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Can Prolonged Incubation of Negative Blood Cultures Show Fungal Positivity?

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ABSTRACT
Objective: Standard duration of one set blood culture (BC) is a maximum of 5-7 days. The aim of this study was to evaluate "culture-negative" vials with a prolonged incubation (max 30 days) time and to observe any mycological growth. **Materials and Methods:** Routine BCs obtained from adult patients of Balıkesir Atatürk City Hospital for a year period were included. Render BC System BC128 (Render Biotech Co. Ltd., Shenzhen, China) were used. Randomly selected vials were re-incubated for additional three weeks by conventional methods. 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:** A total of 6047 BC sets were obtained and randomly chosen 1040+122 negative sets (A and B groups, respectively) were included. In group A, none of them had fungal growth. In B (ongoing empiric antifungal ≤48h), only 2 sets showed significant fungal growth, which were observed in 7±2 days, and all strains were identified as *Candida glabrata* complex and these patients were on empiric fluconazole (200 mg/day). One isolate was susceptible dose dependent; the other one was resistant for fluconazole. Latter sets of these fungemia patients showed positive signals in routine incubation period. **Conclusion:** Invasive fungal infections are increasingly encountered and isolation capacity and optimization of BCs are crucial. In this study, it was obviously observed that standard incubation period is satisfactory in order to detect almost all fungemia.
Keywords: Invasive Fungal Infections, Fungi, *Candida*, Sepsis.

Negatif Kan Kültürlerinin Uzatılmış İnkübasyonu Fungal Pozitiflik Verebilir Mi?

ÖZ
Amaç: Standart kan kültürü azami 5-7 günde sonuçlanmaktadır. Çalışmanın amacı, kültür negatif şişelerin uzatılmış inkübasyonu ile mikolojik üreme olup olmayacağının araştırılmasıdır. **Gereç ve Yöntem:** Balıkesir Atatürk Şehir Hastanesi'nde bir yıl boyunca erişkin hastaların kan kültürleri Render BC Sistemi (Render Biotech Co. Ltd., Çin) ile çalışmaya dahil edildi. Kan kültürlerinden randomize seçilmiş şişelerin (A grubu) inkübasyonu haftalık geleneksel ekimlerle 30 güne tamamlandı. Muhtemel/Olası invazif fungal enfeksiyonu (IFI) olan, ampirik/preemptif antifungal alanların negatif şişeleri ayrıca değerlendirilmiştir (B grubu). Üremelere Phoenix™ 100 sistemi (Becton Dickinson, ABD) ve mısır unu tween 80 agarla (RTA Laboratories, Türkiye) tanımlama, CLSI disk difüzyonla antifungal duyarlılık yapılmıştır. **Bulgular:** Toplam 6047 kan kültürü seti (%23.06 pozitif) işlendi. Randomize seçilmiş 1040+122 negatif set (sırasıyla A ve B grupları) çalışmaya alındı. A grubunda fungal üreme olmazken, B grubunda (flukonazol-200 mg/gün≤48 saat) 2 sette anlamlı fungal üreme oldu (7-9. günler) (*Candida glabrata* kompleks). Flukonazol için, bir izolat doza bağımlı duyarlıyken, diğeri dirençliydi. Takip eden ikinci setleri normal sürede pozitif verdi. **Sonuç:** Kan kültürlerinin izolasyon kapasitesi ile optimizasyonu IFI tanısında kritik önemdedir. Mikroorganizmaların %99'unun tespiti için uygun hacimde en az iki set kan kültürü alınması gerektiğinden, takip eden setlerin normal süreçte pozitif vermesi sebebiyle, üremeler "uzamış pozitif" olarak değerlendirilmedi. Standart inkübasyon süreci tatmin edici olarak kabul edildi.
Anahtar Kelimeler: İnvazif Fungal Enfeksiyonlar, Mantarlar, *Candida*, Sepsis.

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INTRODUCTION

Invasive fungal infections (IFIs) are observed in an increasing trend with high mortalities. Approximately 300 million individuals are affected worldwide and over than 1.5 million individuals are lost, annually. Admission to intensive care units (ICUs), elder ages, intensive usage of antibiotics, mechanic ventilation and other invasive procedures such as catheters and application of immunosuppressant agents are major predisposers. However, there are various cases reported without any such underlying conditions (Arikan-Akdağlı et al, 2019; Gülmez et al, 2021; Seagle et al, 2021). Fungemia is one of the most mortally serious conditions, and by far, the most frequent etiologic agents are *Candida* spp. Geographic location, clinical status and underlying diseases directly affect the type of the causative agent, but the worldwide top five agents are *Candida albicans*, *Candida glabrata* complex, *Candida parapsilosis* complex, *Candida tropicalis* and *Candida krusei* (Gülmez et al, 2021; Seagle et al., 2021).

