Evaluation of Media for Selective Isolation of Yeasts from Oral, Rectal, and Burn Wound Specimens

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Six media were evaluated to determine their ability to isolate yeasts and inhibit bacteria. The media included the following: Snyder, Snyder tellurite, Sabouraud tellurite, Littman-gentamicin, molybdate, and Mycosel (BBL). Doses of mixed intestinal gram-negative bacilli and enterococci were most effectively inhibited by Snyder tellurite agar. Klebsiella pneumoniae was the most common bacterial contaminant of the other media. All six media were comparable in isolating yeasts while preventing the growth of the oral bacterial flora. The selection of a basal fungal growth medium for tellurite incorporation to inhibit bacteria but permit growth of yeasts was affected by pH. The bacteriostatic effect of tellurite was decreased with increasing pH of media while fungistatic action was increased. The arbitrary selection of Snyder and Littman agars to isolate yeast from burn wound cultures demonstrated the need to include a selective medium for these specimens. Blood, phenylethyl alcohol blood agar, and Columbia CN blood agar were all inadequate for isolating yeasts from burns. Growth of a variety of filamentous saprophytic and pathogenic dimorphic fungi grew adequately on four of five selective media tested.

A variety of media are available for the selective isolation of fungi from clinical specimens (1, 4, 5, 11, 15, 16). The addition of cycloheximide to fungal growth media will also inhibit numerous saprophytic and some pathogenic fungi (10). Unfortunately, the compromised host can become infected with a variety of organisms including fungi of relatively low pathogenicity (7). The latter fungi might include some that would be overlooked if specimens containing them were inoculated on cycloheximide media. Specimens which also contain large numbers of bacteria may obscure isolation of fungi on media-containing antibiotics such as chloramphenicol, which do not always inhibit bacteria (3, 5).

Burn patients represent one group that may experience yeast infection (6, 8) but at the same time may be infected or colonized with various bacteria. This study was conducted to evaluate the efficacy of several media to inhibit bacteria and selectively isolate yeast or possibly other fungi regardless of their pathogenic potential. Virtually any organism isolated from burn patients may be contributing to infection, and it is desirable to monitor burn patients as one means of infection control.

MATERIALS AND METHODS

Specimens. Rectal swabs from burned children were collected and inoculated on media by the method of Smith and Dayton (13). Swabs of the oral cavity of burned children and normal adults were taken by swabbing the oral mucosa, tongue, teeth, and gingiva. Swabs were rinsed in 4 ml of FM dilution broth (Difco), and 0.1-ml amounts were spread on plate media with glass rods. Enterococcusag lar (BBL) and MacConkey agar (BBL) were inoculated to detect the presence of enterococci and gram-negative bacilli in specimens, respectively. Surface burn wound cultures were taken by methods previously described (12). Burn wound surface swab cultures have unpredictable type and quantity of microorganisms. Swabs were rolled over one quadrant of a series of plates, followed by streaking each plate with a loop. Fungal media and their final pH, which was taken potentiometrically, were as follows: Snyder agar with 2% dextrose, pH 4.8; Snyder agar with 500 µg of filter-sterilized potassium tellurite per ml, pH 5.0; Sabouraud 4% dextrose agar with tellurite, pH 5.8; Littman oxgall medium with 40 µg of gentamicin per ml, pH 7.0; Mycosel chloramphenicol-cycloheximide agar, pH 7.0; and molybdate medium (2), pH 5.3. The molybdate medium was prepared with Sabouraud agar, Mycosphil agar, pH 4.8, and nystatin assay agar,
pH 6.0. Fungal media were also compared to Columbia sheep blood agar and either phenylethyl alcohol blood agar or Columbia-CN blood agar. All of the above basal or complete media were obtained from Baltimore Biological Laboratories, Cockeysville, Md. Fungal media were incubated at 30 or 37 C and observed daily for 3 days.

