An outbreak of candidemia due to *Candida parapsilosis* in an adult intensive care unit

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SUMMARY

Candida spp. is one of the most common causes of nosocomial bloodstream infections, and *Candida parapsilosis* is an emerging pathogen that is associated with nosocomial outbreaks. We aimed to characterize an outbreak of candidemia due to *C. parapsilosis* in our hospital's adult intensive care unit to investigate the clonal relationship of isolates. This is a retrospective study designed to investigate an outbreak of *C. parapsilosis* bloodstream infections (BSIs) which developed during an 11-week period from July to October 2012. Thirteen outbreak isolates and five isolates from the previous five sporadic cases were included in the study. Identification and antifungal susceptibilities of the isolates were determined by using MALDI-TOF MS (VITEK MS, bioMérieux, France) and by Etest (bioMérieux, France) on RPMI 1640-2% glucose agar (bioMérieux, France) at the Clinical Microbiology Laboratory. Clonal relationships were investigated by repetitive sequence-based PCR (rep-PCR) (DiversiLab, bioMérieux, Marcy L'Étoile, France).
The mean age of the cases, seven of which were female, was 61 years. The mean *Candida* score was 3, the mean length of stay in the intensive care unit (ICU) before infection developed was 33 days. A microbiological cure was obtained in nine (69.2%) patients with appropriate antifungals and catheter removal. Six patients died in a mean of 24 days. All of these isolates were obtained from blood culture, three being also obtained from CVC tips culture. Sixteen isolates were *C. parapsilosis*, and two isolates were *C. orthopsilosis*. All of the isolates were susceptible to amphotericin B, voriconazole, and caspofungin. Three isolates were resistant to fluconazole, and two isolates were dose-dependent susceptible to fluconazole. Out of the 13 outbreak isolates and five previous isolates, 11 and three, respectively, showed the same rep-PCR genotypic profile (genotype 1). Two isolates were the second same genotypic profile (genotype 2), and two isolates were the third same genotypic profile (genotype 3). The outbreak was under control in 11 weeks. The sporadic cases occurred in a subsequent three-month period. Our study shows that if *C. parapsilosis* isolates are present in a unit, it can become colonized in the unit and can spread clonally and rapidly, being able to cause a nosocomial outbreak. Moreover, even one isolate of *C. parapsilosis* in a unit can trigger an outbreak. Molecular typing methods are essential in order to illustrate the epidemiology of hospital outbreaks. Early detection of outbreaks is crucial for the implementation of infection control measures such as disinfection and isolation.

Keywords: *C. parapsilosis*, outbreak, molecular typing, antifungal susceptibility.

INTRODUCTION

Candida spp. is one of the most common causes of nosocomial bloodstream infections (BSIs). In the USA, *Candida* spp. is the fourth leading pathogen cause of nosocomial BSIs [1]. The mortality rate for *Candida* BSIs is approximately between 45-50% [2, 3]. Candidemia causes an increased length of hospital stay as well as increased hospital costs [4, 5].
Over the past decade, the incidence of *Candida parapsilosis* has dramatically increased in some areas [6]. *C. parapsilosis* is the second most common species found in patients with candidemia

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and is widely recognized as being associated with central venous catheters (CVCs), parenteral nutrition, and receiving a long duration of broad-spectrum antibiotic (previous 30 days of candidemia diagnosis) [3, 7].
C. parapsilosis is an emerging pathogen that is associated with nosocomial outbreaks, particularly in neonates [8]. The horizontal transmission of *Candida* spp. can be responsible for the presence of the outbreaks of candidemia [9, 10].
In this study, we aimed to characterize an outbreak of candidemia due to *C. parapsilosis* in our hospital's adult intensive care unit (ICU) between July 2012 and October 2012 to investigate the clonal relationship of isolates by molecular methods and to compare the clonal relationship retrospectively with the *C. parapsilosis* isolates causing blood-stream infections (BSIs) in the same ICUs in different previous times.

PATIENTS AND METHODS

Our hospital is a 680 bedside tertiary care training hospital that includes a 31-bed-side capacity ICU. In total, 13 patients developed *C. parapsilosis* BSIs during the 11-week period between July and October 2012. A retrospective study was designed to investigate the outbreak. The clonal relationships of *C. parapsilosis* outbreak isolates with *C. parapsilosis* isolates causing BSIs in the same ICUs in different times before are investigated retrospectively.

