Comparison of Three Blood Culture Media for Recovery of Anaerobic Bacteria

JOHN A. WASHINGTON II AND WILLIAM JEFFERY MARTIN
Mayo Clinic and Mayo Foundation, Rochester, Minnesota 55901

Received for publication 11 September 1972

Comparison of an anaerobic brain heart infusion broth with tryptic soy broth and Thioglycollate medium failed to demonstrate significant differences in isolation rates of anaerobic bacteria from blood.

Previous studies from this laboratory have shown equivalent recoveries of anaerobic bacteria from blood in tryptic soy broth (Difco) and Thiol broth (Difco) (7) and in Trypticase soy broth (Becton, Dickinson, and Co.) and Thioglycollate-135C (Becton, Dickinson, and Co.) (8). Because of the frequency with which anaerobic bacteria were isolated in those media, a study was undertaken to compare the rates of recovery of anaerobic bacteria from blood in prereduced brain heart infusion (BHI) broth containing 0.025% sodium polyanethol-sulfonate (SPS) (supplemented with vitamin K and hemin mixture and with yeast extract; Robbins Laboratories), Thioglycollate (Thio) medium with SPS (Difco), and tryptic soy broth (TSB) with SPS (Difco).

MATERIALS AND METHODS

Blood, collected aseptically from patients suspected of having bacteremia, was inoculated (10%, v/v) into one bottle each of the three media, as described previously (7, 8). The bottles with TSB and Thio each contained 100 ml of media under vacuum with added CO₂, whereas the bottle of BHI contained 90 ml of anaerobic media. All bottles were incubated unvented at 35 C and were examined daily for 14 days. Routine subcultures of bottles without apparent growth were made at 24 hr to chocolate blood agar plates which were incubated in an atmosphere of 10% CO₂ for 48 hr.

Identification of anaerobic bacterial isolates was based on procedures and principles described by Dowell et al. (2–4) and by Cato et al. (1). Identification procedures for other isolates were as described previously (7).

RESULTS

There were 103 positive blood cultures (Table 1), including 28 in which at least one of the three media inoculated contained anaerobic bacteria of the following species: Bacteroides fragilis, B. melaninogenicus, Fusobacterium fusiforme, Eubacterium cylindroides, Peptostreptococcus, and Peptococcus. In 15 blood cultures, B. fragilis was recovered from all three media in each set (Table 2). Two additional isolates were recovered from two of the three media in each set, and the remaining ones were recovered from one of the three media only.

B. melaninogenicus, F. fusiforme, and E. cylindroides were each recovered once from TSB only. B. melaninogenicus was recovered from a second blood culture in all three media. The single isolate of Peptostreptococcus was recovered from both TSB and Thio, whereas the single Peptococcus was isolated from all three media.

Mean time intervals to positivity of B. fragilis in each medium were not substantially different (Table 3).

BSH was not a satisfactory medium for routine isolation of aerobic and facultatively anaerobic bacteria because, in 35 instances, such organisms were isolated from TSB or Thio but not from BHI; in only five instances were aerobes or facultative anaerobes isolated from BHI and not from either of the other two media.

DISCUSSION

In the previous two studies (7, 8) of blood culture media disregarding presumed contaminants, anaerobic bacteria accounted for approximately 12% of the positive cultures and for approximately 20% of the patients. In neither of those studies did the media contain SPS; in this study, all three media contained SPS. It is of interest that, of a total of 103 positive cultures (presumed contaminants deducted), 28 (27%) contained anaerobic bacteria, a rate which is slightly more than double that previously reported. That this increment
**Table 1. Clinically significant organisms recovered from 103 positive blood cultures**

| Organism                        | No. of times present |
|---------------------------------|----------------------|
| Staphylococcus aureus           | 13                   |
| Streptococcus, group D          | 10                   |
| Streptococcus, viridans group   | 7                    |
| Peptococcus                     | 1                    |
| Peptostreptococcus              | 1                    |
| Lactobacillus                   | 1                    |
| *Eubacterium* cylindroides      | 1                    |
| Candida albicans                | 1                    |
| Escherichia coli                | 27                   |
| Citrobacter freundii            | 1                    |
| Klebsiella pneumoniae           | 11                   |
| Enterobacter aerogenes          | 1                    |
| Proteus mirabilis               | 4                    |
| Pseudomonas aeruginosa          | 4                    |
| Pseudomonas spp.                | 3                    |
| Mima polymorpha                 | 1                    |
| Haemophilus influenza           | 1                    |
| Bacteroides fragilis            | 22                   |
| *B. melaninogenicus*            | 2                    |
| Fusobacterium fusiforme         | 1                    |
| Unidentified gram-negative bacilli | 2              |

*Single cultures containing *Bacillus, Corynebacterium* (aerobic and anaerobic), and *S. epidermidis* are not included. Nine cultures contained two or more bacteria.

**Table 2. Numbers of anaerobic isolates in positive cultures**

| Organism                        | No. positive | By medium* | By combination of media* |
|---------------------------------|--------------|------------|--------------------------|
|                                 |              | TSB        | Thio         | BHI | TSB + Thio | Thio + BHI | TSB + BHI |
| Bacteroides fragilis            | 18           | 19         | 19           |     | 1          | 1          | 5          |
| *B. melaninogenicus*            | 2            | 1          | 1            |     | 1          |            | 1          |
| Fusobacterium fusiforme         | 1            |            |              |     |            |            | 1          |
| *Eubacterium cylindroides*      | 1            |            |              |     |            |            | 1          |
| Peptococcus                     | 1            | 1          | 1            |     |            |            | 1          |
| Peptostreptococcus              | 1            | 1          | 1            |     |            |            | 1          |

*TSB, Tryptic soy broth; Thio, Thioglycollate; BHI, brain heart infusion.

**Table 3. Time interval to positivity for Bacteroides fragilis**

| Medium | No. | Days, mean |
|--------|-----|------------|
| TSB    | 18  | 3.1        |
| Thio   | 19  | 2.7        |
| BHI    | 19  | 2.5        |

is not attributable to the addition of a preduced medium may be seen in the results summarized in Table 1. To what extent the substantially smaller sample in this study may have influenced the results is uncertain. Whether or not recovery of anaerobes would be further enhanced by using an osmotically stabilized anaerobic broth medium, as described by Sullivan et al. (6), remains to be evaluated.

It is evident from the results presented in Table 1 that anaerobic bacteremia occurs with considerable frequency. That the vast majority of those due to the anaerobic gram-negative bacilli in particular are of clinical significance has been well documented in two studies with an aggregate of 190 patients seen at the Mayo Clinic and affiliated hospitals (5, 9).

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