Trends of Antifungal Drug Susceptibility Profile in Candida and Aspergillus Isolates in Respiratory Samples Obtained from Immunocompetent and Immunocompromised Patients in Aligarh District in Northern India

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ABSTRACT

Introduction: Incidence of invasive fungal infections is now rising. An estimated 4.7 million HIV-1–infected persons are living in Asia. The aim of the study was to know the anti fungal susceptibility profile of Candida spp. and Aspergillus spp.in northen India region.

Material and methods: In this study we took 150 patients attending outpatient department and admitted in the wards of T.B. and Respiratory Diseases, along with those attending antiretroviral treatment clinic and ICTC (Department of Microbiology), in J. N. Medical College, AMU.

Results: Amongst Candida isolates, resistance to fluconazole was seen in 6.9% isolates of *C. albicans*. 50% of *C. dubliniensis* and 20% of *C.glabrata* were resistant to fluconazole. Also, resistance to ketoconazole was observed in 25% isolates of *C. dubliniensis*. Only 1 isolate was resistant to AMB which was of *C.glabrata* (20%) and no isolate was resistant to Caspofungin. Resistance to Amphotericin B was seen in 11.8% of *A. fumigatus*, 10% of *A. flavus* and 33.3% of *A. niger*. Resistance to Itraconazole was found in 11.8% of *A. fumigatus*, 20% of *A. flavus* and 33.3% of *A. niger*. Resistance to Ketoconazole was seen in 11.1% of *A. fumigatus*, 14.2% of *A. flavus* and 100% of *A. niger*. No resistance was seen against Caspofungin against any species of Aspergillus.

Conclusion: There is gradual increase in the antifungal resistance among higher drugs reported from other regions, is a major concern for today.

Keywords: Antifungal Drug Susceptibility, Immunocompetent and Immunocompromised.

INTRODUCTION

Respiratory and systemic mycoses are nowadays globally emerging as major problems in infectious diseases. Fungal spores are representing more than 50,000 spores per cubic meter of air during the fungal season.[1,2] Lungs are vulnerable organs for fungal infections as they are the initial portal of entry for fungi causing deep mycoses.[3] Although most of the antifungal resistance occurs in Candida species, resistance in other types of fungi, such as Aspergillus, is also an emerging problem. The full spectrum of the problem is still not known, but the global prevalence of azole resistance in Aspergillus is estimated to be approximately 3 to 6 percent.[4]

Keeping the above sensitive issues and constraints in perspective, we undertook this study. The aim of the study was to know the anti fungal susceptibility profile of Candida spp. and Aspergillus spp.in northen India region so that the prevention and management could be done appropriatly within time and further problem related to drug resistance could be avoided which is on rise due blind anti fungal therapy by clinicians.

MATERIAL AND METHODS

The present study was done on the patients attending outpatient department and admitted in the wards of T.B. and Respiratory Diseases, along with those attending antiretroviral treatment clinic and ICTC (Department of Microbiology), in J. N. Medical College, AMU during the period of January 2015 to October 2016.

Selection of cases

Study group: 150 patients were divided amongst 2 subgroups:

i Immunocompetent – patients with clinical suspicion of lung carcinoma and chronic lung diseases like interstitial lung disease, chronic obstructive pulmonary disease etc.

ii Immunocompromised patients – patients with decreased immunity i.e, with significant neutropenia <500 neutrophils/µl for more than 10 days. These include AIDS, cancer and transplant patients who are on corticosteroids, certain immunosuppressive drugs; and patients with inherited diseases that affect the immune system (e.g., congenital agammaglobulinemia, congenital IgA deficiency). Cases were recruited from the outpatient departments, wards, Intensive Care Units (ICU), Antiretroviral treatment clinic, J. N. Medical College Hospital, A.M.U., Aligarh.

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Antifungal susceptibility testing

Yeast

i. Disc diffusion method

The CLSI 2009 document M44-A2 for disc diffusion testing was followed. The antifungal drugs tested were Amphotericin B, Nystatin, Ketoconazole, Clotrimazole, Fluconazole, Itraconazole and Caspofungin.

The medium used was yeast –nitrogen base-glucose (YNBG) agar, except for susceptibility testing of azoles, when 1.5% l-asparagine (HiMedia Laboratories) was added to the YNBG agar.