Appropriate early diagnosis and treatment are crucial for prognosis of IFIs. Microbiological diagnosis of fungemia mainly depends on blood culture (BC) and so, sensitivity of BC vials is very important. There are only a few automated BC systems worldwide, and some of them also provide mycological vials. Routine BC samples were obtained with inoculation into one anaerobic and one aerobic vial (one set). On the other hand, mycological growth in all these vials is seriously affected because of several reasons such as species type, sample volume and sort of vial (De Plato et al, 2019; Lamy et al, 2016). Furthermore, fungemia cases are generally reported as monomicrobial, but recent reports indicated polymicrobial fungal positivities (Gülmez et al, 2020).

Standard duration of one set BC is a maximum of 5-7 days (Lamy et al, 2016). On the other hand, standard mycological BC evaluation may extend up to 30 days (La Rocco, 2010). This condition creates a question whether it is possible to miss some of the "actually positive" cultures due to 7-day-limited incubation period. The aim of this study was to evaluate "culture-negative" 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 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.

Procedures

Randomly selected vials from all services (Group A) including ICUs were re-incubated in 35°C for additional three weeks after negative signaling at the end of one-week incubation (Four weeks total). In the first day of re-incubation and then once weekly, gram staining and inoculations onto 5% sheep blood agar, eosine methylene blue agar, chocolate agar, sabouraud dextrose agar (SDA), 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 35-37°C, 5% CO_2 atmosphere and for 48h. In case of any growth, identifications were done by Phoenix^{TM} 100 automated system (Becton Dickinson, MA, USA) with cornmeal tween 80 agar (RTA Laboratories, Kocaeli, Türkiye).

Negative vials of patients that had clinically possible/probable IFI status or that were on empirical, preemptive antifungal therapy (Group B) were separately evaluated. All procedures were identical, except, additional antifungal susceptibility testing (AFST) were 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, in case of any fungal growth (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 Istanbul Medipol University. Date: 05.08.2021 Number: 2021/795.

RESULTS

A total of 6047 BC sets were obtained after excluding samples of same episodes of the same patients. Different episodes of the same patients were included. Among these, 23.06% (n=1395) had at least one positive vial, which were excluded from the study. Negative samples as group B were separated (n=122). Randomly chosen 1040 negative sets as group A were included to the study. During 30-day incubation, any growth of bacteria or moulds (except *Fusarium* spp., *Paecilomyces* spp.) was considered as contamination.

Among group A, 9 sets showed contaminant growth (e.g.; gram positive bacilli) within 30-day incubation, none of them had fungal growth. Among group B, only 2 sets showed significant fungal growth. All growths were observed in 7-9 days among total incubation time, and all strains were identified as *C. glabrata* complex. One isolate was susceptible dose dependent (SDD) for fluconazole (≥15mm), the other isolate was resistant (R)

(≤14mm). Caspofungin and voriconazole susceptibility could not be interpreted due to lack of interpretive zone diameters, however they were ≥30mm and ≥20mm, respectively.

All patients with this fungal growth were already in the first 48 hours of their empiric fluconazole treatment (200 mg/day until at least two consecutive BCs were sterile) at the time of sampling due to their underlying conditions (at least one of; COVID-19, invasive catheters, malignancy, severe diabetes mellitus, chronic renal insufficiency, elder ages – 65+, etc.) and clinical symptoms such as persistent fever. It can also be noted that the latter sets of these fungemia patients showed positive signals in routine incubation period.

DISCUSSION

It is obvious that bloodstream infections (BSIs) are a major threat to public health globally, are one of the top-most causes of death, indicating an occurrence of more than two million episodes annually, which cause 13% to 20% case-fatality rate. Fungi were relatively ignored probably because of their difficult-to-detect nature. Nevertheless, mortality of fungemia can reach to over 70%. By far, *Candida* spp. are the most frequently isolated strains from BCs, on the other hand, *Cryptococcus* spp., *Rhodotorula* spp, and yeast-like organisms such as *Trichosporon* spp. can be causative agents (Kotey et al, 2021).

