

Assessment of the types of catheter infectivity caused by *Candida* species and their biofilm formation. First study in an intensive care unit in Algeria

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Abstract: Nosocomial candidiasis remains a potential risk in intensive care units (ICUs), wherein *Candida albicans* is most responsible for its occurrence. Equally, non-*C. albicans* species, especially *C. glabrata*, are also involved. These infections are frequently associated with biofilms that contaminate medical devices, such as catheters. These biofilms constitute a significant clinical problem, and cause therapeutic failures, because they can escape the immune response and considerably decrease sensitivity to antifungal therapy. The diagnosis of catheter-related candidiasis is difficult; however, the differentiation between an infection of the catheter (or other medical implant) and a simple contamination is essential to start an antifungal treatment. Among the methods used for this type of study is the Brun-Buisson method, but this method only examines the infectivity of catheters caused by bacteria. For this reason, we wanted to adapt this method to the yeast cells of *Candida* spp. To assess the various types of infectivity of catheters (contamination, colonization, or infection) and their corresponding rates, as well as the responsible yeast species, we conducted our study, between February 2011 and January 2012, in the ICU at the University Hospital Center of Sidi Bel Abbas, Algeria; during this study, we took photographic images of the tongue of one patient and of that patient's implanted orobronchial catheter. In addition, catheters contaminated by *C. albicans* biofilms were observed by scanning electron microscopy.

Keywords: ICU, contamination, colonization, infection

Introduction

Nosocomial invasive candidiasis is a major problem, associated with significant mortality,^{1,2} that affects 15% of nosocomial infections in patients hospitalized in intensive care units (ICUs).³ Clinical signs of such infections appear late, rendering them difficult to diagnose.^{3,4} Many yeasts are opportunistic pathogens,⁵ and *Candida albicans* is the most common of these to colonize, but non-*albicans* species of *Candida* are increasingly involved in infection,⁶⁻⁸ in particular, *C. glabrata*, an emerging species associated with high mortality.⁹ Compared with *C. albicans* fungemia, the impact of *C. glabrata* fungemia on patients in the ICU is not well known.¹⁰

Candidemia is frequently associated with the colonization of medical devices, such as catheters.¹¹ In addition, a factor that is generally predisposing to invasive candidiasis in the ICU is the alteration of skin and mucus barriers caused by the use of medical devices (catheters, probes, intubation, etc).¹² Further, the resistance of *Candida* spp. to antifungal agents increases with the formation of biofilms into medical implants.¹³⁻¹⁵

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Indeed, *Candida* colonization appears to be a prerequisite for *Candida* infection;¹⁶ therefore, it is important to distinguish between simple contamination, colonization, and infection of catheters,^{6,17} because this differentiation remains crucial in initiating treatment.¹⁸ A threshold of significance, $\geq 10^3$ colony-forming units (CFU)/mL with or without the appearance of systemic symptoms (fever $> 38^\circ\text{C}$) or local symptoms (purulent opening, insertion-site or route inflammation), determines the type of infectivity of the catheter.¹⁹ The removal of the catheter is preferable when it is a gateway to candidemia. Analysis of the culture of the catheter after its ablation is necessary in confirming infection.^{20,21}

Since there is no specific technique by which to study the different types of infectivity of catheters contaminated by *Candida* spp., our study in the ICU at the University Hospital Center of Sidi Bel Abbe, Algeria aimed to adapt the Brun-Buisson technique and previous specific bacterial definitions for the assessment of the different types of fungal infectivity of medical implants, and also to isolate and identify the involved species and calculate their corresponding rates. In addition to this, we aimed to investigate the formation of biofilms on catheter surfaces, using electronic imagery.

Materials and methods

Based on previous results obtained from a prevalence survey that was done in June 2009 by the Epidemiology Department of the University Hospital Center of Sidi Bel Abbes (unpublished data), we conducted this study in the ICU between February 2011 and January 2012. The samples of the implanted medical devices were taken from patients hospitalized for 48 hours or more. Candidemia is considered nosocomial when it first occurs 48 hours or more after hospital admission.^{7,22} Additionally, other samples were taken by swabbing the surface of the tongue. To transport the samples to the laboratory, a refrigerant bag was used.

Patients

The samples were taken directly from patients who presented the most important candidiasis risk factors (implanted medical devices, broad-spectrum antibiotic therapy, long stay in the ICU, etc). For every patient, the following data were registered: age, sex, antibiotic treatment, body temperature during the sampling, and presence or absence of inflammation and/or pus along the route of the catheter.

