www.jmscr.igmpublication.org
Impact Factor (SJIF): 6.379
Index Copernicus Value: 79.54
ISSN (e)-2347-176x ISSN (p) 2455-0450
crossrefDOI: https://dx.doi.org/10.18535/jmscr/v6i9.175

IGM Publication
Journal Of Medical Science And Clinical Research
An Official Publication Of IGM Publication

Research Article

Fluconazole Susceptibility of Candida Species

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Abstract
Aim: *The aim of this study was to evaluate the susceptibility profile of Candida species isolated from clinically relevant specimens by micro broth dilution method to fluconazole.*
Setting of the study: *The study was conducted in Department of Microbiology, Govt. Medical College, Kozhikode, Kerala over a period of one year.*
Materials and Methods: *Antifungal susceptibility of 117 relevant Candida isolates were done by microbrothdilution methods according to CLSI guide lines*
Results: *96.6% of all Candida isolates were sensitive to fluconazole and 3.4% were found to be resistant.*
Keywords: *Candida albicans, Non albicans Candida, Fluconazole.*

Introduction

Candida causes a diverse spectrum of opportunistic infections ranging from mild superficial mucocutaneous infections to life threatening invasive candidiasis.[1]The major pathogens include *C.albicans, C.tropicalis, C.parapsilosis, C.kefyr, C.krusei, C.glabrata, C.guillermondi, C.famata, C.lusitaniae and C.lipolytica*. With widespread use of antibiotic since 1940s, infections due to Candida came to be noticed. Most important predisposing factor for Candida infection and especially to disseminated candidiasis is iatrogenic. Candida species have been recognised as the fourth common cause of nosocomial invasive infections.[2] Among Candida species *C.albicans* is the most common pathogen, but Non albicans Candida species infections are increasing.[3] Polyenes, azoles, flucytosine, echinocandins are the major groups of antifungals used for the treatment of candidiasis. But indiscriminate use may lead to emergence of azole resistant species by selective pressure. In contrast to primary resistance, strain to strain variations in antifungal susceptibility profiles and cross - resistance are always possible. So, it is of great importance to know the species of Candidaresponsible for the infection as well as its susceptibility patterns. Conventional methods like

Macrobroth dilution method, microbroth dilution or disk diffusion methods and commercially available systems like Sensitive yeast one test panel (Trek Diagnostics systems Inc, Westlake, OH), Fungitest (sanofi Dignostics), E-test, VITEK R 2 antifungal susceptibility methods are used for antifungal susceptibility testing.

This study was undertaken to know the antifungal susceptibility of clinically relevant Candida isolates to fluconazole by broth microdilution methods.

Materials and Methods

Susceptibility testing was carried out on 117 Candida isolates from a variety of sources (blood, urine, body fluids, swabs, nail clippings, skin scrapings, gastric aspirates etc) were included in this study. These consisted of *Candida albicans* (46 isolates), *Candida tropicalis* (57 isolates), *Candida parapsilosis* (12 isolates), *Candida kefyr* (1 isolate) and one unidentified species.

The culture was done on Sabouraud's dextrose agar (SDA) in accordance with the standard methods. Yeast isolates were identified on the basis of colony characteristics and further by germ tube production, morphology on corn meal agar, Hi Chrome Candida agar (Hi Media), urease test, carbohydrate fermentation and assimilation tests as per standard recommended procedures (Forbes *et al.*, 2002; Koneman *et. al.*, 1997).[4] Azoles, especially fluconazole is the most widely used antifungal agent in our institution, so the susceptibility of all Candida isolates to fluconazole was determined to formulate an empirical therapy. Antifungal susceptibility testing was performed by broth micro dilution modification method as per CLSI M 27-A3 Document, Third edition.Vol.28, No: 14.[6]

Serial dilutions of fluconazole (from 128 µg/ml to 0.25µg/ml) were prepared in RPMI 1640 broth and then dispensed into micro-dilution test panels. Inoculum was prepared by picking 5 distinct colonies from SDA culture, and the turbidity adjusted to 0.5 McFarland standards. Each well is inoculated with100µL of inoculum suspension. The trays were incubated at 35°C in ambient air for 24 to 48hrs and the plates read both at 24 and 48hrs for the presence and absence of visible growth. MICs for fluconazole were recorded as the lowest concentrations in which prominent decrease in turbidity was observed.

