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Regular Article

Occurrence of Drug Resistant *Candida* Species in the Sputum of TB Clinic Attendees in and Around Chidambaram, Tamilnadu, India

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ABSTRACT: Three years constant study was performed in order to screen the drug resistant Candida presence in the sputum of TB clinic attendees in and around Chidambaram, Tamilnadu, India. Totally 3100 sputum specimens were screened by using the routine mycological procedures. We could able to obtain 20.5% of Candida strains from 3100 TB clinic attendees and *Candida albicans*, *Candida tropicalis*, *Candida dubliniensis* and *Candida krusei* was identified. Overall based on our study report we could able to report 48%, 25% and 22% antifungal resistance by Candida strains towards the antifungal drugs Nystatin, Azole drugs and Amphotericin B. From this we can conclude and suggest that the occurrence of drug resistant Candida strains in the sputum/ respiratory tract of the patients with respiratory tract infections is not uncommon. Therefore the periodical survey of the drug resistant Candida strains is essential not only to for the development of new antifungal drugs and in the appropriate antifungal drug selection for the earliest treatment for the patient care, but also equally important for the prevention of these drug resistant Candida transmitted from the patients to the public.

Key words: *Candida*, Drug resistance

Introduction

Candida species are yeast like fungi that can form pseudohyphae. For most of the part Candida species are confined to human and animal reservoirs; however they are frequently recovered from the hospital environment, including on the foods, counter tops, air conditioning vents, floors, respirators. They are also normal commensals of skin and mucosal membranes of the GIT, genito urinary and respiratory tracts.[1,2,3]

Candidiasis is one of the most frequent opportunistic infection found in human beings and animals. Commonly it is seen in immunocompromised as well as immunocompetant hosts. The incidence rate of fungal infections rose significantly on past two decades[4]. While *Candida albicans* is considered as primary agent of these diseases. Other species like *Candida dubliniensis*, *Candida tropicalis*, *Candida krusei* etc has also been shown to produce severe systemic infection[5].

The respiratory tract Candidiasis is mainly seen as 1) Laryngeal Candidiasis 2) Candida tracheo bronchitis and 3) Candida pneumonia. Candida laryngitis may occur in the absence of oropharyngeal or esophageal Candidiasis, presenting as hoarseness in a patient receiving antibiotics. Sore throat and dysphagia are more readily attributable to accompanying pharyngitis or oesophagitis. On Endoscopy shallow ulcerations or grey members are seen on an erythematous larynx. Pulmonary Candidiasis is more of an autopsy than a clinical entity. Pulmonary lesions araise from hematogenous seeding, causing diffuse recticulonodular streaking which is difficult to see on chest X ray unless another pulmonary disorder is also present, such as adult respiratory distress syndrome or congestive heart failure.[5]

During the first two decades of this century, many researches have been made extensive studies on mycoses caused by yeasts. Tea tasters cough has been considered to be an early account of broncho pulmonary Candidiasis. They suggested that yeast species other than monilia albicans might be involved in Candidiasis and made the first descriptions of the species currently known as *Candida guillermondii, Candida krusei, Candida kefyr, and Candida tropicalis.*

In India, it seems to be limited data available on antifungal susceptibility of Candida species especially isolated from the sputum. The present study aim to focus on the isolation of different Candida species present in the sputum of the TB clinic attendees in order to perform the in vitro antifungal susceptibility pattern of these isolates and to screen the drug resistant Candida strains if any associated with these TB clinic attendees of various clinics in and around Chidambaram.

Even though Candida has been considered as one of the organisms source from endogenous site eg: skin, oral cavity, genito urinary tract, gastro intestinal tract, few studies has been performed. The study related to the isolation and identification of the drug resistant Candida strains from the human sputum is seems to be rare and proved their presence outside, yet the study related to the sources of infection concern to Candida, it has not proven or justify the human as the sources of Candida infection especially the spread among the human respiratory tract to human respiratory tract.

