

## ORIGINAL ARTICLE

## IN VITRO ANTIFUNGAL SENSITIVITY OF FLUCONAZOLE, CLOTRIMAZOLE AND NYSTATIN AGAINST VAGINAL CANDIDIASIS IN FEMALES OF CHILDBEARING AGE

**Fouzia Khan, Rakhshanda Baqai**

Department of Microbiology, University of Karachi, Karachi, Pakistan

**Background:** Vaginal candidiasis is the most common infection of females. A large variety of antifungal drugs are used for treatment. The objective of this study was isolation and identification of *Candida* from high vaginal swabs and in vitro antifungal activity of Clotrimazole, Fluconazole and Nystatin against *Candida*. **Methods:** Two hundred and fifty high vaginal swabs were collected from females reporting at different hospitals of Karachi. Wet mount was performed to observe the budding cells of *Candida*. Vaginal swabs were cultured on Sabouraud's dextrose agar with added antibiotics. Plates were incubated at room temperature for seven days. Chlamydozoospores of *Candida albicans* were identified on corn meal agar. Species of *Candida* were identified on Biggy agar. In vitro antifungal activity of Clotrimazole, Fluconazole and Nystatin was performed by MIC (Minimum inhibitory concentration), well diffusion method and disc diffusion method. **Results:** Out of 250 high vaginal swabs, *Candida* species were isolated in 100 (40%) of cases. Out of 100, *C. albicans* 30 (30%), *C. tropicalis* 21 (21%), *C. parapsilosis* 10 (10%), *C. parakrushi* 8 (8%), *C. glabrata* 8 (8%), *C. krusei* 3 (3%) were isolated. In vitro antifungal activity indicated Clotrimazole (MIC 16 and 8 µg/ml) effective against 68 (70%) of *Candida* SPP, Fluconazole (MIC 64 and 32 µg/ml) effective against 29 (36.2%) and Nystatin disc (100 units) was 51 (63.5%) effective. **Conclusion:** *C. albicans* was mainly isolated. Clotrimazole was more effective as compared to Fluconazole and Nystatin. Antifungal susceptibility testing should be determined before therapy to avoid treatment failures.

**Keywords:** Vaginal candidiasis, Antifungal sensitivity, Species of *Candida*

### INTRODUCTION

Vulvovaginal candidiasis is one of the most common gynaecological disorders among females in our country. Approximately 75% of women experience vulvo vaginal candidiasis during their life, and about 40% to 50% of them suffer from multiple episodes.<sup>1,2</sup> Antifungal susceptibility testing represents a means of predicting therapeutic concentrations of antifungal drugs used to treat vaginal candidiasis.<sup>3,4</sup> National Committee for Clinical Laboratory Standards (NCCLS) published its guidelines for a standardized broth macro- and microdilution assay for in vitro testing of antifungal susceptibilities.<sup>5</sup> The most commonly prescribed treatment for vaginal candidiasis has been the topical application of Clotrimazole, an imidazole antifungal agent.<sup>6</sup> Fluconazole has emerged as the primary treatment option for virtually all forms of susceptible *Candida* infections in both immune competent and immune compromised hosts.<sup>7-12</sup> Nystatin is known to be effective *in vitro* against a variety of *Candida* species.<sup>13</sup>

The objective of this study was isolation and identification of *Candida* species and in vitro antifungal activity of Clotrimazole, Fluconazole and Nystatin against *Candida* species isolated from vaginal cultures.

### MATERIAL AND METHODS

This was case study, 258 high vaginal swabs of female patients of child bearing age group with main

presenting complain of abnormal vaginal discharge were collected from different hospitals of urban areas of Karachi. Detailed clinical history (included medical history, presenting complains, past history, drug history and menstrual history) was recorded and informed consent was taken. Sterilised speculum was used to collect vaginal secretion. Amount, colour and odour of secretion were noted

Vaginal samples were inoculated on Sabouraud Dextrose Agar (SDA) with added antibiotics and incubated 48–72 hours at room temperature.