One patient agreed to photography of the tongue; this was a diabetic woman of 64 years who suffered from meningo-encephalitis. She was treated by antibiotics, gentamicin and cephalosporin three times a day at dose levels of 100 mg/day

and 3 g/day, respectively, and acyclovir (900 mg \times 2/day), vitamin K (10 mg), paracetamol (1 g \times 3/day), and antidiabetic treatment (insulin, 3 \times 8 IU/day). This patient died of a cardiac attack.

Samples and yeast isolates

We took samples based on the method of Boucherit-Atmani et al.¹⁵ Briefly, the implanted medical devices (catheters and other invasive medical devices) were taken directly from patients and placed in sterile Sabouraud liquid medium. After cutting the distal ends of the catheters, the tubes were opened in front of a torch, then rapidly flamed and sealed to avoid contamination. The tubes were then agitated in a vortex mixer for 1 minute, and purified strains were identified by a yeast identification system (API 32-C System; bioMérieux, Marcy L'Etoile, France) and microculture (germ-tube and chlamyospore formation).

Tongue samples were collected by scraping the dorsum of the tongue with a sterile cotton swab, which was returned into the tube immediately after sampling. Then, the tube was agitated, and the different forms (yeast cells, hyphae, and pseudohyphae) were verified using light microscopy. In fact, scraping the surface of the tongue is a reliable method for detecting *Candida* spp.²³

Types of catheter infectivity

The type of infectivity of each collected medical implant was assessed using the quantitative method of Brun-Buisson²⁴ with some modifications. For this reason, the samples were vortexed for 1 minute, and then the yeasts were counted using a Thoma cell. To this end, a volume of the sample (after vortexing) was placed between the Thoma cell and the coverslip, and yeast cells were counted by light microscopy (magnification \times 40). Then, the calculation was reported in CFU/mL. When cell counting was difficult due to high density of the milieu, dilutions were performed.

The different types of catheter infectivity were determined by referring to the significance threshold, $\geq 10^3$ CFU/mL, and to the systemic and/or local clinical symptoms.¹⁹

Biofilms observation with SEM

In order to highlight the formation of biofilms on the catheter surfaces, a central vascular catheter (CVC) and an orobronchial catheter (OBC) were removed from the aforementioned female patient after exposure times of 8 and 3 days, respectively. Biofilm samples were randomly selected and preserved with glutaraldehyde fixative (Sigma-Aldrich, St Louis, MO, USA), and sent to the SEM laboratory.

Catheter-segment fixation was done according to the methods of Chandra et al.²⁵ Briefly, biofilms of *C. albicans* on catheter segments were fixed with 2.5% (v/v) glutaraldehyde in phosphate-buffered saline (PBS) (0.1 M, pH 7.4) and were introduced in sterile Eppendorf tubes that were sealed with an adhesive film, numbered, placed in another vial, and left for 12 hours at 4°C before shipment to the scanning electron microscope (SEM) laboratory for observations.

SEM preparation of the catheter's pieces was performed according to Chandra et al.²⁵ The samples were examined using a Hitachi S-4800 (Hitachi Ltd, Tokyo, Japan) field-emission SEM by Dennis Kunkel Microscopy Inc (Kailua, HI, USA).

Results and discussion

The prevalence survey, which was conducted in June 2009 by the Epidemiology Department of the University Hospital Center of Sidi Bel Abbes, showed that 60% of the patients hospitalized in the ICU were victims of nosocomial infection, and the principal cause was the use of any type of catheter. In addition, this survey revealed widespread use of antibacterial agents, while antifungal agents were prescribed only in rare cases.

According to Avila-Aguero et al,¹¹ previous use of antibiotics, endotracheal intubation, and use of central devices constitute the risk factors for candidemia infection. Effectively, catheters are the leading cause of bloodstream infection in patients in the ICU;²⁶ half of all cases of candidemia occur in the ICU.²⁷

In the present study, a total of 63 samples were taken from different implanted medical devices at the ICU during

the study period. Twelve strains (19.04%) of *Candida* spp. were isolated during the study period; nine strains of *C. glabrata* and only three strains of *C. albicans* were identified (Table 1).

Based on the results displayed in Table 1, *C. glabrata* was predominant compared with *C. albicans*; a ratio of 3:1 of *C. glabrata* versus *C. albicans* was observed.

The strains of *C. albicans* were isolated from the OBC and tongue of the female patient and from the CVC of another patient. The strains of *C. glabrata* were taken from different medical devices implanted into other patients.

