Available online at www.jibresearch.com; ISSN: 2348-6473 (online)

Journal of Innovative Biology

Original Research

September 2014 Vol. 1, Issue 3, P. 142-146

In-vitro isolation and identification of pathogens causing opportunistic mycoses in India

Seema Rani, Pankaj Kumar Jiloha*

Department of Microbiology, Kurukshetra University, Kurukshetra, 136119 (India)

Abstract: The frequency of invasive opportunistic mycoses has increased significantly over the past decades especially in immuno-compromised patients. Invasive mycoses are emerging as an important public health problem worldwide. The emergence of non-*albicans Candida* species and in particular *C. tropicalis*, has been documented. Opportunistic invasive fungal infections (IFIs) have a significant impact on public health in the region, and early diagnosis and appropriate treatment remain important. The incidence of IFI in India is increasing because of the expanded population of immuno-suppressed patients resulting from advances in medical technology, such as treatment for cancer and transplantation, as well as the impact of human immunodeficiency virus. During a study, performed in the Dept. of Microbiology of SRL diagnostic center Gurgaon, a total of 400 samples were collected and analyzed. For isolation of the pathogens, samples were directly inoculated to SDA and BHI agar. Loopful sample should be inoculated to each tube of both medium i.e. two tubes of SDA and BHI. Among the various samples, 107 were positive (26.2%). 107 (99%) fungal isolates were recovered from these samples i.e. *Candida* species, *Aspergillus* sp. and *Penicillium* sp. All the isolates were identified to their species level using germ tube test, growth characteristics, mold slide culture and Microscan walkway 40s1 identification panel. Out of isolated pathogen, *Candida* 94 (87.90%) was most predominant followed by *Aspergillus* 08 (7.5%) *and Penicillium* 05 (4.6%). In vitro study determines that *Candida albicans* (53.1%) dominating over *C. tropicalis* (22.3%).

Keywords: Opportunistic mycoses, non- *albicans*, mutation, immuno-suppressed patients.

Received: 16 July 2014 / Accepted: 04 August 2014 / Published Online: 13 August 2014

A mycosis is a fungal infection of animals, including humans. It is common and a variety of environmental and physiological conditions can contribute to the development of fungal diseases. Inhalation of fungal spores or localized colonization of the skin may initiate persistent infections; therefore, mycoses often start in the lungs or on the skin. Over the past few decades, the incidence of fungal infections has increased dramatically (Hidalgo et al. 2006). Individuals with weakened immune systems are also at risk of developing fungal infections as in the case of people with HIV/AIDS, under steroid treatments, and under chemotherapy. People with diabetes also tend to develop fungal infections. Very young and very old people also are groups at risk so increase in morbidity (Wirk, 2011). Opportunistic mycoses are infections due to fungi with low inherent virulence which means that these pathogens constitute an almost limitless number of fungi. These organisms are common in all environments.

Pathogens causing infectious diseases show an important public health problem worldwide and represent one of the main causes of morbidity and mortality due to incidence of multiple antibiotic resistance in human pathogens (Sharma et al. 2014). Opportunistic fungal pneumonia pathogens: *Candida* sp. causing candidiasis. *Aspergillus* sp. causing aspergillosis., *Mucor* sp. causing mucormycosis., *Cryptococcus neoformans* causing cryptococcosis. Now a day's *C. albicans* is the most common cause of invasive fungal infections and the growing number of new infections from non-albicans *Candida* species is increasingly recognized as a major source of infection (Miceli et al. 2011). *Candida* infections are categorized into two groups i.e. superficial and systemic (Thevissen,

Quick **R**esponse **CODE**:
Rani et al., 2014

The article may be access online @
http://www.jibresearch.com

QR CODE

Corresponding Author:
Jiloha PK, (✉) Department of Microbiology, Kurukshetra University, Kurukshetra-136119, India
Email: pkpkmicro632@gmail.com

2005). The upward trend in the diagnoses of opportunistic mycoses reflects increasing clinical awareness by physicians, improved clinical diagnostic procedures and better laboratory identification techniques. The accurate species identification of *Candida* is important for the treatment, as not all species respond to the same treatment (Saranya et al. 2014). Another important factor contributing to the increasing incidence of infections by fungi that have not been previously known to be pathogenic has been the rise in the number of immuno-compromised patients who are susceptible hosts for the most uncommon agents.

