YEASTS IN SPUTUM

C.S. CHIN & S.C. ONG

INTRODUCTION

YEASTS are often implicated as primary, secondary or opportunistic pathogens in human infections (Conant *et al.*, 1971; Emmons *et al.*, 1971). They cause infections that can be life-threatening, particularly in debilitated individuals, and thus cause much concern in the management of such patients. Of greater concern, perhaps, is the noted increase in prevalence in such infections and their association with medical practices such as the use of antibiotics, corticosteroids, cytotoxic drugs, catheters (Keye & Magee, 1956; Seelig, 1966; Mazunder & Marks, 1975).

Yeasts are often isolated from clinical specimens of sputum and they present a diagnostic problem to the mycologist. Their isolations often confuse the diagnostic work-up on a patient because, while they may cause infections, they are more commonly present as commensal flora and contaminants in sputum. In this paper, results are presented on a study into the incidence of yeasts in sputum and the difficulties met in making a culturally based diagnosis of yeast infections. The relative prevalence of the different yeast types were also studied to offer some insight into the common yeast types that could be expected in the examination of clinical specimens of sputum. Particular interest was focussed on species of *Candida* other than *Candida albicans* because reports of their roles in human infections are increasing. In addition, the presence of factors that could predispose to the isolations of yeasts were sought.

MATERIALS AND METHODS

One hundred and twenty-five sputum specimens from 116 patients were examined over a period of 13 months and were subjected to investigation for yeasts microscopically and culturally.

Bacteriology Division
Institute for Medical Research, Kuala Lumpur.

CHIN C.S. and ONG S.C.

Microscopic examination for yeasts was carried out on Gram-stained smears and wet mounts in 20% potassium hydroxide. Each specimen was cultured for yeasts on 2 tubes each of Sabouraud's dextrose agar, sabouraud's dextrose agar with the incorporation of 0.036% potassium tellurite and mycobiotic agar (Difco). All culture tubes were kept for 3 weeks before discard.

Yeast colonies grown on culture tubes were identified according to methods in Mycology texts (Conant *et al.*, 1971; Emmons *et al.*, 1971 ; Hazen *et al.*, 1973; Lodder, 1974). Identification was carried out irregardless of the amount of growth on culture tubes.

RESULTS

Yeasts were isolated from 80 of the 125 specimens examined and these positive specimens were from 76 patients. A total of 82 yeast isolates were obtained from the positive specimens, with simultaneous occurrence of *Candida albicans* and *Candida tropicalis* in one specimen, and *Candida krusei* and *C. albicans* in another. Three genera of yeasts and 4 species of *Candida* were identified. The frequencies of their isolations are presented in Table I.

Table I
Yeast types and their frequencies of isolation

Yeast types	Numbe of isolates
Candida albicans	62
Candida tropicalis	11
Candida krusei	5
Candida parapsilosis	1
Cryptococcus neoformans	1
Trichosporon sp.	2

The amount of growth of yeast on culture tubes was recorded as follows:- sparse, where growth of colonies occurred only at the original site of inoculation, or were less than 20 in number otherwise; moderate, where colonies grew on subsequent streaks, were approximated to be greater than 20 in number and with predominantly isolated colonies; confluent, where the density of growth was so heavy that most colonies merged and counting was impossible. By these definitions, the growth of 34 isolates was confluent, 12 moderate and 36 sparse (Table II).

Table II
Growth density of yeast isolates

Yeast types	Growth density		
	confluent	moderate	sparse
Candida albicans	21	10	31
Candida tropicalis	9	1	1
Candida krusei	2	0	3
Candida parapsilosis	1	0	0
Cryptococcus neoformans	0	0	1
Trichosporon sp	1	1	0

Of the 46 specimens that yielded moderate to confluent growth of yeasts on culture tubes, 23 were specimens that were received for culture within a day from the collection of specimens and these were described as "acceptable" specimens (Table III). The other 23 specimens were "delayed" specimens that were received more than a day after the collection of specimen. The delay in the times of transport ranged from one to 7 days.

Table III
Acceptability of specimens with moderate and confluent growth of yeasts in terms of the time of transport to the laboratory.

Isolates	"ACCEPTABLE" specimens	"DELAYED" specimens
C.albicans	15	16
C.tropicalis	6	4
C.krusei	1	1
C.parapsilosis	0	1
Trichosporon sp.	1	1

Because of the high incidence of yeasts obtained in the study, a retrospective search for predisposing factors, such as underlying infections and antibiotic treatment, was carried out based on clinical histories submitted together with the positive specimens. Reasons for fungal investigation in these positive specimens included one or more of the following:- radiological evidences (30 cases), chronic cough (10), cough (4), hemoptysis (6), lung infection with no response to antibiotics (7), bronchopneumonia (4), pyrexia of unknown origin (5), bronchiectasis (1), dyspnea (2), chest pain (2), bronchial asthma (1) bronchoscopic evidence of a nodule (1). The underlying infections found that could perhaps predispose to the carriage of yeasts were carcinoma in 4 cases, pulmonary tuberculosis in 2 cases and bronchial asthma in one case. There were 4 old cases of pulmonary tuberculosis while 8 cases had antibiotic therapy specifically mentioned. Five cases had no histories submitted at all while the others had no mention of any underlying infections or predisposing factors. These figures are indicated in Table IV.