In general, fungal infection in critically or seriously ill patients is a significant problem,¹⁶ and the use of indwelling devices, such as catheters, is a source of nosocomial infection.⁵ In addition, the vast majority of the cases of candidemia occur in hospitalized patients;²⁸ commonly affect patients in the ICU; and carry a high mortality risk.²⁹

C. glabrata was previously thought to be a primarily non-pathogenic organism, but, in the past couple of decades, it has been increasingly recognized as a pathogen of problematic etiological origin in bloodstream infections.^{30–32} Additionally, this yeast has recently emerged as an important nosocomial pathogen,^{9,33,34} yet little is known about its epidemiology.³³ In fact, *C. glabrata* has been suggested to be the second most important human pathogen responsible for candidemia.³⁵

According to Develoux and Bretagne,⁶ among the episodes of ICU candidemia, *C. glabrata* was the second most common causative species.¹⁰ However, a recent study found that no difference in mortality was associated with *C. albicans* and *C. glabrata* bloodstream infections.^{32,36} Trick et al³⁷ have demonstrated that, among the *Candida* spp.,

Table 1 Results of the counting and identification of yeast strains isolated from implanted devices in the ICU

Patients			Sampling sites						Count and identification	
Sex, age	ATB	T (°C) ^a	CVC	PVC	TP	UP	OBC	Swab ^b	Count (cells/mL)	Isolated strains
F, 18	Gent + Ampi	36.7			X				<10 ³	<i>C. glabrata</i>
M, 31	Cefa + Cipr	37.3			X				<10 ³	<i>C. glabrata</i>
M, 71	Gent + Cipr	38.1			X				>10 ³	<i>C. glabrata</i>
F, 64	Gent + Cipr + Acclv	37.1					X		>10 ³	<i>C. albicans</i>
								X	–	<i>C. albicans</i>
M, 23	Gent + Cipr	38.7				X			>10 ³	<i>C. glabrata</i>
				X					<10 ³	<i>C. glabrata</i>
F, 17	–	37.6	X						<10 ³	<i>C. glabrata</i>
F, 57	Cefa + Oxa	38.4	X						<10 ³	<i>C. glabrata</i>
M, 66	Amp + Cefo	37.3				X			<10 ³	<i>C. albicans</i>
F, 41	Cefa + Oxa	38.0		X					>10 ³	<i>C. glabrata</i>
F, 46	–	37.0		X					>10 ³	<i>C. glabrata</i>

Notes: ^aPatient's temperature at the time of sampling; ^bswabbing of the patient's tongue.

Abbreviations: F, female; M, male; ATB, antibiotic therapy; Gent, gentamicin; Amp, ampicillin; Cefa, cefazolin; Cipr, ciprofloxacin; Acclv, acyclovir; Oxa, oxacillin; Cefo, cefotaxime; CVC, central venous catheter; PVC, peripheral venous catheter; TP, tracheotomic probe; UP, urinary probe; OBC, orobronchial catheter; *C. glabrata*, *Candida glabrata*; *C. albicans*, *Candida albicans*.

the rate of *C. glabrata* has increased as a cause of bloodstream infection in American ICUs since 1993.

Types of catheter infectivity

Figure 1 shows the results of the types of catheter infectivity. In total, three different types were observed: six (9.52%) cases were contaminations, two (3.17%) cases were colonizations, and three (4.76%) cases were infections. The distinction between these different infectivity types was made according to previous definitions,^{19,24} and the counting of the yeast cells was accomplished using Thoma cells and the collected clinical data. These same observations were also distinguished in all the other medical departments of the University Hospital Center mentioned above (Seddiki et al, unpublished data, 2012).

Similarly to Carrière and Marchandin,¹⁹ three different types of catheter infectivity were reported: contamination, colonization, and infection. The rate of contamination of catheters was higher than the rates of colonization and infection; this explains, in part, the difficulty in diagnosis of catheter-related candidiasis.

The increase in acquired fungal infections in hospital, particularly in candidiasis, has been attributed to the ability of fungi to adhere to the invasive devices that are used for treatment.³⁸ According to Bleichner et al,³⁹ the frequency of isolation of *Candida* spp. in catheter-related infections ranges from 2% to 5%.³⁹ However, catheter-related infection is preceded by the colonization of the distal end of the catheter, and quantitative culture must be used to confirm that the catheter is the source of the signs of infection.¹⁹

Figure 2 shows the OBC colonized by *C. albicans* in the previously described patient. The count of yeast cells isolated from this catheter was higher than 10^3 cells/mL (Brun-Buisson technique). The patient's temperature during the sampling was 37.1°C; however, the patient was given an infusion solution of paracetamol, which prevented an increase of temperature,

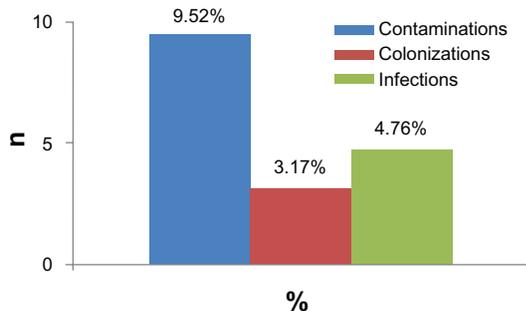


Figure 1 Rates of different types of infectivity of implanted medical devices observed in the intensive care unit.

