Evolution of candidemia incidence and susceptibility testing of Isolated *Candida* strains during a decade in a tertiary general hospital in Greece

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Summary

Recent studies reveal an increase in the incidence of candidemia and changes in the species of the isolated Candida strains, as well as in their drug susceptibility. The aim of this study was to evaluate the isolated *Candida* strains from blood cultures and their susceptibility in a tertiary hospital in Greece, during two periods of three years each (Period 1(P1): 1/1/2004-31/12/2006 and Period 2 (P2): 1/1/2012-31/12/2014). During the study periods blood cultures were sent to the laboratory for diagnosing bloodstream infections. The origin of the strains was for Period 1: Internal Medicine 32/71 (45.1%), Surgical Department 24/71 (33.8%), Intensive Care Unit (ICU) 15/71 (21.1%) and for Period 2: 88/152 (57.9%), 35/152 (22.4%), 29/152 (19.1%), respectively. According to our results, *Candida* strains isolated in Period 1 were 71 (2.7%) and in Period 2, 152 strains (4.5%). In Period 1 *C. albicans* was the predominant strain (29/71, 41%), followed by *C. parapsilosis* (15/71, 21%), *C. glabrata* (13/71, 18%), *C. tropicalis* (7/71, 10%) and *C. famata* (2/71, 3%). In contrary, in Period 2 *C. parapsilosis* was the predominant strain (63/152, 41.4%), followed by *C. albicans* (53/148, 34.9%), *C. glabrata* (14/152, 9.2%), *C. famata* (9/152, 5.9%) and *C. tropicalis* (5/152, 3.3%). Resistance

rate in Period 1 was: fluconazole 8.8%, voriconazole 1.2%, flucytosine 1.2%. Resistance rate in Period 2 was: fluconazole 10.8%, voriconazole 1.3%, flucytosine 1.3%. All *Candida* strains were fully susceptible to caspofungin and amphotericin B in both periods. In the two study periods a significant increase (p: 0.0258), approximately double, in the incidence of candidemia was observed. Noticeable, was the predominance of *C. parapsilosis* in Period 2 (p: 0.0014), unlike with the predominance of *C. albicans* in Period 1. No significant differences were observed in the resistance rate in the two periods concerning fluconazole, flucytosine and voriconazole. Echinocandins and amphotericin B seems to be the most effective treatment in our hospital. Taking into consideration, the different response of *Candida* species in antifungal agents in vivo, identification of *Candida* strains in species level and assess of their susceptibility patterns seems to be a necessity.

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Introduction

Candidemia is defined as bloodstream infection (BSI) caused by *Candida* species. *Candida* in a blood culture should never be considered as a contaminant.[1] The incidence of candidemia seems to vary a lot in different regions and countries, with some authors claiming increase of the incidence and others a decrease.[2-3] However, *Candida*, undoubtfully, remains one of the most common pathogens isolated from blood cultures, fourth in the row in the USA and among the ten most frequent isolated pathogens in Europe.[4-5] One of the major threats of candidemia is the high mortality of the infection which is estimated approximately 30-40%, with a crude mortality as high as 50-60%.[6-7]

C. albicans is still accounted for the majority of BSIs, but BSIs due to non-*albicans* species are, constantly, increasing.8, 9 This, consists an additional problem, as the non-albicans species present resistance to some antifungal agents. Thus, beside *C. glabrata* and *C. krusei*, fluconazole resistance has also been revealed in *C. tropicalis* and *C. parapsilosis*, and echinocandine resistance has started to occur, possibly, due to their broad clinical use.[10]

During a decade, 2004-2014, the incidence of candidemia due to *C. parapsilosis* was increased. This observation triggered the present study which aim was to compare the isolated *Candida* strains from blood cultures and their susceptibility in a tertiary hospital in Greece, in two different study periods consisting from 3-years each, Period 1 (P1): 01/01/2004-12/31/2006 and Period 2 (P2): 01/01/2012-12/31/2014.

Materials and Methods

During the study periods P1 and P2, blood culture vials from 15,723 and 20,626 patients were sent to the laboratory, respectively. BACTEC 9240 (BD) and BacT/ALERT 3D (bioMerieux) automated systems were used for the incubation of the vials. Positive samples from aerobic vials were cultured on McConkey, blood and chocolate agar while positive samples from anaerobic vials were cultured on blood and anaerobic blood agar. Sabouraud dextrose agar was added in cases where the presence of yeasts was confirmed in a blood sample of the positive vial by the Gram stain. The strains were identified by API-ID32C and/or VITEK II automated system (bioMerieux). Susceptibility testing was performed by VITEK II system and/or on RPMI agar by E-test method (bioMerieux), according to CLSI instructions.

