Involvement of yeast species in fungaemia – an investigation of clinical data

Implicarea speciilor de levuri în episoade de fungemie - corelare cu date clinice

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Abstract

Invasive fungal infections have become increasingly prevalent in the recent decade and they have been associated with significant morbidity and mortality. Retrospective evaluation of mycological, clinical and epidemiological features of fungaemia episodes is important for the optimization of the diagnosis and treatment.

Material and methods: *Between August 2006 - 2008, 3284 blood cultures were processed using BacT/Alert Automated System (BioMérieux). We detected 23 fungaemia episodes from 23 patients (with 28 positive blood cultures sets). The mycological diagnosis was based on phenotypical analysis. A detailed retrospective-descriptive study was undertaken including 20 patients with available medical data.*

Results: *The 23 fungaemia episodes were caused by: Candida albicans (n=8), Candida parapsilosis (n=7), Candida krusei (n=3), Candida glabrata (n=1), Candida norvegiensis (n=1), two other non-albicans Candida species and Trichosporon asahii (n=1). Isolates were detected from patients hospitalized in intensive care unit (ICU) (n=13), adult haematology unit (n=6), paediatric haematology unit (n=3), interventional cardiology unit (n=1). Encountered risk factors were as follows: prior antibiotics and acid-controlling agents (H_2 antagonists and proton pump inhibitors) use, more than four days presence in ICU, central venous catheters, mechanical ventilation and administration of immunosuppressive drugs. Seven patients with severe marrow aplasia developed fungaemia. The infection with C. parapsilosis was definitely catheter-related in four children. At the time when fungaemia was detected five patients were already receiving antifungal treatment. Death occurred in 14 out of the 20 fungaemic patients.*

Conclusions: *1. This study demonstrates the increased involvement of non-albicans Candida species and the emergence of an opportunistic fungal pathogen: Trichosporon asahii in our hospital fungaemia episodes. 2. As adequate treatment is essential, rapid species diagnosis and in vitro susceptibility testing to antifungal*

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agents by standardized methods is important. 3. Aggressive antibiotic treatment preceded fungaemia in all patients, therefore we consider that implementation of an adequate policy is necessary in order to optimize antibiotic use.

***Keywords**: fungaemia, Candida spp., diagnosis, epidemiology*

Rezumat

Infecţiile fungice invazive sunt tot mai frecvent raportate, fiind însoţite de creşterea morbidităţii şi a mortalităţii. Evaluarea retrospectivă a caracteristicilor micologice, clinice şi epidemiologice ale episoadelor de fungemie este importantă în vederea optimizării diagnosticului şi a conduitei terapeutice.

***Material şi metode:** În perioada august 2006-2008, dintr-un număr total de 3284 hemoculturi procesate în sistemul automat BacT/Alert (BioMérieux), am identificat 23 episoade de fungemie la 23 de pacienţi (cu 28 de seturi de flacoane de hemocultură pozitive). Diagnosticul micologic a fost stabilit prin metode fenotipice. Am efectuat o analiză detaliată retrospectiv-descriptivă la 20 dintre bolnavii menţionaţi, cu date medicale disponibile.*

***Rezultate:** Cele 23 fungemii confirmate au fost cauzate de: Candida albicans (n=8), Candida parapsilosis (n=7), Candida krusei (n=3), Candida glabrata (n=1), Candida norvegiensis (n=1), alte 2 specii de Candida non-albicans şi Trichosporon asahii (n=1). Izolatele au provenit de la pacienţi internaţi în secţii cu profil de terapie intensivă (n=13), hematologie adulţi (n=6), hematologie copii (n=3) şi cardiologie intervenţională (n=1). Dintre factorii de risc recunoscuţi cei mai frecvent întâlniţi au fost: tratamentele anterioare cu antibiotice şi antisecretoare gastrice, internarea peste patru zile în secţie de terapie intensivă, ventilaţia asistată, utilizarea cateterelor venoase centrale şi a imunosupresoarelor. Şapte pacienţi cu aplazie medulară severă au dezvoltat fungemie. Relaţie definită cu cateterul venos central a fost înregistrată la patru copii, implicată fiind C. parapsilosis. La cinci pacienţi fungemia a apărut în cursul unui tratament antifungic anterior instituit. Decesul a survenit la 14 din cele 20 de cazuri.*