Case definition
The case was defined as the isolation of *C. parapsilosis* from at least one blood culture in a patient admitted to the ICU at least 48 hours before. Only the first isolate from each case patient was included in the study.

Descriptive characteristics
During the study, age, gender, ICU admission diagnosis, length of stay in the ICU before BSI, mechanical ventilation in the last two weeks before BSI, the use of a CVC catheter, urinary catheterisation, the use of TPN, the use of enteral feeding, hemodialysis applications, the use of H2-receptor antagonists, treatment with broad spectrum antibiotics in the last two weeks, *Candida* spp. colonization in the different anatomic cites, a surgical operation in the last one month before BSI, immunosupression, malignancy, receiving antifungal treatment, time of removing the catheter, microbiological recovery, and the outcome within 60 days of the data were recorded using a standardized form.

Microbiological studies
The identification and antifungal susceptibilities of the isolates were determined using matrix-assisted laser desorption/ionization-time-of-flight mass spectrometry (MALDI-TOF MS) (VITEK MS, bioMérieux, France) and by Etest (bioMérieux, France) at the Clinical Microbiology Laboratory. Each isolate from a 24-48 h culture on Sabouraud dextrose agar at 35°C was submitted to MALDI-TOF identification. Protein extraction was performed by spotting a colony on a plate of 0.5 µl of 28.9% formic acid (bioMérieux), as recommended by the manufacturer's instructions. Identification was determined using the database described in the manufacturer's instructions.
Etest agar diffusion testing was performed using RPMI 1640-2% glucose agar (bioMérieux, France). The agar surface was inoculated with a sterile swab dipped in an inoculum suspension adjusted to the turbidity of a 0.5 McFarland Standard. After the surface of the agar was completely dry (15 min at room temperature), each Etest strip was applied to each inoculated plate, and they were incubated at 35ºC and were read at 24 h. The MIC was taken as the lowest concentration of antifungal agent at which the inhibition zone of growth intersected the strips [11]. When the trailing endpoints occur, the MIC was read at the first point of significant inhibition of growth, approximately at 80% inhibition for azoles and caspofungin. The MIC was the endpoint at complete inhibition of growth for amphotericin B according to the bioMérieux, Etest antifungal susceptibility testing package insert, and Etest antifungal reading guide. MIC values of the isolates were evaluated using CLSI breakpoints. The fluconazole susceptibility breakpoints for *C. parapsilosis* were ≤2 µg/ml, S; 4 µg/ml, SDD; ≥8 µg/ml, R [12, 13].

Molecular typing
Clonal relationships were investigated by repetitive sequence-based PCR (rep-PCR) (DiversiLab, bioMérieux, Marcy L'Étoile, France). The DNA was isolated by the UltraClean microbial DNA isolation kit (Mobio Laboratories, USA),

and the procedures were performed according to the manufacturer's recommendations. The DNA concentration was measured and set between 35-80 ng/µl, and the DNA was amplified using the DiversiLab fingerprinting kit for *Candida* spp. (bioMérieux, France) according to the manufacturer's recommendations. The detection of rep-PCR products was made using the Diversilab™ DNA LabChip (Biomeriéux, France). The data was entered into the DiversiLab software system and analyzed with the system (version 3.4), which uses the Pearson correlation coefficient and the unweighted-pair group method with arithmetic averages to determine the distance matrices. The software system was also used to create dendrograms with a similarity matrix and gel image of the fingerprint for DNA. The reports were automatically generated. The isolates with a similarity of >97% were regarded as undistinguishably similar, with a similarity of 95-97% considered as similar and with a similarity of <95% regarded as different.

Ethical considerations

The Ethical Committee of the Istanbul Kartal Dr. Lütfi Kırdar Training and Research Hospital, Istanbul, Turkey, approved the study with a waiver of informed consent (2019/514/146/10).

RESULTS

During the 11-week period between July and October 2012, thirteen cases (patient sample IDs 1, 2, 3, 4, 5, 6, 7, 8, 9, 13, 16, 17, 18) were defined. Two test organisms (*C. parapsilosis* ATCC 22019 and *C. krusei* ATCC 6258) were included in the study. A total of 18 isolates, which were the isolates of the cases and 5 isolates (patient sample IDs 10, 11, 12, 14, 15) from the previous 5 sporadic cases in which *C. parapsilosis* was isolated between January 2011 and June 2012 and were investigated for a clonal relationship.