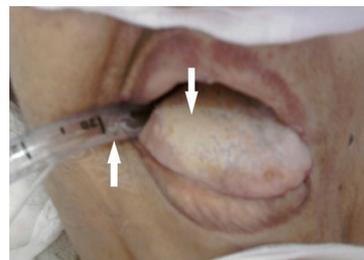


Figure 2 Microbial propagation between the patient's tongue and the implanted orobronchial catheter.

Note: Arrows indicate a white carpet of *Candida albicans* on the infected patient's tongue and the observed *C. albicans* biofilm on the orobronchial catheter.

and the same patient was also treated with broad-spectrum antibiotics. We also observed a white carpet on the patient's tongue, from which the isolate strain revealed *C. albicans*.

The observation of the sample (swabbing of the tongue surface) under an optical microscope revealed the existence of yeast cells and elongated hyphal forms. Interestingly, these different forms exist in the biofilms of *C. albicans*;²⁵ thus, it is possible that this carpet was a biofilm of *C. albicans*, and it may possibly have indicated a dissemination of this yeast between the tongue and the OBC implanted in the neighboring area.

Fungi have the ability to adhere not only to human tissues, but also to any invasive devices.³⁸ In fact, the acquisition of *C. albicans* in the ICU seems to be mainly endogenous.^{40,41} Oropharyngeal candidiasis is a common opportunistic infection occurring in immunocompromised patients.⁴² It appears as a white, elevated, mossy substance attached to the tongue surface or oral mucosa.^{43,44} It has been reported that oral *C. albicans* colonization increases remarkably in diabetic patients, especially in women with diabetes.⁴⁵ In addition, biofilms contribute to the pathogenesis of oral candidiasis,⁴⁶ and its presence on the surface of medical implants may constitute a risk factor for persistent candidiasis.^{15,47}

In our study, most of the patients (including this one) had been treated with broad-spectrum antibacterial agents. *Candida* colonization, indeed, is strongly linked to the receipt of antibiotics, particularly broad-spectrum ones.⁴⁸

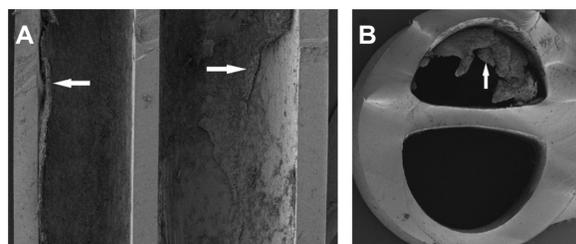


Figure 3 SEM of (A) longitudinal and (B) lateral cuts performed on the CVC.

Notes: Arrows indicate a layer of *Candida albicans* biofilm attached to the inner surface of the CVC. Magnification $\times 35$.

Abbreviations: SEM, scanning electron microscope; CVC, central venous catheter.

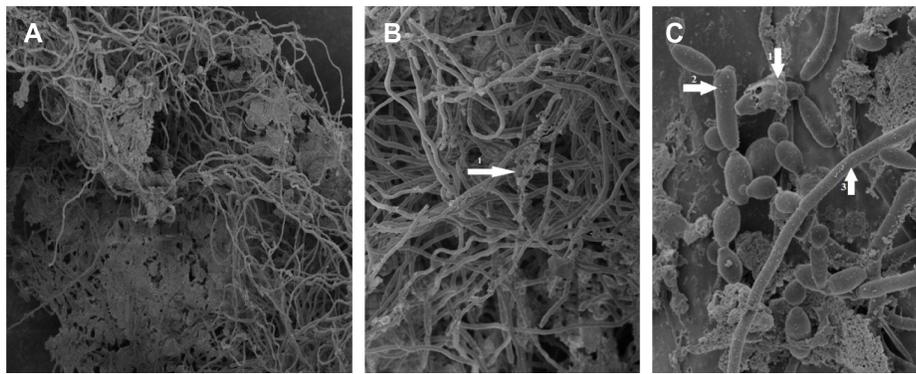


Figure 4 SEM of a mixture of morphological forms of *Candida albicans* biofilm developed on the inner surface of the CVC. Magnification: (A) $\times 450$; (B) $\times 1100$; (C) $\times 4500$.
Notes: Arrows: 1 = ECM; 2 = pseudohypha; 3 = hypha.
Abbreviations: SEM, scanning electron microscope; CVC, central venous catheter; ECM, extracellular matrix.

