

Emergence of non-*albicans* *Candida* species

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Abstract: A steady rise in incidence of systemic candidiasis over the past two decades resulting in higher morbidity, mortality and length of stay of hospitalized patients has been well documented worldwide. The gravity of the situation is further compounded by the emergence of non- *C. albicans* *Candida* (NAC) species as both colonizer and pathogen. Several sentinel and population-based surveillance programmes have noted an increase in the proportion of bloodstream infections (BSI) by NAC species, especially *C. glabrata*, *C. parapsilosis*, *C. tropicalis* and *C. krusei* in tertiary care centres. A few outbreaks due to unusual NAC species are also reported. Certain risk factors such as neutropenia, acute leukaemia, antineoplastic chemotherapy, surgery, intravascular catheter and antifungal prophylaxis have been specifically associated with NAC species causing catheter-related fungaemia. Accurate and rapid species level identification of NAC species is necessary because of differences in epidemiology and antifungal susceptibilities among various *Candida* species. The inherent resistance of a few NAC species to commonly used systemic antifungals such as fluconazole and amphotericin B can pose a therapeutic challenge in the management of NAC candidaemia. New drugs including broad-spectrum triazoles and echinocandins may be used as therapeutic alternatives in such a situation. Overall, higher mortality (35%-100%) due to NAC candidaemia demands strict implementation of preventive strategies and search for new broad-spectrum antifungal drugs.

Introduction

In recent years, a rise in the incidence of invasive candidiasis has been observed in the nosocomial setting, possibly contributed by the advances in medical technology, chemotherapeutics, cancer chemotherapy and bone marrow and organ transplantation. The infection is associated with high morbidity, mortality and an increase in the length of hospital stay.^{1,2} In a study from the US hospitals, it was found that *Candida* species accounted for 8%-15% of nosocomial bloodstream infections (BSI), 38% attributable mortality and a 30-day median increase in hospitalization.² The excess cost attributable to candidaemia in the US is estimated to be \$ 1 billion/year.³ According to the National Nosocomial Infection Surveillance (NNIS) system, USA, *Candida* species is the sixth most common pathogen (7.1%) causing infection and fourth most common isolate (10.1%) in patients in the intensive care unit (ICU). The Surveillance and Control of Pathogens of Epidemiologic Importance (SCOPE) study estimated that *Candida* species is the fourth most common bloodstream pathogen in US hospitals. The annual incidence of candidaemia in the USA ranges from 6-8 per 100 000 population.⁴ However, the picture in developing countries is not clear. According to the limited data available in India, *Candida* species accounted for 16.4%-34.7% of neonatal sepsis⁵ and a burden of 2 per 1000 discharges in a tertiary care hospital.⁶ In the Postgraduate Institute of Medical Education and Research, Chandigarh, India, the second half of the 1980s witnessed an 11-fold increase in candidaemia cases followed

by a further 18-fold rise in 1995 compared with 1991, and the rate was doubled again in 1996 and 1997. However, a 2-3 fold decrease was observed in 1998-2000 after administration of antifungal prophylaxis to high-risk patients.⁷

The overall increase in candidaemia is further complicated by the emergence of non- *C. albicans* *Candida* (NAC) species as both colonizers and pathogens causing nosocomial fungal BSI. A distinct increase in the proportion of cases due to NAC species has been observed.⁷

Incidence of candidaemia due to NAC species

Wingard, in a comprehensive review of all published reports during 1952 through 1992, found that 12 reports showed higher (>50%) proportion of isolation of NAC species.⁸ The common NAC species isolated were *C. tropicalis*, *C. glabrata*, *C. krusei* and *C. parapsilosis*. Other species such as *C. guilliermondii*, *C. lusitaniae*, *C. dubliniensis*, *C. kefyr*, *C. lipolytica* and *C. pelliculosa* were occasionally isolated. A rank order of species distribution has been obtained from population-based and sentinel surveillance data on candidaemia (Table 1).⁴ The earliest population-based surveillance study conducted in 1992-1993 by the Centers for Disease Control and prevention (CDC), USA reported *C. albicans* as the commonest species, followed in order by *C. parapsilosis*, *C. tropicalis* and *C. glabrata*. Subsequent surveillance programmes have noted an increase in the proportion of *Candida* BSI by NAC species and especially an increase in the frequency of BSI due to *C. glabrata* (Table 1). In contrast, surveillance data from other countries continue to reflect the importance of *C. parapsilosis* over *C. glabrata*.⁹

