of reproductive age and above.

**Culture on Selective Media:**

Samples were cultured on appropriate agar plates following laboratory protocols to facilitate growth and phenotypic identification. To isolate *Candida* species, samples were inoculated on BIGGY agar and incubated at 35°C, with daily examinations for up to three days. Colony morphology was used for preliminary identification: *C. albicans* as glistening brown, *C. tropicalis* as brown with a metallic sheen, *C. krusei* as dry, flat brown, and *C. glabrata* as white colonies. Identification was confirmed using the germ tube test, chromogenic agar, Mycosel, and the Dalmau method.

**Antifungal Susceptibility Testing:**

Antifungal susceptibility was assessed using the disk diffusion method following CLSI guidelines. Zones of inhibition were measured to categorize isolates as Susceptible, Susceptible Dose-Dependent, or Resistant based on clinical breakpoints. *Candida albicans* ATCC 14053 and *Candida parapsilosis* ATCC 22019 were quality control strains. *C. krusei,* inherently resistant to fluconazole, was classified as resistant.

**Results**:

Out of 187 specimens, 138 turned out to be *C. Albicans* (73.8%), 41 *C. Glabrata* (21.93%), 5 were *C. Krusei* (2.67%), and 3 isolates were *C. tropicalis* (1.6%). Out of 138 *C. albicans* isolates, 4 were resistant to fluconazole (2.9%). Of 41 *C. Glabrata* isolates, 37 were dose-dependent (90.24%), and 4 were resistant (9.76%). *C. Krusei* is inherently resistant to fluconazole. All 3 (100%) isolates of *C. tropicalis* were sensitive to fluconazole.

**Conclusions**:

These findings highlight an alarming rise in fluconazole resistance among *Candida* species, underscoring the urgent need to integrate routine antifungal susceptibility testing (AST) into clinical practice. Such a proactive approach is pivotal for guiding targeted therapy, minimizing treatment failures, and addressing the escalating threat of antifungal resistance. Strengthening surveillance and stewardship efforts is essential to safeguard the efficacy of existing antifungal agents and improve patient outcomes globally.