Gentamicin Blood Agar Used as a General-Purpose Selective Medium

WILLIAM A. BLACK1 AND FRANCES VAN BUSKIRK

Department of Microbiology, St. Joseph's Hospital, London, Ontario, Canada

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The potential value of a blood agar medium containing a final concentration of 5.5 μg of gentamicin per ml was assessed in a diagnostic laboratory over an 8-week period. The medium gave increased isolation rates of beta-hemolytic streptococci, other streptococci, Bacteroides, clostridia, and yeasts. It also proved valuable in detecting gentamicin-resistant gram-negative bacilli when these were present in heavy mixed culture.

RESULTS

Two hundred twenty-four of the specimens failed to grow on either routine culture media or GBA. The results are based on the remaining 742 specimens which comprised 282 vaginal or cervical specimens, 64 specimens from the perineal region, 150 specimens from abdominal abscesses or wounds, 212 specimens from superficial skin ulcers or wounds other than abdominal, and 34 specimens from other sites.

Organisms growing on any routine culture medium were recorded as positive findings in the columns of Table 1, which compares the isolation rate on GBA or the routine culture media, or both, and also analyzes the organisms isolated only on GBA. Other gram-positive or gram-negative bacteria isolated only on GBA included 4 (31%) strains of Clostridium perfringens and 17 (30%) strains of yeasts. Gentamicin-resistant gram-negative bacteria isolated only on GBA included 20 (26%) strains of Bacteroides and 10 (77%) strains of Providence. Gentamicin resistant gram-negative bacteria isolated on both GBA and routine culture media included three strains of Klebsiella-Enterobacter, and six strains of Serratia marcescens. GBA inhibited the growth of most strains of staphylococci and Klebsiella-Enterobacter, and all strains of Escherichia coli and Pseudomonas.

DISCUSSION

The availability of several selective media for

1 Present address: Department of Microbiology, University Hospital, London N6G 2K3, Canada.
the inhibition of gram-negative bacilli suggests that none is entirely satisfactory. Some (2, 3, 6) include several inhibitors, which makes preparation and quality control unnecessarily tedious. Others (1, 4) fail to inhibit *Pseudomonas* which is frequently encountered in mixed culture. Phenylethanol agar (no. 0504, Difco), which was used in this laboratory, alters both the colonial morphology and hemolysis of gram-positive isolates and also fails to kill *Proteus* strains which frequently reappear on subculture.

These problems prompted the introduction of GBA after early trials supported the hypothesis that the medium would be easy to prepare and would inhibit staphylococci in addition to a wide range of gram-negative bacilli. Initially introduced with a final gentamicin concentration of 5.5 μg/ml for the isolation of beta-hemolytic streptococci from mixed culture, GBA soon proved valuable also for the selective isolation of pneumococci, enterococci, anaerobic streptococci, clostridia, *Bacteroides*, and a variety of yeasts and yeastlike fungi. It was sufficiently nutritious to allow initial reading of plates to be made after overnight anaerobic incubation.

Only three beta-hemolytic streptococci failed to grow on GBA, whereas strains isolated on GBA alone accounted for 25% of all beta-hemolytic streptococci isolates (Table 1). The streptococci grown only on GBA were not Lancefield group A and almost 50% were Lancefield group B. The latter workers concluded that kanamycin would be a preferable selective medium for the isolation of *Bacteroides* group. The beta-hemolytic streptococci isolated on the routine media during the period were Lancefield group A. No evidence from previous work in this laboratory to suggest that the GBA isolates differed in distribution of Lancefield groups from organisms isolated on routine media.

GBA was effective in the selective isolation of other streptococci, *C. perfringens*, yeasts, and *Bacteroides* (Table 1). The failure of 16 (21%) *Bacteroides* species to grow on GBA supports the findings of Finegold and Sutter (5) that resistance to gentamicin is variable in the *Bacteroides* group. *Bacteroides* species isolated only on GBA accounted for 26% of the total *Bacteroides* isolates in the present survey.