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Table 1. *Candida* species distribution as reported by sentinel and population-based surveillance programmes (modified from Pfaller and Diekema⁴)

Surveillance programme ^a	Years	Percentage of total (%)					Other species
		<i>C. albicans</i>	<i>C. glabrata</i>	<i>C. parapsilosis</i>	<i>C. tropicalis</i>	<i>C. krusei</i>	
CDC	1992-1993	52	12	21	10	4	1
NEMIS	1993-1995	56	15	15	10	0	4
SCOPE	1995-1998	53	20	10	12	3	2
CDC	1998-2000	45	24	13	12	2	4
EIEIO	1998-2001	58	20	7	11	2	2
SENTRY	1997-2000	54	16	15	10	2	3

a: CDC: Centers for Disease Control; NEMIS: National Epidemiology of Mycoses Study; SCOPE: Surveillance and Control of Pathogen of Epidemiologic Importance; EIEIO: Emerging Infections and the Epidemiology of Iowa Organisms; NNIS: National Nosocomial Infection Surveillance System.

Table 2. *Candida* species distribution in adults and neonates as reported by different surveillance programs in USA (Modified from Pfaller and Diekema⁴)

Study population	Surveillance program ^a	Percentage of total (%)				Total NAC (%)
		<i>C. glabrata</i>	<i>C. parapsilosis</i>	<i>C. tropicalis</i>	<i>C. krusei</i>	
Adults	NEMIS	24	5	19	0	52
	NNIS	12	10	11	NA ^d	41
	CDC ^b	25	12	14	NA	52
	SENTRY	23	12	10	2	50
Neonates	NEMIS	6	29	0	0	37
	NNIS	2	38	4	0	46
	CDC ^c	0	45	0	0	47
	SENTRY	3	24	7	0	40

^aSee footnote of Table 1 for abbreviations

^b CDC study of 1998-2000

^c CDC study of 1992-1993

^d NA : data not available

The importance of the age of the patient in determining the rank order of *Candida* species causing BSI has also been noted (Table 2).⁴ The predominance of *C. albicans* and *C. parapsilosis* and the lack of *C. glabrata* and other NAC species have been observed in neonatal age groups. In contrast, *C. glabrata* becomes an increasingly important pathogen with increase in age. Knowledge of the above trends can have important implications in nosocomial infection control strategies as well as for prophylaxis and dosages of systemic antifungals.¹⁰ Similar to the western world, the rise in frequency of isolation of NAC species has been observed in tertiary care centres in India as well, with isolation rates ranging from 52.4% to 96%.^{5,6} The dramatic increase in NAC candidaemia (20% in 1991 to 99% in 1997) has been documented in one centre.⁷ In India, *C. tropicalis* is the commonest NAC species isolated followed by *C. guilliermondii*, *C. krusei*, *C. glabrata* and *C. parapsilosis*. An outbreak due to *C. pelliculosa* (*Pichia anomala*) involving 379 neonates and children over a period of 23 months was reported.¹¹

Risk factors for NAC species

Epidemiological studies have successfully identified several significant risk factors for nosocomial candidaemia: acute leukaemia, leucopenia, burns, gastrointestinal surgery, prematurity, prior antibiotic use (vancomycin, imipenem), haemodialysis, intravascular catheters and previous colonization with *Candida* species.^{4,8} However, certain specific factors and patient populations have been observed to be significantly associated with NAC species (Table 3). Preponderance of NAC species, especially *C. glabrata*, has