Results

During P1, 2,605 blood cultures out of a total of 15,723 (16.6%), from equal number of patients, were found positive and during P2 3,406 blood cultures out of 20,626 (16.5%) were found positive. *Candida* strains were isolated in 71 out of 2,605 (2.7%) positive cultures for P1 and in 152 out of 3,406 (4.5%) positive cultures for P2. Candidemia in both study periods was the eighth most common cause of BSIs in our hospital, following in order of priority: *Staphylococcus epidermidis, E. coli, Klebsiella pneumoniae, Acinetobacter baumannii, Pseudomonas aeruginosa, Enterococcus faecalis* and *Enterococcus faecium*. Clinical sample distribution among hospital Units for both study periods appears on Figure 1. In the two study periods a significant increase (p: 0.0258), approximately double, in the incidence of candidemia was observed. The incidence of *Candida* species is shown on Table 1. Noticeable, was the predominance of *C. parapsilosis* in Period 2 (p: 0.0014), unlike with the predominance of *C. albicans* in Period 1 (Table 2). The susceptibility pattern and its changes during the two periods, of *C. albicans* and *Candida* non-*albicans* strains was studied in five antifungal agents: amphotericin B, flucytosine, fluconazole, voriconazole and caspofungin, out of which resistance was observed only in flucytosine, fluconazole and voriconazole, while in amphotericin B and caspofungin all strains were susceptible, as it is shown in Figure 2.

Discussion

The incidence of candidemia which was 2.7% and 4.5% during P1 and P2, respectively, remains moderately low in our hospital, according to international data, where the incidence ranges from 6-10% of the BSIs.[11-13] However, a significant increase, approximately double, was observed during the decade. The increase mainly concerns the Internal Medicine wards (from 45% to 58%), while in ICU remained the same and in Surgical Departments a decrease was observed. In an attempt to interpret this result, a hypothesis that can be made is that the attendance of patients has been increased in our hospital, especially, in the Internal Medicine wards, with a subsequent increase in hospitalization and a prolongation of stay (data esy.net, Greece, 2013). Furthermore, patients undergoing abdominal surgery, suffering from haematological malignancies and residing in ICUs are well known high risk groups. To these it must be add the use of central line catheters, parenteral nutrition, steroids, antibiotic treatment, renal replacement therapy and diabetes mellitus.[12, 14] Among the previous risk factors, a main concern in Greek reality is the high antibiotic consumption.[15]

Another noticeable change in the incidence of candidemia in this study was the predominance of *C. parapsilosis* in P2, unlike with the predominance of *C. albicans* in P1 which led to a small increase of *Candida* non-*albicans* strains in P2. Worldwide, the incidence

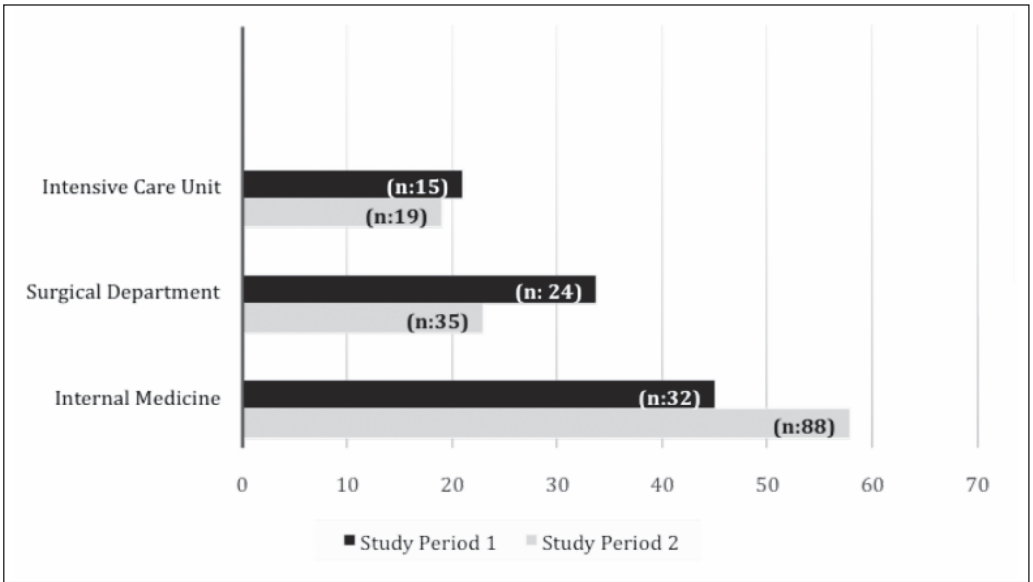

Figure 1 Origin of *Candida* strains (%) during the two study periods (Period 1: n=71, Period 2: n=152).
n: number of strains

