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Original Article

Characterization of candida species isolated from cases of lower respiratory tract infection

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Abstract

Objectives: (1) To identify and characterize the Candida species isolates from lower respiratory tract infection. (2) to determine the rate of isolation of Candida species from sputum samples.

Methods: This study was carried out in the Department of Microbiology, Manipal Teaching Hospital, Pokhara, Nepal from June 2002 to January 2003. A total of 462 sputum samples were collected from patients suspected lower respiratory tract infection. The samples were processed as Gram staining to find out the suitability of the specimen, cultured on Sabouraud's Dextrose Agar (SDA) and also on blood agar and chocolate agar to identify the potential lower respiratory tract pathogens. For the identification of Candida, sputum samples were processed for Gram stain, culture, germ tube test, production of chlamydospore, sugar fermentation and assimilation test. For the identification of bacteria, Gram stain, culture, and biochemical tests were performed by standardized procedure.

Result: Out of 462 samples, 246 (53.24%) samples grew potential pathogens of lower respiratory tract. Among them *Haemophilus influenzae* 61(24.79%) and *Streptococcus pneumoniae* 57 (23.17%) were the predominant bacterial pathogens. Candida species were isolated from 30 samples (12.2%). The majority of Candida species amongst the Candida isolates were *Candida albicans* 21(70%) followed by *Candida tropicalis* 4(13.33%). *Candida krusei* 3(10%), *Candida parapsilosis* 1(3.33%) and *Candida stellatoidea* 1(3.33%). The highest rate of isolation of Candida was between the age of 71 and 80.

Conclusion: Candida isolation from sputum samples is important as found in the present study in which Candida species were the third most common pathogen isolated from patients with lower respiratory tract infection.

Key words: *Candida albicans*, Pulmonary candidiasis, Nepal

Candida is a Gram positive, oval, budding yeast cell that produces pseudohyphae both in culture and in tissues and exudates[1]. It is a member of the normal flora of the mucous membranes in the gastrointestinal, upper respiratory, and female genital tracts[2,3,4] . A rapid presumptive identification of *Candida albicans* can be made by placing the organism in serum and observing germ tube formation [5]. The increase in the incidence of Candida species over the past two decades is significant and non-albicans Candida species continue to replace *Candida albicans* at most of the clinical sites like bloodstream infections[5]. Due to the commensal nature of Candida species, most of the times it is found to be a cause of endogenous infection [5]. Nevertheless, invasive lung infection by Candida species is rare in immunocompetant subjects. The criteria for the diagnosis of pulmonary candidiasis are still controversial. The isolation of Candida from culture of sputum, endotracheal aspirates, bronchoscopic samples, percutaneous lung needle aspirates and even from lung tissue may only represent colonization of the tracheobronchial tree. Despite the debate about the diagnosis of pulmonary candidiasis, the definite diagnosis of pulmonary candidiasis still rest on histological demonstration of the yeast in lung tissue with associated inflammatory changes .[6]

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Material and method

This study was carried out in the Department of Microbiology, Manipal Teaching Hospital, Pokhara, Nepal. Sputum samples showing less than 10 squamous epithelial cells and 25 or more polymorphonuclear leukocytes per low power field (100×) were included in this study.

A total of 462 sputum samples were collected from June 2002 to January 2003. All sputum specimens were processed for Gram staining, culture, germ tube test, detection of chlamydospore and sugars fermentation and assimilation tests.

Gram stain: Smears made from the most purulent or mucopurulent part of the sputum. Gram stained smears were used to look for presence of gram positive budding yeast cells with pseudohyphae. Specimen was considered as acceptable when 25 or more polymorphonuclear leukocytes were seen per low power field (100×) with few (less than 10) squamous epithelial cells.

Culture: The suitable samples were cultured on plain Sabouraud's Dextrose Agar (SDA) and also SDA containing chloramphenicol and cycloheximide. SDA slants were incubated at 37^{0C} and examined twice a week to look for growth to cream coloured pasty colonies suggestive of Candida species. The slants were incubated for one week and discarded if no growth occurred by then.

Germ tube Test: A single colony was inoculated in human serum and incubated at 37^{0C}. After 2-4 hours, wet mount was prepared and examined under the microscope to look for the presence of germ tube.

Chlamydospore formation: All Candida isolates were tested for the production of chlamydospores in corn meal agar with Tween 80. The Candida strains were inoculated in corn meal agar (CMA) and then incubated at 25^{0C}. After 72 hours, the plates were examined under the microscope for the presence of chlamydospores.

Sugar fermentation test: All Candida isolates were subjected to carbohydrate fermentation test. Carbohydrate solutions used were 6% solution of dextrose, maltose, lactose and sucrose with basal media.

