INTRODUCTION

Candida albicans is among the gut flora. Up to 75% of healthy individuals carry Candida as part of their normal commensal oral microflora. However, it is an opportunistic pathogen which can cause infection in certain individuals. C. albicans is often present in small amounts in vagina, mouth, digestive tract, and on the skin. Candida and the other normal flora in the vagina keep each other in balance. However, the conditions like use of antibiotics, pregnancy, diabetes and obesity increases the number of C. albicans, leading to vaginal candidiasis.

The usual symptoms include abnormal vaginal discharge, painful urination, and inflammation of vulva, vaginal and labial itching and burning. Clotrimazole and miconazole are topically used agents while fluconazole is given orally in severe infections. Candida species are generally assumed to be susceptible to most antifungal agents. The recent increased indiscriminate use of antifungal therapy has raised the potential for the emergence of resistance of Candida to antifungal agents.

The incidence of vaginal candidiasis is fairly common in this part of country. It is usually associated with pregnancy and disorders like diabetes or obesity. Its treatment may become difficult because of resistance to availableazole antifungal agents. The present study was designed to determine the in vitro susceptibility of vaginal Candida species isolates from patients of Peshawar, Khyber Pakhtunkhawa (KPK), with the vaginal candidiasis complaints against commonly used azole antifungal agents.

MATERIAL AND METHODS

This cross sectional descriptive study was conducted from February 2013 to July 2013. One hundred samples from eligible patients were collected at Khyber Teaching Hospital, Peshawar, while their processing was jointly done at Microbiology Laboratory, Sarhad University and Pharmacology Laboratory, PMC, Peshawar. The ethical committee of PMC approved the protocol. Married and sexually active either pregnant or non-pregnant women between 18-45 years of age who presented to the tertiary health care centre with self-reported symptoms of vaginal discharge and/or genital itching and/or dysperunia during the study period were included. Women who were immunocom-
primised, diabetic, using antibiotics, corticosteroids and/or oral contraceptive pills were excluded from the study. Women who were sexually menstruating, never been sexually active or had a hysterectomy were also excluded. Written informed consent was obtained from each participant woman. Detail history proforma was completed in all subjects.

Using a sterile cotton tipped swab, high vaginal swab was taken from each participant woman by a trained gynaecologist. The swabs taken from the patients were labeled and kept at cool temperature (2-8°C), which were transported to Microbiology Laboratory, Sarhad University, within 2 hours of collection for culturing. The collected samples were streaked on Sabouraud’s Dextrose agar supplemented with 0.005% (w/v) Chloramphenicol (MM1067, HiMedia Lab. Ltd, India). After incubation period of 48 hrs at 37°C plates were observed for presence of fungal growth. Positive cultures were differentiated to species level by germ tube test9 and HiCrome candida differential agar (M1297A, HiMedia Lab. Ltd, India).

The suspensions of isolated Candida spp were prepared by suspending them in sterile saline and adjusted to match the turbidity of 0.5 McFarland12. Test antifungals included Clotrimazole, Ketoconazole and Itraconazole (Janseen, Beerse, Belgium) and Fluconazole (Pfizer, Surrey, UK). The stock solutions were diluted, such that six dilutions for each antifungal agents; Fluconazole (256-16 µg/ml), Ketoconazole (16-1µg/ml), Clotrimazole (0.24-0.015 µg/ml) and Itraconazole (4-0.25 µg/ml) were obtained. The MIC of each isolate was determined by broth micro dilution method recommended by NCCLS M27-A guide lines12. Data analysis was conducted with statistical software (SPSS).

RESULTS

Out of 100 samples 40 were positive for the presence of Candida spp. After species differentiation, C. albicans was the dominant isolate (n = 21) followed by C. glabrata (n = 13) and C. krusei (n = 6).

Susceptibility and resistance profile of candida spp. isolated from vaginal candidiasis patients against Clotrimazole, In quantitative terms C. albicans isolates showed good susceptibility for Clotrimazole with 20 isolates responding at concentration <0.015 µg/ml and just 1 isolate showing resistance (> 0.24 µg/ml). Similarly, C. krusei (n=5) also showed good susceptibility for Clotrimazole (<0.015 µg/ml) with just 1 isolate showing resistance (0.06 µg/ml). However, C. glabrata offered greater resistance with 9 out of 13 isolates not even responding at 0.24 µg/ml. Overall, 29 Candida isolates responded to this drug, while 11 showed resistance.