Quality control strains were *Candida albicans* ATCC 24433, *C. parapsilosis* ATCC 22019, and *C. krusei* ATCC 6258 were included as the control organisms each time with every drug.

ii. Broth microdilution method

Broth micro dilution method taken in this study as per CLSI (2008) guidelines based on document no.M27-A3.[5]

The antifungal drugs tested were,

- Amphotericin B (Hi-media Laboratories)
- Fluconazole (Hi-media Laboratories)
- Ketoconazole (Hi-media Laboratories)
- Caspofungin (Sigma-Aldrich)

Quality control

ATCC 24433 *Candida albicans*, *C. parapsilosis* ATCC 22019 and *C. krusei* ATCC 6258 were included as the control organisms each time with each drug.

Molds

i. Broth microdilution method

The CLSI document M38-A2 for microtiter mold testing was followed for processing(CLSI, 2008).[5] The medium used for sensitivity testing was RPMI-1640 buffered with 0.165 mol/L MOPS (Hi-Media Laboratories).

The antifungal agents used were,

- Amphotericin B (Hi-media Laboratories)
- Itraconazole (Hi-media Laboratories)
- Caspofungin (Sigma-Aldrich)

ii. Disc diffusion methii. od

M51-A for mold disk diffusion testing was followed (CLSI, 2010).[6]

RESULTS

The MIC values of both Candida and Aspergillus isolates were calculated by Broth Micro Dilution method (BMD) and resistance was also checked by the disc diffusion method. Amongst Candida isolates, resistance to fluconazole was seen in 6.9% isolates of *C. albicans.* 50% of *C. dubliniensis* and 20% of *C.glabrata* showed resistance to fluconazole. Also, resistance to ketoconazole was seen in 25% isolates of *C. dubliniensis*. Only 1 isolate came out resistance to AMB which was of *C.glabrata* (20%) and no isolate was found to be resistant to Caspofungin.

Resistance to Amphotericin B was seen in 11.8% of *A. fumigatus*, 10% of *A. flavus* and 33.3% of *A. niger*. Resistance to Itraconazole was seen in 11.8% of *A. fumigatus*, 20% of *A. flavus* and 33.3% of *A. niger*. Resistance to Ketoconazole was observed in 11.1% of *A. fumigatus*, 14.2% of *A. flavus*

Candida spp.	No. of isolates	Clotrimazole		Flucanozole		Ketoconazole		Amphotericin B		Itraconazole		Nystatin		Caspofungin	
		S	R	S	R	S	R	S	R	S	R	S	R	S	R
C.albicans	14	12(93.1)	2(6.8)	13(92.8)	1(7.1)	13(92.8)	1(7.1)	14(100)	0	14(100)	0	14 (100)	0	14 (100)	0
C.dubliniensis	4	2(71.4)	2(50)	2(71.4)	2(50)	4(100)	0	4(100)	0	4(100)	0	4(100)	0	4(100)	0
C.glabrata	5	5(100)	0	4(80)	1(20)	5(100)	0	4(80)	1(20)	5(100)	0	5(100)	0	5(100)	0
C.parapsilosis	3	3(100)	0	3(100)	0	2(66.6)	1(33.3)	3(100)	0	3(100)	0	3(100)	0	3(100)	0
C.tropicalis	3	3(100)	0	3(100)	1(33.3)	3(100)	0	3(100)	0	3(100)	0	3(100)	0	3(100)	0
Total	29	25(86.2)	4 (13.8)	25(86.2)	3 (10.3)	27 (93.1)	2 (6.9)	28 (96.5)	0	29(100)	0	29 (100)	0	29 (100)	0

Figures in parenthesis indicate percentage

Table-1: Antifungal susceptibility patterns of the drugs obtained by Disk Diffusion method for Candida (M44-A2).

Aspergillus spp.	No. of isolates	Amphotericin B		Itraconazole		Ketoconazole		Caspofungin	
		S	R	S	R	S	R	S	R
A. fumigatus	17	15(88.2)	2(11.7)	15(88.2)	2(11.7)	16(94.1)	1(5.8)	17(100)	0
A. flavus	10	9(90.0)	1(10.0)	8(80.0)	2(20.0)	9(90.0)	1(10.0)	10(100)	0
A. niger	3	2 (66.6)	1(33.3)	2(66.6)	1(33.3)	0	3(100)	0(0)	0
Total	30	26(86.6)	4(13.3)	25(83.3)	5(16.6)	25(83.3)	5(16.6)	30(100)	0

*Figures in parenthesis indicates percentage

Table-2: Antifungal susceptibility patterns of the drugs obtained by Disk Diffusion method for Aspergillus (M51-A).

and 100% of *A. niger*. No resistance was observed against Caspofungin against any species of Aspergillus.

