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Original Article

Candida albicans outbreak associated with total parenteral nutrition in the neonatal unit

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Abstract

Background: The most frequently isolated fungi in patients using TPN belongs to the *Candida* genus. Various infections including venous catheter infections, fungemia, endocarditis and ophthalmitis may be encountered. **Objective:** Upon growth of *Candida* in the blood cultures from the pediatric (neonatal) unit of our hospital, a surveillance was performed in this unit and involving the health care workers. Clonal relationships of the isolates were investigated with molecular tests. **Methods:** Blood samples obtained from the patients in pediatric neonatal unit were studied with automatized blood culture [BacT/Alert (Bio Mérioux, France)]. Yeast isolates from environmental surveillance cultures (TPN solutions, hands of healthcare personnel, étagère, etc) and patients were identified as *C. albicans* with conventional methods and ID 32 C and ATB™ Fungus 3 (Biomerieux, France) kits. Clonal similarity was determined by using AP-PCR as initial method and we have also typified all strains by the method of REP-PCR (diversilab system,bioMérieux). Finally; Pulsed Field Gel Electrophoresis (PFGE) was used for confirmation. **Results:** *C. albicans* was isolated in blood cultures of seven patients. Similar antifungal susceptibility patterns were observed in all isolates. AP-PCR and REP-PCR showed that the *C. albicans* isolates grown in the TPN solution and from the patients' blood cultures were clonally same strains. PFGE analysis further confirmed this clonality. **Conclusion:** According to results of the molecular methods, we thought that a *C. albicans* outbreak had occurred in the neonatal pediatric unit, due to contamination of TPN solution.

Key words: *Candida albicans, fungal infection, neonatal unit, parenteral nutrition*

Introduction

Candida species are the microorganisms that are normally found in human skin and mucosal flora. About 30–50% of normal individuals have *Candida* in mouth and gastrointestinal tract. The transmission occurs during birth or shortly after birth in neonates, and *Candida* replaces the normal flora.[1] Treatment is not needed in the absence of clinical symptoms when only a few *Candida* species are isolated from sputum, urine, faeces and vagina. However, it should be taken more seriously if *Candida* species are isolated from sterile body fluids (SBF) or tissue samples. There is also clinical significance when cultures grow large numbers of colonies.[2]

The medical and technological advances in Neonatal Intensive Care Units (NICUs) have led to lower mortality, particularly for the babies with very low birth weight and/or congenital abnormalities. However, nosocomial infections (NIs) are still a serious health problem.[3] *Candida* takes the third place in late-onset infections in NICUs and may lead to serious NIs.[4]

Moreover, *Candida* species are the most common fungal agents in the patients receiving Total Parenteral Nutrition (TPN). Common risk factors for *Candida* infections include immunosuppression, low birth weight, intravenous lines, TPN, broad spectrum antibiotic use and major surgical procedures.[4] Catheter-associated infections in such patients may result in candidaemia and fungal endocarditis.[5]

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Candida species produce a biofilm which is an important factor in the development of infections.[5,6] In particular, due to its lipid content, TPN solutions increase the budding and biofilm formation of *Candida albicans*,[7] consequently leading to sepsis after detaching from the biofilms.[8] Therefore, *Candida* species can cause hospital outbreaks by using the contaminated medical devices and/or TPN solutions.

We studied *Candida* samples that were isolated from the blood cultures of the patients and the samples collected from the healthcare workers and equipment in our hospital. We used molecular methods to perform epidemiologic analysis and investigate the genetic similarities between the strains.

Materials and Methods

The study was conducted at our Medical School Hospital, which has a 13-year history as a 600-bed research and application hospital. The clinic holds 33 beds, including 26 beds in the Paediatrics Clinic and 7 beds in the Paediatric Intensive Care Unit. The unit also has 7 patient rooms and 1 Intensive Care Unit, and it is equipped with three full-time nurses and three caregivers.

The days the blood samples were transported to our laboratory were as follows: The sample of the index patient on June 8, the sample of the second patient on June 28 and the other samples on July 6, 8, 11, 16 and 21, respectively. All the samples were identified within the 2 days after arrival. The same TPN solution was used for all the samples. The blood samples obtained from the patients hospitalised in Paediatric–Neonatal Intensive Care Unit were collected in blood culture bottles (BacT/Alert PF Pediatric) and processed in automated blood culture device (BacT/Alert, Bio Mérioux, France). No *C. albicans* species were detected in the blood samples obtained from the patients hospitalised in the Intensive Care Unit and the patients receiving no TPN.