Germ Tube Test was performed by taking 2ml of serum in a test tube and mixed with 2–3 colonies of *Candida* and then incubated at 37 °C for 2 hours aerobically. After incubation small amount of sample were taken on slide and observed under microscope for germ tube formation for *C. albicans*.

Identification of *C. albicans* on Corn meal agar was done by taking few colonies from Sabouraud dextrose agar and streaked on corn meal agar then incubated aerobically for 48–72 hours at room temperature. After incubation, chlamydozoospore was observed under microscope to identify *C. albicans*.

Biggy agar was used to differentiate species of *Candida*. *Candida* were inoculated on Biggy agar and incubated for 48–72 hours at room temperature. Colonies of different species of *Candida* showed different morphological appearance.

According to National Committee for Clinical Laboratory Standards (NCCLS) antifungal susceptibility testing were done by using disc diffusion methods, MIC (minimum inhibitory concentration), well diffusion method and broth dilution method.<sup>14</sup> Three antifungal drugs Fluconazole, Clotrimazole and Nystatin were used.

RPMI-1640 medium were used for making dilutions of antifungal drugs. It was buffered with MOPS (3-[N-morpholino] propanesulfonic acid) and 2% glucose. Candida were streaked on Sabouraud dextrose agar and incubated for 48–72 hours at room temperature.

Filter paper was used to make discs, these discs immersed in different concentration of Fluconazole and Clotrimazole. After incubation the zones of susceptibility and inhibition were observed.

Commercially prepared disc of 100 units were used. Mueller hinton agar containing 2% glucose and 0.5 µg/ml methylene blue were streaked with Candida, disc were placed on it and incubated for 48–72 hours at room temperature then checked the zones for susceptibility and inhibition.

Statistical analysis was done using SPSS-14. Chi-square test was used in analysing comparison among antifungal drugs, calculated by finding the difference between each observed and theoretical frequency for each possible outcome. Friedman test was used to analyse susceptibility of Fluconazole, to detect differences in treatments across multiple test attempts. McNemar test is non-parametric method was applied to analyze efficacy of Clotrimazole.

**RESULTS**

Table-1 shows distribution of vaginal infections in female patients. Candida 100 (40%) was the most common infection. Followed by *G. vaginalis* 50 (20%), *T. vaginalis* 30 (12%), group B streptococci 20 (8%) were reported in low prevalence.

**Table-1: Distribution of vaginal infections in patients**

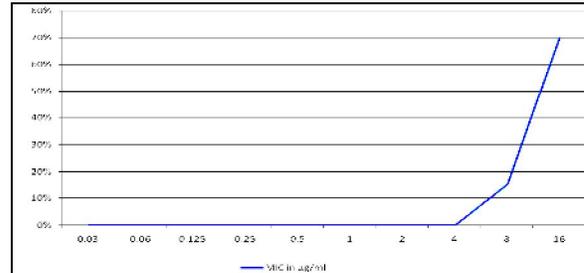
Vaginal Infections	Percentages
Candida	40%
<i>G. vaginalis</i>	20%
<i>T. vaginalis</i>	12%
Polymicrobial Infections	10%
Group B streptococci	8%

Table-2 shows Different species of Candida isolated from female patients. *C.albicans* (40%) was commonly isolated followed by *C. tropicalis* (21%), *C. parapsillosis* (10%), *C. glabrata* (8%), *C. parakrusei* (8%) and *C. krusei* (3%). The most prevalent pathogen was *C. albicans* identified from vaginal candidiasis.

**Table-2: Frequency distribution of Candida species**

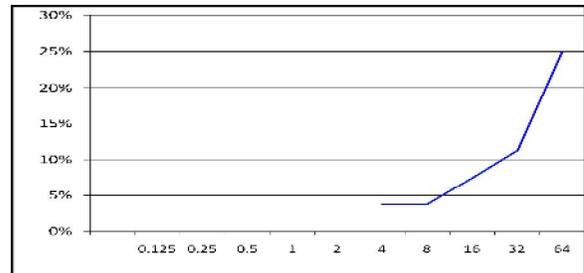
Species of Candida	Percentages
<i>C. albicans</i>	30%
<i>C. tropicalis</i>	21%
<i>C. parapsillosis</i>	10%
<i>C. parakrusei</i>	8%
<i>C. glabrata</i>	8%
<i>C. krusei</i>	3%

Figure-1: Demonstrated MIC of Clotrimazole against Candida species. MIC 8 µg/ml 15% and MIC 16 µg/ml were 70% efficient with significant P-value (<0.01). MIC less than 8 µg/ml were not efficient. This study showed Clotrimazole with MIC 16 µg/ml was known to be effective.