***Concluzii:** 1. Studiul obiectivează implicarea în fungemiile din spitalul nostru mai ales a speciilor de Candida non-albicans, dar şi a unui agent fungic oportunist emergent: Trichosporon asahii. 2. În scopul instituirii unei conduite terapeutice adecvate, considerăm importantă stabilirea precoce a diagnosticului de specie în corelaţie cu testarea susceptibilităţii la antifungice prin metode standardizate. 3. Toţi pacienţii au beneficiat de tratamente antibacteriene înainte de dezvoltarea fungemiei, din această cauză considerăm o necesitate adoptarea unei politici adecvate în domeniul antibioterapiei.*

***Cuvinte cheie**: fungemie, Candida spp., epidemiologie*

Introduction

Invasive fungal infections pose a serious threat to hospitalized patients worldwide [1]. They are associated with high morbidity, mortality, extended hospital stay and increase of the cost of medical care [30]. Systemic mycoses are a relatively rare condition, occurring primarily as a terminal event in patients with debilitating, neoplastic, immunosuppressive diseases and following organ transplantation [30].

Fungaemia was defined as the isolation of any pathogenic species of fungus from at least one blood culture specimen from a patient with signs and symptoms of infection. The recovery of fungus species from the bloodstream was considered to be a significant observation even in the absence of clinical signs or symptoms, especially if the patient was immunosuppressed, or receiving corticosteroid therapy [4].

Candidaemia is the fourth most important cause of nosocomial bloodstream infection in U.S.A. [10] and Candida species are the most frequent agents of opportunistic mycoses worldwide [21, 22]. Essentially all forms of invasive candidiasis begin as an episode of candid-

aemia. From a clinical point of view, four forms of invasive candidiasis are described:

- catheter-related candidaemia;
- acute disseminated candidiasis;
- chronic disseminated candidiasis;
- deep organ candidiasis.

Also, in the last two decades many studies mention the emergence and significant increase in incidence of non-albicans Candida species, Cryptococcus, Trichosporon, Malassezia, Rhodotorula, Hansenula species isolated from bloodstream infections [13, 17, 21]. Several reasons have been proposed including the extensive use of broad-spectrum antibiotics and antineoplastic agents, intravenous catheterization, prosthetic devices and grafts. Increased number of patients with neutropenia, immunosuppression and more aggressive surgery were also reported. The widespread use of fluconazole may account for the relative decrease in recovery of *C. albicans* from blood cultures compared to other Candida species [30].

The aim of these investigations was to evaluate the mycological, clinical and epidemiological features of fungaemia episodes in our hospital for the last two years.

Material and methods

Between 1st August 2006 and 31st July 2008, 3284 blood cultures were processed using BacT/Alert Automated System (BioMérieux) in the Department of Microbiology. A total of 28 positive blood cultures were analyzed from 23 fungaemic patients. The mycological diagnosis was based on the following tests:

- Gram-stained smear from blood cultures;
- growth on Columbia blood agar, Sabouraud's dextrose agar (SDA), chromogenic medium: Candiselect 4 (BioRad) at 37° C;
- germinative-tube formation;
- API Candida (BioMérieux);
- Vitek 2 Compact System (BioMérieux);
- *In vitro* susceptibility testing to antifungal agents was performed only for some isolates, by ATB™ Fungus 2 (BioMérieux) or by E-test (AB BIODISK) on RPMI 1640.

A detailed retrospective-descriptive analysis was undertaken with 20 patients whose medical data were available. We collected the following details: age, gender, date of admission, ward, date of candidaemia, underlying condition, antibiotics and/ or antifungal agents use prior microbiological confirmation of fungaemia. Duration of hospitalization and neutropenia, exposure to invasive medical procedures and other possible predisposing factors were also investigated**.**

Results

During the study period we detected 23 fungaemia episodes, which were caused by: *C. albicans* (n=8; 34.78%), *C. parapsilosis* (n=7; 30.43%), *C. krusei* (n=3; 13.04%), *C. glabrata* (n=1; 4.34%), *C. norvegiensis* (n=1; 4.34%), other species of non-albicans Candida (n=2; 8.69%) and *Trichosporon asahii* (n=1; 4.34%).