DISCUSSION

The MIC values of both Candida and Aspergillus isolates were calculated by Broth Micro Dilution method (BMD). An ideal method of, susceptibility testing must be easy, reproducible, accurate and cost-effective. BMD of antifungal susceptibility is time consuming and labor intensive. So, the antifungal susceptibility pattern of these isolates was also tested by disc diffusion method according to their respective CLSI documents.

The MIC values were calculated for fluconazole, ketoconazole and amphotericin B in all the candida isolates. The susceptibility pattern to seven antifungal agents, i.e., Fluconazole, Ketoconazole, Clotrimazole, Itraconazole, Amphotericin B, Nystatin and Caspofungin were also observed by disk diffusion method. Susceptibility was observed as 86.2% to fluconazole and clotrimazole, 93.1% isolates to ketoconazole, 96.5% to amphotericin B and all the isolates (100%) were susceptible to each nystatin, itraconazole and caspofungin.

Resistance was seen in 13.8% isolates to fluconazole and clotrimazole, 6.9% isolates to ketoconazole and 3.4% isolates to amphotericin B. These findings were similar with a study conducted by Xess *et al*., who reported 11.7% resistance to fluconazole and Belet N et al., as (8.5%).[22,7] In contrast to our study, Kotwal *et al.,* found a higher rate of fluconazole resistance (26%).[8]

Azole group of antifungal agents exhibited a higher rate of resistance as compared to amphotericin B which is similar to the study by Changdeo SA.[9] Azole resistance in Candida spp. is of concern because these drugs are frequently used as therapeutic alternatives to amphotericin B. Azole group of antifungal agents are preferred because they are easy to administer and are less nephrotoxic.

Resistance to fluconazole was seen in 6.9% isolates of *C. albicans*. Similar susceptibility of *C.albicans* isolates was also reported by M.W. Rizvi et al.[10] As compared to *C.albicans*, most of the NAC usually showed a reduced susceptibility to the common antifungal agents, especially *C.glabrata* which exhibits decreased fluconazole susceptibility.[11] Our study also demonstrated a slightly higher rate of fluconazole resistance among NAC (13.8%) as shown by Deorukhar S *et al*.,.[12] Among NAC, 50% of *C. dubliniensis* and 20% of *C.glabrata* showed resistance to fluconazole. Also, resistance to ketoconazole was observed in 25% isolates of *C. dubliniensis*. Maheshwari M *et al*., in a study on HIV positive patients from Delhi, showed that *C. dubliniensis* was one of the most common isolates and resistance was also significant.[13] Only 1 isolate came out resistant to AMB which was of *C.glabrata* (20%) among cancer patients, *C. glabrata* was one of the most common Candida species isolated by Hachem R *et al*., and; Slavin MA *et al*.,.[14,15] which is also the main species exhibiting multiazole, echinocandin, and multidrug resistance (resistance to at least 2 classes of antifungal drugs).[16,17] However, Roildes *et al.,* from Greece reported that all the isolates were susceptible to AMB, and 97.5% were susceptible to azoles.[18] Not a single isolate was found resistant to Caspofungin. Caspofungin was shown to be equivalent to (and less toxic than) amphotericin B in the treatment of patients with invasive candidiasis.[19] Moreover, they exhibit potent activity against fluconazole-resistant Candida spp.[20] Table:1

Among all the 30 Aspergillus isolates, MIC ranges for Amphotericin B, Itraconazole, Caspofungin were identified. Resistance to Amphotericin B was seen in 11.8% of *A. fumigatus*, 10% of *A. flavus* and 33.3% of *A. niger*. Resistance to Itraconazole was observerd in 11.8% of *A. fumigatus*, 20% of *A. flavus* and 33.3% of *A. niger*. Resistance to Ketoconazole was seen in 11.1% of *A. fumigatus*, 14.2% of *A. flavus* and 100% of *A. niger*. No resistance was observed against Caspofungin against any species of Aspergillus. Caspofungin Resistance in Aspergillus has not been reported from India still.[21] This could be because Caspofungin is still one of the least used antifungal in India.Table:2

CONCLUSION

The need of the hour is to undertake more studies on trends of antifungal susceptibility patterns especially in a country with a rising population like ours where both the rural and urban masses are potentially at risk.