C. albicans is the most common yeast causing oro-pharyngeal candidiasis. It is considered to be one of the most common opportunistic fungal diseases in patients of HIV/AIDS worldwide and occurring an estimated of 80-95% of patients with HIV disease (Priscilla et al. 2002; Hodgson et al. 2002). Nowadays, non-*albicans Candida* species (e.g., *C. tropicalis* and *C. galabrata*) exhibit more resistant and they have reported other parts of the world (Oberoi, 2012; Pfaller and Diekema, 2007). Patients with primary immuno deficiencies are susceptible to mycotic infections particularly when cell-mediated immunity is compromised. In addition, several types of secondary immuno deficiency may be associated with an increased frequency of fungal infections.

Most *Candida* infections are mucocutaneous and do not cause mortality (Njunda et al. 2012). Under favourable conditions, it appears that over 20 *Candida* species can cause human infections (Surain and Aneja, 2014). Fungi may cause lung disease through direct infection of pulmonary tissue, through infection of pulmonary air spaces/lung cavities, or through their ability to trigger an immunological reaction when fungal material is inhaled. The later mechanism is involved in cases of allergic broncho-pulmonary aspergillosis, *Aspergillus*-induced asthma and extrinsic allergic alveolitis due to fungi (eg, maltworker's lung, farmer's lung). Among various *Aspergillus* isolates, *A. fumigatus* is most common species (50–60%), followed by *A. flavus*, *A. niger* and *A. terreus* (10–15% each), Whereas *A. nidulans*, *A. ustus* and other rare *Aspergillus* sp. represent <2% of isolates (Patterson et al. 2000). The aim of this study was to isolate, identify and to study the various pathogens causing opportunistic mycosis in Human being.

MATERIALS AND METHODS

Study area

The study took place in the SRL diagnostic Center, Gurgaon and in the Dept. of Microbiology, Kurukshetra University, Kurukshetra.

Study design and Sample collection

This report is a study that was performed during a 3 month period (from Feb to April 2014). According to standard techniques, 400 specimens (Bronchoalveolar lavage (BAL), hair, tissue, sputum, urine) were collected from different patients. The samples were obtained in SRL diagnostic, Gurgaon. Specimens were cultured on Sabouraud dextrose agar (SDA) and brain heart infusion agar (BHIA), was performed following procedures described by the National Committee for Clinical Laboratory Sciences.

Isolation and identification of samples

For isolation of the pathogens causing infection, samples were taken in 15 ml conical flask around 1 ml and adding 1 ml mixture of NAL (N-acetyl L-cysteine) and sodium citrate and vortex the whole mixture gently. Incubate for 15 min at room temperature, add buffer up to 14 ml and then centrifuge. Remove the supernatant with sterile capillary pipette. Use sediment to inoculate the culture medium. Then samples can be directly inoculated to SDA and BHI agar (Saranya et al. 2014). Loopful sample should be inoculated to each tube of both medium i.e. two tubes of SDA and BHI with antibiotics and two of BHI and SDA without antibiotics. They should be held for the entire incubation period to ensure that the slow growing fungi are not over looked. The wet mounts using lacto phenol cotton blue is made before releasing preliminary and final culture report. Place a small drop of lacto phenol cotton blue on a clean microscopic slide. Remove aseptically a small portion of growth midway between the colony center and edge. Place the removed colony in the lacto phenol drop. Tease the fungus with 2 dissection needles so as to have a thin spread out. Gently place a cover slip at the edge of the drop of the mounting fluid. Examine the slide under microscope. This technique is followed when it is difficult to identify molds with tease mounts. In a petri dish, place V-shaped glass rod, microscope slide and a cover slip. Aseptically cut 1cm sq. agar block from CMA. Transfer the agar block onto the slide in the set up. Transfer very small amount of mold to the four sides of agar block. Add 1 to 1.5 mL of sterile water to the petri dish and incubate (Aneja, 2003). The isolates were identified to their sp. level using germ tube test, growth characteristics, mold slide culture and microscan walkway 40S1 identification panel (Salehei et al. 2012). Plates were examined before first day report after observing wet mount and then after 7th day because mostly yeast grow within 2-3 day. Yeast report was made after isolate in SDA plates and then microscan walkway used for their identification. Routine fungal cultures must be incubated in duplicate at 37°C and BOD incubator at 25°C for three weeks before they can be discarded. Cultures should not be discarded when a fungus is first isolated. For mold, the slide culture at 30°C for 3-5 days when mature conidia or spores are observed lactophenol cotton blue for observing under microscope (Aneja, 2003).