Isolates were recovered from 23 patients, 12 male and 11 female. Their median age was 38.43 years (range 2 – 69 years old). Candidaemia episodes occurred in patients hospitalized in intensive care unit (ICU) (n=13; 56.52%), adult haematology ward (n=6; 26.08%), paediatric haematology ward (n=3; 13.04%) and interventional cardiology unit (n=1; 4.34%).

Only 20 patients with available medical data were included in our study.

The most important underlying conditions were haematological malignancies (n=8), neurological diseases (n=3), liver diseases (n=3) and traumatisms (n=3) (*Table 1*).

Ninety percent of the 20 fungaemic episodes occurred 48 hours after admission to hospital. Candidaemia was generally a late complication during hospital stay, occurring at a median of 23.35 days after admission (range 1 – 115 days).

Each analyzed patient was subject to at least one risk factor. All patients received multiple broad-spectrum antibiotics prior to the fungal bloodstream infection (mean duration: 18.9 days, range 4 – 118 days). Fifty percent of the patients had more than four days presence in an ICU before the onset of fungaemia. Other coexisting factors, which are known to be predisposing to fungaemia episodes, were present in our patients, as follows: acid-controlling agents (H_2 antagonists and proton pump inhibitors) use (95%), indwelling central venous catheters (50%), corticosteroid agents (50%), chemotherapy (45%), mechanical ventilation (45%), surgical interventions (40%), severe marrow aplasia (35%) and metronidazol treatment longer than four days (20%).

In four patients, a bacteraemic episode preceded Candida spp. isolation, while in two subjects bacteraemia was diagnosed simultaneously with candidaemia.

C. parapsilosis was responsible for fungaemia in seven patients, four of which were children. Provided no other source of infection could be documented, the bloodstream infection with *C. parapsilosis* was definitely catheter-related in all four children (in cultures of the catheter tips obtained by roll-plate technique grew more than 15 CFU of the same Candida species).

Fever (range 37.2° C – 39.2° C) was the most frequent clinical manifestation of fungaemia. It appeared in 19 out of 20 patients (95%). The single afebrile patient (36.8° C) had haematological malignancy and neutropenia.

Simultaneously with candidaemia detection, 12 subjects had neutrophil count between 11.000/ µL and 37.000/ µL. We identified seven patients with severe marrow aplasia (leukocyte count < 1000/µL and neutrophil count < 500/µL). The mean duration of severe neutropenia before the development of the first fungaemic episode was 9.42 days (range 0 – 20 days).

Prior to the candidaemic episode, colonization with the same Candida species was microbiologically documented in five cases (from oropharyngeal samples, uroculture or bronchial aspirate) and in one patient the gastroscopic examination revealed the possible diagnosis of Candida oesophagitis.

In the last six months before the fungaemic episode, 65% of patients received systemic antifungal drugs, especially fluconazole. Five patients had been receiving fluconazole for 7 to 15 days leading to the day of Candida spp. isolation from a blood culture. The curative antifungal treatment was not administered to three patients (15%) because they died before the first positive blood culture. Fluconazole was the most frequently used drug as primary treatment (n=14), followed by voriconazole (n=5), caspofungin (n=2) and nystatin (n=1). Six subjects survived after candidaemia episodes and they were treated with antifungal drugs for a median duration of 12.66 days (range 7 – 21 days).

The crude mortality rate in our study was 70% (14 of 20 patients died). Six patients (30%) died within 3 days from the incident candidaemia, half of the six patients were diagnosed with septic shock and three patients did not receive curative treatment with antifungal agents. The rest (n=8) of patients died between the 12 and 39-day after positive fungal blood culture.

Discussion

Our study highlights the importance of fungaemia among hospitalized patients, developing in the presence of well-known risk factors.

The ARTEMIS global study revealed that *C. albicans* accounted for only 43% of Candida spp. isolates obtained from 14.887 blood cultures (134 institutions in 40 countries). *C. glabrata* and *C. tropicalis* accounted for 14%, respectively for 12% of all bloodstream isolates of Candida spp. [21]. The SENTRY Surveillance Program has shown that the only not-

Table 1. Diagnostic, clinical and epidemiological features of candidaemic patients in our hospital

