STUDY OF YEAST SPECIES ISOLATED FROM CLINICAL SPECIMENS

Lt Col P SENGUPTA ^{*}, Brig VC OHRI ⁺

ABSTRACT

Opportunistic yeast infections are very common in patients with malignancy or some form of immuno-suppression. Conventional methods of yeast identification are time consuming and labour intensive whereas commercial methods are expensive and beyond reach of most laboratories. Disk diffusion method offers a relatively simple, inexpensive and rapid method of identifying yeasts. 63 specimens of yeasts were isolated and identified from 68 patients by conventional methods. With disk diffusion method 54 of 63 species (85.7%) gave the same identification.

MJAFI 1999; 55 : 319-321

KEY WORDS: Disk diffusion method; Yeast.

Introduction

Incidence of opportunistic yeast infection has registered a tremendous increase in recent years. This is because of the improvement of methods for long term management of immunosuppressed, cancerous and other severely debilitated patients who are increasingly being put on steroids, cytotoxic drugs, irradiation and broad spectrum antibiotics. Although *Candida albicans* is the pathogen isolated in most cases, other genera and species are also implicated in human pathogenicity. Identification of yeast species by conventional methods requires careful and prolonged studies of gross, microscopic, morphologic and biochemical reactions. As the conventional methods of yeast identification are time consuming and labour intensive and various commercial methods viz. API 20C, Minitek yeast system, uni-yeast tek are expensive and beyond the reach of most laboratories, alternative methods are worthy of investigation. Yeasts are known to be sensitive to dyes, affording a possible basis for differentiating species by disk-diffusion method [1].

Material and Methods

Sixty eight patients of clinically manifested yeast infections were selected for study from various wards and OPDs of Command Hospital (SC) Pune. Specimens included oral and vaginal swabs, urine and skin scrapings.

One part of each specimen was subjected to direct microscopy and Gram stain for yeast cell and pseudohyphae, CSF, in addition was put for biochemical examination, cell count and capsule stain with India ink. Second part of each specimen was inoculated onto Sabouraud's dextrose agar (SDA) with 5 µg/ml gentamicin and incubated at 37° for 48 hours.

Isolated colony on SDA with gentamicin, after noting colony morphology and Gram stain was subjected to the following battery

of tests: germ tube test [2], urease test [3], chlamydospore production in corn meal agar with Tween 80 [4] growth in Sabouraud's broth for pellicle formation and deposit, nitrate assimilation [4] sugar assimilation and sugar fermentation tests [5] Particular yeast species was identified on the basis of yeast identification scheme recommended by Al-Doori [4].

In addition to the conventional methods described above, disk-diffusion method [1] was also applied for identification of yeast species. Disks, 6 mm in diameter and 1 mm thick, containing janus green (150 ug), ethidium bromide (37.5ug) 2,3,5 triphenyl tetrazolium chloride (150 ug), brilliant green (1.5 ug) cycloheximide (15ug) and rhodamine 6G (60ug) were applied in a set pattern numbered serially 1-6 on SDA which was inoculated by isolated yeast species by flooding the surface of the plate. After incubation at 37°C for 24-48 hours, depending on species, zone of inhibition of growth (mm) were measured. Strains were recorded as sensitive or resistant to six chemicals in disks 1 to 6 and sensitivity profile recorded. For example, a strain of *Candida albicans* of code 100406 was inhibited by chemicals 1,4,6 (janus green, brilliant green and rhodamine 6G) but resistant to chemicals 2,3,5 (ethidium bromide, 2,3,5 TTC and cycloheximide).

Result

Clinical profile of 68 patients selected for study is shown in Table-1.

TABLE I
Clinical profile of patients under study

Disease	No. of patients	Percentage
Malignancy (solid tissue tumour, haematological)	50	73.53
Vaginal discharge (diabetic, pregnant)	6	8.80
New born with oral thrush	3	4.40
Others (on long term steroid treatment, non-bacterial cystitis, meningitis, fungal skin lesion)	9	13.27
Total	68	100.00

^{*} Classified Specialist (Pathology), Military Hospital, Yol 176052, ⁺ Commandant, 167 Military Hospital, C/O 56 APO.