**Identification and pure culture studies.** Clinically isolated yeasts were identified by conventional morphological and biochemical methods (10). Gram-negative bacilli were identified with the API-20 Profile recognition system (Analytab Products, Inc., Carle Place, N.Y.). Enterococci were identified by Gram stain, lack of catalase activity, and by growth and esculin hydrolysis on bile-esculin agar (Difco). Staphylococci were identified by Gram stain, catalase activity, and coagulase tests (12). The effect of pH on tellurite inhibition of bacteria and yeasts was determined by measuring the minimal inhibitory concentration of potassium tellurite at pH 4.8, 5.7, and 7.3. Trypticase soy dextrose broth was used for bacteria, and Sabouraud 2% dextrose broth (BBL) was used for yeasts. Cultures were diluted to the Kirby-Bauer turbidity standard, and 0.05 ml of inoculum was added to tubes containing 2 ml of twofold serial dilutions of tellurite in broth. Since filamentous fungi were not encountered in clinical specimens, a group of saprophytic and pathogenic molds was tested for growth on selective media using the agar plug inoculation technique (9). Mycosel agar with cycloheximide was not included because many saprophytic fungi are inhibited from growing on the medium. Media were incubated at 28 C, and the diameters of colonial growth were measured after incubation ranging from 2 to 14 days depending upon the growth rates of the organisms. All fungi used in this experiment were kindly supplied by the Texas State Health Department, Austin, Tex.

**RESULTS**

The antibacterial properties of six fungal selective media were compared by inoculating the media with saline rinses of 44 rectal and 20 oral swab cultures (Table 1). The combined group of specimens yielded 20 yeasts which consisted of 12 Candida albicans, 4 C. tropicalis, 2 C. parapsilosis, 1 C. pseudotropicalis, and 1 Torulopsis glabrata. Bacteria from rectal specimens produced confluent growth on Snyder agar. Yeasts were isolated more frequently from Snyder tellurite agar, followed by Littman and molybdate media. The most common cause of bacterial contamination of the fungal media was *Klebsiella pneumoniae*. Five species of gram-negative bacilli resistant to gentamicin grew on Littman medium, but occurred in

| Specimen and no. tested | Yeast medium | No. and types of microorganisms isolated |
|------------------------|--------------|----------------------------------------|
|                        | Yeasts       | Enterococci                            | Gram-negative bacilli |
| Rectal, 44             | -            | 12                                     | 26 | 44 (confluent growth, none was isolated) |
|                        | Snyder       | 1                                      | 0  | 2 *Klebsiella pneumoniae* |
|                        | Snyder-tellurite | 9                                    | 1  | 10 (Escherichia coli, K. pneumoniae, Proteus mirabilis, Citrobacter sp., Pseudomonas aeruginosa) |
|                        | Littman oxgall with gentamicin | 7                                      | 0  | 0 |
|                        | Sabouraud tellurite | 3 (PG)*                              | 22 | 13 (K. pneumoniae) |
|                        | Molybdate    | 7                                      | 0  | 34 (K. pneumoniae, E. coli, Enterobacter cloacae) |
|                        | Mycosel      | 3                                      | 0  | 0 |
| Oral, 20               | -            | 8                                      | 2  | 5 (K. pneumoniae) |
|                        | Snyder       | 6                                      | 0  | 4 (K. pneumoniae) |
|                        | Snyder-tellurite | 8                                    | 0  | 0 |
|                        | Littman oxgall with gentamicin | 8                                      | 0  | 1 (K. pneumoniae) |
|                        | Sabouraud tellurite | 1 (PG)*                              | 1  | 2 (K. pneumoniae) |
|                        | Molybdate    | 6                                      | 0  | 5 (K. pneumoniae, Enterobacter agglomerans) |
|                        | Mycosel      | 8                                      | 0  | 2 (K. pneumoniae) |

* - Total number of yeasts and certain bacteria that were found in specimens. Yeast selective media were incubated at 30 C and observed daily for 3 days. Bacterial contaminants which grew on yeast selective media were isolated and identified.

*PG, Poor growth.*
numbers insufficient to obscure the growth and isolation of yeasts. Eight yeasts were recovered from oral swab specimens, and five of the six media were comparable in isolating them. Gram-negative bacilli were less frequently found in the oral specimens and, when present, occurred in small numbers. Sabouraud medium was not used alone to isolate yeasts because previous experiments demonstrated that gram-positive and gram-negative bacilli grew rapidly on the medium. The addition of 500 µg of tellurite per ml to Sabouraud agar inhibited fecal gram-negative bacilli but not enterococci. Yeasts, however, grew poorly or not at all on Sabouraud tellurite agar.

