

Detection of Fungi in Blood Cultures

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In a retrospective study covering the period January 1972 to June 1974, recovery rates of bacteria and of fungi were generally equivalent with tryptic soy broth, Thiol, thioglycolate, and Columbia broth media (all under vacuum with carbon dioxide and sodium polyanetholesulfonate). An additional biphasic medium, consisting of brain heart infusion broth and a brain heart infusion agar slant, which was inoculated only where fungal sepsis was suspected clinically, yielded significantly higher recovery rates of fungi. There were 29 instances of cultures with fungi in both the biphasic and broth media, 80 instances of cultures with fungi only in the biphasic medium, and no instances of fungi only in the broth media. The isolates were as follows: *Candida albicans*, 74; *C. parapsilosis*, 20; *C. tropicalis*, 16; *Torulopsis glabrata*, 18; *Torulopsis* sp., 1; *Cryptococcus neoformans*, 12; *C. laurentii*, 2; and *Histoplasma capsulatum*, 16. Despite routine subcultures of the broth media to chocolate blood agar within 24 h of inoculation and after 5 days of incubation, detection of fungemia was significantly improved by the use of a biphasic medium.

There has been an increase in the incidence of opportunistic infections in debilitated patients and, consequently, an increase in the number of cases of fungal sepsis. However, there have been few reports describing methods for recovery of fungi in blood cultures (1). In the present study, the rate of recovery of fungi in a specific fungal blood culture medium was compared with that in standard bacterial blood culture media during the period January 1972 to June 1974.

MATERIALS AND METHODS

In retrospective fashion, blood culture data from January 1972 to June 1974 were evaluated for the recovery of fungi. Only those cultures of specimens collected from the same patient and inoculated simultaneously into fungal and bacteriological media were compared. Not all bacterial blood cultures had a matching fungal blood culture (fungal blood cultures at the Mayo Clinic and affiliated hospitals are performed only by physician's order in cases clinically suspected to be fungal sepsis).

Fungal blood cultures. The standard fungal blood culture bottles prepared and used at the Mayo Clinic resemble the Castañeda bottles used for brucella cultures. Each contained a slant of 50 ml of brain heart infusion agar overlaid with 60 ml of brain heart infusion broth (Difco). After inoculation of 10 ml of blood into the culture bottles, they were vented with a sterile cotton-plugged needle and incubated in an upright position for 30 days at 30 C. Cultures were examined daily for visual evidence of growth, and each examination was followed by a gentle mixing of the blood-broth mixture over the agar slants. Visual growth was stained and subcultured onto appropriate media for identification.

Bacterial blood cultures. Throughout the period of this study, tryptic soy broth (Difco) (TSB) was used as the standard blood culture medium; however, several other media were evaluated including Thiol broth (Difco), thioglycolate broth (Difco), Columbia broth (Difco), and TSB enriched with 15% sucrose (Difco). Blood (10%, vol/vol) was inoculated in parallel into one bottle of TSB and into one of the various media under evaluation at a given time. Each bottle contained 100 ml of medium and 0.025% sodium polyanetholesulfonate (SPS). Each medium was under vacuum with CO₂ and no bottles were vented during incubation. Cultures were incubated for 2 weeks at 35 C and were observed daily for 7 days and again after 14 days for visual evidence of growth. Each was subcultured, within 24 h after collection and at 5 days, onto chocolate agar plates that were incubated for 48 h at 35 C under 10% CO₂. Previous studies in this laboratory, of approximately 6,000 bacterial blood cultures, failed to demonstrate any value in a routine 14-day subculture for recovery of fastidious microorganisms, including yeasts.

RESULTS

During the study period, 50,362 bacterial blood cultures and 7,425 fungal blood cultures were performed in our clinical microbiology laboratories. Of these cultures, 12 were considered to harbor fungal contaminants, including *Penicillium*, *Aspergillus*, and *Alternaria* species. The pathogenic fungi recovered from 7,425 fungal blood cultures are as follows: *Candida albicans*, 74; *Candida parapsilosis*, 20; *Candida tropicalis*, 16; *Cryptococcus neoformans*, 12; *Cryptococcus laurentii*, 2; *Histoplasma capsulatum*, 16; *Torulopsis glabrata*, 18; *Torulopsis*

sp., 1. Members of the genus *Candida* were most frequently isolated, with *C. albicans* being the most common organism. The only filamentous organism isolated was *Histoplasma capsulatum*. *Nocardia asteroides*, a rather uncommon bacterium seen in blood cultures, was also isolated in four fungal cultures.

The standard fungal blood culture medium yielded significantly higher recovery rates of fungi than did the bacterial blood culture media. There were 29 instances of cultures in both media positive for fungi, 80 instances of positive cultures in the fungal medium only, and no instances of positive fungal cultures in the bacterial culture media only.

There were no statistically significant differences in the recovery of fungi in the different bacterial blood culture media used throughout the study.

DISCUSSION

In this study, the biphasic fungal blood culture medium of brain heart infusion agar and broth appears to be superior ($P < 0.001$) to the bacterial blood culture media evaluated in detecting fungemia. Several possible reasons for this advantage are: (i) the fungal blood culture medium is more enriched, (ii) the fungal blood cultures are vented to provide an aerobic environment, (iii) mixing the blood-broth mixture over the agar surface provides a daily subculture, and (iv) the agar slant allows growth to

occur in the aerobic portion of the bottle above the liquid surface.

The biphasic fungal blood cultures were incubated for 30 days; however, bacterial blood cultures were kept for only 14 days. A recent study of 32 fungal blood cultures positive for yeast showed that the mean recovery time was 5.3 days (range, 2 to 14 days). Six *H. capsulatum* cultures had a mean recovery time of 16 days (range, 12 to 24 days). The 14-day incubation period for the bacterial blood culture appears to have been adequate because most of the organisms were recovered within this period in the fungal blood culture medium.

A preliminary study in our laboratory with simulated blood cultures containing a small inoculum of yeasts suggests that venting culture bottles (Difco) greatly increases the recovery of fungi from bacterial blood culture media; however, further studies to confirm these findings are being performed. Moreover, we do not presently know the extent to which these characteristics might be applicable to blood culture bottles from other manufacturers.

At the current time, we recommend that fungal blood cultures be considered when a request for a blood culture is received and that consideration be given to inoculating a vented biphasic medium; this should increase the chances of detecting fungemia.

LITERATURE CITED

1. Komorowski, R. A., and S. G. Farmer. 1973. Rapid detection of candidemia. *Am. J. Clin. Pathol.* **59**:56-61.