JOURNAL OF CLINICAL MICROBIOLOGY, July 1975, p. 18-20
Copyright © 1975 American Society for Microbiology
Vol. 2, No. 1
Printed in U.S.A.

Recovery of Yeast from Vented Blood Culture Bottles

GLENN D. ROBERTS, CARLYLE HORSTMEIER, MARSHA HALL, AND JOHN A. WASHINGTON II*
Department of Laboratory Medicine, Mayo Clinic and Mayo Foundation, Rochester, Minnesota 55901

Received for publication 6 May 1975

Rates of isolation of yeasts from blood cultures were significantly enhanced by venting vacuum blood culture bottles in studies of both simulated and patients' blood cultures; however, the time interval to detection of positivity of yeasts in the clinical studies was significantly ($P < 0.01$) shorter in a vented bottle with biphasic brain heart infusion medium than in a vented bottle with soybean-casein digest broth. The mean time intervals to detection of positivity were 2.6 days in the former and 5.2 days in the latter.

Recent observations with simulated blood cultures and with cultures of clinical specimens by Gantz and co-workers (3) and by Blazevic and co-workers (1), respectively, have demonstrated decreased and delayed recovery of yeasts from unvented vacuum blood culture bottles. Since these studies also demonstrated decreased and delayed recovery of *Bacteroides* spp. from vented vacuum blood culture bottles, it was concluded that it is necessary to inoculate blood into both vented and unvented vacuum blood culture bottles. Studies at the Mayo Clinic comparing isolation rates of yeasts from patients' blood in vented and unvented vacuum bottles and in a biphasic medium, similar to that described by Ruiz Castaneda (6), prompted this report.

MATERIALS AND METHODS

Clinical blood cultures. Cultures of blood from patients suspected of having bacteremia were collected and processed according to techniques described elsewhere in detail (8). In such cases, blood was inoculated (10%, vol/vol) at the patient's bedside into each of two vacuum bottles of tryptic soy broth (TSB) with CO_2 and 0.025% sodium polyanetholsulfonate. Prior to their incubation at 35 C, one of the two bottles was transiently vented with a sterile, cotton-plugged needle to release its vacuum. Both bottles were inspected daily for 7 days and again after 14 days; both were routinely subcultured onto chocolate blood agar within the first 24 h and after 5 days of incubation. In cases with suspected fungemia, a bottle containing brain heart infusion (BHI) broth and a BHI agar slant was inoculated with an additional 10 ml of blood (5). This bottle was permanently vented with a sterile, cotton-plugged needle prior to incubation in an upright position at 30 C for 4 weeks. Cultures were examined daily for visible evidence of growth, after which the blood-broth mixture was gently mixed over the agar slant. All yeast isolates were identified with procedures described elsewhere (2).

Simulated blood cultures. Cultures of *Candida ablicans*, *C. tropicalis*, *C. parapsilosis*, and *Cryptococcus neoformans* were prepared so that approximately 100 colony-forming units could be inoculated into bottles containing the following media: biphasic BHI, biphasic Trypticase soy (TSBS), and TSB. The TSBS was commercially prepared (BBL) and bottled under vacuum with CO_2 and sodium polyanetholsulfonate. Each organism was inoculated in parallel into six bottles of each medium. Each of the media was then studied under three venting conditions (unvented, transient, and permanent) and at two incubation temperatures, 30 and 35 C.

RESULTS

Clinical blood cultures. Only those cases were analyzed in which the two bottles with TSB and the bottle with biphasic BHI were inoculated in parallel with blood from a single venipuncture. The results are summarized in Tables 1 and 2. In most instances the insolates were multiple positive cultures from a few patients, including 4 with *C. albicans*, 3 with *C. tropicalis*, 1 with *C. parapsilosis*, and 1 with *Cryptococcus neoformans*. The differences in isolation rates between the biphasic BHI and the vented TSB are not statistically significant in contrast with those between the biphasic BHI and the unvented TSB ($P = 0.008$). In cultures in which matched pairs were positive, the mean detection times in biphasic BHI and in vented TSB were 2.6 and 5.2 days, respectively, a statistically significant difference ($P < 0.01$) by the sign test. In matched positive pairs of biphasic BHI and unvented TSB the mean detection times were 3.2 and 9.0 days, respectively, also a statistically significant difference ($P = 0.028$) by the delta-t test.

