YEAST INFECTIONS AND ANTIFUNGAL SUSCEPTIBILITY PATTERNS IN MALTA

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Summary

During the period 1 May to 31 July 1995, a total of 130 different yeast isolates were cultured from 122 specimens taken from 94 patients (54 males and 37 females; no data was available for 3 patients). The three most common specimens were sputa (50.8%), urines (19.7%) and pus samples (10.7%). *Candida albicans* (70.0%), *Candida glabrata* (14.6%), *Candida tropicalis* 1 (6.9%), *Candida parapsilosis* (3.9%) were isolated. Some yeast isolates (4.6%) could not be identified with the assimilation test kit used. All isolates were sensitive to amphotericin B and nystatin. There was intermediate sensitivity to 5-fluorocytosine (1.5%), miconazole (11.5%), econazole (14.6%) and ketoconazole (31.5%). Additionally, some yeast isolates were resistant to 5-fluorocytosine (7.7%), ketoconazole (0.8%) and miconazole (0.8%).

Keywords

Candida, yeast infections, amphotericin B, 5-fluorocytosine, azoles

Introduction

Candida species are yeastlike fungi that normally occur as commensal organisms in the gastrointestinal tract. They can colonize healthy mucosa or induce various clinical syndromes in susceptible patients. One of the major tasks in the prevention and therapy of candidiasis is the difficulty in differentiating colonization from systemic infections (2). These may remain localized (oesophagitis, abdominal abscess) or may disseminate in the bloodstream and cause fungemia. However, isolation media for blood cultures have a high failure rate in detecting fungi, even in clinically proven fungemia. This increases the risk of false negative diagnosis (5).

Although over 100 species of *Candida* have been identified, only a few have been isolated from humans, *C. albicans* and *C. tropicalis* being the two species most commonly isolated from clinical specimens. The most frequent causative agent of fungemia and hematogenously disseminated candidiasis is *C. albicans*. However, recent studies have shown an increase

in infections caused by *C. tropicalis*, *C. parapsilosis*, *C. krusei* and *C. lusitaneae* (4, 7-9). Both *C. albicans* and *C. tropicalis*, are emerging as significant pathogens, capable of causing local and systemic infections in seriously-ill immunocompetent patients and also in severely immunocompromised patients (1, 3, 6).

The aims of this preliminary study, which is part of a larger two-year study, were:

i. to assess the incidence of yeast infections and prevalent species in patients admitted to St. Luke's Hospital, and,
ii. to determine if there are emerging resistant species.

Patients and Methods

The study population consisted of 94 patients, all of whom were hospitalized at St. Luke's Hospital, with nine exceptions (2 from St. Vincent de Paule's Residence, 3 from Zammit Clapp Hospital, 2 from Boffa Hospital, 1 from the Gozo General Hospital and 1 from Mosta Health Centre). Sixty-four patients were over 60 years old while only four were in the age group ranging from <1 to 20 years.

All samples (except cerebrospinal fluid and high vaginal swabs) received by the Microbiology Laboratory, St. Luke's Hospital for analysis, were screened for the presence of yeasts. The general procedure adopted for all specimens involved the following steps:

1. All samples were cultivated on Sabouraud Dextrose Agar with chloramphenicol (incubated at 30°C for 24 hours).

2. Pure cultures were examined by performing:
   a gram stain
   a germ tube test to differentiate between *C. albicans* and otherspecies.
   assimilation tests to determine the yeast's metabolic profile (API 20CAux, BioMérieux)
   slide cultures to study yeast morphology.

3. Antifungal susceptibility tests were performed (ATB Fungus, BioMérieux).

4. Patient clinical data was collected and analyzed. The clinical records of the patients were also examined. Data collected included underlying medical conditions, possible risk factors for fungal infections, any recent operations, therapy schedules and outcome of hospitalization.

Results

Of 122 samples, 130 yeasts were isolated. For some patients, more than one sample was sent for culture and often, more than one yeast species was isolated from the same patient. This accounts for the difference between patient and sample number and number

of isolated yeasts. The majority of the patients were over 60 years old (Table 1).

Table 2 shows various presenting clinical conditions of the patients involved in this study. Respiratory tract conditions were the commonest (47%); of these, chest infections (20.2%) and cough (10.6%) ranked highest. Other conditions presented at smaller frequency.