An additional benefit of GBA was the isolation of three strains of *Proteus*, three strains of *Klebsiella-Enterobacter*, and six strains of *Serratia* which were unexpectedly resistant to gen-

| Organism                        | No. of isolates on both GBA and routine culture media | No. of isolates on GBA only (% of total isolates from all sources) | No. of isolates on routine culture media only | Total no. of isolates from all sources |
|---------------------------------|-------------------------------------------------------|-------------------------------------------------------------------|-----------------------------------------------|---------------------------------------|
| Beta-hemolytic streptococci     | 62                                                    | 22 (25%)                                                          | 3                                              | 87                                    |
| Enterococci                     | 30                                                    | 31 (41%)                                                          | 14                                             | 75                                    |
| Anaerobic streptococci          | 33                                                    | 10 (18%)                                                          | 13                                             | 56                                    |
| *Streptococcus viridans*        | 10                                                    | 15 (47%)                                                          | 7                                              | 32                                    |
| *Bacteroides* sp.               | 40                                                    | 20 (26%)                                                          | 16                                             | 76                                    |
| *Clostridium perfringens*       | 7                                                     | 4 (31%)                                                           | 2                                              | 13                                    |
| Yeasts                          | 27                                                    | 17 (30%)                                                          | 13                                             | 57                                    |
| *Serratia marcescens*           | 6                                                     | 1                                                                  | 1                                              | 7                                     |
| Staphylococci                   | 4                                                     | 205                                                               | 2                                             | 209                                   |
| *Proteus* sp.                   | 3                                                     | 50                                                                 | 1                                              | 53                                    |
| *Pseudomonas* sp.               | 4                                                     | 45                                                                 | 3                                              | 45                                    |
| *Providence* group              | 4                                                     | 78                                                                 | 3                                              | 78                                    |
| *Escherichia coli*              | 3                                                     | 78                                                                 | 3                                              | 78                                    |
| *Klebsiella-Enterobacter*       | 3                                                     | 78                                                                 | 3                                              | 78                                    |
| “Coliforms”*                    | 6                                                     | 78                                                                 | 3                                              | 78                                    |
| Others*                         | 6                                                     | 11 (16%)                                                          | 51                                             | 66                                    |

*Lactose-fermenting organisms in either pure or mixed growth. Considered commensal flora and not fully identified.

*A heterogeneous group which included isolates of lactobacilli, diphtheroids, pneumococci, *Acinetobacter*, and *Flavobacterium*.

### Table 1. Organisms isolated on GBA or routine culture media, or both, from 742 specimens during the 8-week period of study

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tamicin. The survival of Providence on GBA confirms earlier findings that at least 40% of Providence isolates in this hospital are resistant to gentamicin. The higher isolation rate of Providence on GBA in this series was due to selective isolation of strains which were obscured on the routine media by a heavy overgrowth of gentamicin-sensitive gram-negative bacilli.

The increased isolation rate on GBA of the potential pathogens previously mentioned is explicable by the difficulty in recognizing these organisms when they are present in mixed culture, particularly with gram-negative bacilli or staphylococci. Since the trial has ended, the use of GBA as an additional primary medium has, on several occasions, alerted technologists to the presence of unsuspected pathogens which have almost invariably been isolated after a reexamination of the cooked-meat broth. There is thus no evidence that discrepancies observed during the trial were attributable to deficiencies in the routine culture media.

In the study, GBA was used under test conditions which could most easily be simulated in a busy diagnostic laboratory. Thus, GBA plates were poured in the routine preparation room, and commercially available gentamicin was used rather than the pure gentamicin powder containing 53.9% active base (supplied by the Schering Corp.) which had been used in the initial trials. Again, only one-quarter of a plate of GBA was inoculated from each specimen, and plates were incubated only anaerobically and read after overnight incubation. There seems little doubt that even better results would have been obtained by using one plate per specimen and a longer incubation period. The trial, however, was intended to evaluate the practical usefulness of the medium rather than its absolute properties and, for this purpose, overnight incubation seemed most appropriate.

Occasional strains of potential pathogens failed to grow on GBA. Although this precludes its use as a sole primary diagnostic medium, it is less important than the fact that the organisms cited in Table 1 as having been isolated only on GBA would not have been detected at all had the later medium not been used. The use of a less inhibitory concentration of gentamicin was found to be unsatisfactory, since it did not yield a higher isolation rate of streptococci Bacteroides, clostridia, or yeasts but did permit a noticeable increase in overgrowth of gram-negative bacilli. Similar considerations weighed against the use of less inhibitory aminoglycosides with a narrower antibacterial spectrum such as neomycin. In practice, GBA has proven valuable in this laboratory as an additional primary diagnostic medium for specimens from sites where a mixed flora is common, such as wound swabs from the abdominal or perineal areas or swabs taken from burns or contaminated superficial skin ulcers. It has also proved to be the medium of choice for the subculture of a mixed bacterial growth thought to contain any of the pathogens which have been shown to selectively grow on the medium.

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