RESULTS AND DISCUSSION

During a study performed in the Dept. of Microbiology of SRL Diagnostic center Gurgaon, a total of 400

samples were collected and analyzed. For isolation of fungi and yeasts, samples were directly inoculated to SDA and BHI agar. Loopful sample should be inoculated to each tube of both medium i.e. two tubes of SDA and BHI. Among the various samples, 107 were positive (Table 1). 107(99%) fungal isolates were recovered from these samples i.e. *Candida* sp., *Aspergillus* sp. and *Penicillium* sp.

Table1 Positivity of patients from various samples

TOTAL	POSITIVE	NEGATIVE
400	107(27%)	293 (73%)

All the isolates were identified to their species level using germ tube test, growth characteristics, mold slide culture and Microscan walkway 40s1method (Fig 1 and 2). Out of isolated pathogen, *Candida* 94 (87.9%) was most predominant followed by *Aspergillus* 08 (7.5%) *and Penicillium* 05 (4.6%), respectively (Fig 4). In vitro study determines that *C. albicans* (53.1%) dominating over *C. tropicalis* (22.3%), respectively (Fig 5).

The microscan walkway 40 S1 is a rapid identification panel used for rapid identification of yeast and yeast like species using enzymatic reactions. Chromogenic and modified conventional tests are used for the identification of yeast isolated from clinical specimens. The rapid yeast species isolated from clinical specimens. The rapid yeast identification is 96 well micro dilution panel that utilizes 27 dehydrated substrates. A heavy suspension of yeast in autoclaved water is used to rehydrate the substrate. Identification is obtained after incubation of the panel at 35-37°C for 4 hours. During study, amino acids HPR, PRO, GLAR, and NGAL were found to be positive for *C. albicans*. TRY, GGLY, GLAR, GLPR, AGL 1, AGL 2, NGAL, GLY, and IDX show positive for *C. tropicalis* by microscan walkaway 40SI method. On the basis of amino acids we can distinguish between different species of yeast (Table 2

Figure 1 Isolated *Candida* sp. **(A and B)** isolated mold in DTM and SDA medium **(C and D)**

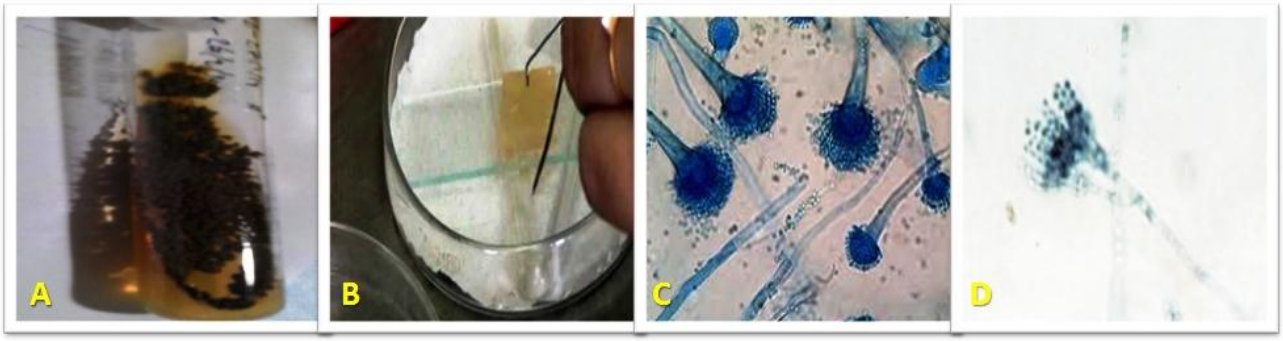

Figure 2 isolated molds in BHI **(A)**, Slide culture technique **(B)**, Spores of *Aspergillus* sp. **(C)**, *Penicillium* sp. **(D)**