The most frequent species isolated was *C. albicans*. It was isolated from all the specimens submitted except the nail sample. *C. glabrata* was isolated only from sputa, urines, pus samples and the single nail specimen. *C. tropicalis* 1 was present in sputa, urines and bronchial lavages while *C. parapsilosis* was isolated from ear swabs, bronchial lavages and pus samples (Fig. 1).

C. albicans was the predominant species isolated (70.0%) (Fig. 2). Out of the non-*albicans* species, *C. glabrata* was the most prevalent (14.6%), followed by *C. tropicalis* 1 (6.9%) and *C. parapsilosis* (3.9%). 4.6% of the yeasts isolated could not be identified.

Figure 3 shows that all yeast isolates were sensitive to the polyenes (amphotericin B and nystatin). Intermediate sensitivity was exhibited to 5-fluorocytosine (1.5%), miconazole (11.5%), econazole (14.6%) and ketoconazole 31.5%). There was also some resistance to 5-fluorocytosine and two of the azoles (ketoconazole and miconazole).

Of the resistant yeasts, ten isolates of *C. albicans* were resistant to 5-fluorocytosine while 3 isolates of *C. tropicalis 1* separately showed resistance to either 5-fluorocytosine or miconazole or ketoconazole (Fig. 4).

Discussion

Candida species isolated from blood, mid-stream urine specimens, bronchial lavages, bronchial and oesophageal brushings, pus and ear swab samples were considered as clinically significant. In the case of sputum, the presence of *Candida* is significant only after elimination of primary causes of pulmonary or upper respiratory disease. This must also be supported by the patient's general clinical picture and any risk factors present which predispose the patient to colonization.

The throat swab isolates were held to be inconclusive due to probable salivary contamination. It is also difficult to evaluate the significance of the *C. albicans* isolated from stools, since *Candida* is ubiquitous in healthy persons. Moreover, candidemia has not been demonstrated in patients with *Candida* diarrhoea.

It is interesting to note that *C. glabrata* was the second most common yeast isolate from sputa and the only species isolated from nail specimens. This data is in accordance with international studies which report an increase in the frequency of infections caused by *C. glabrata*. In fact, both *C. glabrata* and *C. parapsilosis* are emerging as pathogens worldwide. Most authors

consider *C. tropicalis* as the second most frequent isolate in proven clinical infections; however, this is not so in our case.

The absence of resistance to the polyenes is consistent with the results of other studies. As expected, there was resistance to 5-fluorocytosine. An isolate of *C. tropicalis* 1 was resistant to ketoconazole while another strain of the same species showed resistance to miconazole. Due to the short duration of the study, it is still early to conclude that there is emergence of azole resistant non-*albicans* species. It is hoped that better conclusive results will be obtained at the end of the major study.

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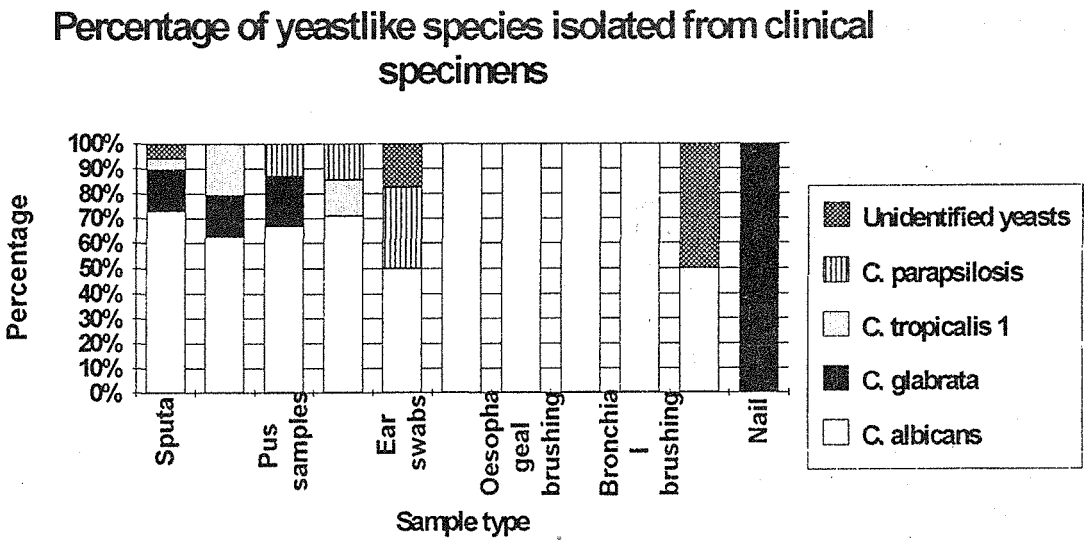