Patient	Age (years)/ Sex	Underlying illness	Risk factors	Species	Curative antifungal therapy	Outcome
1. P.I.	60/M	Liver disease, Diabetes mellitus	A, B, C, G	*C. albicans*	nystatin	Died
2. H.S.	52/F	Neurological disease	A, B, C, G, E, K	*C. albicans*	fluconazole	Died
3. W.E.	67/F	Liver disease, Renal failure	A	*C. albicans*	-	Died
4.K.M.	60/F	Liver disease, Diabetes mellitus	A, B, C, G, H, J	*C. albicans*	fluconazole	Died
5. C.I.	51/F	Traumatism	A, B, C, G, H, J	*C. parapsilosis*	fluconazole	Died
6. C.C.	39/M	Traumatism	A, B, C, G, H, K	*C. parapsilosis*	fluconazole/ voriconazole	Survived
7. K.L.	21/M	Neurological disease	A, B, C, G, E, K	*C. norvegiensis*	-	Died
8. M.C.	12/M	Traumatism	A, B, C, E, G, H, K	*C. glabrata*	fluconazole	Died
9. R.M.	57/F	Autoimmune disease, Diabetes mellitus	A, B, C, D, E, G	*C. krusei*	fluconazole/ voriconazole	Died
10.D.M.	69/M	Haematological malignancy	A, B, D, F, I	*C. albicans*	-	Died
11. N.A.	66/M	Cardiac disease	A, B, E, H, K	*C. parapsilosis*	fluconazole/ voriconazole	Survived
12. S.K.	3/F	Haematological malignancy	A, B, D, E, F, I,	*C. parapsilosis*	fluconazole	Survived
13. P.S.R.	15/M	Haematological malignancy	A, B, D, E, F	*C. parapsilosis*	fluconazole	Survived
14. C.D.	2/M	Haematological malignancy	A, B, D, E, F, I	*C. parapsilosis*	fluconazole	Survived
15. P.E.	34/F	Neurological disease	NDA	*C. krusei*	NDA	NDA
16. E.C.	37/F	Haematological malignancy	A, B, D, F, I	*C. albicans*	voriconazole/ caspofungin	Died
17. V.Z.	24/M	Haematological malignancy	A, B, D, F, I	Non-*albicans Candida*	fluconazole	Died
18. B.V.	18/M	Haematological malignancy	A, B, D, F, I, J	*C. krusei*	caspofungin	Died
19. I.N.	56/F	Autoimmune disease	A, B, C, D, E, F, H	*C. albicans*	fluconazole	Survived
20. K.J.	40/M	Neurological disease	A, B, C, E, G, J, K	*C. albicans*	fluconazole	Died
21. T.M.	45/F	Haematological malignancy	A, B, D, F, I	*T. asahii*	fluconazole/ voriconazole	Died
22. V.R.	41/M	Abdominal disease	NDA	Non-*albicans Candida*	NDA	NDA
23. I.I.	15/F	Neurological disease	NDA	*C. parapsilosis*	NDA	NDA

Legend: A – broad-spectrum antibiotics; B – acid-controlling agents; C – presence more than four days in ICU; D – corticosteroid agents; E –indwelling venous catheters; F – chemotherapy; G – mechanical ventilation; H – surgical interventions; I – severe marrow aplasia; J – metronidazol treatment; K – nasogastric catheter; NDA – no data available

able geographic difference in the species distribution among three continents (North America, Latin America and Europe) was a higher frequency of *C. glabrata* as a cause of bloodstream infections in the United States of America [22]. In Europe (106 institutions in seven countries) *C. albicans* was identified in 56.4% of cases, followed by *C. glabrata* 13.6%, *C. parapsilosis* 13.3% and *C. tropicalis* 7.2% [26]. Species distributions, among 712 cases of candidaemia detected during recent prospective sentinel surveillance in Brazil, were as follows: *C. albicans* (40.9%), *C. tropicalis* (20.9%), *C. parapsilosis* (20.5%), *C. pelliculosa* (6.2%), *C. glabrata* (4.9%), *C. krusei* (1.1%) [8]. In our study, non-albicans Candida species and *T. asahii* were responsible for a total of 65.21% of fungaemia cases. We detected 30.43% *C. parapsilosis*, 13.03% *C. krusei*, one case of candidaemia induced by *C. glabrata* and no *C. tropicalis* strains were identified.

The increased use of invasive medical procedures as well as the prophylactic and empirical use of antifungal drugs, especially those of azolic derivates, has been mentioned for the emergence of non-albicans Candida species [19,30].