Table 1 Incidence of species of *Candida* strains during the two study periods (Period 1: 1/1/2004-31/12/2006 and Period 2: 1/1/2012-31/12/2014)

Species	Study Period 1 n:71 (%)	Study Period 2 n: 152 (%)
C. albicans	29 (40.8)	53 (34.9)
C. parapsilosis	15 (21.1)	63 (41.4)
C. glabrata	13 (18.3)	14 (9.2)
C. tropicalis	7 (9.8)	5 (3.3)
C. famata	2 (2.8)	9 (5.9)
C. lusitaniae	1 (1.4)	
C. krusei	1 (1.4)	2 (1.3)
C. dubliniensis	1 (1.4)	1 (0.6)
C. guilliermondii		1 (0.6)
C. lipolytica	1 (1.4)	
C. kefyr		1 (0.6)
C. pelliculosa		1 (0.6)
C. rugosa		1 (0.6)
C. sphaerical		1 (0.6)
Candida spp	1 (1.4)	

Table 2 Changes in the origin and in the incidence of Candida species during the two study periods [Period 1 (P1): 1/1/2004-31/12/2006 and Period 2 (P2): 1/1/2012-31/12/2014]

Species	Internal Medicine		Surgical Department		ICU	
	P1 (n:32)	P2 (n: 88)	P1 (n:24)	P2 (n:35)	P1 (n:15)	P2 (n:29)
C. albicans	14	27	9	12	6	14
C. parapsilosis	7	39	5	15	3	9
C. glabrata	6	9	4	4	3	1
C. tropicalis	4	3	2	1	1	1
C. famata	1	5		1	1	3
C. lusitaniae			1			
C. krusei		1	1	1		
C. dubliniensis		1			1	
C. guilliermondii		1				
C. lipolytica			1			
C. kefyr						1
C. pelliculosa				1		
C. rugosa		1	1			
C. sphaerical		1				

of *C. albicans* and *Candida* non-*albicans* strains are approximately 50% each, but the species of Candida non-albicans varies a lot in different geographical parts. In USA and North Europe *C. albicans* remains the predominant strain (ranging from 45% to 58%). In opposition, *C. parapsilosis* fungemia is more frequent in South Europe (22% - 30%), South America (20% - 22%), Australia (20%) and Asia (20% - 50%) and, especially, in Japan (39%).[16-17] Albeit, fact remains that *C. parapsilosis* is, mainly, isolated from blood and indwelling medical devices as it is capable to form biofilms that increases the virulence.[18] Beside, according to in-