Figure 1

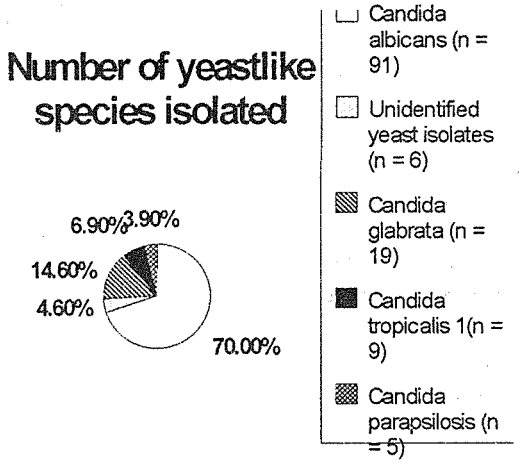

Figure 2

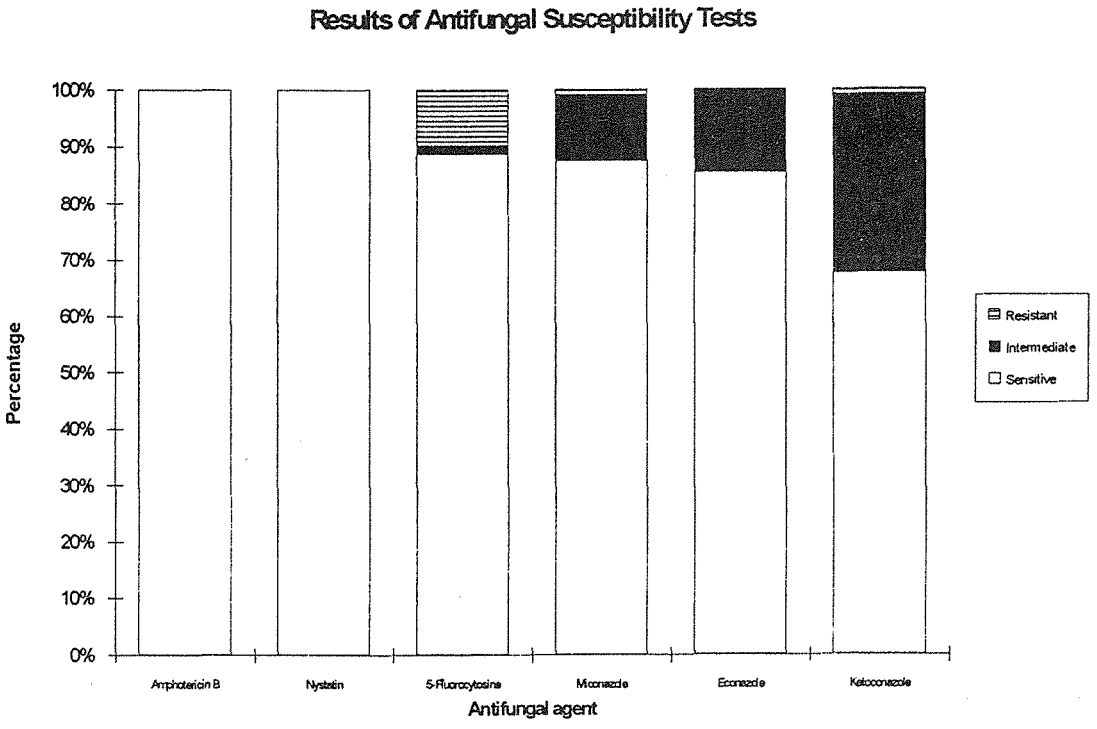
Results of Antifungal Susceptibility Tests
100%
90%
80%
70%
60%
50%
40%
30%
20%
10%
0%
Percentage
Amphotericin B
Nystatin
5-Fluorocytosine
Miconazole
Econazole
Ketoconazole
Antifungal agent
Resistant
Intermediate
Sensitive