Positive samples were determined in the automatised system, and the environmental samples were cultured on sheep blood agar and Saburoud Dextrose Agar. The plates were kept at 37°C for 24–48 h. The microscopic analysis and Gram-staining were performed on plates with colonial growth. The identification of the yeast and antifungal susceptibility of the samples were determined by using ID 32 C and ATB™ Fungus 3 (Bio Merieux-France) kits, respectively.

Three surveillance cultures were obtained from the rectum, skin and patient bed of each of the 28 patients hospitalised at the paediatrics clinic. A total of 96 surveillance cultures were collected from TPN fluids, shelves, examination tables, patient beds, infusion pump sets, intravenous fluids, stethoscopes, tables, keyboards, sinks, air conditioners and baby incubators in neonatal care unit with sterile saline-soaked swabs. In addition, skin cultures were obtained from the hands of 12 health care workers and 15 significant others for the investigation of *Candida* colonisation.

Sterile cotton-tipped swabs were used for the collection procedure after moistening them with sterile distilled water. On the day of collection, the collection swabs were collected from all the staff working in the shift of the collection day by rubbing the swabs all over the palmar surface, fingers and webs of both hands.[9] The conventional methods used for the blood samples were also used for the identification of the surveillance cultures.

The inclusion criteria for the study were >72-h of hospitalisation, receiving TPN and absence of *Candida* colonisation. The exclusion criteria included long-term antibiotic use, use of intravascular catheter, malignancy, *Candida* colonisation, candiduria, history of gastrointestinal surgery, neutropenia, diabetes mellitus and use of ventricular assist device.[10]

Clonal relations of the isolates were investigated by using the arbitrarily primed polymerase chain reaction (AP-PCR) analysis and repetitive sequence-based PCR (REP-PCR). The isolates were identified as "same type" or "subtype." Afterwards, the pulsed field gel electrophoresis (PFGE) method was used for the confirmation of the genetic relationships between the strains isolated from the blood culture and surveillance samples positive for *C. albicans*.

Molecular typing

Arbitrarily primed polymerase chain reaction analysis

Genomic DNA was extracted from each of the eight isolates by using QIAamp DNA mini kits with yeast protocol (Qiagen, USA). AP-PCR was performed and PCR amplification was performed in a 50 µl master mix containing approximately 50 ng of template DNA, ×1 amplification buffer, 0.4 mM dNTP mix, 4 mM MgCl 2, 2.5 U Taq DNA polymerase and 10 pmol M13 (5'-GAGGGTGGCGGTTCT-3') primer. The reaction mixture was amplified in a PalmCycler (Corbett Life Science, Sydney, Australia) using the following programme: 15 min at 95°C and then 40 cycles of 30 s at 95°C, 1.5 min at 40°C and 1.5 min at 72°C and final extension, 10 min at 72°C. The amplification products were electrophoresed in 2% agarose gel and then stained, observed and photographed under ultraviolet (UV) illumination. The analysis of AP-PCR band profiles was performed using the GelCompar software version 6.0 (Applied Maths, Courtrai, Belgium). If the Dice similarity coefficient value was below 90%, the isolates were accepted as different genotypes. If the value was between 90% and 95%, the isolates were identified as subtypes of the same strain; if the value was between 96% and 100%, the isolates were identified as the same genotype.

DiversiLab analysis

The isolates were cultured on Sabouraoud agar at 35°C for 24–48 h. Extraction of *C. albicans* DNA was performed using the UltraClean Microbial DNA Isolation Kit (Mo Bio Laboratories, Inc., Carlsbad, CA, USA) following the instructions of the manufacturer. A 5 U/µl enzyme of zymolyase (Zymo Research Corporation, Irvine, USA) was used and treated with sonicator for 15 min for shredding proteins and then quantified spectrophotometrically (MaestrogenNano, Taiwan, China) and diluted to 35 ng/µl with molecular grade water. REP-based PCR of extracted DNA was made using the DiversiLab *Candida* fingerprint kit (bioMérieux, Marcy l'Etoile, France). Afterwards, 35 ng of genomic DNA, 2.5 U AmpliTaq polymerase, 2.5 µl 10 PCR buffer (Applied Biosystems) and 2µl primer mix were added to the REP-PCR master mix in a total volume of 25 µl per reaction. Thermal cycling parameters were as follows: Initial denaturation at 94°C for 2 min, followed by 35 cycles of denaturation at 92°C for 30 s, annealing at 50°C for 30 s and extension at 70°C for 90 s, with a final extension at 70°C for 3 min. Amplified fragments of various sizes and intensities were separated and detected with DNA chip (bioMérieux, Marcy l'Etoile, France) on Agilent 2100 Bioanalyzer (Agilent Technologies, Palo Alto, California, USA). The results were demonstrated as dendrogram (with a Pearson correlation similarity matrix) including a virtual gel image of the fingerprint for each DNA sample. Strain-level discrimination was indicated between the samples of the same species with >95% similarity and minor band differences.