**Figure-1: MIC of Clotrimazole against Candida species**

Figure-2 describes MIC of Fluconazole against Candida species. MIC 4 µg/ml and 8 µg/ml were 4% effective against Candida species, MIC 16µg/ml 7.5%, MIC 32 µg/ml 11.2% and MIC 64 µg/ml 25% were found to be effective with significant P-value (<0.01). Maximum positive responses were obtained on MIC 64 µg/ml while MIC less than 4 µg/ml showed no response.



**Figure-2: MIC of Fluconazole against Candida Species**

Table-3: Showed the comparison of different antifungal drugs. Clotrimazole was found to be most effective antifungal drug showed (70%) efficacy followed by nystatin (63.5%) and fluconazole (36.2%) with significant p-value <0.01.

**Table-3: Efficiency of Antifungal drugs**

Antifungal Drugs	Sensitive	P-value
Clotrimazole	70%	<0.01
Nystatin	63.5%	<0.01
Fluconazole	36.2%	<0.01

## DISCUSSION

The most common infections in females of child bearing age is vaginitis. About 30–50% of vaginal episodes are due to *Candida species* and two thirds of all women experience at last one acute episode of vaginal candidiasis during their life time. Vaginal candidiasis occurs as a result of a shift in the vaginal flora. The mechanism whereby *Candida* induces inflammation is not yet fully established.

In the present study vaginal candidiasis was found to be most prevalent infection. *C. albicans* showed higher prevalence followed by *C. tropicalis*, *C. parapsilosis*, *C. glabrata*, *C. parakrusei* and *C. krusei*. Other study stated that clinical vaginal candidiasis was detected in 12.1% of the cases. *C. albicans* was isolated in 80.2% of patients and other species in 19.8%.<sup>15</sup> In Pakistan there is increase in vaginal candidiasis, study showed 61.2% *C. albicans* in vaginal discharge.<sup>16</sup> *C. glabrata* was the second most frequently occurring fungus.<sup>17</sup> Over the last thirty year the incidence of non-albicans species have steadily increased and *C. glabrata* being the most common species.<sup>18</sup> With the rising frequency of fungal infections, as well as increase of resistance to antifungal agents, it is imperative that clinical applicable antifungal susceptibility testing should be available.<sup>19</sup> Fluconazole is widely prescribed drug used to treat vaginal candidiasis.<sup>20</sup> Other study reported Amphotericin-B, Itraconazole, Fluconazole, Nystatin and Ketoconazole for vaginal candidiasis.<sup>21</sup> In present study antifungal susceptibility testing was performed by MIC, disc diffusion method and well- diffusion methods. Other study showed the efficacy of Fluconazole against isolates of *Candida spp.* by the E-test, disc diffusion, and broth micro dilution-based reference methods.<sup>22</sup> Isolates of *Candida species* were evaluated simultaneously by broth dilution method (NCCLS Method) and well diffusion method.<sup>23</sup> Different concentration of Fluconazole and Clotrimazole were tested against *Candida species*. Fluconazole gave maximum response 25% at MIC 64 µg/ml against *Candida species*. The efficacy of Fluconazole against vaginal isolates obtained from 556 women with complicated vaginal candidiasis were determined by evaluating two Fluconazole treatment regimens. Of 393 isolates of *Candida spp* 377 (96%) were highly susceptible to Fluconazole (MICs, <8 µg/ml) and 14 (3.6%) were resistant (MICs, ≥64 µg/ml).<sup>24</sup> Clotrimazole showed highest response (68.75%) of susceptibility at MIC 16µg/ml. Other study showed 94.3% to 98.5% efficacy of Clotrimazole against *Candida species* at MIC >1 µg/ml.<sup>25</sup> In present study Nystatin indicated second highest response of susceptibility against *Candida species* after Clotrimazole. Study conducted in India carried out antifungal susceptibility testing showed 9.5% resistant strains of *Candida* against Nystatin and 16.7% were resistant

against Fluconazole.<sup>26</sup> Resistance of vaginal *Candida* isolates to Nystatin and Clotrimazole were infrequent.<sup>27</sup>