Since Snyder-tellurite agar was effective in inhibiting bacteria while allowing yeasts to grow, an experiment was conducted to determine the effect of pH on the minimal inhibitory concentration of potassium tellurite on bacteria and yeasts (Table 2). Acidic pH enhanced the bacteriostatic effect of tellurite, whereas alkaline pH enhanced the fungistatic action of tellurite. Two pure cultures of each of 10 species of yeasts were streaked on molybdate agar prepared with three basal media—MycoPhil, Sabouraud, and nystatin assay agar. The final pH values of these media were 4.8, 5.3, and 6.0, respectively. The yeasts included seven species of the genus Candida, Cryptococcus neoformans, Trichosporon cutaneum, and Torulopsis glabrata. Growth of these yeasts on the three media were comparable.

Burn wound specimens were then cultured on combinations of selective and nonselective media in two experiments to determine the need to include a fungal selective medium with these specimens (Table 3). In group 1, 25 burn specimens contained yeasts and all were isolated on Snyder agar, none were isolated on phenylethyl alcohol blood agar, and only six yeasts were isolated on plain blood agar. In most cases however, the burn specimens contained a variety of staphylococci, enterococci, and Pseudomonas aeruginosa. The growth of these bacteria on both blood agars obscured growth and isolation of yeasts. In group 2, 23 yeasts were isolated on Littman agar. Columbia-CN blood agar, which inhibited growth of all gram-negative bacilli, aided in permitting growth and recognition of yeasts more than blood agar.

Growth by most of the filamentous or dimorphic fungi on Littman agar was the slowest or most retarded (Table 4). Snyder, Snyder tellurite, Sabouraud tellurite, and molybdate media were generally comparable in supporting growth of these organisms. There was some variation in growth on tellurite and molybdate media, ranging from retarded to stimulated growth.

**DISCUSSION**

Several of the media studied, such as Littman and Mycosel agars, are known and can be expected in some capacity to isolate fungi and inhibit bacteria. The ability of these or other media to perform as indicated may be dependent upon the nature of the specimen being cultured and the condition of the patient. Nei-

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**Table 2. Effect of pH on potassium tellurite inhibition of bacteria and yeasts**

| Organism tested      | MIC of tellurite |
|----------------------|-----------------|
|                      | pH 4.8 | pH 5.7 | pH 7.3 |
| **Bacteria**         |        |        |        |
| Streptococcus faecalis | 15     | 125    | >500   |
| Staphylococcus aureus | 15     | 250    | 500    |
| Escherichia coli      | 15     | 31     | 125    |
| Klebsiella pneumoniae | 15     | 15     | 15     |
| Enterobacter cloacae  | 15     | 125    | 500    |
| Proteus mirabilis     | —b     | 15     | 50     |
| Salmonella enteritidis| —      | 15     | 62     |
| S. epidermidis         | —      | 15     | 250    |
| **Yeasts**            |        |        |        |
| Candida albicans      | >500   | 500    | 250    |
| C. krusei             | >500   | 500    | 250    |
| C. stellatoidea       | >500   | 250    | 125    |
| Trichosporon cutaneum | >500   | 500    | 250    |

*Bacteria were tested in Trypticase soy broth; yeasts were tested in modified Sabouraud 2% dextrose broth. Minimal inhibitory concentration (MIC) values were determined after 24 h of incubation at 35 C.*

*Organism did not grow in control broth at pH 4.8 without tellurite added. All other organisms grew in broths without tellurite.

**Table 3. Isolation of yeasts from burn wound cultures on selective and nonselective media**

| Group | Total no. of yeasts isolated* | No. of yeasts isolated on each medium |
|-------|-------------------------------|--------------------------------------|
| 1     | 25                            | Blood, 6                             |
|       |                               | Phenylethyl alcohol blood, 0         |
|       |                               | Snyder, 25                           |
| 2     | 23                            | Blood, 8                             |
|       |                               | Columbia-CN blood, 14                |
|       |                               | Littman, 23                          |

*Plates were incubated for 72 h at 35 C and observed daily.

*Total number of yeasts isolated by combined isolation on all media.
ther Littman medium with gentamicin, nor Mycosel medium with chloramphenicol, were sufficient to inhibit gram-negative bacilli from the intestinal tract. Higher concentrations of antibiotics in the media, or use of more dilute specimens, might have affected the efficacy of these media. Gentamicin is used extensively, however, in this hospital for the treatment and prevention of septicaemia, and it was observed that a variety of gentamicin-resistant, gram-negative bacilli were isolated from the feces of some patients.