Descriptive characteristics

All of these isolates were obtained from blood culture, and three of these isolates were also obtained from CVC tips culture. The age, gender, and ICU admission diagnosis of the cases are shown chronologically in Table 1. The mean age of the cases was 61 years (range 19 to 89 years), and 7 of them were female. The characteristics of the cases are shown in Table 2. The mean *Candida* score was 3 (range, 1 to 5), and the mean length of stay in ICU before infection developed was 33 (range, 3 to 84 days) days. A total of four cases had a surgical operation (two abdominal surgeries). All of the patients, except one, had received wide-spec-

Table 1 - The ICU admission diagnosis of outbreak cases with chronological order.

Sample ID of patients	*Date of isolation (day/month/year)*	*Age (years) / sex*	*ICU admission diagnosis*
8	24/07/12	19/M	2°-3° Burn (30% total body surface area)
5	26/07/12	38/F	Multiple trauma and intracerebral haemorrhage
7	30/07/12	79/F	Lung cancer and shortness of breath
1	14/08/12	80/F	Intra-abdominal surgery
3	15/08/12	45/M	Multiple trauma, intracerebral haemorrhage and pneumothorax
9	15/08/12	49/F	Toxic epidermal necrolysis
2	24/08/12	83/F	Diaphragmatic hernia
6	24/08/12	89/F	Septic shock
4	03/09/12	37/M	Intra-abdominal surgery, pneumonia and alcohol abuse
13	28/09/12	70/M	Gastrointestinal bleeding
17	01/10/12	85/F	Cerebrovascular disease
16	04/10/12	46/M	Intracerebral haemorrhage
18	12/10/12	74/M	Coronary artery disease, pneumonia and shortness of breath

trum antibiotics in the previous two weeks. Five cases received carbapenem plus colistin therapy, three cases received carbapenem plus glycopeptide therapy, one case received colistin plus tigecycline therapy, two cases received carbapenem therapy alone, and one case received third-generation cephalosporin therapy alone. The mean time of removing the CVC catheter in place after the diagnosis of 12 patients was 1.5 days (range, 1 to 2 days). A microbiological cure was obtained in 9 (69.2%) patients with appropriate antifungals and catheter removal. Nevertheless, despite the early and appropriate empirical antifungal treatment, six (46.2%) patients with severe main diagnosis to the ICU admission died in a mean of 24 (range, 3 to 54 days) days.

Table 2 - The characteristics of outbreak cases.

Risk factors for candidemia	*n*
MV* in the last two weeks before BSI	12
Use of CVC**	12
Urinary catheterisation	13
Use of H2- receptor antagonists	13
Use of TPN***	10
Use of enteral feeding	3
Treatment with broad spectrum antibiotics in the last two weeks	12
Candida colonization in the different anatomic cites	6
Malignancy	2
Immunosuppression	2
Burn	1
Neurologic disorders	3
Surgical operation	4
Haemodialysis	1
Antifungal treatment	
Fluconazole	9
Caspofungin, fluconazole	2
Caspofungin	1
Caspofungin, anidulafungin	1
CVC removal	12
Outcome	
Microbiological recovery	9
Survival	7

*Mechanical ventilation, **Central venous catheter, ***Total parenteral nutrition".

Identification and antifungal susceptibility tests
Sixteen isolates were *C. parapsilosis*, and two isolates were *C. orthopsilosis* (patient sample IDs 11, 14). All of the isolates were susceptible to amphotericin B, voriconazole, and caspofungin. Three isolates were resistant to fluconazole (patient sample IDs 1, 4, 8), and two isolates were dose-dependent susceptible to fluconazole (patient sample IDs 10, 17).