TABLE 1. *Yeast isolates from blood by medium*

TSB, vented	Biphasic BHI, vented	
	Positive	Negative
Positive	10	8
Negative	13	

TABLE 2. *Yeast isolates from blood by medium*

TSB, unvented	Biphasic BHI, vented	
	Positive	Negative
Positive	4	3
Negative	14	

Simulated blood cultures. The results of these studies are presented in the figures. All *Candida* species tested were detected macroscopically in the vented bottles within their first 48 h of incubation (Fig. 1 and 2). No differences between the two types of venting were noted. Indeed, there were only five instances in which their detection required longer than 24 h, and these all occurred in the TSBS bottle. Macroscopic detection of *C. neoformans* in the vented bottles required approximately 1 to 2 days longer than did *Candida*. When unvented, detection times were markedly delayed or even absent in TSB, slightly delayed in TSBS, and unchanged in BHI (Fig. 3). The temperature of incubation did not generally affect the time interval to detection of positivity.

DISCUSSION

It has been established beyond any reasonable doubt that the detection of yeasts is delayed or absent in unvented vacuum blood culture bottles. The decrease in 1971 noted by Gantz et al. (3) in isolation rate of *Candida* spp., from 3.5% to somewhat less than 1% of patients with positive blood cultures, may have been due to the introduction of vacuum blood culture bottles in that year; however, it is not possible to determine whether or not this difference is statistically significant from their data or whether or not other factors may have contributed to it. Some of the other factors which may influence the frequency of occurrence of fungal sepsis were reviewed by Maki et al. (4) and by Ryan et al. (7) and were related to contamination of fluids used for total parenteral nutrition and the manner of insertion of an intravenous

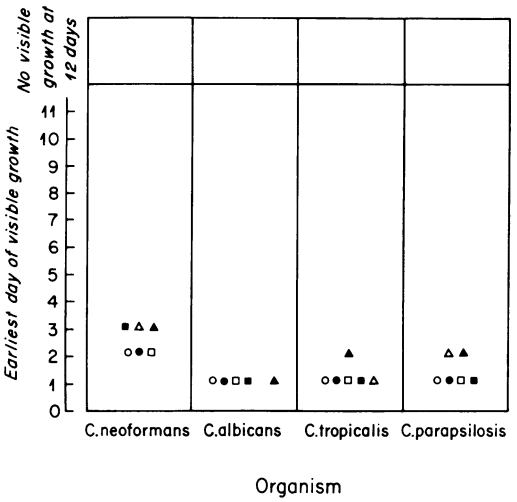

FIG. 1 *Earliest day of visible growth by four species of yeasts in each of three different types of permanently vented blood culture bottles. Symbols: ○, TSB at 30 C; ●, TSB at 35 C; □, BHI at 30 C; ■, BHI at 35 C; △, TSBS at 30 C; ▲, TSBS at 35 C.*

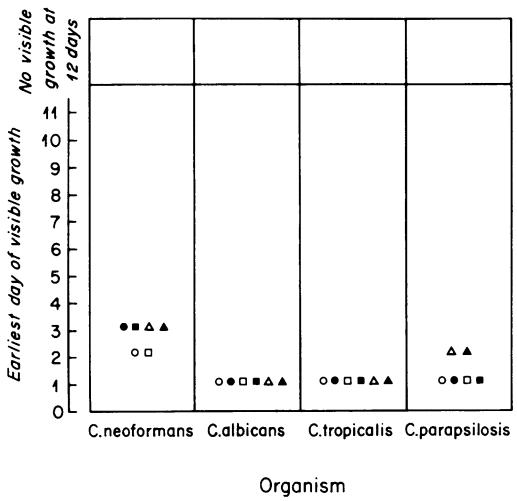

FIG. 2. *Earliest day of visible growth by four species of yeasts in each of three different types of transiently vented blood culture bottles. Symbols same as in Fig. 1.*

catheter, as well as its subsequent care. In our own experience, for example, there has been a substantial reduction during the past 2 years in the rate of isolation of yeasts from fungal blood cultures in association with the initiation of a routine catheter care program by the nurse epidemiologists for patients receiving total parenteral nutrition.