A total of 64 specimens of oral and vaginal swabs were taken for direct microscopy and culture. 56 showed yeast cell and pseudohyphae on Gram stain. Of the remaining 8 samples, 3 showed yeast cells but no pseudohyphae. 5 samples showed Gram negative bacilli only and no yeast cell. Direct microscopic examination of only specimen of CSF showed yeast cell and capsule in India ink preparation. Microscopic examination of centrifuged deposit of urine showed presence of round yeast cell. Skin scraping mount with KOH, demonstrated presence of yeast cells and pseudohyphae. Of the total 68 samples, 63 yielded positive yeast isolates on culture, of them 42 were germ tube positive and 21 germ tube negative. 42 isolates, which were germ tube positive also showed chlamydospore production in CMA with Tween-80. The yeast from CSF was positive for urease test.

Based on chlamydospore formation in CMA with Tween 80, germ tube production and pseudohyphae in direct microscopy, 42 isolates were identified as *Candida albicans*. For further identification of remaining isolates, sugar fermentation, sugar assimilation and nitrate assimilation tests were put up. Distribution of yeast species among isolates are shown in Table-2.

TABLE 2
Distribution of yeast species among isolates

Species	No. identified	Percentage
<i>Candida albicans</i>	42	66.6
<i>C. tropicalis</i>	9	14.3
<i>C. pseudotropicalis</i>	2	3.2
<i>C. guilliermondii</i>	1	1.6
<i>C. parapsilosis</i>	4	6.3
<i>C. krusei</i>	2	3.2
<i>T. glabrata</i>	2	3.2
<i>C. neoformans</i>	1	1.6
Total	63	100

63 clinical isolates were also identified by disk diffusion method as outlined by Sobezak [1]. For 54 isolates both the methods gave the same identification, an agreement of 85.7% (Table-3).

Discussion

Clinical profile of patients in this study showed 50 out of 68 cases studied suffered from some form of

TABLE 3
Behaviour of 54 yeast isolates in disk-diffusion method

Species	Inhibition with chemicals						Code	No. of isolates
	JG	EB	TTC	BG	CY	RG		
<i>C. albicans</i>	+	-	-	+	-	+	100406	32
	+	+	-	+	-	+	120406	6
<i>C. tropicalis</i>	+	+	+	+	+	+	123456	6
<i>C. pseudotropicalis</i>	+	+	+	+	-	+	123406	2
<i>C. guilliermondii</i>	+	+	-	+	+	+	120456	1
<i>C. parapsilosis</i>	-	+	-	+	+	+	020450	2
<i>C. krusei</i>	-	+	+	+	+	+	023456	2
<i>T. glabrata</i>	-	+	+	-	+	-	023050	1
<i>C. neoformans</i>	+	+	-	+	+	-	120450	1

Note : JG : Janus green; CY : Cycloheximide; EB : Ethidium bromide; RG : Rhodamine 6 G; TTC : Triphenyl tetrazolium; BG : Brilliant green

malignancy (Table-1). Routine oral examination of cancer patients has revealed a greater incidence of *Candida* infection than most other patients [6]. The literature has verified that opportunistic *Candida* infections occur in both solid tumour patients and those with leukaemia and lymphoma, as well as naturally and artificially immunosuppressed hosts. Out of 50 malignant cases in this study, 11 cases were carcinoma of oropharynx and laryngopharynx receiving radiotherapy. Radiotherapy administered for oral neoplastic disease has profound effect on oral flora. One of the effects is to cause increase in number of yeast colonies isolated from oral mucosa and saliva [9]. Studies have shown *Candida* spp are usually present in oral infection with radiotherapy although *Aspergillus* and *Torulopsis glabrata* are sometime also associated [9].

Vaginal swab was taken from 6 patients with vaginal discharge, 3 of them diabetic and 3 pregnant. *Candida* infection has long been associated with pregnancy. The rate is highest in third trimester when vaginal pH is lowest [10]. Besides pregnancy, diabetes, antibiotic therapy and oral contraceptives also predispose to vaginal candidiasis. In our series 4 out of 6 vaginal swab specimens yielded *C. albicans*, the other two showing *C. tropicalis* and *T. glabrata* respectively.