Species-level identification is very important to start an appropriate therapy, however such identification requires BC positivity, growth on conventional media and identification procedures via like API ID32C (bioMérieux, Marcy-l'Étoile, France), Vitek 2 (bioMérieux, Marcy-l'Étoile, France) and BD Phoenix™ systems (Becton Dickinson, NJ, USA), which takes a minimum of 3-4 days (Lin et al, 2019). This delay indicates limits of presumptive treatment for fungemia, so societies like European Confederation of Medical Mycology (ECMM) and The European Society of Clinical Microbiology and Infectious Diseases (ESCMID) published guidelines to manage such conditions as empiric, preemptive and prophylactic treatments (Cornely et al., 2012; Hope et al, 2012; Ullmann et al, 2012). Regardless, early detection of the etiologic agent and source of infection are crucial, since time-to-detection was found to be a good prognostic factor, especially in adjunction with bedside antifungal stewardship applications (Butta et al, 2019; Dudakova, 2022). In addition, BC and conventional culture still remain to be major parts of gold standards, because of lack or inefficiency of indirect diagnostic procedures (serology, etc.) (Cuenca-Estrella et al, 2012). Therefore, it is clear that sensitivity and specificity are very important for diagnostic capacity of BCs in fungemia. In our study, with prolonged incubation, there was a very limited number of "delayed" positive vials which were all already on empirical antifungal therapy, indicating that BCs were totally effective in diagnosis. This result was compatible with several previous studies and recommendations for optimal recovery in guidelines (Lamy et al, 2016).

Several studies suggested that 5 days of incubation via automated systems is enough, whereas the incubation period over 5 days seems to increase contaminations. Marginson et al. (Marginson et al., 2014) found that 2.7% of positive BC vials gave signal between 5 and 7 days, but only 0.5% of them were clinically significant. On the other hand, clinically significant fungi are mostly low-level CO_2 producers, or slowly growing microorganisms. Although there is antimicrobial binding resin in vials, antifungal application prior to sampling may reduce and/or delay positivity, since concentration of microorganism has critical role (Burduniuc et al, 2019; Lamy et al, 2016). Another study of Bourassa et al. (Bourassa et al., 2019) reported only one *Fusarium* spp. growth with prolonged incubation over a 4.5-year period. Similar results were obtained not only from fungal side, but also bacterial perspective, indicating even 4-days of incubation was sufficient (Baron et al., 2005; Ransom et al., 2021). Our study supported these data and prior-to-sampling fluconazole therapy in our B group might be considered as "delayer" in our positive vials, despite high MICs.

Notably, the "prolonged" isolates were *C. glabrata* complex (one resistant to fluconazole). It has several differences from other *Candida spp*. including genetic pattern, virulence factors and susceptibility profile (Hassan et al., 2021). Its flexibility on environmental adaptation also seems to be remarkable (Carreté et al, 2019). On the hand, a recent study on immune relations showed this strain to have relatively much more decreased survival and more rapid elimination (Kämmer, 2020). However, in cases of invasive *C. glabrata* infections, morbidity and mortality is approximately 40–60%, which might be because of intrinsic low susceptibility to the most commonly used azoles (Hassan et al., 2021). In our study, both "prolonged-positive" sets were the first samples of the patients, and the patients immediately become "usual" positive in the second sampling. This might be because of empiric therapy in combination with immune response, even if the strains were SDD and R. Hence, microbiological susceptibility does not always correlate with clinical resistance (Morio et al., 2017; Rex et al., 2002). Wider multicenter studies are required to prove this hypothesis.

Limitations and Strengths of Study

Our study has several limitations. First, there was not any standardization in BCs, including volume, sampling procedures, etc. Particularly volume is a major indicator for false negative results; however, we believe it was substantially ruled out by repetitious conventional inoculations. On the other

hand, international guidelines recommend at least 4 bottles (2 sets) with adequate volume for each one to detect 99% of the pathogens (Lamy et al, 2016). Since our two isolates were actually in this spectrum and latter sets were "usual" positive for both patients, the isolates should not be considered as "prolonged positive". Secondly, we could not measure preliminary fungal concentrations in the vials which is labor-intensive and not routinely used. These BC systems are well validated recently, so we do not think that it is a corruptive issue. Thirdly, pre-device period and environmental temperature are major factors for false result (Lamy et al, 2016). 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. Finally, we could not perform broth microdilution, could not share MICs of the isolates including fluconazole and so, we could not evaluate epidemiologic cut-off values for antifungals. However, the patients were already on only fluconazole therapy, and we performed CLSI disk diffusion for this antifungal, thus we do not think this was a disruptive issue.