Molybdate agar has been evaluated as a selective and differential medium for yeasts (2). The differential features of the medium were apparent in this study but not evaluated. In two previous studies (3, 5), the selective properties of molybdate agar were examined using spumut specimens and also challenged by inoculation with pure cultures of bacteria. The medium was also compared to malt agar containing chloramphenicol. The latter medium was inferior to molybdate medium in inhibiting bacteria. In the present study, K. pneumoniae was found to grow abundantly on molybdate agar, but this particular organism is very commonly found in the intestinal tract of burned children (14).

Snyder agar was of no value in inhibiting intestinal gram-negative bacilli, but the addition of 500 μg of tellurite per ml to the medium provided an effective antibacterial agent. Since Snyder agar has a pH of 4.8, a lower concentration of tellurite can be expected to inhibit bacteria. Snyder agar can also be prepared without autoclaving. Such a medium has both practical and economic advantages. For example, growth of yeasts on Sabouraud tellurite agar having a higher pH would inhibit yeasts, and Littman agar supplemented with gentamicin is very costly. The choice of a fungal medium for isolating yeasts from burns, as indicated in this study, is rather arbitrary, but essential. It was found that the use of blood, phenethyl alcohol, blood, or Columbia-CN blood agar for isolation of yeasts from burns would have been inaccurate. Littman and Snyder agars were both comparable in isolating yeasts from burn cultures, but Snyder agar is preferred in this laboratory because it is easier and less expensive to prepare than media with gentamicin. Snyder tellurite agar is a promising new selective medium for yeasts and additional studies are underway to continue its evaluation. Molybdate agar is also relatively simple and inexpensive to prepare and, with the possible exception of K. pneumoniae, will inhibit the growth of gram-positive and gram-negative bacteria from the upper respiratory tract and intestinal tract.

### Table 4. Comparative growth of various fungi on 5 selective media

| Time of incubation (days) | Organism            | Snyder | Snyder tellurite | Littman gentamicin | Sabouraud tellurite | Molybdate |
|--------------------------|---------------------|--------|------------------|--------------------|---------------------|-----------|
| 2                        | Mucor sp.           | 85     | 50               | 4*                 | 50                  | 50        |
|                          | Trichoderma sp.     | 65     | 60               | 25                 | 35                  | 70        |
|                          | Aspergillus fumigatus | 15     | 20               | 4                  | 20                  | 30        |
|                          | Penicillium sp.     | 30     | 20               | 20                 | 30                  | 20        |
|                          | Aspergillus niger   | 50     | 40               | 20                 | 40                  | 50        |
|                          | Absidia sp.         | 35     | 30               | 10                 | 45                  | 25        |
|                          | Paecilomyces sp.    | 35     | 30               | 10                 | 20                  | 35        |
| 5                        | Pullularia pullulans| 40     | 20               | 10                 | 35                  | 45        |
|                          | Fusarium sp.        | 45     | 50               | 40                 | 50                  | 60        |
|                          | Alternaria sp.      | 85     | 85               | 15                 | 35                  | 35        |
|                          | Curvularia sp.      | 50     | 50               | 20                 | 50                  | 50        |
|                          | Scopulariopsis sp.  | 60     | 35               | 30                 | 60                  | 27        |
| 14                       | Trichophyton tonsurans | 26     | 31               | 15                 | 30                  | 20        |
|                          | Sporothrix schenckii| 20     | 20               | 10                 | 22                  | 20        |
|                          | Cladosporium sp.    | 50     | 55               | 22                 | 50                  | 45        |
|                          | Fonsecaea pedrosi   | 12     | 20               | 15                 | 15                  | 20        |
|                          | Microsporum canis   | 35     | 38               | 40                 | 60                  | 15        |
|                          | Microsporum audouinii| 45     | 45               | 30                 | 50                  | 40        |

* Media were incubated at 28 C and diameter of colonial growth was measured to the nearest millimeter.
* Initial colony plug inoculum was 4 mm in diameter. Snyder agar (pH 4.8) was used as a growth control because it lacked a specific antibacterial inhibitor.
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