Biofilm observation with SEM

C. albicans biofilm from the inner surface of the CVC segments was examined and imaged with SEM.

Longitudinal and lateral cuts performed on the CVC (Figure 3A and B, respectively) revealed a dense layer of *C. albicans* biofilm attached to the inner surface of the CVC; however, its external surface was not affected by the biofilm.

With higher magnifications of SEM, the structure of the biofilm could be demonstrated. Figure 4A–C shows a mixture of morphological forms of *C. albicans* biofilm. This biofilm revealed a dense network of yeasts, pseudohyphae, and hyphae. The extracellular matrix was also observed; it coated cells and hyphae.

The OBC of the female patient was contaminated by *C. albicans* biofilm. This is shown clearly in Figure 5, which also shows the complex structure of the biofilm. *C. albicans* biofilm was formed by yeast cells, pseudohyphae, hyphae, and a visible matrix.

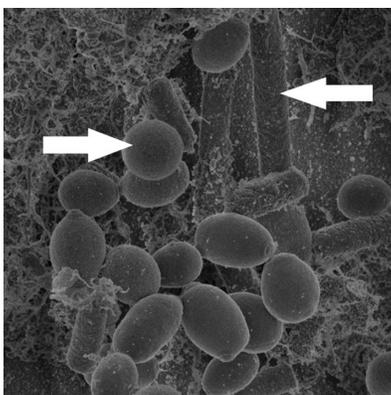


Figure 5 SEM of the contaminated OBC showing *Candida albicans* biofilm.
Notes: Arrows indicate a yeast cell and hypha. Magnification $\times 6000$.
Abbreviations: SEM, scanning electron microscope; OBC, orobronchial catheter.

In Andes et al,⁴⁹ the SEM of the biofilm on catheter segments demonstrated more clearly the heterogeneous architecture of the biofilm structure. In their work, SEM of in vivo *C. albicans* biofilm showed a mature biofilm that consisted of a dense network of yeasts cells, hyphae, and pseudohyphae. Further, the same results were observed by Hawser and Douglas⁵⁰ 10 years before.

In addition, biofilms of *C. albicans* consist of matrix-enclosed microcolonies containing yeasts, hyphae, and pseudohyphae. The biofilm is arranged in a bilayer structure⁵¹ consisting of a thin yeast layer and an upper hyphal layer.⁵² In a recent study, the structure of the biofilm was investigated using SEM, and this study, indeed, revealed an extracellular matrix material coating the cells.⁵³ In fact, adjacent to the catheter surface, yeast cells were densely embedded in an extracellular matrix.⁴⁸

Conclusion

Progress in the medical field is constantly evolving; however, clinical, epidemiological, and microbiological studies have so far failed to prevent nosocomial infections and, in particular, those of fungal origin. The current study is, to the authors' knowledge, the first survey of an ICU in Algeria. It highlights the nosocomial candidemia related to medical implants (catheters, probes, intubations, etc) by calculating their rates and by determining the causative strains and the various types of catheter infectivity. These infections, in fact, are serious additional risks acquired by critically ill patients who require significant intensive care.

According to the literature, there is no accepted method by which to evaluate the types of catheter fungal infectivity. Within this frame, our study has shown that the Brun-Buisson method seems to be suitable for these studies. Our results revealed three different types

of catheter infectivity: contaminations (9.52%), infections (4.76%), and colonizations (3.17%). In addition, the results prove that *C. glabrata* was strongly involved in the contamination of catheters; this species was more frequently isolated from the implanted medical devices than *C. albicans*.

On the other hand, SEM revealed the formation of biofilms of *C. albicans* on the surfaces of the catheters. Consequently, biofilms constitute a major problem in the diagnosis of candidiasis.

To conclude, it is highly recommended that vigilance be exercised when using medical implants to prevent fungal infections, especially in ICUs. To this end, in developing countries, the surveillance of nosocomial infections caused by fungi remains an open discussion, and further studies are warranted in this field.

Acknowledgments

The authors express great appreciation for the support provided by Prof Bertrand Dupont. We also wish to thank Mr Brahim Yala, teacher of English, for reviewing and correcting this manuscript.

Disclosure

The authors report no conflicts of interest in this work.

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