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Can Blood Culture Contamination Cloud Fungal Positivity?

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ABSTRACT

Objective: Blood culture (BC) is gold standard for diagnosis of fungemia. Contamination of BC is a major problem worldwide, since it may delay actual diagnosis. The aim of this study was to evaluate "contaminant" vials with a prolonged incubation (max 30 days) with antimicrobial supplemented media to observe any mycological growth. **Materials and Methods:** Routine BCs obtained patients of Balıkesir Atatürk City Hospital for a year period were included. Render BC System BC12-8 (Render Biotech Co. Ltd., Shenzhen, China) were used. Contaminated vials were re-incubated and conventionally inoculated weekly for four weeks total. In case of any growth, identifications were done by Phoenix™ 100 system (Becton Dickinson, MA, USA) with cornmeal tween 80 agar (RTA Laboratories, Kocaeli, Türkiye). Antifungal susceptibility testing was applied with CLSI disk diffusion method. **Results:** 3.9% (235 sets and additional 138 vials) of total 6047 BC sets (23.06% positive) were contaminated. Only one vial from central venous catheter showed fungal growth within the first week of conventional inoculation (>8th day of total incubation). The isolate was identified as *Candida guilliermondii* complex and susceptible to caspofungin. Latter set of this patient were positive for the same fungi in the 3rd day of incubation. **Conclusion:** International guidelines recommend <3% contamination rate. In this study, our single strain was isolated from catheter vial with prolonged incubation and following set was routinely positive for the same fungi. For optimal isolation at least 2 following sets were required and thus, prolonged incubation was not beneficial. It was found that strict following of the rule of "2 following sets" was enough for optimal isolation of fungemia agent.
Keywords: Invasive Fungal Infections, Blood Circulation, Infections, Contamination, *Candida*.

Kontamine Kan Kültürleri Fungal Pozitifliği Gölgeleyebilir Mi?

ÖZ

Amaç: Kan kültürü kontaminasyonu dünyada ciddi bir sorundur ve kan dolaşımı enfeksiyonu tanısını geciktirebilmektedir. Bu çalışmanın amacı, kontamine şişelerin uzatılmış inkübasyonu ile mikolojik üreme elde edilip edilemeyeceğinin araştırılmasıdır. **Gereç ve Yöntem:** Balıkesir Atatürk Şehir Hastanesi'nde bir yıl boyunca erişkin hastaların kan kültürleri Render BC128 Sistemiyle (Render Biotech Co. Ltd., Çin) çalışmaya alındı. Kontamine kan kültürü şişelerinin inkübasyonu geleneksel yöntemlerle haftalık ekilerek 30 güne tamamlandı. Üremelere Phoenix™ 100 sistemi (Becton Dickinson, ABD) ve mısır unu tween 80 agar (RTA Laboratories, Türkiye) ile tanımlama, CLSI disk difüzyon ile antifungal duyarlılık yapıldı. **Bulgular:** Toplam yıllık 6047 kan kültürü seti (%23.06 pozitif) işlendi ve bunların %3.9'u (235 set ve ek 138 şişe) kontamineydi. Sadece santral venöz kateterden alınan bir şişede anlamlı fungal üreme oldu (toplam inkübasyonun 8. Gününden sonra). Kaspofungine duyarlı *Candida guilliermondii* kompleks olarak tespit edildi. Bu hastanın takip eden ikinci seti 3'üncü gün pozitif sinyal verdi. **Sonuç:** Uluslararası rehberler, kontaminasyonu <%3 olarak önermektedir. Bu çalışmada, kateterden alınan kan kültürü şişesinde uzatılmış inkübasyonla fungal üreme oldu ve takip eden set aynı mikroorganizma için rutin inkübasyonda pozitifti. Optimal izolasyon için en az 2 takip eden set alınması gerektiğinden, kontaminasyon tanıyı geciktirmedi ve kontamine şişelerin uzatılmış inkübasyonu fayda sağlamadı. "2 takip eden set" kuralına uyum ile optimal düzeyde fungemi etkeninin izole edilebileceği sonucuna varıldı.
Anahtar Kelimeler: İnvazif Fungal Enfeksiyonlar, Kan Dolaşımı, Enfeksiyonlar, Kontaminasyon, *Candida*.

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INTRODUCTION

Invasive fungal infections (IFIs) show an increasing trend. They have high mortality, and nearly 2 million individuals are lost annually due to these infections. Predisposing factors including hospitalization to intensive care units (ICUs), elder ages, mechanic ventilation and other invasive procedures, usage of immunosuppressing drugs are common, however, there are cases reported without any such conditions (Arıkan Akdağlı et al., 2019; Gülmez et al., 2021; Seagle et al., 2021). Fungemia is a serious IFI condition and the most prevalent causative organisms are *Candida* spp. Organism varies due to geographic location, clinical status and underlying diseases, *Candida albicans*, *Candida glabrata* complex, *Candida parapsilosis* complex, *Candida tropicalis* and *Candida krusei* stand at the top (Gülmez et al., 2021; Seagle et al., 2021).

