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Demonstration and utility of clustered pseudohyphae on Gram-stained smears from *Candida albicans*-positive blood cultures

Mehmet Doymaz[a,*], Carmen Yampierre[b], Georgio Tarchini[c], William Riley[b]

[a] *Microbiology Laboratory, Saint Vincent Catholic Medical Centers, New York, NY 10011, USA*
[b] *Microbiology Laboratory, Beth Israel Medical Center, New York, NY 10003, USA*
[c] *Infectious Disease Department, Beth Israel Medical Center, New York, NY 10003, USA*

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Abstract

The presence of clustered and branched pseudohyphae was investigated on Gram-stained smears of 78 consecutive yeast-positive blood cultures. The accuracy of the method was 96.1%, with a positive predictive value of 96.6% for *Candida albicans*. These findings demonstrate that the presence of clustered and branched pseudohyphae on Gram stain may be used for the rapid and presumptive identification of *C. albicans* from yeast-positive blood culture bottles.
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Rapid and accurate identification of *Candida albicans* from positive blood cultures has critical importance in candidemias (Rentz et al., 1998). Significant therapeutic decisions are based upon the Gram stain report and presumptive identification of the yeast in positive blood cultures. The majority of *C. albicans* isolated from sterile sites are susceptible to the azole class of antifungals. Fluconazole, the relatively inexpensive antifungal, is the choice of treatment (Ostrosky-Zeichner et al., 2003; Pfaller et al., 1998; Shawn et al., 2006). Susceptibility of other yeast species to fluconazole, however, varies significantly (Pappas et al., 2004), thereby justifying the rapid, reliable, and accurate differentiation of *C. albicans* from other yeasts.

Candida species are among the most frequently isolated pathogens from nosocomial blood stream infections with an incidence rate approximating 9% (Diekema et al., 2003; Hajjeh et al., 2004; Wisplinghoff et al., 2004). Mortality rates from these infections are significant and among the highest in all blood stream infections reaching up to 47%, with significantly higher rates in some patient subsets, for example, neutropenic subjects (Diekema et al., 2003; Wisplinghoff et al., 2004).

Until recently, the germ tube test was the most rapid presumptive identification test. The advent of chromogenic agars has provided considerable speed and accuracy to the yeast identification (Grace et al., 2005). In recent years, peptide nucleic acid fluorescence in situ hybridization techniques (PNA FISH) have been adapted to meet the clinical needs for rapid identification (Forrest et al., 2006). Variety of other methods including molecular-based tests is constantly explored in this field for possible utility.

In a recent study with the Bactec 9240 (Labequip LTD, Markham, Ontario, Canada), Harrington et al. (2007) observed clustered pseudohyphae on 97% of all initial Gram-stained smear preparations from cultures that were subsequently confirmed as *C. albicans*. The current report investigates whether the observation of clustered and branched pseudohyphae from initial Gram stain smears as a predictor for *C. albicans* could be extended to our blood culture system.

This retrospective study represented 78 unique and consecutive patients with candidemia from Beth Israel Medical Center, New York, NY. Archived yeast-positive smears from the period of December 2006 and April 2008 were retrieved and analyzed for the presence of clustered

* Corresponding author. Tel.: +1-212-604-8415; fax: +1-212-604-8573.
E-mail address: mdoymaz@svcmcny.org (M. Doymaz).

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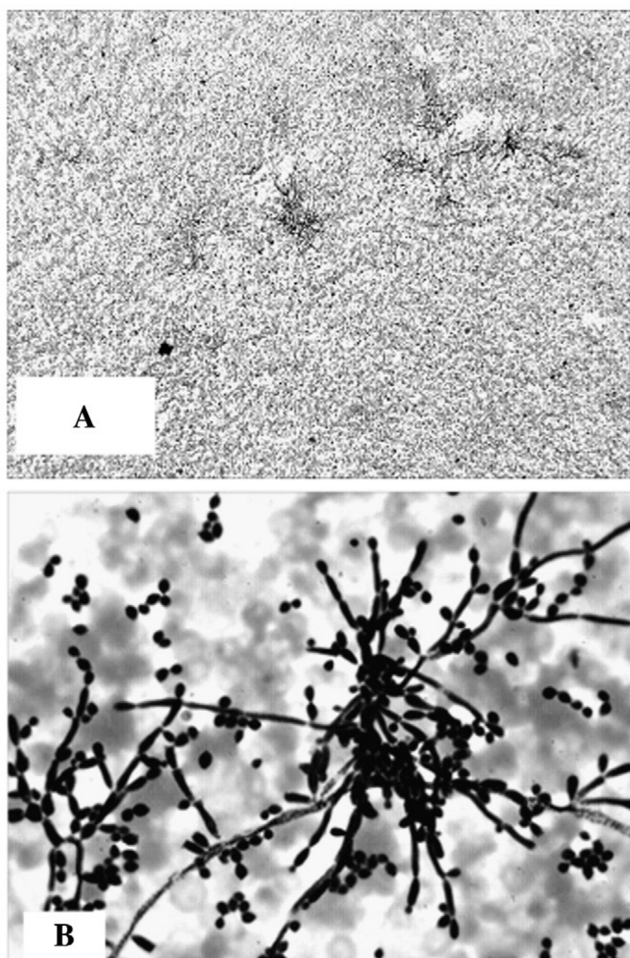

Fig. 1. (A) Gram-stained preparation showing clustered and branched pseudohyphae (10×). This magnification is suitable for rapidly screening Gram stains for pseudohyphae. (B) Fine details of the pseudohyphae are discerned (100×). This magnification is typically used for reading Gram stains.

pseudohyphae (Fig. 1A and B). The median age of the study population was 64 years (range, 4 months to 98 years), representing 40 females and 38 males. Specific identification of these 78 isolates is presented in Table 1. One culture representing a mixed yeast infection (*C. albicans* and *Candida glabrata*) was excluded from the study.