Table 3. Risk factors for non albicans *Candida* infections

<i>Candida</i> species	Risk factors
<i>C. tropicalis</i>	Neutropenia, acute leukaemia, antibiotics, antineoplastic chemotherapy
<i>C. glabrata</i>	Solid tumours, surgery, azole therapy, long hospital stay, antibiotics, old age
<i>C. parapsilosis</i>	Intravascular catheters, neonates, prosthesis
<i>C. krusei</i>	Neutropenia, azole therapy, old age
<i>C. lusitanae</i>	Neutropenia, polyene use

been reported in surgical operation theatres and the ICUs.¹² Wingard reported that NAC species were more frequently isolated in leukaemia and bone marrow transplant patients ($p < 0.001$).⁸ In another study, haematological malignancies ($p < 0.03$), neutropenia ($p < 0.01$) and antifungal prophylaxis were documented to be significantly associated with NAC fungaemia.⁴ Breakthrough (BT) candidaemia (candidaemia after 5 days of systemic antifungal therapy) is more often caused by NAC species, with isolation rates of 65%-80% against 45%-55% in non-BT episodes.⁴ Previous colonization with NAC species ($p < 0.001$) has also been reported to be predictive for NAC candidaemia. The role of antifungal prophylaxis in increased isolation of NAC species has been intensively investigated and a highly significant association of prior azole therapy with rise in incidence of NAC candidaemia (especially *C. krusei*) has been identified. *C. parapsilosis* has been increasingly associated (17%-45%) with candidaemia in neonates with significant risk factors including gestational age (<32 weeks); 5-minute Apgar score <5; shock; disseminated intravascular coagulation; prior use of intralipid; total parenteral nutrition; central venous catheter; intubation and use of H² blockers.⁴

Pathogenicity of NAC species

The virulence of NAC species has been suggested to differ from those of *C. albicans*. Though most NAC species have been reported to be less virulent compared with *C. albicans in-vitro* and also in animal models, severe infections in humans with fatal outcomes have been attributed to NAC candidaemia. Hence, the pathogenicity of NAC species *in-vitro* or in animal models cannot be extrapolated to humans. In neutropenic patients, higher isolation of *C. tropicalis* and *C. krusei* with a grave prognosis is noted. The reason is not clear. However,

biofilm production has been implicated as a potential virulence factor for NAC species responsible for catheter-related fungaemia. *C. tropicalis* and *C. parapsilosis* tend to frequently colonize indwelling catheters frequently in individuals receiving intravenous hyperalimentation¹³. Comparison of clinical bloodstream isolates, representing different *Candida* species, with each other and with those from other anatomical sites, revealed that biofilm production was most frequent with *C. tropicalis* (80%), followed by *C. parapsilosis* (73%), *C. glabrata* (28%) and *C. albicans* (8%).¹⁴

Diagnosis

The emergence of NAC species as important human pathogens demands rapid species-level identification for prompt institution of appropriate antifungal therapy. Accurate diagnosis is of paramount importance because of differences in epidemiology and in antifungal susceptibilities of various species. Diagnosis of systemic candidiasis can be established either directly after isolation of the yeast or indirectly by detection of antibodies, antigens, metabolites and nucleic acids. Indirect tests such as antibody, antigen (mannan, enolase, HSP 90, proteinase) and metabolite (arabinitol) detection tests can be successfully used in diagnosis of systemic candidiasis but species-specific identification is difficult. Conventional methods of yeast identification after isolation in culture rely heavily on Wickerham or Delft assimilation/fermentation characters, which are cumbersome, and often beyond the range of expertise available in non-specialized clinical microbiology laboratories. Various commercially available rapid identification phenotypic tests, which can be used after primary isolation of the yeast on culture, have been developed. These include: (1) rapid methods (requiring less than 5 hours): enzymatic tests after primary isolation, immunological tests with polyclonal and monoclonal antibodies, biochemical and enzymatic panels, both manual and automated; (2) methods requiring 15 hours or more: biochemical and enzymatic panels, both manual and automated; and (3) physico-chemical methods: cellular fatty acid analysis by gas-liquid chromatography and spectroscopic techniques (Fourier transformed-infrared spectroscopy, pyrolysis mass spectrometry).

The incorporation of fluorogenic or chromogenic substrates directly into growth media agar to reveal species-specific enzyme activity allows easier discrimination of some *Candida* species and high sensitivity (85%-100%) and specificity (90%-100%) are reported for species identification.¹⁵ The Iatron serological *Candida* check kit (Iatron Laboratories Inc., Tokyo, Japan) can also rapidly identify *C. albicans*, *C. tropicalis*, *C. guilliermondii*, *C. krusei*, *C. kefyr*, *C. glabrata* and *C. parapsilosis* but its use is limited because of low specificity.