CONCLUSION

Diagnosis of BSIs are mainly based on BCs. Fungi are increasingly encountered as causatives of BSIs due to various changes in patient populations. In addition, they currently have a widening species spectrum (Gülmez et al, 2021). Within this scope, isolation capacity and optimization of BCs are crucial. In this study, it was obviously observed that standard incubation period is satisfactory in order to detect almost all fungemia. Escalation/de-escalation methods, effects of MICs and usage of mycological vials were beyond the scope of this study, however it is clear that more studies are required discussing such issues.

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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, DÖY, BGS, MG; **Material, methods and data collection:** AKS, DÖY, BGS, MG; **Data analysis and comments:** AKS, DÖY, BGS, MG; **Writing and corrections:** AKS, DÖY, BGS, MG.

REFERENCES

Arikan-Akdagli, S., Gülmez, D., Doğan, Ö., Çerikçioğlu, N., Dereli, M. D., Birinci, A., & et al. (2019). First multicentre report of in vitro resistance rates in candidaemia isolates in Turkey. *Journal of Global Antimicrobial Resistance*, *18*, 230-234. https://doi.org/10.1016/j.jgar.2019.04.003.

Baron, E. J., Scott, J. D., & Tompkins, L. S. (2005). Prolonged incubation and extensive subculturing do not increase recovery of clinically significant microorganisms from standard automated blood cultures. *Clinical Infectious Diseases*, *41*(11), 1677-1680. https://doi.org/10.1086/497595.

Bourassa, L., Kates, O., & Liu, C. (2019). Limited diagnostic utility of extended aerobic blood culture incubation for fungal pathogen detection. *Open Forum Infectious Diseases*, *6*(Suppl 2): S140.

Burduniuc, P. O., Bunescu, I., Sofronie, O., Bivol, M., Craciun, O., Burduniuc, A., & Balan, G. (2019). The importance of blood cultures in the effective management of bloodstream infections. *Moldovan Medical Journal*, *62*(3), 28-37. https://doi.org/10.5281/zenodo.3404096 .

Butta, H., Sardana, R., Mendiratta, L., Sibal, A., Gupta, V., Chawla, R., & Jafri, A. A. (2019). Time to detection of yeast isolates in pediatric and adult patients with fungemia and its relevance to clinical profile and outcome. *Indian Journal of Critical Care Medicine*, *23*(1): 27-30. https://doi.org/10.5005/jp-journals-10071-23108.

Carreté, L., Ksiezopolska, E., Gómez-Molero, E., Angoulvant, A., Bader, O., Fairhead, C., & Gabaldón, T. (2019). Genome comparisons of *Candida glabrata* serial clinical isolates reveal patterns of genetic variation in infecting clonal populations. *Frontiers in Microbiology*, *10*, 112. https://doi.org/10.3389/fmicb.2019.00112.

Clinical and Laboratory Standards Institute (CLSI). Method for antifungal disk diffusion susceptibility testing of yeasts, 3rd edition. CLSI Document M44. Wayne, Pennsylvania, USA, 2018.

Clinical and Laboratory Standards Institute (CLSI). Performance standards for antifungal susceptibility testing of yeasts, 1st edition. CLSI Supplement M60. Wayne, Pennsylvania, USA, 2017.

Cornely, O. A., Bassetti, M., Calandra, T., Garbino, J., Kullberg, B. J., Lortholary, O., & et al. (2012). ESCMID guideline for the diagnosis and management of *Candida* diseases 2012: non-neutropenic adult patients. *Clinical Microbiology and Infection*, *18*, 19-37. https://doi.org/10.1111/1469-0691.12039.

Cuenca-Estrella, M., Verweij, P. E., Arendrup, M. C., Arikan-Akdagli, S., Bille, J., Donnelly, J. P., & et al. (2012). ESCMID guideline for the diagnosis and management of *Candida* diseases 2012: diagnostic procedures. *Clinical Microbiology and Infection*, *18*, 9-18. https://doi.org/10.1111/1469-0691.12038 .

De Plato, F., Fontana, C., Gherardi, G., Privitera, G. P., Puro, V., Rigoli, R., & et al. (2019). Collection, transport and storage procedures for blood culture specimens in adult patients: recommendations from a board of Italian experts. *Clinical Chemistry and Laboratory Medicine (CCLM)*, *57*(11), 1680-1689. https://doi.org/10.1515/cclm-2018-1146 .

Dudakova, A., Blei, C., Groß, U., & Schulze, M. H. (2022). Impact of routine bedside infectious diseases service on clinical management and prognosis of patients with *Candida* fungemia–an example for Antifungal Stewardship at university level in Germany. *International Journal of Infectious Diseases*, *119*, 150-159. https://doi.org/10.1016/j.ijid.2022.03.054.