Clinical involvement of urinary tract with *Candida* is reported in association with disseminated candidiasis, diabetes, pregnancy, use of antibiotics and unclean catheters. Diagnosis is difficult to make as *Candida* is frequently cultured in urine. Counts in excess of 1000 colonies/ml are indicative of active infection [11]. Our patient with UTI presented with frequency and urgency of micturition. Repeated culture for bacteria was negative. Centrifuged deposit of the specimen showed yeast cell.

Blood sample was taken from a patient with septicaemia who was terminally ill from disseminated tes-

ticular carcinoma. He was on chemotherapy. *C. quilliermondii* was isolated from blood. Candida septicæmia is an increasingly encountered infection, particularly as a terminal event to an underlying disease. *C. albicans* and *C. tropicalis* are isolated in most cases [12].

Cryptococcus neoformans was isolated from the only CSF specimen taken from a patient of meningitis. Clinically he had fever and headache.

Nuchal rigidity, Brudzinksky's sign and Kernig's sign was absent. CSF showed slight turbidity, pleocytosis (WBC 500/cmm). Biochemical profile showed low sugar (20 mg/100 mL), high protein (100 mg/100 ml), Gram stain of centrifuged deposit did not show any bacteria. Culture on chocolate/blood agar failed to grow any bacterial pathogens. India ink staining of CSF showed few capsulated organisms.

Disks containing chemicals or antibiotics have long been used for routine identification of Gram negative anaerobic bacilli. Sobczak (1985) developed a simple disk-diffusion method for identification of yeasts that depended on their different but distinct susceptibilities to different chemicals. API 20C auxano gramme was used as standard method for comparison. For 594 of 623 routinely isolated yeast, the disk-diffusion and commercial API 20C auxonogramme test gave the same identification, an agreement of 95.3 percent, only 8 of 1052 isolates from clinical specimens were not identified by disk-diffusion method. The result presented in our study with disk-diffusion reveals that 54 (85.7%) of 63 isolates have been identified correctly. In another Indian study, disk diffusion method correctly identified 93% yeasts in 18-24 hours and 100% in 48 hours [12]. The method described is straight forward and gives result within 24 hours. The

disks remain stable upto one year and results are not subject to minor technical variation in application of the system.

The disk-diffusion method offers, therefore a reliable means for the identification of yeasts from clinical specimens. Potential of the test is high, specially because it can be applied in small laboratories, without the need for extensive or expensive facilities.

REFERENCES

1. Sobczak H. A simple disc-diffusion test for differentiation of yeast species. *J Med Microbiology* 1985;20:307-16.
2. Reppon JW. Medical Mycology, The pathogenic fungi and the pathogenic actinomycetes. 2nd ed. Philadelphia, WB Saunders Co. 1982:774-80.
3. Dolan DT. A practical approach to identification of yeast-like organisms. *Am J Clin Path* 1971;55:580-90.
4. Al Doory Y. Laboratory Medical Mycology. Philadelphia. Lea & Febiger. 1980:247-58.
5. Haley L, Callaway CS. Laboratory methods in medical mycology, 4th edn. Washington DC HEW 1978:348-50.
6. Armstrong D, Young LS, Meyer RD, Blevins AH. Infections complications of neoplastic disease. *Med Clin North Am* 1971;5:729-45.
7. Viola MV. Acute leukemia and infection. *JAMA* 1967;201:923-6.
8. Bodey GP. Fungal infections complicating acute leukemia. *J Chronic Dis* 1966;19:667-87.
9. Bodey GP. Infection in cancer patients. *Cancer Treatment Review* 1974;2:89-94.
10. Goldberg PK, Kozinn P, et al. Incidence and significance of candiduria. *JAMA* 1979;241:582-4.
11. Wingard JR, Mery WG, Rein Saral. Candida tropicalis: A major pathogen in immunocompromised patients. *Ann Intern Med* 1979;91:539-43.
12. Devi B, Mohan N, Mohan U. Identification of yeast using disk diffusion test. *Indian J of Med Microbiology* 1997;15(4):157-8.