In the present study Clotrimazole was most effective (68.75%) as compare to nystatin (62.5%) and Fluconazole (55%). Fluconazole found to be drug of choice.<sup>28</sup> Other study suggested that the treatment of vaginal candidiasis with oral Fluconazole would be effective. The rates of clinical effectiveness were 80% or 76% in the single oral Fluconazole group, and 72% or 58% in the intravaginal Clotrimazole group.<sup>29</sup>

## CONCLUSION

It is concluded that antifungal susceptibility testing should be performed before prescribing treatment. Fluconazole is widely used drug against vaginal candidiasis but showed high resistance it leads to treatment failure.

## REFERENCES

- Lanchares JL, Hernande ML. Recurrent Vaginal Candidiasis changes in etiopathogenical pattern. Int. J Gynecol and Obstet 2000;71(Suppl-1):S29–35.
- Ferrer J. Vaginal candidosis: epidemiological and etiological factors. Int J Gynecol Obstet 2000;71(Suppl-1):S21–7.
- Ghannoum MA. Susceptibility testing of fungi and correlation with clinical outcome. J Chemother 1997;9(1):19–24.
- Ghannoum MA., Rex, J N. Galgiani. Susceptibility testing of fungi: current status of correlation of in vitro data with clinical outcome. J Clin Microbiol 1996;34:489–95.
- CLSI. Reference Method for Broth Dilution Antifungal Susceptibility Testing of Yeasts; Approved Standard-Second Edition. CLSI document M27-A2 [ISBN 1-56238-469-4]. Pennsylvania, USA; CLSI; 2002.
- Pavelic Z, Skalko-Basnet N, Jalsenjak I. Characterisation and in vitro evaluation of bio adhesive liposome gels for local therapy of vaginitis. Int J Pharm 2005;301:140–8.
- Meis J, Petrou M, Bille J, Ellis D, Gibbs D. A global evaluation of the susceptibility of *Candida species* to fluconazole by disk diffusion. Ugeskr Laeger 2000;162:1907–8.
- Mian UK, Mayers M, Garg Y, Liu QF, Newcomer G, Madu C, et al. Comparison of fluconazole pharmacokinetics in serum, aqueous humor, vitreous humor, and cerebrospinal fluid following a single dose and at steady state. J Ocul Pharmacol Ther 1998;14:459–471.
- Pfäller MA., Diekema DJ, Jones RN, Sader HS, Fluit AC, Hollis RJ, et al. International surveillance of bloodstream infections due to *Candida species*: frequency of occurrence and in vitro susceptibilities to fluconazole, ravuconazole, and voriconazole of isolates collected from 1997 through 1999 in the SENTRY antimicrobial surveillance program. J Clin Microbiol 2001;39:3254–9.
- Pfäller MA., Messer SA, Hollis RJ, Jones RN, Doern D, Brandt ME, et al. Trends in species distribution and susceptibility to Fluconazole among blood stream isolates of *Candida species* in the United States. Diagn Microbiol Infect Dis 1999;33:217–22.
- Rex JH, Walsh TJ, Sobel JD, Filler SG, Pappas PG, Dismukes WE, et al. Practice guidelines for the treatment of candidiasis, Infectious Diseases Society of America. J Parenter Enteral Nutr 2000; 24:119–25.