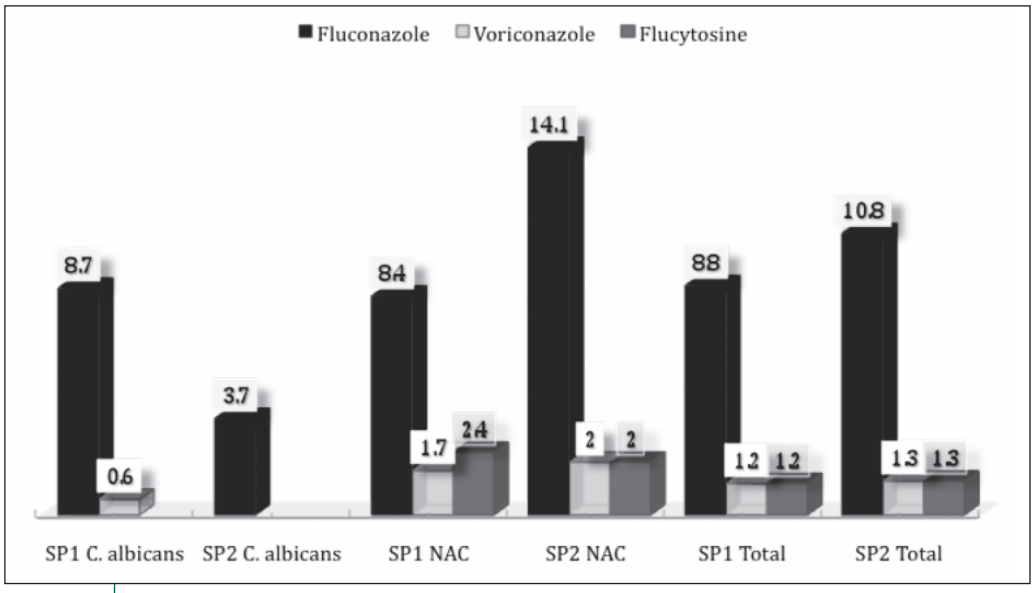

Figure 2 Resistance of *C. albicans* and non-*albicans Candida* (NAC) strains in antifungal agents during the two study periods [Period 1 (P1): 1/1/2004-31/12/2006 and Period 2 (P2): 1/1/2012-31/12/2014] (%).
SP1: Study Period 1, **SP2:** Study Period 2, **NAC:** non-*albicans Candida*
None of the strains exhibit resistance to amphotericin B and caspofungin.

ternational bibliography, *C. parapsilosis* incidence in BSIs seems to be increasing in correlation of an extensive use of echinocandines, most likely because the certain species is less susceptible to treatment with the specific drug.[19]

Regarding the resistance, no significant differences were observed in the two study periods. Resistance, mainly, concerned three antifungal agents, fluconazole, flucytosine and voriconazole, while no resistance was observed in echinocandines and amphotericin B. None of the above drugs exhibited an average resistance higher than approximately 10%. The higher rate was observed in fluconazole and *Candida* non-*albicans* were found to be more resistant than *C. albicans*. According to CDC's data, approximately 7% of all *Candida* BSIs isolates are resistant to fluconazole, most of which are *C. glabrata*, but the proportion of resistant strains remained almost the same over the past twenty years. Echinocandin resistance, on the contrary, seems to be on the rise with approximately 1% of all *Candida* strains tested at CDC showing echinocandin resistance which is, mainly, correlated with *C. parapsilosis*.[20-21] Additionally, echinocandin resistance is thought to be acquired from prior exposure to the drug.[22]

Nowadays, susceptibility testing for *Candida* spp is strongly recommended for all bloodstream isolates and other invasive or clinical significant candidiasis. Exposure to antifungal drugs consists an environmental stress that develops resistance, especially, in echinocandins[(14)]. The incidence of *C. albicans* resistance remains low and, mainly, concerns fluconazole where it ranges between 0.3% to 2%.[23]. Generally, *C. albicans* is susceptible to amphotericin B and echinocandins, although, non-susceptible to echinocandins strains has been reported.[24]

In our study, the most common non-*albicans Candida* strains were *C. parapsilosis*, followed by *C. glabrata* and *C. tropicalis*.

C. parapsilosis is usually susceptible to most antifungals but there are reports which reveal resistance to fluconazole, ranging from 2% to 6%.[23] In vitro data of *C. parapsilosis* susceptibility show MICs to echinocandins higher than all other *Candida* species; but the clinical significance of this fact is yet to be investigated.[25] Since 2005, *C. parapsilosis* has been renamed to *C. parapsilosis* complex which is composed from three distinct species: *C. parapsilosis, C. orthopsilosis* and *C. metapsilosis*. The differentiation between species is not possible in routine laboratories as it can be performed only molecularly. Nevertheless, *C. parapsilosis* remains the main isolated species while *C. metapsilosis* is considered as an environmental organism[(16)]. Some scientists propose the exact identification in species level, as *C. parapsilosis* exhibits higher MICs and resistance to echinocandins than the other two species.[21]

C. glabrata's resistance to fluconazole is well known and it is mostly due to changes in drug efflux.[14] Cross-resistance among the azoles is a common phenomenon for *C. glabrata* and, usually, concerns voriconazole.[26]

Although, echinocandins are the drug of choice for the treatment of candidiasis due to *C. glabrata*, references of resistance to this antifungal class are increasing, especially, after exposure to them.[27] In addition, there are reports of *C. glabrata* strains which are resistant to fluconazole and voriconazole and can become non-susceptible to echinocandins.[28]

C. tropicalis, which is usually seen in neutropenic patients, is mostly susceptible to all antifungal agents. Nevertheless, rare isolates resistant to caspofungin, in haematological patients, and to fluconazole have been reported.[23-24]

In summary, the epidemiology of candidemia and the susceptibility to antifungal agents seems to be a necessity when taking under consideration the different response to treatment of different *Candida* species.