And it is well known fact the transmission of the pathogen from the human respiratory tract through the droplet nuclei or by aerosols and the spread of respiratory tract infection are coming fast when the already affected individuals serves as the source of infection either as patients or carriers.

There are sufficient scientific publications available for the reference of respiratory tract infection and the association of Candida but there are very few or occasional publications are available regarding the carriage rate of Candida in the respiratory tract of the TB clinic attendees. To best of our knowledge, there is no evidenced publication available pertaining to the antifungal susceptibility pattern of the Candida species isolated from the sputum of the TB clinic attendees and the evaluation of the drug resistance among them.

Since Candida involves in opportunistic infection with debilitated persons and also causes secondary infection even among immuno competent persons and also developing drug resistance, it is necessary to give importance to the evaluation of the drug resistant Candida strains present in the respiratory tract of the patients suffering from the respiratory tract infections.

In our present study, in order to evaluate the occurrence of drug resistant Candida present in the respiratory tract of the individuals suffering from the respiratory tract infection, we selectively chosen the TB clinic attendees since these cases contains both TB + ve and TB – ve individuals with clinically proven respiratory tract infections.

Materials and methods

Subjects included and source

Patients with signs and symptoms of chronic respiratory tract infection attended the out patient TB clinics at Rajah Muthiah medical College and Hospital, Chidambaram, Tamil Nadu, India, Mahatma Gandhi Medical College & Research Institute, Puducherry and also various private nursing homes in and around Chidambaram, India were included in this study. A total of 3100 patient's samples collected during January 2007 to December 2009.

Selection criteria

The fresh untreated cases with signs and symptoms of respiratory tract infection attended the TB clinics were included in this study. Prior to specimen collection, patients were enquired about the antibiotic treatment if they were undergoing or underwent and it was recorded.

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Specimens Collection
Three consecutive days early morning saliva and sputum was collected from 3100 TB clinic attendees in separate sterile container.3 days early morning saliva were collected in a sterile container after gargling mouth with sterile normal saline. Patients were instructed to collect sputum samples after the deep cough in order to correlate Candida infection.

Direct microscopic examination
10% KOH wet mount
Both saliva and sputum samples were screened for yeast cells with or with out pseudohyphae.

Gram's Staining
Uniform smears from saliva and the purulent, muco-purulent or blood stained portion of the sputum was prepared and subjected to Gram stain and examine for the presence of gram positive budding yeast cells with or with out pseudohyphae.

Candida isolation
The sputum and the saliva collected from the TB clinic attendees were subjected for the candida isolation by inoculating on to two sets of SDA , plates with antibiotics (Chloramphenicol 50mg/lit), one set incubated at 37°C, and the other set at 25-30°C(BOD) and observed for one week. The typical creamy, smooth, pasty colonies with yeasty odour were noted and recorded. The isolated Candida strains were furtherly identified as Candida species by the following methods ie, identifying pseudo mycelium, blastospore and chlamydospore formation on corn meal agar, germ tube production and also on growth on chrom agar (Hi-Media, Mumbai).

Antifungal susceptibility test
Antifungal susceptibility test was performed by disc diffusion method with commercially available antifungal discs- AmphotericinB 100 units, Clotrimazole 10 mcg, Fluconazole 10 mcg, Ketoconazole 10 mcg, Nystatin 100 units, Voriconazole 1 mcg, Itraconazole 30 mcg and Miconazole 50 mcg all are supplied by Hi- Media pharmaceuticals, Mumbai. Inoculum was prepared by picking five distinct colonies of Candida from fresh 24 hour old culture gown on SDA and incubated at 37º C. Colonies are suspended in 5ml sterile 0.85% normal saline and adjusted to the turbidity to yield 10^6 cells per ml (ie 0.5 McFarland standards.)