Table IV
Analysis of predisposing factors found in clinical histories of cases positive for yeasts

Clinical histories	Number of specimens	
	positive for yeasts	total examined
Antibiotic therapy	8	10
Bronchial asthma	1	5
Carcinoma	4	4
Previous pulmonary tuberculosis	4	11
Current pulmonary tuberculosis	2	4
Predisposing factors absent	50	72
No histories provided	11	19

DISCUSSION

In this study, 64% specimens were found positive for yeasts and 65.5% of patients had one or more positive specimens. Of the 82 isolates of yeasts, the predominating genus was *Candida*, accounting for 79 isolates. In earlier studies, Baum (1960) isolated *Candida* species from 55% of patients while Jen *et al.* (1967) found an incidence of 30.16% in pulmonary cases. The incidence obtained in normal healthy students in these two

studies were 10% and 20% respectively. Comparing these results, the high isolation rate obtained in this present study is not unexpected since all specimens investigated were from patients with presenting pulmonary complaints and who had reasons for investigation.

Among the isolates, members of the genus *Candida* were most frequently encountered (Table I). C. albicans was the specie most frequently isolated, accounting for 62 isolates. The other remaining isolates comprised 11 *C. tropicalis*, 5 *C.krusei* and 1 *Candida parapsilosis*. While the pathogenic role of *C.albicans* in human infections has been well documented and accepted, the same cannot be said of the other *Candida* species. In fact the view previously held by many that defined *C.albicans* as the only pathogenic specie in the genus has hampered the acceptance of etiological involvements of the other species in human infections. Although their degree of pathogenicity may be less than that of *C.albicans*, their ability to cause disease has nevertheless been proven by reports of species such as *C.tropicalis, Candida visvanathii, Candida pseudotropicalis, Candida stellatoidea, Candida guilliermondii, C.krusei, C.parapsilosis* as causes of bronchopulmonary candidiasis, endocarditis, meningitis, cutaneous and corneal candidiasis (Skinner, 1947; Conn *et al.*, 1959; Manchester & Georg, 1959; Winner & Hurley, 1964; Louria *et al.*, 1967; Painter & Isenberg, 1973; Sandhu & Sandhu, 1976; Mosur *et al.*, 1977). In face of this, laboratory tendencies towards the search and reporting of *C.albicans* only should be corrected and rightful attention be rendered to other *Candida* species during laboratory investigation of sputum specimens. In this present study, although no definite etiological significance could be ascribed to such isolates due to reasons to be discussed later, their presence in sputum could nevertheless be deemed significant on at least two counts. Where these organisms were present in abundance and collection and transport satisfactory, there is a possibility of etiological involvement (Winner & Hurley, 1964). Where they were present in insignificant amount, probably as commensals, then their importance lay in the threat they pose as sources for infections should conditions favour the transition from commensalism to parasitism (Winner, 1969). From table II and table III 22 isolates appear to satisfy the first count while 35 satisfy the second count.

No definite etiological involvement could be claimed in this study because species of *Candida* are known commensals in the oropharyngeal regions of many healthy individuals and contamination during sputum collection could not be ruled out. Indeed the isolation of *Candida* in sputum often confuses rather than enlightens a diagnostic work up. Critieria proposed to distinguish patients with *Candida* present as a respiratory pathogen from those with *Candida* as part of their normal flora have included chronicity of positive cultures, absence of other infective and non-infective causes, specific speciation, presence of mycelia as opposed to yeast alone, quantitative sputum culture, serology (Winner & Hurley, 1964; Drake & Maibach, 1973; Masur *et al.*, 1977), but none is completely satisfactory without the histologic demonstration of the fungus in lung tissue or a positive culture of a transthoracic biopsy specimen. A further drawback to a laboratory diagnosis of bronchopulmonary candidiasis is the delay and improper transport of specimens to the laboratory in this study. One criterion set by Winner & Hurley (1964) to ensure a reasonable certainty that organisms recovered from sputum were grown from organisms in the lung, was that specimens must be cultured within an hour or two after collection or they must be refrigerated. In the present study, of 46 specimens that yielded moderate to confluent growth, only 22 were isolated from "acceptable" specimens while another 22 were from specimens delayed for more than a day after collection and which were not refrigerated during transport (Table III). Therefore, although their significant amount of growth satisfies the criterion of quantity, these latter isolations have to be discounted so far as the diagnosis of bronchopulmonary candidiasis is concerned because of the delay in culture. Even among the "acceptable" specimens, it is probable that some specimens were received at the laboratory more than 2 hours after collection, but because the times of collection of specimens were not specified, these specimens could not be determined. The problem of delayed specimens is unfortunately here to remain until such times when mycological examination is readily available at or to each hospital. To minimize the problem, provisions for the refrigeration of specimens during transport must be catered for, and the consequences of the lack of refrigeration appreciated. In view of the problem of contamination by

commensals and delay in transport of specimens, it is urged that a diagnosis of bronchopulmonary candidiasis be made within the total framework of a clinical, roentgenological and laboratory picture; the mere presence of *Candida* in sputum should not lead to a halt in the search for a more likely pathogen, nor should its presence completely be ignored either.