Our data in simulated blood cultures substan-

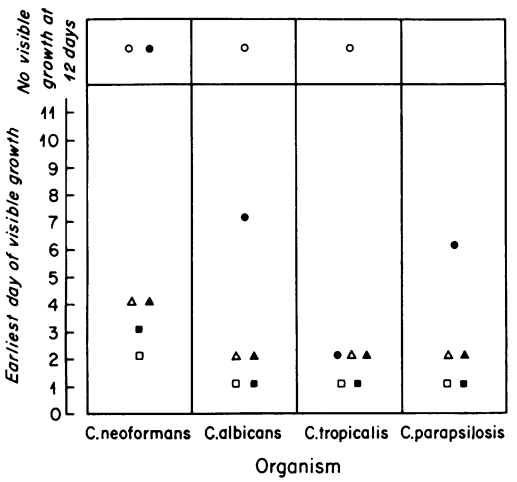

FIG. 3. *Earliest day of visible growth by four species of yeasts in each of three different types of unvented blood culture bottles. Symbols same as in Fig. 1.*

tiates those reported by Gantz et al. (3). Blazevic et al. (1) have demonstrated significantly ($P < 0.01$) higher isolation rates of *Candida* spp. from blood in vented bottles containing Columbia broth than in unvented bottles containing the same medium. Preliminary results from a separate study at the Mayo Clinic comparing vented (transient) and unvented TSB bottles have demonstrated significantly ($P < 0.001$) higher isolation rates of *Candida* spp. from the vented bottle (Table 3). In another study, by Roberts and Washington (5), it was found that significantly ($P < 0.001$) more isolates of fungi were recovered from blood in the biphasic BHI bottle than in the unvented TSB bottle. It was, therefore, of interest to compare isolation rates of fungi in the biphasic medium bottle with those in the vented (transient) TSB bottle. The clinical data, although limited in numbers, demonstrated statistically significant differences between the time intervals to detection of positivity in these two blood culture systems, despite the fact that the vented TSB bottle was subcultured routinely within 1 day and 5 days after its inoculation.

Commercial sources of satisfactory biphasic media in the United States are very limited. For this reason we included one such bottle (TSBS) in our simulated blood culture studies. This bottle would routinely require venting because of the fact that its contents are under vacuum with CO_2. An additional problem is that there are no published data comparing the isolation rates of fungi from clinical specimens inoculated onto BHI agar and soybean-casein digest agar (e.g., Trypticase [BBL]). Such studies are currently under way in our laboratory.

TABLE 3. *Yeast isolates from blood by medium*

TSB, unvented	TSB, vented	
	Positive	Negative
Positive	7	0
Negative	20	

It would, therefore, appear from these data that, although a vented vacuum blood culture bottle is generally satisfactory for recovering yeasts from blood, their prompt detection is best accomplished in a vented bottle with biphasic media.

ACKNOWLEDGMENT

We are indebted to Duane M. Ilstrup, Department of Medical Statistics and Epidemiology, Mayo Clinic, for statistical analysis of the data.

LITERATURE CITED

1. Blazevic, D. J., J. E. Stemper, and J. M. Matsen. 1975. Effect of aerobic and anaerobic atmospheres on isolation of organisms from blood cultures. J. Clin. Microbiol. 1:154–156.
2. Dolan, C. T., and G. D. Roberts. 1974. Identification procedures: yeast and yeastlike organisms, p. 153–164. *In* J. A. Washington II (ed.), Laboratory procedures in clinical microbiology. Little, Brown and Co., Boston.
3. Gantz, N. M., J. L. Swain, A. A. Medeiros, and T. F. O'Brien. 1974. Vacuum blood-culture bottles inhibiting growth of *Candida* and fostering growth of *Bacteroides*. Lancet ii:1174–1176.
4. Maki, D. G., D. A. Goldmann, and F. S. Rhame. 1973. Infection control in intravenous therapy. Ann. Intern. Med. 79:867–887.
5. Roberts, G. D., and J. A. Washington II. 1975. Detection of fungi in blood cultures. J. Clin. Microbiol. 1:309–310.
6. Ruiz Castaneda, M. 1947. A practical method for routine blood cultures in brucellosis. Proc. Soc. Exp. Biol. Med. 64:114–115.
7. Ryan, J. A., Jr., R. M. Abel, W. M. Abbott, C. C. Hopkins, T. M. Chesney, R. Colley, K. Phillips, and J. E. Fischer. 1974. Catheter complications in total parenteral nutrition: a prospective study of 200 consecutive patients. New Engl. J. Med. 290:757–761.
8. Washington, J. A., II. 1975. Blood cultures: principles and techniques. Mayo Clin. Proc. 50:91–98.