Anti mycotic sensitivity agar (Hi- Media) which had a depth of 4mm was prepared. A sterile non toxic cotton swab dipped in the standard inoculum and streaked the entire agar surface of the plate 3 times; turning the plate at 60^0 angle between each streaking. Then apply the discs using aseptic technique with a distance of at least 24mm, kept the plates at 37^0 c for 20-24 hrs. If it showed insufficient growth; read only after 48 hrs. The zone of inhibition around the discs were noted and recorded.

Results

A total of 3100 saliva and sputum samples were screened for Candida, among that, the direct microscopic examination of the sputum specimens revealed 44% and 91% yeast cells presence with 10% KOH and Gram's stain examination respectively whereas out of 3100 saliva only 09% and 15% had shown yeast cells. Since the percentage of Candida isolates from the saliva (0.6%) was statistically insignificant, for better convenience, the sputum specimens alone processed for further test proceedings. And 365 (11.7%) of the sputum were found to be AFB smear positive. On sputum culture we obtained 638 (20.5%) Candida strains. The predominant species isolated was *Candida albicans* 561 strains (88%) followed by *Candida tropicalis* 38 (6%), *Candida dubliniensis* 26 (4%) and *Candida krusei*13 (2%).

In detail totally 638 Candida strains isolated from the sputum of the TB clinic attendees from which 160 Candida strains belongs to AFB smear positive sputum and 478 Candida strains from AFB smear negative sputum

Out of 365 smear positive AFB cases, 160 of their sputum yielded Candida growth. *Candida albicans* (n=131) was the predominant species among all other Candida species followed by *Candida tropicalis* (n=14), *Candida dubliniensis* (n=11) and *Candida kruseii* (n=4).

The sputum (n=2735) of the patients with other respiratory tract infections yielded 478 (17.4%) Candida growth. On speciation the 478 Candida strains were recorded as *Candida albicans* (n= 430), followed by *Candida tropicalis* (n= 24), *Candida dubliniensis* (n= 15) and *Candida krusei* (n= 9) (Table1).

Table1: Candida species isolated from the sputum of TB clinic attendees

Candida Species	**Sputum Smear 3100**	
	AFB +ve 365	**AFB-ve 2735**
C. albicans	131 (35%)	430 (15.8%)
C. tropicalis	14 (3.8%)	24(0.9%)
C. dubliniensis	11 (3%)	15(0.5%)
C. kruseii	4 (1%)	9(0.3%)
Total	160 (43.8%)	478 (17.4%)

From our three years constant research we could able to record the antifungal resistance pattern of the Candida strains as follows, 25% antifungal resistance was expressed to Clotrimazole, Fluconazole and Ketoconazole 22% and 48% resistant to the antifungals Amphotericin B and Nystatin respectively.

In detail out of 561 *Candida albicans* strains, (Table 2 & Figure 1) 147 (26%) shown resistance to the antifungals Clotrimazole, Fluconazole, Ketoconazole and 24% and 51% resistance was recorded with the drugs Amphotericin B and Nystatin.

Among 38 numbers of *Candida tropicalis* 13% resistance was recorded with Clotrimazole and Fluconazole while 16% resistance with Ketoconazole, 5% and 13% resistance with the antifungals Amphotericin B and Nystatin.

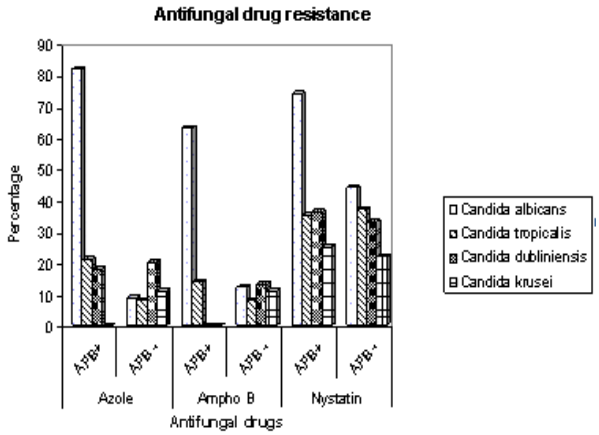
About 26 *Candida dubliniensis* strains, 19% resistance was observed towards the drugs Clotrimazole, Fluconazole and Ketoconazole 8% and 35% with Amphotericin B and Nystatin.