Gülmez, D., Sığ, A. K., Akar, N., & Duyan, S. (2021). Changing trends in isolation frequencies and species of clinical fungal strains: what do the 12-years (2008-2019) mycology laboratory data tell about?. *Mikrobiyoloji Bulteni*, *55*(1), 53-66. https://doi.org/10.5578/mb.20156.

Gülmez, D., Alp, S., Gursoy, G., Ayaz, C. M., Dogan, O., Arikan-Akdagli, S., & Akova, M. (2020). Mixed fungaemia: an 18-year report from a tertiary-care university hospital and a systematic review. *Clinical Microbiology and Infection*, *26*(7), 833-841. https://doi.org/10.1016/j.cmi.2020.03.030.

Hassan, Y., Chew, S. Y., & Than, L. T. L. (2021). *Candida glabrata*: pathogenicity and resistance mechanisms for adaptation and survival. *Journal of Fungi*, *7*(8), 667. https://doi.org/10.3390/jof7080667.

Hope, W. W., Castagnola, E., Groll, A. H., Roilides, E., Akova, M., Arendrup, M. C., & et al. (2012). ESCMID* guideline for the diagnosis and management of *Candida* diseases 2012: prevention and management of invasive infections in neonates and children caused by Candida spp. *Clinical Microbiology and Infection*, *18*, 38-52. https://doi.org/10.1111/1469-0691.12040.

Kämmer, P., McNamara, S., Wolf, T., Conrad, T., Allert, S., Gerwien, F., & et al. (2020). Survival strategies of pathogenic *Candida* species in human blood show independent and specific adaptations. *Mbio*, *11*(5), e02435-20. https://doi.org/10.1128/mBio.02435-20.

Kotey, F. C., Dayie, N. T., Tetteh-Uarcoo, P. B., & Donkor, E. S. (2021). *Candida* bloodstream infections: changes in epidemiology and increase in drug resistance. *Infectious Diseases: Research and Treatment*, *14*, 11786337211026927. https://doi.org/10.1177/11786337211026927.

Lamy, B., Dargère, S., Arendrup, M. C., Parienti, J. J., & Tattevin, P. (2016). How to optimize the use of blood cultures for the diagnosis of bloodstream infections? A state-of-the art. *Frontiers in microbiology*, *7*, 697. https://doi.org/10.3389/fmicb.2016.00697.

La Rocco M. Processing specimens for fungal culture. In: Garcia LS (editor), Clinical Microbiology Procedures Handbook, 3th Edition. ASM press, USA, 2010.

Lin, S. Y., Lu, P. L., Tan, B. H., Chakrabarti, A., Wu, U. I., Yang, J. H., & et al. (2019). The epidemiology of non-*Candida* yeast isolated from blood: the asia surveillance study. *Mycoses*, *62*(2), 112-120. https://doi.org/10.1111/myc.12852.

Marginson, M. J., Daveson, K. L., & Kennedy, K. J. (2014). Clinical impact of reducing routine blood culture incubation time from 7 to 5 days. *Pathology*, *46*(7), 636-639. https://doi.org/10.1097/PAT.0000000000000167.

Morio, F., Jensen, R. H., Le Pape, P., & Arendrup, M. C. (2017). Molecular basis of antifungal drug resistance in yeasts. *International journal of antimicrobial agents*, *50*(5), 599-606. https://doi.org/10.1016/j.ijantimicag.2017.05.012.

Ransom, E. M., Alipour, Z., Wallace, M. A., & Burnham, C. A. D. (2021). Evaluation of optimal blood culture incubation time to maximize clinically relevant results from a contemporary blood culture instrument and media system. *Journal of Clinical Microbiology*, *59*(3), e02459-20. https://doi.org/10.1128/JCM.02459-20.

Rex, J. H., & Pfaller, M. A. (2002). Has antifungal susceptibility testing come of age?. *Clinical Infectious Diseases*, *35*(8), 982-989. https://doi.org/10.1086/342384.

Seagle, E. E., Williams, S. L., & Chiller, T. M. (2021). Recent trends in the epidemiology of fungal infections. *Infectious Disease Clinics*, *35*(2), 237-260. https://doi.org/10.1016/j.idc.2021.03.001.

Ullmann, A. J., Akova, M., Herbrecht, R., Viscoli, C., Arendrup, M. C., Arikan-Akdagli, S., & et al. (2012). ESCMID guideline for the diagnosis and management of *Candida* diseases 2012: adults with haematological malignancies and after haematopoietic stem cell transplantation (HCT). *Clinical Microbiology and Infection*, *18*, 53-67. https://doi.org/10.1111/1469-0691.12041.