C. parapsilosis seems to have a special affinity for paediatric settings. This Candida species is part of the endogenous microbiota of human beings and is a commensal organism, which penetrates blood by the skin. The yeast is capable of biofilm production in glycosylated solutions and adheres to synthetic materials, such as catheters. Over the last few years, intravascular devices and total parenteral nutrition solutions have been related to *C. parapsilosis* fungaemia [7, 9]. Catheter-related candidaemia had a better outcome than did non-catheter-related candidaemia. Not surprisingly, removal of the catheter significantly ameliorates the disease [15].

In Italy, Bassetti et al. reported that *C. parapsilosis* surpassed the other non-albicans Candida spp. to become the most common species isolated after *C. albicans* [2]. The high incidence of *C. parapsilosis* candidemia has been previously reported in South American hospitals. The increased proportion of candidaemias due to *C. parapsilosis*, a yeast species almost always susceptible to fluconazole, is not readily explained by increased fluconazole use. It is likely that changes in the proportion of fungaemias due to *C. parapsilosis* reflect nosocomial acquisition of this species [2]. In our study, *C. parapsilosis* was predominant from all non-albicans Candida species (30.43%) and was identified from four children with indwelling central venous catheters.

C. krusei and *C. tropicalis* were more frequently recovered from patients with neutropenia who had lymphoma or leukaemia [30]. *C. krusei* and *C. glabrata* fungaemia were associated with previous exposure to azoles [8, 27]. Sixty-five percent of our patients received systemic fluconazole treatment prior to fungaemic episode and 40% of them had haematological malignancies.

After fluconazole received US Food and Drug Administration approval in 1990, it has been shown that receipt of fluconazole can promote *C. glabrata* colonization in a select patients' population [16, 29]. Also, several authors reported an increasing frequency of occurrence of *C. glabrata* with increasing patient age [22, 26]. Why *C. glabrata* is unusual in our hospital bloodstream infections is not clear, but the wide geographic variability of species distribution suggests that factors other than the use of fluconazole may be important, including demographic characteristics and the use of antibiotics.

As previously reported, we detected one fatal episode of fungaemia with *T. asahii* which occurred in a patient with severe marrow aplasia [11]. It is considered an emerging life-threatening opportunistic fungal pathogen. The crude mortality in invasive trichosporonosis is about 80% in patients with persistent neutropenia [12].

High rates of fungal infections in ICU were reported in many articles [5, 27]. This data

may be explained by the fact that these patients are usually considered at high risk, depending on life support, thus subject to multiple invasive procedures which make them more susceptible to a rapid fungal invasion. Most of the patients involved in our study (n=13) were from ICU.

Nosocomial fungal infections can originate from endogenous strains brought into the hospital environment by the patients themselves; alternatively, exogenous strains can be transmitted to the patients from contaminated infusates, biomedical devices, and the hands of health care workers [1]. Ninety percent of our 20 fungaemic episodes occurred 48 hours after admission to hospital.

The risk factors assessed in our study are also quoted by several authors [6, 8]. In our analysis it was seen that prior antibiotics and acid-controlling agents use, central venous catheters, corticosteroid agents, cytostatic drugs, mechanical ventilation, neutropenia were all present. The information about nasogastric and vesical catheters were not complete in our patients' medical histories.

Appearance of fungaemia may reflect the disturbance of the normal gut flora caused by the increased use of more potent broad-spectrum antibiotics. This is in agreement with our results where all patients had received more than three combined antibiotics prior to the onset of fungaemia. Restoration of the normal gut microbial ecology is a fundamental prerequisite for the control of the fungal infections [25, 28]. This requires limiting the use of antibiotics such as β-lactams and β-lactamase inhibitor antibiotics, fluoroquinolones, and carbapenems, all known to have an impact on gut ecology.

The clinical manifestations of fungaemia range from nothing more than fever to overt and life-threatening sepsis. Clinical picture of the patients with candidaemia caused by *C. albicans* and non-albicans Candida species are indistinguishable [6]. It is important to note that trichosporonosis may appear similar to disseminated candidiasis both in its clinical and histological appearance [12].

In five of our seven neutropenic patients we observed the implication of non-albicans Candida species and *T. asahii.* Only two of these patients survived (their central venous catheter was removed, they recovered from severe marrow aplasia and received adequate antifungal treatment). The rest of neutropenic subjects died (the severe marrow aplasia persisted).