Out of 13 *Candida krusei* strains, 13% resistance was recorded with Nystatin and 8% resistance to rest of the antifungal drugs (Table2).

Table 2: Antifungal drug resistance pattern of Candida strains

Candida sp	Sputum samples n= 3100		Antifungal Drug Resistance					
	AFB + n=365	AFB –ve n= 2735	Azole %		Ampho B %		Nystatin %	
			AFB sputum smear					
			+ve	-ve	+ve	-ve	+ve	-ve
C.albicans	131	430	82	9	63	12	74	44
C. tropicalis	14	24	21	8	14	8	35	37
C. dubliniensis	11	15	20	20	0	13	40	33
C. krusei	4	9	0	11	0	11	33	22

Figure 1: Antifungal drug resistance



Among the 160 Candida strains isolated from the AFB smear positive sputum, 70% of them had shown resistance to the drugs Clotrimazole, Fluconazole and Ketoconazole, 53% and 66% resistance to the antifungals Amphotericin B and Nystatin. While out of 478 Candida strains isolated from the AFB smear negative sputum 45 of them shown resistance to Clotrimazole and Fluconazole, 10% to Ketoconazole, 12% and 43% to the drugs Amphotericin B and Nystatin respectively.

Out of 160 Candida strains isolated from the AFB smear positive sputum, 49(30%) strains found as multi drug resistant (MDR) strains while out of 478 Candida strains isolated from AFB smear negative sputum, 23 (4%) were recorded as MDR strains. The resistance pattern focused in Figure 3.

The MDR Candida strains were tested with the newer antifungals (Table 3). All the species found to be sensitive to the drugs Itraconazole, Micanozole and Voriconazole except *Candida albicans* to which 49% resistance was recorded with Itraconazole and Voriconazole respectively, 44% resistance to Miconazole (Table 3).

Table 3: 2nd Line Susceptibility Testing with Voriconazole, Miconazole and Itraconazole for MDR Candida

Candida Species	No: of strains		Antifungal Drug Resistance					
	AFB Smear +ve	AFB Smear -ve	Vorico		Mico		Itraco	
			AFB Smear					
			+ve	-ve	+ve	-ve	+ve	-ve
C.albicans	48	20	40	9	39	5	40	09
C. tropicalis	01	02	0	0	0	0	0	0
C. dubliniensis	00	01	0	0	0	0	0	0
C. krusei	00	00	0	0	0	0	0	0

Figure 2: Multi drug resistant candida strains isolated

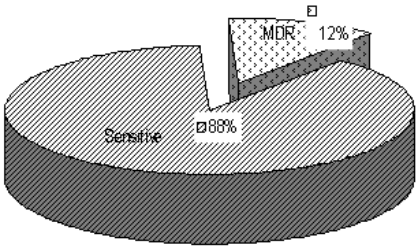


Figure 3: MDR Candida

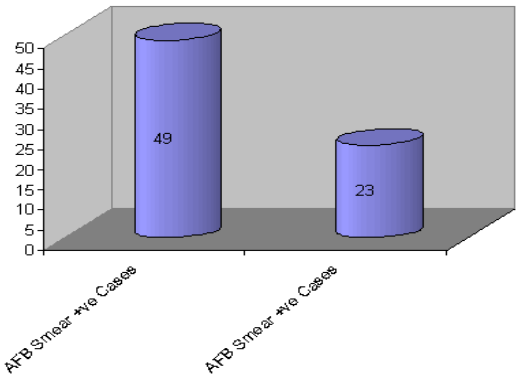
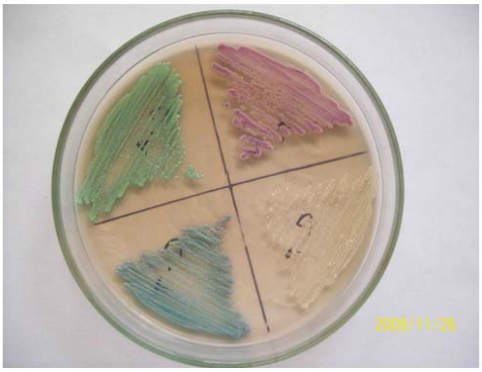