Results

96.6% of all Candida isolates were sensitive to fluconazole and 3.4% were found to be resistant.

Among the different species, 100% of *C.albicans*,. 96.5%.*C.tropicalis* and 91.7% of *C.paraspilosis* were sensitive to fluconazole. One unidentified Non albicans Candida species was resistant to fluconazole.

Table: 1 Percentage of clinically relevant Candida species isolated

Candida species	Number	Percentage
C. albicans	46	39.3
C. tropicalis	57	48.7
C.parapsilosis	12	10.2
C. kefyr	1	0.9
Non albicans Candida-un identified	1	0.9
Total	117	100

Table: 2 Fluconazole susceptibility patterns of Candida species:

Species	Fluconazole susceptibility		Total
	Sensitive (S)	Resistant (R)	
C. albicans	46(100%)	0	46
C. tropicalis	55(96.5%)	2(3.5%)	57
C.parapsilosis	11(91.7%)	1(8.3%)	12
C. kefyr	1(100%)	0	1
Unidentified	0	0	1

Discussion

Fluconazole is the most widely used triazole since its approval in 1990. Its availability in both oral and parenteral formulations, led to widespread use of fluconazole for both treatment and prophylaxis in candidiasis. The increased use of this drug has caused increasing rate of resistance among *Candida* spp, mainly *C. glabrata* and *C. krusei* isolates. Use of prophylaxis with azoles remains controversial in most high risk population.[7] Empirical use of fluconazole in febrile patients at

high risk for invasive candidiasis is a common therapeutic strategy, there should be concern about the extensive use of fluconazole due to a possible shift towards non albicans species as the cause of infection. A study by Garnacho Montero et al,2010 reported that prior fluconazole treatment is an independent risk factor for candidemia caused by microbiologically confirmed fluconazole resistant species.[8] Although, fluconazole is most frequently used antifungal agent in the treatment of systemic yeast infections, resistance rates have been reported for *C.albicans* (5.7–5.8 %) and *C. tropicalis* (6.2–9.8 %) by Fothergill AW. Zhang L andLockhart SR.[9,10,11] Globally, *C. glabrata* showed the higher resistant rates (7.7–11.9 %) than other *Candida* species.[12]

In the present study 96.6% of the total Candida isolates were sensitive to fluconazole and 3.4% were found to be resistant.100% of *C.albicans* and *C. kefyr* was susceptible to fluconazole. Fluconazole resistance in *C.tropicalis* was 3.5% and in *C.parapsilosis* 8.3%. In a study by Sahar Ali Mohamed and Ziab Zakey Al Ahamedey, 2013 reported the resistance rate of 22.5% in Non albicans Candida species and 5% in *C.albicans* isolates.[13] Study by Adhikari et al also showed increase susceptibility to fluconazole[14] by candida. The variation in fluconazole susceptibility of Candida species observed in different studies may be due to the difference in institutional based protocol for the usage of antifungal agents and diversity in the study population. In the present study most of the Candida isolates (96.6%) in general, and 100% isolates of *C.albicans* were susceptible to fluconazole. Species which are intrinsically resistant to fluconazole were not prevalent in our institution, so fluconazole can be used for empirical therapy in clinically susceptible cases of candidiasis.

Conclusion

Fluconazole can be used as an empirical therapy in clinically susceptible cases of candidiasis. But the indiscriminate use of fluconazole should be controlled by good clinico microbiological correlation in order to prevent the emergence of azole resistant species by selective pressure.

Competing interests

There are no competing interests.