Besides *Candida, Trichosporon sp* and *Cryptococcus neoformans* were the other yeasts isolated. *Trichosporon sp* is a common contaminant in clinical specimens, especially sputum (Conant *et al.*, 1971) and its importance in clinical specimens lies more in the differentiation of it from other yeast-like fungi. The one exception is in hair, where *Trichosporon cutaneum* is a known cause of infection. No member of the genus has been implicated in human bronchopulmonary infections. *C.neoformans*, on the other hand, is an important human pathogen known to cause infections that are inevitably fatal without treatment if the central nervous system is involved. Therefore a positive sputum culture had always been accepted as sufficient indication for anticryptococcal therapy, if not to treat the pulmonary infection, then to prevent widespread dissemination and cerebromeningeal cryptococcosis. However, the clinical significance of the one isolate of *C.neoformans* made in this study from a patient under investigation for tuberculoma or aspergilloma, was doubtful because culturally, very insignificant amount of *Cryptococcus* was isolated and histopathologically, the case was confirmed as one of tuberculoma. It was interesting then to note that Weidman (1949), still alive without therapy 15 years after *C.neoforman* was cultured from his sputum concluded that its presence had the same significance as that of *C.albicans*. More convincingly, Tynes *et al.* (1968) and Warr *et al.* (1968) reported patients in whom sputum were positive for *C.neoformans* for up to 6 months without dissemination, and subsequently became negative without any anticryptococcal therapy. Reiss and Szilagyi (1965) and Howard (1973) also reported isolations of *C.neoformans* in patients with no sign of cryptococcosis. These findings bear important implications to the treatment of cryptococcosis, especially when there are not many choices of effective and non-toxic drugs. But whatever the significance of a sputum culture, it is essential that the patient must be studied intensively to ensure that their disease is stable in the lungs and not disseminated.

The search for predisposing factors that could explain the high incidence of yeast isolations in this study was often hindered by inadequate information given in histories provided with the specimens. In 11 cases that were positive for yeasts, no histories were provided at all. From the histories provided, the largest number of specimens received for examination and the largest number positive for yeasts were from patients without any predisposing factors. However, it is believed that, although not stated, most, if not all, of these patients would have had some form of antibacterial therapy prior to fungal investigation. If so, then the high rate of yeast isolations in this group is not surprising since the use of antibiotics has been noted for its predisposing roles in opportunistic infections by yeasts (Keye & Magee, 1956; Seelig, 1966; Mazumder & Marks, 1975). Antibiotic therapy was specifically mentioned with 10 specimens and 8 of these were positive for yeasts. An underlying disease can also predispose to an increased incidence of yeasts isolations either through effects of the disease itself, such as general debilitation and lowered resistance, anatonomical abnormalities, and deficiencies in immune and defence mechanisms, or through effects of the treatment of the disease, such as the use of antibiotics, cytotoxic drugs and steroids. The underlying diseases mentioned in the histories of patients in this study that could perhaps have such effects were carcinoma, pulmonary tuberculosis and bronchial asthma. All 4 specimens with accompanying histories of carcinoma investigated were positive for yeasts. Four specimens which were positive for yeasts were from patients previously treated for pulmonary tuberculosis and 2 were from patients currently receiving treatment. In the one positive specimen with mention of bronchial asthma in the history, it is uncertain whether the condition predisposed the colonization of yeasts or whether the yeast (C.albicans) was the cause of the condition, as has been known to occur (Winner & Hurley, 1964). Although we have sought and attempted to define some factors that probably did play a contributory role towards the high isolation rate of yeasts in the study, the actual extent of their contribution could not be gauged because some of the yeast isolates might have been commensals in the oropharyngeal

region of the patients even prior to effects of predisposing factor.

In conclusion, the proper collection, transport and repeat of sputum specimens must be ensured, with total awareness of the high possibility of contamination, before any significance could be attached to any yeast isolation made since these organisms are such common occurrence in sputum. Any interpretation made on a laboratory isolation must also be done in the light of all clinical, roentgenological and other laboratory investigations.

SUMMARY

125 sputum specimens were examined for the presence of yeasts. A high percentage (64%) were positive. *C.albicans* was the most common isolate. Other species isolated included *C.tropicalis, C.krusei, C.parapsilosis, Trichosporon sp.* and *C.neoformans.* Their importance, the problems in laboratory diagnoses and the presence of predisposing factors were discussed.

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