Figure 4: Multi drug Resistant Candida strain

Figure 4 a: Multi drug Resistant Candida Strain with newer antifungals also

Figure 5: A sensitive strain

Figure 6: Different Candida species on Chrom Agar (Light green- *Candida albicans*, Blue- *Candida tropicalis,* Cream – *Candida parapsilosis* and Pink – *Candida krusei*.)



Discussion

In the 1990s, there was a significant increase in the prevalence of drug-resistant fungal infections due to *Candida* species in patients hospitalized for mucosal or systemic diseases.[6-8] The widespread application of Fluconazole or related azole antifungals is postulated to promote selection of resistant subpopulations by shifting colonization to more naturally resistant species, such as *Candida krusei* or *Candida glabrata.*[9-11] Alternatively, azole-resistant subspecies have arisen in vivo and in vitro that show changes in the target enzyme lanosterol 14-a-demethylase, in expression of multi drug efflux pumps, or in both.[12-14]The azoles, particularly Fluconazole, remain among the most common antifungal drugs, but their intensive clinical use for both therapy and prophylaxis has favored the emergence of resistant strains.

There have been significant changes in the management of candidiasis in the last few years, particularly related to the appropriate use of echinocandins and expanded-spectrum azoles for candidemia, other forms of invasive candidiasis, and mucosal candidiasis. Pre disposing factors include malignant disease, diabetes mellitus, severe burns and cortico steroid therapy. The same background factors are found in the rare patient with Candida sinusitis or otitis media[15].Aspiration of Candida in to the lung is uncommon, but can occur in the agonal stages of illness. Mouth flora and squamous cells are seen independent areas of the lung at autopsy, along with Candida.

Infections due to *Candida* species necessitate the use of Fluconazole, Voriconazole, or the echinocandins because these isolates are frequently intrinsically resistant to AmphotericinB or develop resistance to AmphotericinB while the patient is on therapy and the antifungal therapy regimen needs constant vigil and requires periodical antifungal susceptibility/resistance evaluations.

Resistance pattern of the Candida species to the azoles have been discussed by Jose et al 2010[15] and shown to have resistance to both azoles and Amphotericin B antifungals. So the alternative antifungal regimens are essential and the role of other combinations of antifungals to treat complicated *Candida* infections needs to be evaluated. Though recent research publications indicating the effective role of azoles and Amphotericin B and the newer antifungal drugs, [16, 17] still there seems to be occurrence of drug resistant Candida strains in the stream of certain infection.

Candida species have various degrees of susceptibility to the frequently used antifungal drugs. The epidemiology of Candida infections has been extensively studied in North America and Europe[18], where large surveillance programs exist. In Latin America, these data are limited[17,] with some regional studies in a few medical centers.[18,19] Colombo et al[17] carried out the largest multicenter study in eleven medical centers of nine Brazilian cities.

To best of our knowledge there is no evidence based data available regarding the research studies related to the surveillance of drug resistance of Candida strains isolated from the sputum of TB clinic attendees especially from south India. In this situation we felt it is essential to carry out research study in this particular field.

From our three years constant research, we could able to record the drug resistant Candida strains from the sputum of the pulmonary tuberculosis patients (93%) and 52% from tuberculosis negative patients with other respiratory tract infections respectively.