Ketoconazole, C. albicans isolates showed poor susceptibility for Ketoconazole with just 3 isolates responding at concentration <1 µg/ml while 2 isolates were resistant at 8 µg/ml and 16 isolates showed highest resistance (>16 µg/ml). C. glabrata (n=4) were susceptible to ketoconazole (2 µg/ml) but 9 isolates showed resistance (>16µg/ml). However, C. krusei showed good susceptibility with 1 isolate responding at <1µg/ml and 4 isolates responding at 2 µg/ml, with just 1 isolate offering resistance (4 µg/ml). Overall, 12 Candida isolates responded to this drug, while 28 showed resistance.

Fluconazole, C. albicans isolates showed poor susceptibility for fluconazole (Table 1) with 1 isolate responding at concentration <16µg/ml and 4 isolates at 32µg/ml whereas 4 isolates were resistant at 64µg/ml, with 1 isolate not even responding at 128 µg/ml and 11 isolates were resistant at 256µg/ml. A single C. glabrata isolate was susceptible to fluconazole at <16µg/ml while 3 isolates responded at 32 µg/ml while 9 isolates offered resistance as they were inhibited at 256µg/ml. Similarly, only two C. krusei isolates responded to fluconazole at 32µg/ml while 4 were resistant, out of which three isolates were inhibited at 64 µg/ml and one was controlled at 128 µg/ml. Overall, 11 isolates responded to this agent, while 29 showed resistance.

Itraconazole in quantitative terms C. albicans isolates showed moderate susceptibility for Itraconazole (Figure 4) with 6 isolates responding at concentration <0.25 µg/ml, 2 isolates each at concentrations of 0.25 µg/ml and 0.5µg/ml, respectively while 11 isolates showed resistance, as they were inhibited at 4µg/ml. However, C. glabrata (n=13) showed maximum resistance to Itraconazole even with inhibitory concentration of 4 µg/ml. C. krusei offered greater resistance with two

| Table 1: Susceptibility pattern of Candida isolates against test azole antifungal agents |

<table>
<thead>
<tr>
<th>Clotrimazole</th>
<th>Ketoconazole</th>
<th>Fluconazole</th>
<th>Itraconazole</th>
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<tbody>
<tr>
<td></td>
<td>Sensitive</td>
<td>Resistant</td>
<td>Sensitive</td>
</tr>
<tr>
<td>C. albicans</td>
<td>20</td>
<td>1</td>
<td>3</td>
</tr>
<tr>
<td>(n=21)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C. glabrata</td>
<td>4</td>
<td>9</td>
<td>4</td>
</tr>
<tr>
<td>(n=13)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C. krusei</td>
<td>5</td>
<td>1</td>
<td>5</td>
</tr>
<tr>
<td>(n=6)</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Total</td>
<td>29</td>
<td>11</td>
<td>12</td>
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responding at 1µg/ml, three at 2µg/ml and one at 4µg/ml. Overall, 10 Candida isolates responded to this drug, while 30 showed resistance (Table 1).

DISCUSSION

In this study prevalence of vaginal candidiasis in Peshawar was found to be 40%, which is higher as compared to studies done in India and Holland where prevalence rates range from 18.5-23%. Furthermore, in this study non-albicans vaginitis was 47.5% while albicans vaginitis was 52.5% which is less as compared to the report of Mohanty and others in which non-albicans vaginitis was 64.8%. This can be due to the geographical variation in prevalence of organism in a particular population. Overall percentage of non-albicans vaginitis in present study was higher than the report of university of Iowa vulvovaginitis clinic patients in which 24% of the total, were non-albicans species.

Susceptibility to clotrimazole was very good (72.5%) in vulvovaginal candidiasis patients. C. glabrata expressed resistance against it (69.2%). Finding of this study are in conformity with a Libyan study that gives 100% susceptibility of C. albicans for clotrimazole 7 but our findings of resistance in C. glabrata isolates are contrary to this same study, which reports 100% susceptibility for clotrimazole that may be attributed to development of resistance in this region.