Prognosis of IFI is strongly associated with early diagnosis and immediate treatment. Blood culture (BC) has a crucial role in microbiological diagnosis of fungemia (Arıkan Akdağlı et al., 2019; De Plato et al., 2019; Lamy et al., 2016; Seagle et al., 2021). Contamination of BCs is a major problem worldwide, not only because of clinical issues but also economic burden. The American Society for Microbiology (ASM) recommends contamination rates ≤3%, and discrimination of so-called contaminant microorganisms, whether they are pathogens or not, is a controversial condition (Mataj et al., 2020).

Incubation takes max 5-7 days for a BC vial, however fungal agents may require an incubation period up to 30 days (Lamy et al., 2016; La Rocco, 2010). On the other hand contaminant agents, particulary bacteria, can show positivity in this 7-day duration (Osaki et al., 2020). This might delay detection of fungemia, especially in case of miscatch in microscopy. The aim of this study was to evaluate "contaminant" vials with a prolonged incubation (max 30 days) time and to observe any mycological growth, if exists.

MATERIALS AND METHODS

Study type

This randomized study was conducted through a prospective style.

Study group

Routine BCs obtained from adult and pediatric patients of Balıkesir Atatürk City Hospital for a year period (1st Nov 2020–1st Nov 2021) were included. Render Automated Blood Culture System BC128 (Render Biotech Co. Ltd., Shenzhen, China) were used for BCs. All BCs were inserted to device within two hours (>98%) and it could be followed by our hospital software. Patients with disrupted pre-incubation periods were not included to the study.

Procedures

In routine cultivation, one set were accepted and applied as one aerobic, one anaerobic vial. Vials that gave positive signal and evaluated as contamination were re-incubated in 35°C (Four weeks total incubation). Once weekly, gram staining and inoculations onto SDA medium with chloramphenicol and gentamicin (RTA Laboratories, Kocaeli, Türkiye) and ROSACHROM Candida Agar (Gül Biology Laboratories, Istanbul, Türkiye) were applied. Plates were incubated in 35°C, 5% CO_2 atmosphere and for 48h. Fungal identifications were performed by Phoenix™ 100 system (Becton Dickinson, MA, USA) and cornmeal tween 80 agar (RTA Laboratories, Kocaeli, Türkiye). Antifungal susceptibility testing (AFST) was applied with disk diffusion method (Fluconazole 25µg, Voriconazole 1µg, Caspofungin 5µg; Bioanalyse, Ankara, Türkiye) according to The Clinical and Laboratory Standards Institute (CLSI)-M60 guideline (Clinical and Laboratory Standards Institute, 2017; Clinical and Laboratory Standards Institute, 2018). *Candida parapsilosis* ATCC 22019 and *Candida krusei* ATCC 6258 were used for quality control purposes.

Statistical analysis

This study is a descriptive analysis, ratios were shared.

Ethical considerations

Approved by Ethical Board of İstanbul Medipol University. Date: 05.08.2021 Number: 2021/796.

RESULTS

A total of 6047 BC sets were obtained and samples of same episodes of the same patients were excluded (different episodes were included). 23.06% (n=1395) of sets showed positivity in at least one vial, and 3.9% (235 sets and additional 138 vials) of sets were evaluated as contamination. Contaminant microorganisms were predominantly coagulase-negative staphylococci (84.3%) (CoNS), followed by *Micrococcus* spp., *Corynebacterium* spp, *Bacillus* spp. and other gram-positive bacilli.

Only one vial from central venous catheter (CVC) (other peripheral vial of the set were negative) showed fungal growth within the first week of conventional inoculation (>8th day of total incubation). All positive vials were undergone to Gram staining, this vial was contaminated by *Corynebacterium* spp. and its microscopy was positive for gram positive bacilli. However, yeasts were not observed. The isolate was identified as *Candida guilliermondii* complex. The strain was susceptible to caspofungin (14mm), however susceptibility tests for other antifungal could not be performed due to lack of zone diameters.

Latter set of this patient were positive for the same fungi in the 3rd day of incubation. The patient was not on empiric or preemptive antifungal treatment in the first sampling time; however he had elder age (65+), chronic renal insufficiency, hypertension and diabetes mellitus. He had CVC and was on vancomycin (25mg/kg/day) and meropenem (6g/day) treatment due to his worsening clinical status, altered acute phase reactants and fever (39°C). His radiologic screening did not indicate any pneumoniae, meningitis or other source of infection.

Following the fungal positivity, CVC was removed and caspofungin treatment was initiated to the patient. The patient was accepted as "cure" after two week of caspofungin (70/50 mg) treatment according to ESCMID guide (Cornely et al., 2012).