Isolation of *Candida dubliniensis* for the first time in India was reported in 2003.[20] Distribution of antifungal susceptibilities of Candida species from mucosal sites of HIV positive patients was studied by Parisa et al 2010 and resistance to Fluconazole and Itraconazole was more frequent antifungals shown to have resistance[21]. In our study we could also able to obtain *Candida dubliniensis* (26 out of 636 Candida strains) from the sputum of the TB clinic attendees from which it was recorded as, 11 and 15 from the sputum of pulmonary tuberculosis patients and non pulmonary tuberculosis patients with other respiratory tract infections respectively.

From our study we conclude that the significant percentage (13%) of the resistant Candida strains have been isolated from the sputum of the TB clinic attendees with both the pulmonary TB cases and patients with other respiratory tract infections with out TB. Quite high level percentage (48%) of the resistance towards the antifungal drug Nystatin was observed from all the Candida species followed by Azole drug (24%) and Amphotericin B (22%).

The emergence of Candida antifungal resistance expressed as resistance to single drugs or to multiple drugs is the problem in treating the patients especially the patients those who are under immuno suppressive state and those who are already suffering from some other diseased condition. The failure in the treatment associated with Multi drug resistant which may increase the morbidity and mortality among such patients; therefore it requires prompt diagnosis and adequate antifungal therapy to these individuals.. Although studies demonstrate that antifungal resistance is relatively rare[17,19,22], antifungal drugs have been used intensively either to control such infections or as prophylactic in long-term treatments, creating serious worries that might select for drug resistances, thus greatly harming infection control which was already mentioned by Pfaller et al and Yang et al.[23,24]

Though controversial statement and views existing in this field, the positive look in to our view holds significance, since both tuberculosis negative and positive patients those who are all carrying these drug resistant Candida strains in their respiratory tract are comes under the first line in the spreading of these drug resistant Candida strains to others and to the society. For the public concern, a strong and steady prophylactic measure to be fixed to avoid the danger of spreading these resistant Candida strains. This could not be possible if any field of science or medical research fail to perform the initial step ie the screening of the drug resistant strains and their sources.

From our constant study and the results obtained, we could conclude that the occurrence of drug resistant Candida strains in the sputum/ respiratory tract of the patients with respiratory tract infections is not uncommon and we suggest that the more extensive and elaborative studies in the field of screening the drug resistant Candida strain and its source to be performed, for the same, researchers should be directed in this field by the governing authorities and funding agencies.

Therefore the periodical survey of the drug resistant Candida strains is essential not only to for the development of new antifungal drugs but also equally important in the appropriate antifungal drug selection for the earliest treatment for the patient care. By this we can suggest the related study in this field is essential either to support our study results or to criticize it.