Molecular typing
Out of the thirteen outbreak isolates and five previous isolates, eleven (patient sample IDs 1, 2, 3, 4, 6, 8, 9, 13, 16, 17, 18) and three (patient sample IDs 10, 12, 15), respectively, showed the same rep-PCR genotypic profile using the technique of rep-PCR (rep-PCR genotype 1). Two isolates (patient sample IDs 5, 7) were the second same genotypic profile (rep-PCR genotype 2), and two other isolates (sample ID of patients are 11, 14) were the third same genotypic profile (rep-PCR genotype 3). Fluconazole resistant isolates (patient sample IDs 1, 4, 8), and dose-dependent susceptible isolates (patient sample IDs 10, 17) were the same genotypic profile (rep-PCR 1) (Figure 1).

Outbreak control
The outbreak was under control in 11 weeks by repeated training in order to increase the compliance with following infection control measures in ICUs. We offered recommendations to the health care staff regarding the placement and maintenance of central venous catheters. Patients with candidemia were isolated to prevent transmission to others. Environmental cleanliness was made more efficient, and sterilization and disinfection of the medical devices and equipments as well as fluids of total parenteral nutrition were checked. Intensive care personnel were trained on hand hygiene, ways of transmission of infectious agents, isolation measures, and the use of personal protective equipment. After the outbreak, the sporadic cases occurred in a subsequent three-month period.

DISCUSSION

In this study, we described an outbreak of candidemia due to *C. parapsilosis* in our hospital's adult ICU. Out of the thirteen outbreak isolates and five previous sporadic isolates, eleven and three, respectively, were detected the same genotypic profile by using the technique of rep-PCR.

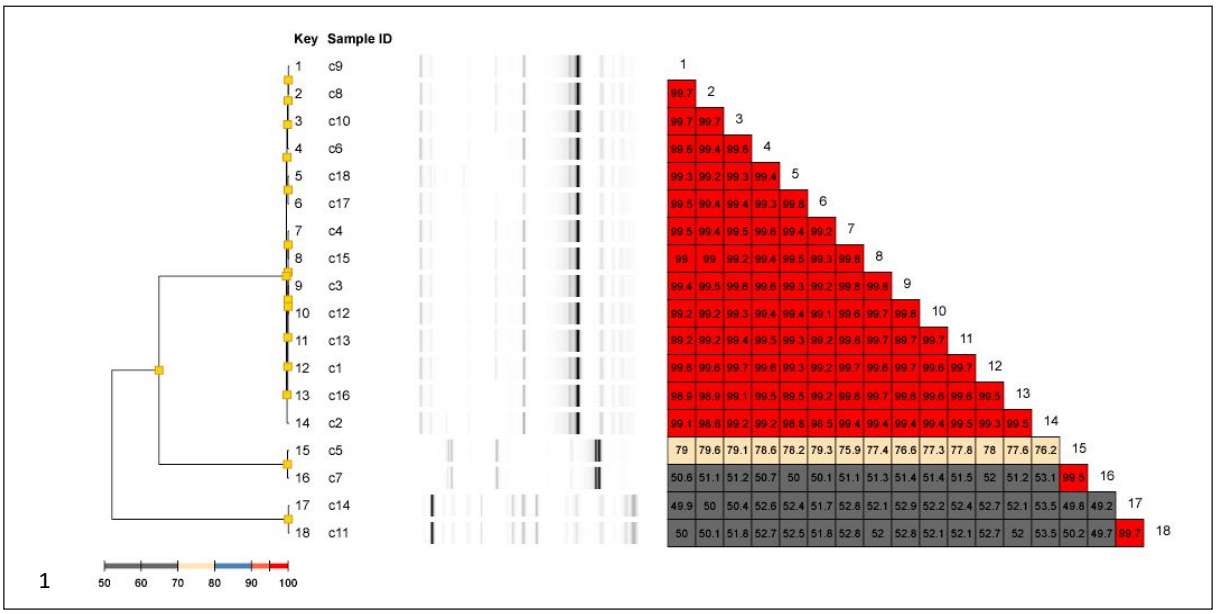

Figure 1 - Genetic analysis of *C. parapsilosis* isolates.

The following risk factors for candidemia have been identified in ICU patients: prolonged use of antimicrobials, central intravenous lipid emulsion, endotracheal tube, high APACHE II score, surgical patients, an indwelling urinary catheter, low birthweight, and prematurity for infants [14-16]. In our study, most of the outbreak cases had severe illness, mechanical ventilation, indwelling urinary catheter, CVCs, the use of broad-spectrum antibiotics in the previous two weeks, and the use of TPN.