DISCUSSION

BSIs are a global threat to public health with 13% to 20% case-fatality rate, however mortality of fungemia seems to be more severe (over 70%). Despite of *Candida* spp. stand at top of the aethiologic agents, other species such as *Cryptococcus* spp., *Rhodotorula* spp. or *Trichosporon* spp. might also cause IFIs (Kotey et al., 2021). Early detection and treatment have proven to have crucial role for prognosis, especially in guidance of antifungal stewardship committees (Butta et al., 2019; Dudakova, 2022). However, indirect diagnostic procedures (serology, etc.) remain to be insufficient for exact diagnosis because of several reasons, thus importance of BC seems to be expanded (Cuenca-Estrella et al., 2012). Species-level identification is crucial for an appropriate therapy, which also requires BC positivity and biochemical and/or automated identification procedures (Lin et al., 2019). These procedures result in a minimum of 3-4 days, which delays a targeted treatment, so empiric, preemptive and prophylactic antifungal managements were defined in European Confederation of Medical Mycology (ECMM) and The European Society of Clinical Microbiology and Infectious Diseases (ESCMID) guidelines (Cornely et al., 2012; Hope et al., 2012; Ullmann et al., 2012). In this study, there was only one contaminated (by *Corynebacterium* spp.) but "delayed" fungus-positive vial of a patient with infectious symptoms. Of note, the patient was on empiric antibiotic therapy with a persistent fever, the sample was obtained from CVC and the latter vials were "usual" positive for the same yeast, as mentioned.

Most clinically significant fungi are relatively slow-growers and low-level CO_2 producers, however several studies indicated that standard 5-day incubation with BC vials is enough for growth of almost all these fungi. Marginson et al. (Marginson et al., 2014) stated that only 0.5% of BC vials of which gave positive signal between 5-7 days, were clinically significant. In addition, prolonged incubation may increase contaminations (Lamy et al., 2016). Similar studies like Bourassa et al. (Bourassa et al., 2019), Baron et al. (2005) and Ransom et al. (2021) pointed that prolonged incubation is an insignificant, not cost-effective and in overall, unnecessary application. Our study supported these data, since the "delayed" positive vial-*C.guilliermondii* complex did not actually provide any advantage in prognosis and/or time of beginning for treatment. In addition, it is controversial to assess this vial as "delayed", since the second set (the following) gave positive signal for the same organism during routine incubation period. BC guidelines strongly recommend at least 2 following sets to detect most of an aethiologic agent (>95%), thus, evaluation of these data should be done in this manner (Lamy et al., 2016). Furthermore, routine "prolongation" requires additional SDA inoculations for every week and this actually caused at least over 800$ additional cost monthly, which were not actually also created any beneficial profit.

Notebly, it should be pointed that the patient was not on ongoing antifungal therapy in time of sampling. Thus, possible delayer effect of antimicrobial usage was discarded. Even so, *C.guilliermondii* complex is a relative mystery in antifungal sesceptibility except echinocandins, of which the isolate was susceptible. Neither The European Committee on Antimicrobial Susceptibility Testing (EUCAST) nor CLSI defined clinical breakpoints for azoles regarding this organism (Clinical and Laboratory Standards Institute, 2017; The European Committee on Antimicrobial Susceptibility Testing, 2022).

Limitations and strengths of study

Our study has a few limitations. First, standardization in BCs, including volume, sampling procedures, etc. could not be verified. Secondly, preliminary fungal concentrations in the vials could not be measured, however, well-validated BC systems are in use, so we think that this was a minor issue. Finally, we did not use mycological vials to focus on fungemia, but in routine cultivation it is not recommended (Lamy et al., 2016) and even used, this might have been a disruptive issue in design of our study.

The main strength of this study is that the study was applied for a year period without any interventions, standard methodologies were used in all applications and was conducted as a prospective blind study, since all microbiological and clinical data were combined at the end of one-year study period.

CONCLUSION

Fungi have an increasing trend as causatives of BSIs and diagnosis of fungemia is mainly based on BCs, thus, widening the detection spectrum of BCs and optimization are crucial approaches. In this study, standard incubation period showed a fulfilling performance, and also, contaminations did not create a negative effect regarding detecting fungemia. On the other hand, contamination still remains to be a major problem for BCs, which should be decreased even in our facility, but is out of scope of this study.

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Conflict of Interest

The authors wish to confirm that there are no known conflicts of interest associated with this publication and there has been no significant financial support for this work that could have influenced its outcome.

Author Contributions

Plan, design AKS, GA, BYA, MG; **Material, methods and data collection:** AKS, GA, BYA, MG; **Data analysis and comments:** AKS, GA, BYA, MG; **Writing and corrections:** AKS, GA, BYA, MG.

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