Pulsed field gel electrophoresis analysis

PFGE analysis was performed using a contour-clamped homogeneous electric field system (CHEF DR-II, BioRad, Richmond, USA) as described previously[11] with minor modifications. Yeast cells were grown on Sabouraud dextrose agar plates for 48 h at 35°C. Colonies were suspended in 2 mL suspension buffer (75 mM NaCl, 25 mM EDTA [pH: 8.6]), and the optical density was adjusted so that it was approximately equivalent to McFarland's Standard No. 5.0. After the digestion of the cells and washing of the plugs, the genomic DNA in the agarose plugs was restricted to 20 U of Sfi I (Promega Corporation, WI, USA). Fragmented DNA was electrophoresed in 1% of pulsed field certified agarose (Bio-Rad Laboratories, CA, USA). The electrophoresis conditions were 14°C at 4 V/cm^2 for 20 h. The initial and final switch times were 5 s and 50 s, respectively. Each gel was stained with ethidium bromide (5 mg/mL) and photographed under UV light. The DNA band profiles were analysed with GelCompar software version 6.0 (Applied Maths, Courtrai, Belgium). Depending on the criteria of Tenover *et al*., the strains were evaluated as indistinguishable, closely related, possibly related or different.[12]

TPN solution ingredients were 3% NaCl (Polifarma Drug Company), 30% dextroz (Eczacıbaşı Baxter), 6% trophamine (Eczacıbaşı Baxter), 10% dextroz (Polifarma) and intra-lipid 20% (Fresenius Kabi).

Results

A positive signal was detected in a blood culture of a newborn girl (index case), and the isolate was identified as *C. albicans*. This patient had multiple medical problems including pre-maturity, respiratory distress syndrome, sepsis, necrotising enterocolitis and patent ductus arteriosus. In addition, *C. albicans* growth was detected in the blood cultures of additional patients in the same unit. Of these, the second patient presented with haemolytic uremic syndrome, invagination and hyponatraemia and was receiving Na treatment; the third patient had been born as oligohydramnios and had anal atresia; the mother had gestational diabetes, and in this patient, the treatment was terminated and then TPN solution was given; the fourth patient started TPN following the termination of oral intake due to asphyxia and convulsion; the fifth patient was hospitalised due to pre-maturity and the patient started enteral feeding but then switched to TPN due to sepsis; the sixth patient started TPN after being hospitalised due to omphalocele and then operated on at the paediatric surgery clinic; the seventh patient started TPN after being hospitalised at the NICU due to birth asphyxia anoxia. Due to the detection of these patients, we collected surveillance cultures from the environment, the patients and the healthcare workers in the unit. No *C. albicans* were detected in the samples obtained from the hands and the bodies of the patients and the healthcare workers and the samples collected from the hospital environment. Nevertheless, only one sample, which was obtained from the TPN solution, was positive for *C. albicans*. This strain showed almost the same antifungal susceptibility pattern with the strains that were previously isolated from the patients. All the isolates were susceptible to amphotericin and flucytosine and resistant to fluconazole, itraconazole and voriconazole [Table 1].

The AP-PCR method showed that the initial isolates were closely related. Therefore, we thought that these consecutive isolations were due to an outbreak. To identify the transmission dynamics, we performed surveillance on the physical environment and healthcare workers. The infection control team increased the preventive measures. As a result, a total of 7 patient isolates and one environmental isolate from the TPN solution were obtained. The strains were revealed as similar clones when typified by AP-PCR [Figure 1] and as same type when typified by REP-PCR [Figure 2]. Finally, the PFGE analysis also confirmed that all the isolates were closely related; however, due to the faint bands, we decided not to include the images into this study since they were low quality.