The adhesion capacity of *C. parapsilosis* to animate and inanimate surfaces and developing biofilms on indwelling medical devices play a basic role in the pathogenesis of colonization and invasion of *C. parapsilosis* [9, 10, 17, 18]. Therefore, *C. parapsilosis* can persist in a hospital environment. Many studies have shown that health care workers can become contaminated with *C. parapsilosis* during patient care activities [10, 16, 19, 20]. Consequently, the horizontal transmission of *C. parapsilosis* can cause outbreaks in hospital wards. Most *C. parapsilosis* outbreaks reported in the literature are in neonatal ICUs [6, 14, 22-25]. However, even less than neonatal ICU outbreaks, adult ICU outbreaks have also been reported [26, 27]. In the literature, in some outbreaks, a source was not found, but outbreaks controlled support of infection control measures [27]. Although we searched for the source or sources of contamination, none were found.

C. parapsilosis is particularly frequent as a cause of CVC infection, and neutropenic patients are relatively more likely than non-neutropenic patients to have *Candida* bloodstream infections that originate in the gut [28]. Central venous catheters (CVCs) should be removed as early as possible in the course of candidemia when the source is presumed to be from the CVC [29]. The median time from diagnosis of candidaemia to catheter removal for our cases was 1.5 days. Consequently, we managed to get the outbreak under control in 11 weeks by repeated training in order to increase the compliance with infection control measures in ICUs.

In recent years, as a result of studies on the development of mycological identification, *C. parapsilosis* was separated into 3 distinct species, namely, *C. parapsilosis*, *C. orthopsilosis*, and *C. metapsilosis*. True species identification is becoming challenging, and expertise in molecular identification is not yet available in each clinical laboratory. MALDI-TOF MS is an alternative to conventional phenotypic methods, due to its short turnaround time, low cost, and the availability of in vitro diagnostic databases [30]. For accurate identification

of outbreak isolates, we used a MALDI-TOF MS system. We found that 2 isolates were *C. orthopsilosis* (patient ID numbers 11, 14), and they have the same genotypic profile (rep-PCR genotype 3). Although broth dilution methods for performing antifungal susceptibility testing of yeasts are recommended by the CLSI or EUCAST guidelines, the Etest is effective for determining susceptibilies to amphotericin B, azoles and echinocandins against *Candida* spp., it is recommended for the purpose of reproducible results, and it is easily used by technical personnel [11, 31, 32]. In some studies, *C. parapsilosis* isolates were reported as being susceptible to amphotericin B, fluconazole, voriconazole, and caspofungin [6, 27]. However, some studies demonstrate reduced susceptibilities to fluconazole and voriconazole [10, 33, 34]. In this study, antifungal susceptibilities of the isolates were determined by Etest. All of the outbreak isolates were susceptible to amphotericin B, voriconazole, and caspofungin. However, three of the outbreak isolates (16.7%) were resistant to fluconazole. Fluconazole-resistant isolates (patient sample IDs 1, 4, 8) and dose-dependent susceptible isolates (patient sample IDs 10, 17) were the same genotypic profile (rep-PCR 1).

The rapid and accurate identification of the etiological agents of BSI is crucial for treatment and identification of the outbreaks. Thus, molecular typing can help us to investigate suspected outbreaks in hospitals and can also help us to understand the source of the infection. Several genotyping methods such as microsatellite, randomly amplified polymorphic DNA [20], DNA fingerprinting, and rep-PCR have been used for the identification of candidemia outbreaks [19, 23, 35, 36]. We discovered the presence of an outbreak of *C. parapsilosis* in the adult ICU in our hospital by using the technique of rep-PCR.

One of the major limitations of this study is that it was unable to confirm antifungal resistance for fluconazole with a reference method and resistance genes using molecular methods (the A395T mutation in *ERG11* that is typical in azole-resistant isolates), while another limitation was its retrospective design.

CONCLUSIONS

We detected an outbreak of candidemia due to *C. parapsilosis* in our hospital's adult ICU. Our study shows that if a *C. parapsilosis* isolate is present in a unit, it can become colonized in the unit and can be spread clonally and rapidly and cause a nosocomial outbreak. Moreover, even one isolate of *C. parapsilosis* in a unit can lead to an outbreak.

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Conflicts of interest

The authors declare no conflicts of interest.

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