Figure 3

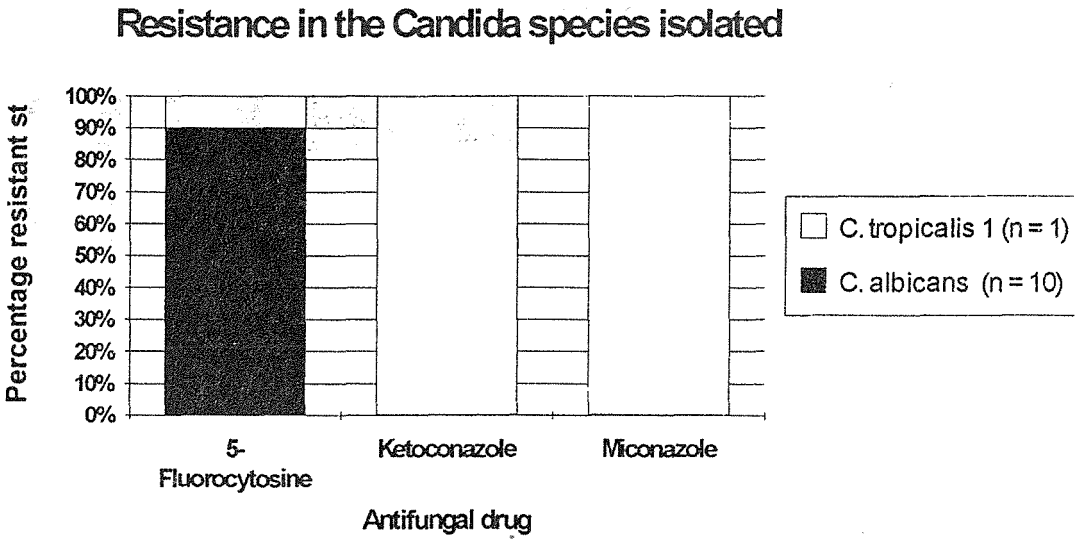
Resistance in the Candida species isolated
Percentage resistant st
100%
90%
80%
70%
60%
50%
40%
30%
20%
10%
0%
5-
Fluorocytosine
Ketoconazole
Miconazole
Antifungal drug
C. tropicalis 1 (n = 1)
C. albicans (n = 10)

Figure 4

Table 1 Anagraphical Data

Characteristic	Number	%
No. of Patients	94	100.0
Sex: Males	54	57.4
Females	37	39.4
ND	3	3.2
Age: 0-20	4	4.3
21-40	8	8.5
41-60	16	17.0
60+	64	68.1
ND	2	2.1

* No data available

Table 2 Presenting Clinical Condition

Clinical Condition	Number of Patients	%
1. CONDITIONS OF THE RESPIRATORY TRACT	**44**	**47.0**
(a) Upper/Lower Respiratory Tract Infections	**34**	**36.3**
Hoarseness	1	1.1
Cough	10	10.6
Whooping cough	1	1.1
Acute bronchitis	1	1.1
Respiratory Tract infection	1	1.1
Chest infection	19	20.2
Pneumonia	1	1.1
(b) Other Respiratory Conditions	**10**	**10.7**
Dyspnoea	1	1.1
Bronchiectasis	1	1.1
Chronic Obstructive Airway Disease	3	3.2
Respiratory Failure	2	2.1
Fibrosing alveolitis	1	1.1
Pleural effusion	2	2.1
2. LOCALISED/SYSTEMIC INFECTIONS (non-respiratory)	**17**	**18. 4**
Oesophagitis	2	2.2
Ear infections	4	4.3
Urinary Tract Infections	5	5.3
Infected wound	2	2.2
Nodal Empyema	1	1.1
Brucellosis	1	1.1
Septic shock	2	2.2
3. CHRONIC OR ACUTE CONDITIONS (non-infectious)	**17**	**18.2**
Diabetes mellitus	3	3.2
Motor Vehicle Accident	1	1.1
Diarrhoea	2	2.1
Pyrexia	5	5.3
Cardiovascular Disease	3	3.2
Alcoholic Liver Disease	1	1.1
Breast Cancer	1	1.1
Hydronephrosis	1	1.1
4. NO DATA AVAILABLE	**16**	**17.0**
Total	**94**	**100.0**