Discussion

Intravascular device use is a common medical treatment modality which also leads to substantial risks in medical

Table 1: Antibiogram and arbitrarily primed-polymerase chain reaction analysis, repetitive sequence-based polymerase chain reaction and pulsed-field gel electrophoresis results of Candida albicans strains grown in total parenteral nutrition and blood samples

Sex and age	Antifungal Susceptibility					Genotyping*
	Flucytosine	Amhotericin	Fluconazole	Itraconazole	Voriconazole	
M (1st month)	S	S	R	R	R	A
F (1st month)	S	S	R	R	R	A
M (1st month)	S	S	R	R	R	A
F (1st month)	S	S	R	R	R	A
M (1st month)	S	S	R	R	R	A
M (1st month)	S	S	R	R	R	A
F (1st month)	S	S	R	R	R	A
TPN	S	S	R	R	R	A

*Common genotyping results (AP-PCR, REP-PCR and PFGE). TPN: Total parenteral nutrition, PFGE: Pulsed-field gel electrophoresis, S: Sensitive, R: Resistance, AP-PCR: Arbitrarily primed polymerase chain reaction analysis, REP-PCR: Repetitive sequence-based polymerase chain reaction, M: Male, F: Female

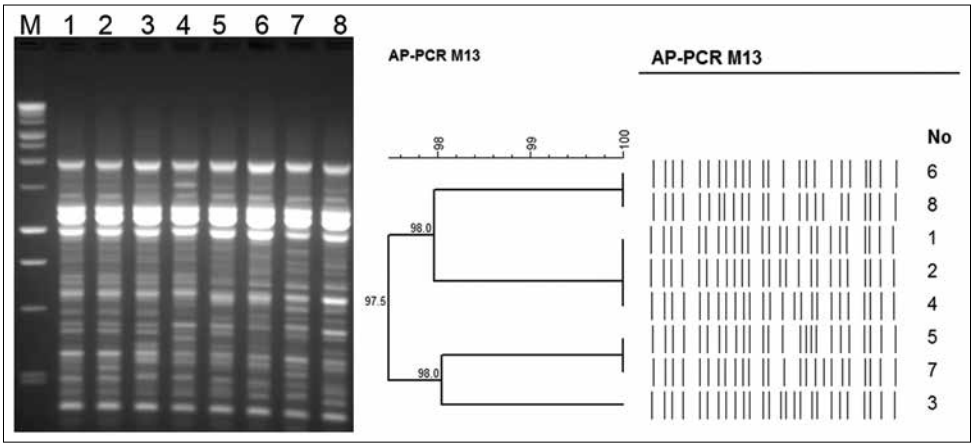

Figure 1: All strains based on Dice similarity coefficient are similar to the rate of 97.5%. Accordingly, all the clonal isolates were either the same or not distinguished

practice. NI risk is one of these risks. Central venous catheter-related bloodstream infections account for 10% of all NIs. These infections are commonly caused by coagulase-negative *Staphylococcus*, *S. aureus*, *Enterobacter* spp., *Candida* spp., *Klebsiella* spp., *Pseudomonas* spp., *Escherichia coli* and *Enterococcus* spp.[13-16]

Sepsis caused by the TPN that is used as a supportive medical modality is a common condition and leads to serious complications.[17] The lipid ingredient in the TPN solutions is held responsible for coagulase-negative Gram-positive cocci, *E. coli* and *S. aureus*, besides *C. albicans*.[18,19] However, the microorganism growth other than *Candida* growth is associated with the pH of the solution. The growth of other microorganisms is inhibited when pH is 5 or less.[18] We believe that since the contaminated TPN solutions in this study had 20% lipid ingredient, they contributed to the growth of *Candida* species. Although the pH of the solution used in this study was unknown, we assumed that the pH was excessively high, based on the *Candida* overgrowth without other microorganism growth.

Candida spp. are the most frequent cause of fungal infections in patients receiving TPN,[8] and *C. albicans* is the leading strain.[17] In a surveillance study, 56% of *Candida* infections were caused by *Candida glabrata* and 23% of the infections by *C. albicans*. In that study, various complications were observed including venous catheter infections, catheter-related candidaemia, fungal endocarditis and fungal ophthalmitis.[5] *Candida* outbreaks due to TPN solution are caused by the contamination from different sources during the preparation phase. In one study, *Candida tropicalis* grew in six out of the 29 blood cultures collected from NICU. Similar organisms were isolated from the nail samples of room service personnel with mild onychomycosis and asymptomatic nurses. The infected patients received long-term multiple antibiotics and TPN solution.[20] In our study, we found that *C. albicans* was the causative agent of blood stream infection in six patients due to the use of TPN solution and the use of broad-spectrum antibiotics that was probably contaminated with this strain. Moreover, *C. albicans* spp. were more commonly isolated in our study compared to *C. glabrata* spp., which indicates