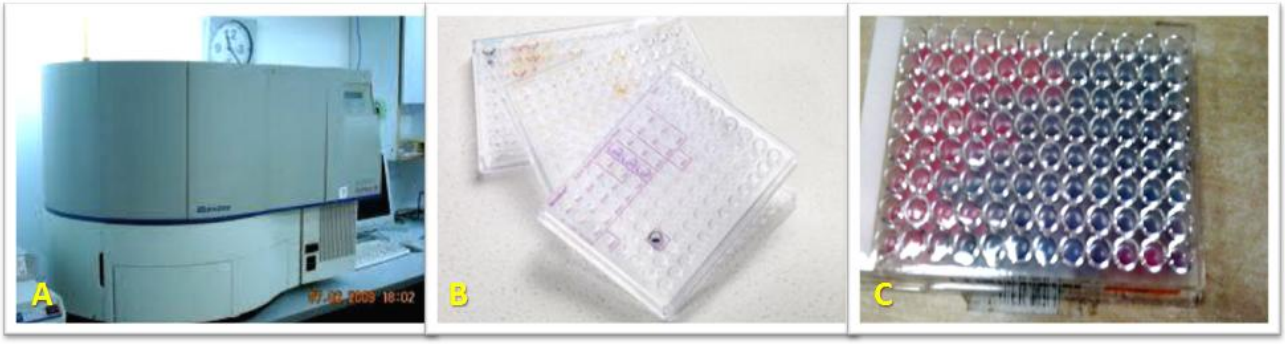

Figure 3 Microscan walkaway 40 SI **(A)**, Microscan rapid yeast identification panel **(B)**, Rapid yeast panel after result **(C)**

Table 2 Amino acids are positive during identification by microscan identification panel

C. albicans	*C. tropicalis*	*C. glabrata*	*C. famata*	*C. parapsilosis*
HPR	TRY	TYR	HPR	HPR
PRO	GGLY	HIS	PRO	PRO
TYR	GLAR	SUC2	AGL 2	TYR
GLAR	GLPR	ALA	NGAL	GLAR
NGAL	AGL 1	ILE	BGL	LYAL
ILE	AGL 2	GGLY	-	GGLY
IDX	NGAL	GLPR	-	AARG

HPR-Hydroxyproline-β-naphthylamide; **ILE**-L-Isoleucine -β- naphthylamide; **PRO**-L-proline- β- naphthylamide; **TYR**-L-tyrosine- β- naphthylamide; **GLY**-Glycine- β-naphthylamide; **GGLY**-Glycglycine- β- naphthylamide; **GLAR**-Glycyl-L-arginine-4 methoxy- β- naphthylamide; **GLPR**-Glycyl-L-proline-4methoxy-β-naphthylamide; **AARG**-L-Arginyl-L-arginine-β- naphthylamide; **ALA**-L-Alanine-4-methoxy-β- naphthylamide; **LYAL**-L-Lysyl-L-alanine-4-methoxy-β-naphthylamide; **URE**- Urea; **IDX**-3-Indoxyl phosphate; **HIS**-L-Histidine- β- naphthylamide; **SUC2**-Sucrose; **TRE**-trehalose; **NGAL**-ρ-Nitrophenyl-N-Acetyl-β-D-galactosamide; **AGL1**-ρ-Nitrophenyl- α-D-glucopyranoside;**AGL2**-ρ-Nitrophenyl- α-D-glucopyranoside

and Fig 3). In our study, the results of *C. albicans* (53.10%) recorded is lower in comparison to study from Thailand (66.6% in adults and 70% in children), Hong Kong (54.8%), Italy (61.9%), Mexico (92%) and India (65.3%) (Njunda et al. 2012). Most published reports concluded that *C. albicans* has been the predominant species isolated from various cases of candidiasis (Kao et al. 1999; Morgan, 2005; Mokaddas et al. 2007). During a study in Kuwait showed that *C. albicans* was