REFERENCES

1. Pashley CH, Fairs A, Free RC, Wardlaw AJ. DNA analysis of outdoor air reveals a high degree of fungal diversity, temporal variability, and genera not seen by spore morphology. Fungal Biol. 2012; 116: 214–24.
2. Denning DW, Pashley C, Hartl D, Wardlaw A, Godet C, Del Giacco S, et al. Fungal allergy in asthma-state of the art and research needs. Clin Transl Allergy 2014;4:14.
3. Panda BN. Fungal infections of lungs the emerging scenario. Indian J Tuberc 2004; 51:63-69.
4. Arendrup MC. Update on antifungal resistance in Aspergillus and Candida. Clin Microbiol Infect 2014;20:42-8.
5. CLSI. Reference Method for Broth dilution Antifungal Susceptibility Testing of Yeasts; Approved standard-Third Edition. CLSI document M23-A3, 2008;28, No 14.
6. CLSI. Method for antifungal disk diffusion susceptibility testing of non-dermatophyte filamentous fungi; Approved Guideline, CLSI document M51-A. Wayne PA. Clinical and Laboratory Standards Institute; 2010.
7. Belet N, Cifti E, Aysev D, Gutriz H, Uysal Z, Tacyildiz N, et al. Invasive Candida Infections in children:the clinical characteristics and species distribution and antifungal susceptibility of Candida spp. Turk J Pediatr 2011;53:489-98.
8. Kotwal A, Biswas D, Sharma JP, Gupta A, Jindal P. An observational study on the epidemiological and mycological profile of Candidemia in ICU patients. Med Sci Monit 2011;17:CR663-CR668.
9. Changdeo SA. Species ditribution, virulence factors and antifungal susceptibility profile of Candida isolated from Oropharyngeal lesions HIV infected paients .Int J

Cur Micobl Ap Sci 2014;3:453-60.
10. Rizvi MW, Malik A, Shahid M, Singhal S. Candida albicans infections in a north Indian tertiary care hospital: antifungal resistance pattern and role of SDS-PAGE for characterization. Biology and Medicine 2011;3:176-81.
11. Eraso E, Moragues MD, Villar-Vidal M, Sahand IH, González-Gómez N, Pontón J, et al. Evaluation of the new chromogenic medium Candida ID 2 for isolation and identification of Candida albicans and other medically important Candida species. J Clin Microbiol 2006;44:3340-5.
12. Deorukhkar S, Saini S. Non albicans Candida species: its isolation pattern, species distribution, virulence factors and antifungal susceptibility profile. International Journal of Medical Science and Public Health 2013;2:533–538.
13. Maheshwari M, Kaur R, Chadha. Candida Species Prevalence Profile in HIV Seropositive Patients from a Major Tertiary Care Hospital in New Delhi, India. J Pathog 2016;2016:6204804.
14. Hachem R, Hannah H, Kontoyiannis D, Jang Y, Raad I. The changing epidemiology of invasive candidiasis: Candida glabrata and Candida krusei as the leading causes of candidemia in hematologic malignancy. Cancer 2008;112:2493-9.
15. Slavin MA, Sorrell TC, Marriott D, Thursky KA, Nguyen Q, Ellis DH. Candidemia in adult cancer patients: risks for fluconazole-resistant isolates and death. J Antimicrob Chemother. 2010;65:1042–51.
16. Alexander BD, Johnson MD, Pfeiffer CD, Jiménez-Ortigosa C, Catania J, Booker R, Increasing echinocandin resistance in Candida glabrata: clinical failure correlates with presence of FKS mutations and elevated MIC. Clin Infect Dis. 2013;56:1724–32.
17. Rodrigues CF, Silva S, Henriques M. Candida glabrata: a review of its features and resistance. Eur J Clin Microbiol Infect Dis 2014;33:673-88.
18. Roilides E, Farmaki E, Evdoridou J, Francesconi A, Kasai M, Filioti J, et al. Candida tropicalis in a neonatal intensive care unit: epidemiologic and molecular analysis of an outbreak of infection with an uncommon neonatal pathogen. J Clin Microbiol 2003; 41:735-741.
19. Mora-Duarte, Betts JR, Rotstein C, Colombo AL, Thompson-Moya L, Smietana J, et al. 2002. Comparison of caspofungin and amphotericin B for invasive candidiasis. N. Engl. J. Med. 347:2020-2029.
20. Pfaller MA, Diekema DJ, Messer SA, Boyken L, Hollis RJ, Jones RN, et al. In vitro activities of voriconazole, posaconazole, and four licensed systemic antifungal agents against Candida species infrequently isolated from blood. J Clin Microbiol. 2003;41:78–83.
21. Shivaprakash MR, Geertsen E, Chakrabarti A, Mouton JW, Meis JF. In vitro susceptibility of 188 clinical and environmental isolates of Aspergillus flavus for the new triazole is avuconazole and seven other antifungal drugs. Mycoses 2011;54:e583-9.
22. Xess I, Jain N, Hasan F, Mandal P, Banerjee U. Epidemiology of Candidemia in a Tertiary Care Centre of North India: 5-Year Study. Infection 2007;35:256-9.

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