Sugar assimilation test: The assessment of the ability of yeast to utilize carbohydrates is based on the use of carbohydrate- free yeast nitrogen base agar and observing for the presence of growth around carbohydrate impregnated filter paper disks after an appropriate period of incubation. Carbohydrates used were glucose, lactose, maltose, sucrose and galactose.

Results

A total of 462 sputum samples were collected from the patients attending the Manipal Teaching Hospital, Pokhara, Nepal. Out of 462 samples, Candida species were isolated from 30 samples (12.1%) (Table 1). The majority of *Candida* species amongst the Candida isolates were *Candida albicans* followed by *C. tropicalis*, *C. krusei*, *C. parapsilosis* and *C. stellatoidea* (Table 2). The youngest patient from whom Candida species was isolated from sputum was 30 years old and the oldest patient was 85 years old. The highest rate of isolation of Candida was between the age of 71 and 80 (Table 3). *Candida* was isolated from 21 male patients and only from 9 female patients. The male to female ratio was 2.3:1 (Fig 1)

Table 1: Organisms isolated from sputum samples

Organism isolated	No. of Isolates	% of isolates
1. *Haemophilus influenzae*	61	24.79%
2.*Streptococcus pneumoniae*	57	23.17%
3. *Candida* species	30	12.19%
4.*Streptococcus pneumoniae+Haemophilus influenzae*	21	8.53%
5. *Moraxella catarrhalis*	19	7.72%
6. *Klebsiella* species	17	6.91%
7. *Pseudomonas aeruginosa*	14	5.69%
8.*Haemophilus parainfluenzae*	13	5.28%
9. *Acinetobacter* species	9	3.65%
10. *Staphylococcus aureus*	3	1.21%
11. *Streptococcus pyogenes*	2	0.81%
Total	246	100

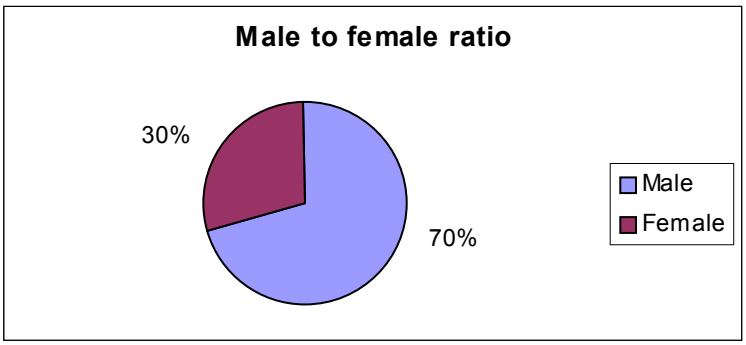
Table 2: Different species of Candida isolates

S.N.	*Candida* species	No. of Isolates	% of Isolates
1.	*Candida albicans*	21	70 %
2.	*Candida tropicalis*	4	13.33 %
3.	*Candida krusei*	3	10 %
4.	*Candida parapsilosis*	1	3.33 %
5.	*Candida stellatoidea*	1	3.33 %
Total		30	100

Table 3: Age distribution among which Candida species isolated

Age(Years)	No. of Isolates	% of isolates
21-30	1	3.33
31- 40	2	6.66
41-50	6	20
51-60	6	20
61-70	2	6.66
71-80	9	30
81-90	4	13.33
Total	30	100

Fig 1: Male to female ratio



Discussion

The diagnosis of pulmonary candidiasis is difficult not because it is difficult to demonstrate the organism in clinical samples but because isolation of Candida species in sputum does not necessarily mean that a person is suffering from pulmonary candidiasis. A good clinical correlation is therefore necessary to ascertain the clinical significance of the isolate.

The commonest bacterial pathogen was *Haemophilus influenzae* (24.7%) followed by *Streptococcus pneumoniae* (23.1%), and Candida species (12.1%) as the third most common organism obtained from sputum specimens (Table 1). The incidence of Candida pneumonia varies among different studies. Data from several studies including different subset of patients show an incidence ranging from 0.23 to 4.5% .[7,8,9] Two studies conducted by Torres A. et al in 1990 and Rello J. et al in 1993, showed the incidence of isolation of fungi and yeasts in ventilator-associated pneumonia to be 4.5 and 3.5% respectively. [10,11]

The total number of Candida species isolated was 30. *Candida albicans* was found to be the commonest with 70%(21/30) of the isolates falling into this species. This was followed by *C. tropicalis* 13.33%(4/30), *C. krusei* 10%(3/30), *C. parapsilosis* 3.33%(1/30) and *C. stellatoidea* 3.33%(1/30) (Table 2).