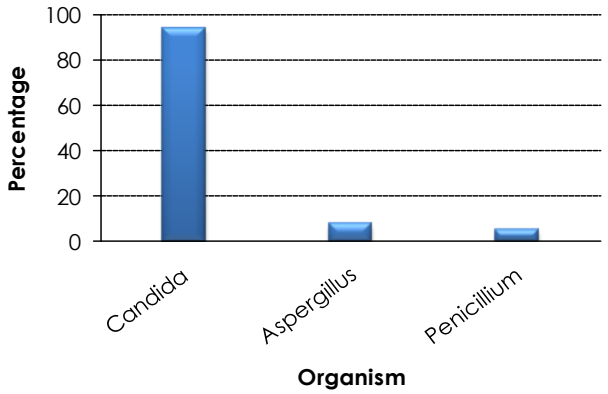

Figure 4 positive rates between different genus

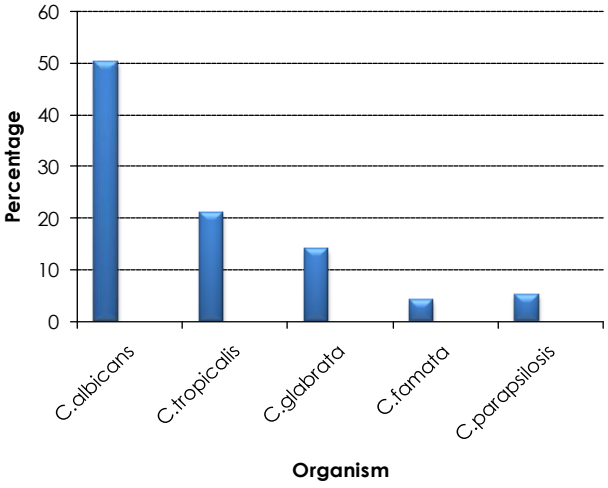

Figure 5 Determine positive rates between *Candida* sp.

the predominant species in bloodstream infections (39.5%), followed by *C. parapsilosis* (30.6%), *C. tropicalis* (12.4%), *C. glabrata* (5.6%) and *C. krusei* (1.6%), respectively (Mokaddas et al. 2007). In Brazil, *C. albicans* accounted for 40.9% of cases followed by *C. tropicalis* (20.9 %), *C. parapsilosis* (20.5 %) and *C. glabrata* 4.9% (Colombo et al. 2006). In our study *C. albicans* and *C. tropicalis* were observed in 53.1% and 22.3% of patients.

Aspergillus sp. does not always reflect invasive disease, because colonization can occur in immuno-compromised patients, and false-positive results that obtained from environmental contamination are occasionally a problem. Refinements in the prop-hylactic use of fluconazole, itraconazole, aerosolized amphoteric B, and the introduction of new formulations of existing antifungals may reduce the incidence of systemic fungal infections in some patient groups. Patients with presumed fungal infection require more intense and accurate monitoring for signs of disseminated infection. Thus early diagnosis may guide appropriate treatment and prevent mortality in such patients.

CONCLUSION

In therapeutic technologies and in particular the development of novel immunosuppressive therapies have prolonged the period of risk for many individuals. *C. albicans* was the predominant isolate followed by *C. tropicalis*. *Aspergillus* species do not always reflect invasive disease, because colonization can occur in immuno-compromised patients, and false-positive results that result from environmental contamination are occasionally a problem. In routine life, there is lots of mutation, so diagnosis of genus is not an easy work. Patients with presumed fungal infection require more intense and accurate monitoring for signs of disseminated infection. Early diagnosis may guide appropriate treatment and prevent mortality.

Acknowledgment: The authors would like to thank the authorities of SRL diagnostic center, Gurgaon for providing extending facilities and Dr. Geeta Dhamija (Ayurvedic Medical Officer, Jindal Hospital, Hisar) for providing the necessary information on the topic.

References

Aneja KR, 2003. *Experiments in Microbiology, Plant Pathology and Biotechnology*. 4th ed. New Delhi: New Age International Publishers.