References

1. Nobel W C - Microbiology of Human skin 2 nd ed. London : Lioyd – Luke, 1980: 263 – 90.
2. Kaufman R H, ed. – Vulvovaginal Candidiasis: A symposium. J. Reprod. Med. 1986; 31: 639 - 72
3. Clayton Y M, Nobel WC – Observation on the epidemiology of Candida albicans. J. Clin.Pathol 1966;19;76 – 78
4. Jawetz E, Mel Nick JL, and Addel berg EA Review of Med Microbiology Lange medical publications 1976:545
5. Chander J et al A text book of medical microbiology, 3rd edition Jan 2009
6. Nguyen MH, Peacock JE Jr, Morris AJ, et al. The changing face of candidemia: emergence of non–*Candida albicans* species and antifungal resistance. Am J Med 1996; 100:617–23.
7. Pfaller MA, Messer SA, Hollis RJ, 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.
8. Pfaller MA, Jones RN, Messer SA, Edmond MB, Wenzel RP. National surveillance of nosocomial blood stream infection due to species of *Candida* other than *Candida albicans:* frequency of occurrence and antifungal susceptibility in the SCOPE Program. SCOPE Participant Group. Surveillance and Control of Pathogens of Epidemiologic Importance. Diagn Microbiol Infect Dis 1998; 30(2):121–9
9. Alexander BD, Perfect JR. Antifungal resistance trends towards the year 2000: implications for therapy and new approaches. Drugs 1997; 54: 657–78.
10. Pfaller MA. Epidemiology of candidiasis. J Hosp Infect 1995; 30(Suppl): 329–38.
11. Rex JH, Rinaldi MG, Pfaller MA. Resistance of *Candida* species to fluconazole. Antimicrob Agents Chemother 1995; 39:1–8.
12. Marr KA, Lyons CN, Rustad TR, Bowden RA, White TC, Rustad T. Rapid, transient fluconazole resistance in *Candida albicans* is associated with increased mRNA levels of CDR. Antimicrob Agents Chemother 1998; 42:2584–9.
13. Sanglard D, Kuchler K, Ischer F, Pagani JL, Monod M, Bille J. Mechanisms of resistance to azole antifungal agents in *Candida albicans* isolates from AIDS patients involve specific multidrug transporters. Antimicrob Agents Chemother 1995; 39:2378–86.
14. Sanglard D, Ischer F, Koymans L, Bille J. Amino acid substitutions in the cytochrome P-450 lanosterol 14alpha-demethylase (CYP51A1) from azole-resistant *Candida albicans* clinical isolates contribute resistance to azole antifungal agents. Antimicrob Agents Chemother 1998; 42:241–53
15. Candidiasis Author: Jose A Hidalgo, MD**,** e-medicine article, wikipedia Updated: Jan 11, 2010
16. Charlier C, Hart E, Lefort A, et al. Fluconazole for the management of invasive candidiasis: where do we stand after 15 years?. *J Antimicrob Chemother*. Mar 2006;57(3):384-410. [Medline]
17. Colombo AL, Nucci M, Park B J, Nouér SA, Arthington-Skaggs B, da Matta DA, Warnock D, Morgan J: Epidemiology of candidemia in Brazil: a nationwide sentinel surveillance of candidemia in eleven medical centers. J Clin Microbiol 2006, 44:2816-2823.
18. Medrano DJ, Brilhante RS, Cordeiro AR, Rocha MF, Rabenhorst SH, Sidrim JJ: Candidemia in a Brazilian hospital: the importance of Candida parapsilosis. Rev Inst Med Trop Sao Paulo 2006, 48:17-20
19. Passos XS, Costa CR, Araújo CR, Nascimento ES, e Souza LK, Fernandes Ode F, Sales WS, Silva Mdo R: Species distribution and antifungal susceptibility patterns of Candida spp. bloodstream isolates from a Brazilian tertiary care hospital. Mycopathologia 2007, 163:145-151.
20. Gugnani HC, Becker K, Fegeler W, Basu S, Chattopadhya D, Baveja U et al. Oropharyngeal carriage of Candida species in HIV infected patients in India. Mycoses 2003;46:299-306
21. Parisa Badiee, Abdolvahab Alborzi, Mohammad Ali Davarpanah, Elaheh Shakiba Distributions and antifungal susceptibility of Candida species from mucosal sites in HIV positive patients. Archives of Iranian medicine, volume 13, number 4, July 2010
22. Aquino VR, Lunardi LW, Goldani LZ, Barth AL: Prevalence, susceptibility nprofile for fluconazole and risk factors for candidemia in a tertiary care hospital in Southern Brazil. Braz J Infect Dis 2005, 9:411-418.
23. Pfaller MA, Diekema DJ, Messer SA, Boyken L, Hollis RJ: Activities of fluconazole and voriconazole against 1,586 recent clinical isolates of Candida species determined by Broth microdilution, disk diffusion, and Etest methods: report from the ARTEMIS Global Antifungal Suscetibility Program, 2001. J Clin Microbiol 2003, 41:1440-1446.
24. Yang YL, Ho YA, Cheng HH, Ho M, Lo HJ: Susceptibilities of Candida species to amphotericin B and fluconazole: the emergence of fluconazole resistance in Candida tropicalis. Infect Control Hosp Epidemiol 2004, 25:60-64.

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