Germain G. St et al 1998, found the distribution of Candida species to be as follows: *C. albicans* 54%, *C. glabrata* 15%, *C. parapsilosis* 12%, *C. tropicalis* 9% and *C. krusei* 3% .[12] A multicentre surveillance study conducted in Quebec, Canada in the year 2001 also reported a similar pattern of distribution of species. They were *C. albicans* 64%, *C. tropicalis* 17%, *C. parapsilosis* 8%, *C. glabrata* 6%, *C. krusei* 2%, *C. lusitaniae* 1% and unidentified *Candida* species 1%.[13] The findings of the present study are more or less similar with the Quebec surveillance study except for the fact that *C. glabrata* was not isolated in its study. This could be due to variation in geographical distribution of various Candida species. The isolation rate of Candida species from sputum was found to be highest in the seventh decade of life followed by the fourth and fifth decade (Table-3,).

A study conducted by Resende Pinho J.C. in 2002, found the isolation rates of Candida species to be high in ages ranging from 60-80 years.[14] In the present study, the isolation rates of Candida species is high in ages ranging from 71-80 years old. So this study is similar to the study conducted by Resende Pinho J.C. in Brazil. In the present study, the medical records of each patient were examined to characterize and survey the main risk factors associated with them. The main risk factors which were found in this study were Chronic Obstructive pulmonary disease (COPD), smoking, tuberculosis, malnutrition, malignancy, diabetes mellitus, HIV infection, and prolonged use of antibiotics. Isolation of Candida was highest in patients with chronic obstructive pulmonary disease (76.65%), followed by patients with tuberculosis (33.3%), malnutrition (16.6%), malignancy (6.6%), diabetes mellitus (6.6%), and HIV infection (3.3%).

The male to female ratio was found to be 2.33:1. Resende Pinho J.C. 2002, found this figure to be 1.12:1.[14] The preponderance of males in our study can be explained by the fact that smoking and chewing tobacco, which leads to various obstructive lung diseases, is more common in males than in females. (Fig-1)

Conclusion

Candida isolation from sputum samples is important as found in the present study in which Candida species were the third most common pathogen isolated from patients with lower respiratory tract infection. Candida isolates from patient needs to be correlated clinically to rule out any skepticism that might arise out of issuing such a report. In some instances it may be better to ask for repeat cultures of clinical samples in order to provide an unequivocal evidence of Candidal infection.

References

1. Jawetz E, Melnick JL and Adelberg EA - Review of Medical Microbiology. 20th ed.: Lange medical publication, 1976: 545.
2. Nobel WC - Microbiology of Human skin. 2nd ed. London: Lioyd-Luke, 1980: 263-90.
3. Kaufman RH, ed. - Vulvovaginal candidiasis: A symposium. J. Reprod. Med.1986; 31: 639-72.
4. Clayton YM, Nobel WC - Observation on the epidemiology of Candida albicans. J.Clin. Pathol 1966; 19: 76-78.
5. Chander J - A textbook of Medical Mycology. 2nd ed. 2002: 212-227.
6. Masur H, Rosen PP, Armstrong D- Pulmonary disease caused by Candida species. Am. J. Med. 1977; 63: 914-25.
7. Odds FC. 1988. Candida and Candidiasis.2nd ed. :Bailliere Tindall, London, 1988.

8. Odds FC. Genital candidiasis. Clinical Exp. Dermatology. 1982; 7:343-54.
9. Ellen JB, Lance RP, Syoney MF. Bailey & Scott's Diagnostic Microbiology. 9th ed.: Mosby,1990: 219-233.
10. Torres A, Aznar R, Gatell MJ et al. Incidence, risk, and prognosis factors of nosocomial pneumonia in mechanically ventilated patients. American Review of Respiratory Disease. 1990; 142:523-528.
11. Rello J, Ausina V, Ricart M, Castella J, and G. Prats. Impact of previous antimicrobial therapy on the etiology and outcome of ventilator associated pneumonia. Chest. 1993; 104:1230-1235.
12. St-Germain G, Laverdiere M, Pelletier R et al. Prevalence and antifungal susceptibility of 442 Candida isolates from blood and other normally sterile sites: Results of a 2- year (1996-1998) multicentre surveillance study in Quebec, Canada. Journal of Clinical Microbiology. 2001; 39: 949-953.
13. St- Germain G. Etude de 100 souches de levures isolees de specimens cliniques normalement steriles. Soc. Fr. Mycol. Med. 1998;17: 309-312.
14. Pinho Resende JC, De Resende MA, Saliba JL. Prevalence of Candida species in hospitalized patients and their risk factors. Mycoses. 2002; 45: 306