Ethical approval and consent to participate:

The ethical approval for study was taken from Institutional Research committee, Govt. Medical College, Kozhikode, Kerala

Funding

State Board Medical Research (SBMR), Kerala

References:

1. Edward JE.Candida species :Mandell GL, Bennet JE, Dolin R, Mandell, Douglas and Bennetts, Principles and Practice of infectious diseases.&7th Ed. Churchil Livingstone;2010 p.3225-40
2. Jacquiline. M. Achkar and Bettina C. Fries, Departments of Medicine and Microbiology and Immunology,Albert Einstein College of Medicine, Bronx, New York, Clinical Microbiology Reviews, Candida infections of genitor urinary tract; American Society for Microbiolgy. Apr. 2010,Vol.23, p.253-73.
3. Mohandas M. Ballal. M. Distribution of Candida Species in Different Clinical Samples and Their Virulence, Biofilm Formaton, proteinase and Phospholipase Production. A Study on Hospitalized Patients in Southern India. J. Glob. Infect.Dis.2011;3 (1) 4-8.
4. Forbes BA, Sahm DF, Weissfeld AS, Laboratory methods in basic mycology In: Wilson L, Wurm E, editors, Bailey & Scott's Diagnostic Microbiology. 12th Ed.Mosby Elsevier,2007. p.700-13.
5. Koneman, E.W., Allen, S.D., Janda, W.M., Schreckenberger, P.C. 1997.Mycology. In: Color Atlas and Textbook of Diagnostic Microbiology, 5th edn. Lippincott Williams & Wilkins, Philadelphia,PA. p. 983-1057.

6. Reference method for Broth Dilution Antifungal susceptibility Testing of Yeast; Approved Standard - CLSI M27-A3 Document 2008,Third edition,vol. 28 No.14 Clinical and Laboratory Standards Institute, Wayne.
7. Varkadas, K Z,G. Samonis, A. Michalo-poulos, E.S. Soteriades and M.E. Falagas. 2006. Antifungal prophylaxis with azoles in high risk, surgical intensive care unit patients, Ameta analysis of randomized placebo controlled trials.Crit.Care.Med.34:1216-24.
8. Jose Garnacho-Montero, Ana Diaz martin, Emiloio Garcia carbera, Critical care and emergency department, intensive care unit, Virgen del Rocio. Univesity hospital risk factors for fluconazole resistant Candidemia: Antimicrobial Agents And Chemo-therapy,Aug. 2010,Vol.54,No.8 p.3149-54
9. Fothergill AW, Sutton DA, McCarthy DI, Wiederhold NP. Impact of new antifungal breakpoints on antifungal resistance in *Candida* species. J Clin Microbiol. 2014; 52: 994–997. doi: 10.1128/ JCM.03044-13. [PMC free article] [PubMed] [Cross Ref].
10. Zhang L, Yang HF, Liu YY, Xu XH, Ye Y, Li JB. Reduced susceptibility of *Candida albicans* clinical isolates to azoles and detection of mutations in the *ERG11* gene. Diagn Microbiol Infect Dis. 2013;77:327–329. doi: 10.1016/j.diagmicrobio.2013.08.018. [PubMed][Cross Ref].
11. Lockhart SR, Iqbal N, Cleveland AA, Farley MM, Harrison LH, Bolden CB, et al. Species identification and antifungal susceptibility testing of *Candida* blood-stream isolates from population-based surveillance studies in two US cities from 2008 to 2011. J ClinMicrobiol. 2012;50: 3435–3442. doi: 10.1128/JCM.01283-12. [PMC free article] [PubMed] [Cross Ref.
12. Pfaller MA, Diekema DJ, Gibbs DL, Newell VA, Ellis D, Tullio V, et al. Results from the ARTEMIS DISK global antifungal surveillance study, 1997 to 2007: a 10.5 year analysis of susceptibilities of *Candida* species to fluconazole and voriconazole as determined by CLSI standardized disk diffusion. J ClinMicrobiol. 2010;48:1366–1377. doi: 10.1128/JCM.02117-09. [PMC free article] [PubMed][Cross Ref.
13. Saharali Mohamed and ZiabZakey Al Ahmadey. Biofilm Formation and Anti-fungal susceptibility of Candida Isolatesfrom Various Clinical Specimens, British Microbiology Research Journal. 2013;3 (4):590-601.
14. Adhikary. R, Joshi.S. Species distribution and antifungal susceptibility of Candidemia at a Multi superspeciality centre in South India. Indian J med microbial 2011;29:309-11.