The automated blood culturing system used in this study was the VersaTREK (Trek Diagnostics Systems, Cleveland, OH 44131). Each set of blood cultures comprised 1 aerobic and 1 anaerobic bottle containing 80 mL of broth to which 5 to 10 mL of blood samples were obtained from the patients according to standard phlebotomy procedures. The overwhelming majority of the analyzed smears were from aerobic bottles.

The definitive identification of each yeast isolate, including *Candida dubliniensis*, is made using several methods, including growth characteristics on various media such as Corn Meal Tween-80 and CHROMagar™ *Candida* (BBL, Becton Dickenson and Company, Sparks, MD 21152) as well as the formation of germ tubes in serum (Larone, 2002). In addition, PNA FISH (Avandx, Woburn, MA 01801) and the Vitek 2 YBC card (bioMérieux, F-69280 Marcy-l'Etoile, France) were used.

The observation of pseudohyphae on Gram stains from blood cultures was the sole criteria used to presumptively identify *C. albicans*. For each stain, a circular area approximately 1 cm^2 was scanned.

Using various mycological tests, we determined that 31 of 78 yeast-positive blood cultures were positive for *C. albicans*. Of these 31 cultures, 29 demonstrated pseudohyphae on the direct Gram-stained smears. Two were negative for pseudohyphae formation, an observation corroborated by each author. The reviewers of the smears were blinded to the final identification of the *Candida* spp. Identification of clustered pseudohyphae elements on Gram stain does not require much more training than those needed for reading regular Gram-stained smears. However, the technologists reading Gram stains from positive blood cultures should be in-serviced about the importance of the clustered pseudohyphae on such smears. Of the 47 other yeast-positive blood cultures, only 1 had structures resembling pseudohyphae on the Gram-stained preparations. This culture was identified as *Candida tropicalis*. Table 2 presents the statistical analysis.

C. albicans form pseudohyphae under a variety of growth conditions. This feature is the basis for the germ tube test, which is widely used in clinical microbiology laboratories for the identification assay for *C. albicans*. Germ tube formation is probably one of the fastest and most practical methods available to clinical microbiology laboratories for the rapid and presumptive identification of *C. albicans*. In our medical center, *C. albicans* are responsible for about half of candidemias. This incidence is followed by *Candida parapsilosis*, *C. glabrata*, and *C. tropicalis* (Table 1). This observation has been noted by others (Hajjeh et al., 2004).

Table 1
Yeast species isolated from blood cultures

Yeast species	Positive
C. albicans	31 (39.7%)
C. parapsilosis	20 (25.6%)
C. glabrata	16 (20.5%)
C. tropicalis	7 (8.9%)
Cryptococcus neoformans	4 (5.1%)

Table 2
Analytic comparison of the methodologies used in the study

	Pseudohyphae on Gram stain	Mycological Identification
C. albicans	29 (93.5%)	31
Yeast, not *C. albicans*	1 (3.3%)	47
Accuracy	96.1%	
Specificity	97.9%	
Sensitivity	93.5%	
Positive predictive value	96.6%	
Negative predictive value	95.9%	

Currently, laboratories have the opportunity to utilize a variety of methodologies ranging from culture-based methodologies to molecular amplification and fluorescence-based methods for the rapid and reliable identification of *C. albicans* from yeast-positive blood cultures. Although these techniques have proved to be beneficial from both clinical and cost perspectives, they may take several hours for completion. In comparison, Gram stain observations are the first reportable results available after detection of positivity from automated blood culture instruments. Thus, the presumptive identification of *C. albicans* from Gram-stained smears would enable clinicians to initiate the appropriate antifungal regimen promptly after the initial smear notification. In addition, the observation of branched pseudohyphae on Gram stain precludes *C. glabrata* from the consideration, which tends to be resistant to fluconazole in most cases. It is important to emphasize to the clinical care team and for the medical record that the presence of clustered pseudohyphae on Gram-stained smears leads to presumptive identification of *C. albicans* and confirmatory studies will follow.

This study determined that investigation of clustered and branched pseudohyphae provided accurate identification in 96% of cases. Positive predictive value of this observation was 96.6% for *C. albicans* in direct Gram-stained smears from positive blood culture bottles. Equally important was the lack of pseudohyphae from smears of blood cultures positive for other yeasts that had a 95.9% negative predictive value. The accuracy of the observations reported in this study is close to PNA FISH studies. However, PNA FISH studies, compared with Gram staining, are time consuming, are expensive, and demand much more technical requirements.

These results support previously published studies and extend these observations to the VersaTREK blood culture system. Furthermore, these results indicate that the rapid and accurate identification of *C. albicans* and this species discrimination from other yeasts are possible directly from positive blood culture bottles with widely available and inexpensive Gram stain.

In conclusion, this study provides further support to the previous observations that pseudohyphae on Gram-stained preparations made directly from yeast-positive blood culture bottles provide a reliable and accurate basis for presumptive identification of *C. albicans*. Studies performed on larger number of samples and in other blood culture systems would provide additional support for these observations. Prospective studies including assessment of clinical outcomes and evaluating the possible reductions in echinocandin usage would also complement the present report.

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