Colombo AL, Nucci M, Park BJ, Noue SA, Arthington- Skaggs B, da Matta DA, Warnock D, Morgan J (2006) Epidemiology of candidemia in Brazil: a nationwide sentinel surveillance of candidemia in eleven medical centers. J Clin Microbiol 44: 2816–2823

Hidalgo DL, Roberts GD, Galgiani JN (2006) Results of a survey of antifungal susceptibility tests in the United States and inter laboratory comparison of broth dilution testing of flucytosine and amphotericin. B J Clin Microbiol 23:298-301

Hodgson TA, Rachanis CC (2002) "Oral fungal and bacterial infections in HIV-infected individuals: an overview in Africa." Oral Dis 2:80-7

Kao AS, Brandt ME, Pruitt WR, Conn LA, Perkins BA, Stephens DS, Baughman WS, Reingold AL, Rothrock GA (1999) The epidemiology of candidemia in two United States cities: results of a population-based active surveillance. Clin Infect Dis 29:1164–1170

Miceli M.H, Díaz JA, Lee SA (2011) Emerging opportunistic yeast infections. Lancet Infect Dis 11:142–51

Mokaddas EM, Al-Sweih NA, Khan ZU (2007) Species distribution and antifungal susceptibility of *Candida* bloodstream isolates in Kuwait: a 10-year study. J Med Microbiol 56: 255–259

Morgan J (2005) Global trends in candidemia: review of reports from 1995–2005. Curr Infect Dis Rep 7: 429–439

Njunda AL, Nsagha DS, Assob JCN, Kamga HL, Teyim P (2012) In vitro antifungal susceptibility patterns of *Candida albicans* from HIV and AIDS patients attending the Nylon Health District Hospital in Douala, Cameroon. J Pub Health Afr 2(2): 4-7

Oberoi JK, Wattal C, Goel N, Raveendran R, Datta S, Prasad K (2012) Non-*albicans Candida* species in blood stream infections in a tertiary care hospital at New Delhi, India. Ind J Med Res 136: 997-1003

Patterson TF, Kirkpatrick WR, White M, Hiemenz JW, Wingard JR, Dupont B, Rinaldi MG, Stevens DA, Graybill JR (2000) Invasive aspergillosis: disease spectrum, treatment practices, and outcomes. I3 *Aspergillus* Study Group. Medicine (Baltimore). 79(4): 250-60

Pfaller MA, Diekema DJ (2007) Epidemiology of invasive candidiasis: a persistent public health problem. Clin Microbiol Rev 20(1):133-63

Salehei Z, Seifi Z, Mahmoudabadi AZ (2012) Sensitivity of vaginal isolates of *Candida* to eight antifungal drugs isolated from Ahvaz, Iran jundishapur J Microbiol 5(4): 574-577

Priscilla LS, Milan EP, Martinez R (2002) Multicenter Brazilian study of oral *Candida* species isolated from AIDS patients. Mem Inst Oswaldo Cruz Rio de Janeiro 97 (2): 253-257

Saranya S, Moorthy K, Malar S.S.A.S, Punitha T, Vinodhini R, Bhuvaneshwari M, Kanimozhi C (2014) Prevalence and antifungal susceptibility pattern of *Candida albicans* from low socio-economic group. Int J Pha Pharma sci 6(2): 1-5.

Sharma C, Aneja KR, Surain P, Dhiman R, Jiloha P, Meashi V, Kaur M (2014) *In vitro* evaluation of anti-microbial spectrum of *Acacia nilotica* leaves and bark extracts against pathogens causing otitis infection. J Innov Biol 1(1):051-56

Surain P, Aneja KR (2014) Anticandidal potential of *Crinum asiaticum* leaves extract against selected oral and vaginal *Candida* pathogens. J Innov Biol 1(1):027-030

Thevissen K (2005) Fungal infections and antifungal strategies. Benth Sci Pub 6:847

Wirk B, Wingard JR (2011) Assessing responses to treatment of opportunistic mycoses and salvage strategies. Curr Infect Dis Rep 13(6):492-503