Identification systems based on direct enzyme detection by providing substrates to determine the yeast's enzymatic profile are available in both manual and automated versions. The API 20 AUX (88.5% accurate) is considered a reference method and

new systems such as Auxacolor (61%-63% accurate) and Fungichrom (81%-91% accurate) have been evaluated in some laboratories. Among the automated systems, ID 32C strip, Vitek yeast biochemical card and Vitek 2 ID-YST system can correctly identify >93% of yeasts and the highest accuracy is observed with the ID-YST system.¹⁵ Many probes have been designed for the various *Candida* species: *C. tropicalis* (Ct3, Ct14), *C. glabrata* (Cg6, Cg12), *C. dubliniensis* (Cd1, Cd24, Cd25), *C. parapsilosis* (Cp3) and *C. krusei* (CkF1, CkF2). These probes have been evaluated for rapid identification but these are available only in select centres. Specific amplification of certain target genes: *LIA1* (encoding lanosterol 1-4 α demethylase), *hsp 90*, actin gene, mitochondrial DNA and ribosomal DNA can also be used for identification of *Candida* species.¹⁵

Management

The inherent resistance of some NAC species to commonly used systemic antifungals can pose a therapeutic problem in the management of NAC candidaemia. A proportion of *C. krusei* and *C. glabrata* isolates are either primarily or secondarily resistant to fluconazole. Resistance to amphotericin B is observed in *C. lusitaniae*, *C. krusei*, *C. rugosa* and *C. guilliermondii* strains.¹⁶ Decreased susceptibility of *Candida* species causing BSI in older patients to both azoles and amphotericin B has been observed due to decrease in *C. albicans* and increase in *C. glabrata* and *C. krusei* candidaemia.

Trends in the susceptibility of *Candida* species BSI isolates to fluconazole over time have been assessed by both population-based and sentinel surveillance programmes. It was observed that resistance to triazoles is still not a frequent event. *C. glabrata* isolates generally exhibit bimodal susceptibility to azoles, some demonstrating frank resistance (MIC >64 μ g/ml), whereas others are susceptible. *C. tropicalis* is generally susceptible to azoles and no significant increase in resistance has been observed.⁷ Many new 'extended-spectrum' triazoles, both licensed and investigational, are being evaluated for efficacy against the various *Candida* species. It has been observed that ravuconazole, posaconazole and voriconazole are more active than amphotericin B, 5-fluorocytosine, itraconazole and fluconazole *in-vitro* against all *Candida* species and are possibly better agents against *C. krusei*.¹⁷

Generally, all *Candida* species are susceptible to amphotericin B except for some strains of *C. lusitaniae* and *C. guilliermondii*. However, recently candidaemia caused by amphotericin B-resistant strains of *C. glabrata* and *C. krusei* have also been reported.^{7,18} Reports of amphotericin B resistance is of special concern because of the paucity of therapeutic alternatives in these patients. Still, amphotericin B should be used for empirical treatment in centres where the incidence of NAC candidaemia is >50%. However, excellent *in-vitro* susceptibility to the newer azoles, particularly voriconazole, can make it the alternative option in the treatment

of *C. krusei* infections.¹⁷ For infections due to *C. glabrata*, dose-dependent responses to azoles are observed. In patients developing BT infection with severe immunosuppression or neutropenia, alternative agents are preferred.¹⁹ Newer drugs, e.g. broad-spectrum triazoles and echinocandins may be used to treat serious *Candida* infections.¹⁹

In spite of increased awareness, the overall mortality due to NAC candidaemia has been reported to be around 35%-100%.^{4,20} Thus, intensive search for newer therapeutic modalities as well as strict implementation of preventive strategies are required. Elimination of specific risk factors associated with particular NAC species, e.g. uncontrolled use of azoles for *C. krusei* and *C. glabrata* infections and intravascular devices for *C. parapsilosis* may be a realistic approach. General infection control measures to reduce nosocomial transmission (handwashing by healthcare workers and antisepsis) must be emphasized.

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