Colonization with the same species of yeast has been reported to be an important independent risk factor for candidaemia [14, 23]. But, a prospective double-blinded study reported that despite a higher incidence of *C. glabrata* colonization in fluconazole neutropenic recipients, only one patient (compared with none in the placebo group) developed an invasive *C. glabrata* infection [16]. We consider that molecular typing of strains would be necessary to prove the implication of colonizing strains in later developing bloodstream infection. Molecular techniques have high discriminatory power in establishing the relatedness of clinically relevant Candida strains.

Laverdiere et al. reported that in neutropenic patients *C. albicans* colonization was reduced from 30 to 10% after fluconazole prophylaxis [16]. The optimal duration of prophylaxis with azoles is currently unknown, but it has been recommended that this should cover the period of neutropenia [20]. The role of fluconazole use in ICUs remains controversial. A recent guideline suggests that fluconazole prophylaxis in ICU may be considered in carefully selected patients if high rates of invasive candidiasis persist despite standard infection-control procedures [20]. Future investigations utilizing the colonization index may clarify the patient populations for whom antifungal prophylaxis may be warranted [16].

Five patients suffered abdominal or neurosurgical intervention before candidaemic episode. In case no. 11, candidaemia appeared after a coronarography procedure.

We performed *in vitro* susceptibility test to antifungal agents only for some isolates. It is well-known that yeast species susceptibilities to antifungal drugs are different. The ARTEMIS multicentric study revealed a consistently high level of resistance to fluconazole among *C. krusei*, *C. glabrata*, *C.guillermondii*, *C. inconspicua*, and *C. norvegiensis* [21]. A particular concern is the increased prevalence of species that are resistant to the azole antifungals. *Candida glabrata*, for example, is often resistant to fluconazole, and its ability to become cross-resistant to newer azole antifungals is a recent concern [8, 18]. *In vitro* sensivity assays for Trichosporon spp. have not been standardized by Clinical Laboratory Standard Institute (CLSI).

We identified three patients that were untreated and died within three days from the incident candidaemia. In our analysis, the most common treatment option administered was fluconazole.

In the Infectious Diseases Society of America (IDSA) guidelines for treatment of candidiasis published in 2004, caspofungin is recommended as the primary therapy for candidaemia in both neutropenic and non-neutropenic patients, as alternative therapy for candidial endocarditis and chronic disseminated candidiasis and as alternative therapy for oropharingeal and oesopharingeal candidiasis [20]. The echinocandins are not active against *Cryptococcus neoformans*, Trichosporon spp., Fusarium spp., or any zygomycetes. Breakthrough *Trichosporon asahii* fungaemia during caspofungin, posaconazole and itraconazole treatment was mentioned in several recent articles. Voriconazole has a potent activity against Cryptococcus spp., Trichosporon spp. [21] and Fusarium spp. IDSA recommends that candidaemia therapy should be continued for two weeks after the last positive blood culture result and resolution of signs and symptoms of infections [20].

Blood cultures have been reported to be negative in up to 50% of all autopsy-proven cases of invasive candidiasis [3]. The necroptic characteristics of the 14 died patients was not investigated in our research.

In our study, the crude mortality rate was 70% (n=14). This aspect is in agreement with several reports that mentioned a mortality rate associated with candidaemia between 35 and 75% [8, 24, 30]. Five patients out of the six survivors had bloodstream infections with *C. parapsilosis*. Candidaemia due to *C. parapsilosis* was associated with a lower mortality rate than the one due to *C. albicans* [8, 20]. The explanation for this effect may be that *C. parapsilosis* is less virulent in experimental animals than is *C. albicans* and does not adhere to and penetrate endothelium as well as *C. albicans*.

Conclusions

1. This study demonstrates the involvement of non-albicans Candida species and an emerging opportunistic fungal pathogen: *Trichosporon asahii* in our hospital fungaemia episodes.

2. As adequate treatment is essential, rapid species diagnosis and *in vitro* susceptibility testing to antifungal agents by standardized methods is important.

3. Aggressive antibiotic treatment preceded fungaemia in all patients, therefore we consider that implementation of an adequate policy is necessary in order to optimize antibiotic use.

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