Περίληψη

Μελέτη της εξέλιξης της συχνότητα των καντινταιμιών και έλεγχος ευαισθησίας των στελεχών *Candida* spp σε διάστημα μιας δεκαετίας σε τριτοβάθμιο γενικό νοσοκομείο

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Πρόσφατες μελέτες αποκαλύπτουν αύξηση στη συχνότητα των καντινταιμιών και αλλαγές που αφορούν τα είδη των απομονωθέντων στελεχών *Candida*, καθώς και της ευαισθησίας τους στα αντιμυκητιασικά φάρμακα. Σκοπός του παρόντος ήταν να μελετήσει τη συχνότητα απομόνωσης και να ελέγξει την ευαισθησία σε αντιμυκητιασικά φάρμακα στελεχών *Candida* spp απομονωθέντων από αιμοκαλλιέργειες τριτοβάθμιου Νοσοκομείου Αθηνών, σε δύο περιόδους, διάρκειας τριών ετών η καθεμία (Περίοδος 1: 1/1/2004-31/12/2006 και Περίοδος 2: 1/1/2012-31/12/2014). Κατά τη διάρκεια της μελέτης αιμοκαλλιέργειες από νοσηλευόμενους ασθενείς στάλθηκαν στο εργαστήριο για διαγνωστικούς σκοπούς. Κατά την Περίοδο 1 απομονώθηκαν 71 στελέχη *Candida* (2,7%) και την Περίοδο 2, 148 στελέχη (4,3%). Η προέλευση των στελεχών στην Περίοδο 1 ήταν: Παθολογικές κλινικές 32/71 (45,1%), Χειρουργικές κλινικές 24/71 (33,8%), Μονάδα Εντατικής Θεραπείας (ΜΕΘ) 15/71 (21,1%) και στην Περίοδο 2: 92/156 (59%), 35/156 (22,4%), 29/156 (18,6%), αντίστοιχα. Στην Περίοδο 1, το επικρατούν στέλεχος ήταν *C. albicans* (29/71-41%) και ακολουθούσαν *C. parapsilosis* (15/71-21%), *C. glabrata* (13/71-18%), *C. tropicalis* (7/71-10%) και *C. famata* (2/71-3%). Αντιθέτως, στην Περίοδο 2, το επικρατούν στέλεχος ήταν *C. parapsilosis* (64/148-43,2%) και ακολουθούσαν *C. albicans* (54/148-36,5%), *C. glabrata* (15/148,10-1%), *C. famata* (10/148-6,7%) και *C. tropicalis* (5/148-3,4%). Τα ποσοστά αντοχής στην Περίοδο 1 ήταν: fluconazole 8,8%, voriconazole 1,2%, flucytosine 1,2% και στην Περίοδο 2: fluconazole 10,8%, voriconazole 1,3%, flucytosine 2%. Όλα τα στελέχη *Candida* ήταν ευαίσθητα σε caspofungin και amphotericin B. Συμπερασματικά, παρατηρήθηκε σημαντική αύξηση, σχεδόν διπλάσια, της συχνότητας της καντινταιμίας κατά τη δεύτερη περίοδο μελέτης (p: 0,0258). Η συχνότητα της καντινταιμίας αυξήθηκε στις Παθολογικές Κλινικές, παρέμεινε παρόμοια στη ΜΕΘ και μειώθηκε στις Χειρουργικές Κλινικές. Αξιοσημείωτη είναι η επικράτηση της *C. parapsilosis* (p: 0,0014) στην Πε-

ρίοδο 2 σε αντίθεση με αυτή της *C. albicans* στην Περίοδο 1. Δεν παρατηρήθηκαν σημαντικές διαφορές στα ποσοστά αντοχής σε fluconazole, voriconazole, flucytosine στις δύο περιόδους, ενώ αποτελεσματική θεραπευτική αγωγή, όσον αφορά το νοσοκομείο μας, φαίνεται να αποτελούν οι echinocandines και amphotericin B. Η παρατηρούμενη αντοχή στα στελέχη *Candida* υπαγορεύει την ανάγκη κατευθυνόμενης θεραπευτικής αγωγής με βάση τον έλεγχο ευαισθησίας στις περιπτώσεις καντινταιμίας.

Λέξεις κλειδιά
καντινταιμία, εξέλιξη, επιδημιολογία, ταυτοποίηση, ευαισθησία, τριτοβάθμιο νοσοκομείο

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