12. Richardson K, Cooper K, Marriott MS, Tarbit MH, Troke PF, Whittle PJ. Discovery of fluconazole, a novel antifungal agent. *Rev Infect Dis* 1990;12:S267–S271.
13. Arikian S, Ostrosky-Zeichner I, Lozano-Chiu M, Paetznick V, Gordon D, Wallace T, *et al.* In Vitro Activity of Nystatin Compared with Those of Liposomal Nystatin, Amphotericin B, and Fluconazole against Clinical *Candida* Isolates. *J Clin Microbiol* 2002;40:1406–12.
14. Rex JH, Pfaller MA, Walsh TJ, Chaturvedi V, Espinel-Ingroff A, Ghannoum MA, *et al.* Antifungal Susceptibility Testing: Practical Aspects and Current Challenges. *Clin Microbiol Rev* 2001;14:643–58.
15. Grigoriou O, Baka S, Makrakis E. Prevalence of clinical vaginal candidiasis in a university hospital and possible risk factor. *Eur J Obstet Gynecol Reprod Biol* 2005;126:121–5.
16. Puri KJ, Madan A, Bajaj K. Incidence of various causes of vaginal discharge among sexually active females in age group 20–40 years. *Indian J Dermatol Venereol Leprol* 2003;69:122–5.
17. Wenjin Q, Yifu S. Epidemiological study on vaginal *Candida glabrata* isolated from pregnant women. *Scand J Infectious Dis* 2006;38:49–54.
18. Philips AJ. Treatment of non-*albicans* *Candida* vaginitis with amphotericin B vaginal suppositories. *Am J Obstet Gynecol* 2005;192:2009–13.
19. Hoffman HL, Pfaller MA. In vitro antifungal susceptibility testing. *Pharmacotherapy* 2001;21:1115–235.
20. Tavallaee M, Rad MM. Fixed drug eruption resulting from fluconazole use: a case report. *J Med Case Reports* 2009;3:7368.
21. Kikani BA, Kikani KM, Pathak SJ. Effects of chemically synthesized azole compounds on clinical isolates of vaginal candidiasis in comparison with commercially prepared antifungal drugs. *Internet J Microbiol* 2008;4(2).
22. Matar MJ, Ostrosky-Zeichner L, Paetznick VL, Rodriguez JR, Chen E, Rex JH. Correlation between E-test, disk diffusion and micro dilution method for antifungal susceptibility testing of fluconazole and voriconazole. *Antimicrob Agents Chemother* 2003;47:1647–51.
23. Magaldi S, Meta-Essayag S, Hartung de capriles C, Perez C, Colella M, Olaizola C. Well diffusion for antifungal susceptibility testing. *Int J Infect Dis* 2004;8(1):39–45.
24. Sobel JD, Zervos M, Reed BD, Hooton T, Soper D, Nyirjesy P, *et al.* Fluconazole Susceptibility of Vaginal Isolates Obtained from Women with Complicated *Candida* Vaginitis: Clinical Implications. *Antimicrob Agents Chemother* 2003;47:34–8.
25. Richter SS, Galask RP, Messer SA, Hollis RJ, Diekema DJ, Pfaller MA. Antifungal Susceptibilities of *Candida* Species Causing Vulvovaginitis and Epidemiology of Recurrent Cases. *J Clin Microbiol* 2005;43:2155–62.
26. Verghese S, Padmaja P, Asha M, Elezabeth SJ, Anith A, Kandawi KM, *et al.* Prevalence, species distribution and antifungal sensitivity of vaginal yeast in infertile women. *Indian J Pathol Microbiol* 2001;43:313–4.
27. Fan SR, Liu XP, Li JW. Clinical characteristics of vulvovaginal candidiasis and antifungal susceptibilities of *Candida* species isolates among patients in southern China from 2003 to 2006. *J Obstet Gynaecol Res* 2008;34:561–6.
28. Bahadori F, Broomand F, Diba K, Yekta Z, Namaki A. Comparison of fluconazole and clotrimazole in the treatment of acute *Candida albicans* vulvovaginitis. *J Family Reprod Health* 2008;2(4):179–83.
29. Mikamo H, Kawazoe K, Sato Y, Hayasaki Y, Tamaya T. Comparative Study on the Effectiveness of Antifungal Agents in Different Regimens against Vaginal Candidiasis. *Chemotherapy* 1998;44:364–8.

---

### Address for Correspondence:

Fouzia Khan, R-19, Zoramini Residency, Scheme 33, Sector 22, University Road Karachi, Pakistan. Cell: +92-321-28181633

Email: fouziakhan@yahoo.com