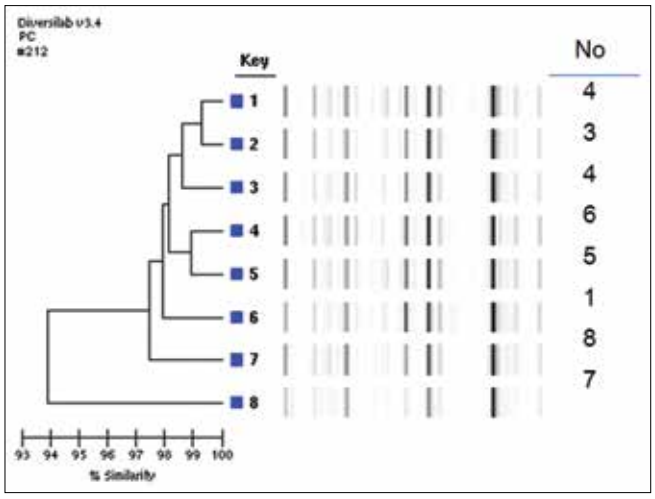

Figure 2: Dendrogram and virtual gel image fingerprints obtained from 8 *Candida albicans* strains using the DiversiLab system. Pearson's correlation coefficient was used to create a pairwise percentage similarity matrix and the dendrogram was derived using unweighted pair group method with arithmetic averages. A 95% similarity threshold (vertical line) was chosen for *Candida albicans*

that *Candida* outbreak is more likely to be caused by TPN solution.

In *Candida* outbreak, *C. albicans* growth was seen in the blood sample of the index case on the 1st week of June and after this, the growth was seen in the blood samples other patients from the final week of June to throughout July. However, no *C. albicans* growth was seen in the samples collected from the environments and bodies of the infants and the hands of the healthcare workers. For this outbreak, TPN solution is commonly used. In our study, the solution was manually prepared by the healthcare workers and given to the patients. The analyses revealed that *C. albicans* growth occurred only in the patients requiring and using TPN but not in the patients not using TPN.

Molecular typing methods are useful in the surveillance and control of nosocomial outbreaks since they can provide information on the clonal relatedness among the isolates, identify reservoirs and determine the routes of transmission. PFGE, due to its high discriminatory power, is the gold standard assay for the molecular typing of many microorganisms. However, PFGE exhibits several major disadvantages including high cost of equipment, labour intensiveness (due to non-automaticity) and the excess time required to analyse the profiles of DNA bands (pulsotypes). Although there are many molecular typing methods based on PCR, the most widely used is REP-based PCR and AP-PCR. Most of the PCR techniques used for molecular typing have none of the limitations of PFGE since they are less costly and labour intensive (such as bioMérieux's DiversiLab system) which are commercially available, rapid, designed with high sensitive technology and widely available in clinical laboratories for pathological and epidemiological analyses,[21] and they also generate DNA profiles that are easier to evaluate, depending on the microorganism. The discriminatory power of PCR is generally lower than or similar to that of PFGE. Both PFGE and PCR require optimal laboratory standardisation to guarantee good reproducibility. PCR methods are preferable in the study of small and time-limited outbreaks, whereas PFGE is preferable in more complex outbreaks with longer duration, in which clonal evolution and dynamics are studied.[22] Therefore, in this study, the similarities between the strains were initially evaluated by AP-PCR and REP-PCR (DiversiLab bioMérieux, Marcy l'Etoile, France) and then confirmed by PFGE.

In this outbreak, we observed that AP-PCR and REP-PCR were rapid methods which could give the initial clues whether the recorded cases resulted from an epidemic or sporadic infection within almost half a day. Moreover, these methods were also cost-effective and reliable and they provide reproducible results. In this outbreak, our first decision was made depending on the results of these methods. Following this, we notified the infection control team and they increased the preventive measures. Following the epidemic, the manual methods used for the preparation of TPN were abandoned that they were replaced by automated methods. After the outbreak had finished, all the strains were studied with PFGE. The three methods showed that all the strains were closely related; suggesting that the outbreak occurred due to clonal spread of an epidemic *Candida* strain and the TPN solution was most likely to be responsible for this outbreak.

Early detection of an outbreak and understanding the ways of dissemination are important to prevent the spread of an epidemic strain. Therefore, we suggest that AP-PCR and REP-PCR (DiversiLab system) can be used at the initial diagnosis of hospital outbreaks. However, since PFGE is still the reference method, further studies are needed to confirm this finding.

Conclusion

C. albicans is one of the most important problems in the hospitalised patients receiving TPN solution. Therefore, the highest attention must be paid to prevent the external contamination of TPN solution since the infections are associated with high mortality.

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Nil.

Conflicts of interest

There are no conflicts of interest.

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