In quantitative terms C. albicans showed high susceptibility towards clotrimazole with 20 isolates responding at <0.015µg/ml and just one isolate showing resistance. Similarly C. krusei (n=5) showed good susceptibility for clotrimazole, and only one isolate showed resistance. This finding is similar to the finding of Richter and cowokers which showed all 420 isolates of C. albicans responding at 0.12µg/ml, all 112 isolates of C. glabrata responding at MIC of 8µg/ml while all 12 isolates of C. krusee controlled at MIC of 1µg/ml. This also favors the finding of Elabib et al which reports Candida species response for clotrimazole to be in MIC range of <0.02-20µg/ml. However, in present study C. glabrata offered greatest resistance, which is contrary to the earlier findings reporting MICs of clotrimazole to be in the range of 0.15-12µg/ml.

In this study C. albicans isolates showed poor susceptibility for ketoconazole with only 3 isolates responding while 18 isolates showing resistance. Only 4 isolates of C. glabrata were susceptible to Ketoconazole but 9 isolates showed resistance. However, C. krusee showed good susceptibility with five isolates responding; while just one isolate offering resistance. Overall, 12 candida isolates (30%) responded to this drug, while 28 showed resistance. This is contrary to the finding of an Iranian study that reported 90.6% sensitivity to ketoconazole for C. albicans, 89.6% sensitivity for C. krusei, and 85% sensitivity for C. glabrata.

C. albicans isolates showed poor susceptibility for fluconazole as, just 5 out of 21 isolates responded the drug while 16 out of 21(76.1%) isolates showed resistance. In case of C. glabrata isolates 4 out of 13 (30.7%) were susceptible while 9 out of 13 (69.2%) were resistant. Similarly, only 2 C. krusee (33.3%) isolates responded while 4 were resistant. Overall, 11 isolates (27.5%) responded to this agent, while 29 showed resistance. This is contrary to earlier report in which none of the Candida isolates were resistant to Fluconazole.

In this study, for itraconazole, only 10 isolates (47.6%) of C. albicans were susceptible while all of the rest including isolates of C. glabrata and C. krusei showed resistance to it. This is in contrast to earlier finding that reported 10% of C. albicans and 88% C. glabrata to be resistant to Itraconazole.

In quantitative terms C. albicans isolates showed moderate susceptibility for itraconazole with 6 isolates responding at concentration <0.25 µg/ml, 2 isolates at 0.25 µg/ml and 0.5µg/ml, respectively while 11 isolates were resistant. However, all of C. glabrata isolates showed maximum resistance to itraconazole. Similarly C. krusei was also resistant. Overall, 10 Candida isolates responded to this drug, while 30 showed resistance. This is contrary to the report by a Brazilian public tertiary care hospital in which out of 212 isolates 43 (20.3%) were resistant to itraconazole. In their study resistance to itraconazole was found (10%) in C. albicans and (88%) in C. glabrata. However, present study parallels previous findings, which reported 33.7% resistance in C. albicans isolates and 86.5% resistance in C. krusei isolates. It is also in agreement with 85% resistance report of itraconazole in C. glabrata isolates.

CONCLUSION

C. albicans is the major organism responsible for vulvovaginal candidiasis and is susceptible to clotrimazole while it has high resistance for ketoconazole. C. glabrata is second common specie responsible for vulvovaginal candidiasis and is susceptible to clotrimazole while it has high resistance for ketoconazole. In case of C. krusei isolates 4 out of 13 (30.7%) were susceptible while 9 out of 13 (69.2%) were resistant. Similarly, only 2 C. krusee (33.3%) isolates responded while 4 were resistant. Overall, 11 isolates (27.5%) responded to this agent, while 29 showed resistance.

This finding is contrary to earlier report in which none of the Candida isolates were resistant to Fluconazole.

REFERENCES


AUTHOR’S CONTRIBUTION
Following authors have made substantial contributions to the manuscript as under:

Zafar S: Main idea.

Khurram M: Manuscript writting.

Usman R: Literature search.

Khan F: Statistics and followup.

Nasim R: Overall supervision.

Authors agree to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

CONFLICT OF INTEREST: Authors declare no conflict of interest

GRANT SUPPORT AND FINANCIAL DISCLOSURE NIL