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Gentamicin Blood Agar Used as a General-Purpose Selective Medium

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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.

After the introduction of a blood-agar base containing gentamicin in this laboratory for the isolation of beta-hemolytic streptococci from mixed culture, it was frequently observed that the medium proved inadvertently useful in the selective isolation of other potential pathogens. The present study was designed to evaluate gentamicin blood agar (GBA) as a general-purpose selective medium, with particular reference to the routine diagnostic work of a service laboratory.

MATERIALS AND METHODS

Medium. The test medium was Columbia blood agar base (Oxoid CM331) containing final concentrations of 10% sheep blood and 5.5 μg of gentamicin per ml. The gentamicin used was a commercial preparation (Schering) containing 40 mg/ml. A working dilution of 110 μg/ml was stable for at least 3 months at 4 C.

Survey material. During an 8-week period from 19 January 1972 to 15 March 1972, 966 consecutive clinical specimens submitted to one section of the laboratory in Amies transport medium no. 0996, Difco) were plated on routine culture media and then on one-quarter of a GBA plate. The routine culture media used in every case included 7.5% sheep blood agar plates for aerobic and anaerobic incubation and a MacConkey plate. Most of the specimens were also inoculated into Robertsons cooked-meat medium. Gram-stained smears were made from every specimen.

The routine plates were incubated for 48 h aerobically and anaerobically before a final reading was made. The GBA plates were read after overnight incubation by a technologist who was unaware of the findings on the routine media. A comparison of both sets of independently recorded results was made retrospectively.

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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 ortal number of isolates in that group. Twenty-two (25%) strains of beta-hemolytic streptococci, 31 (41%) strains of enterococci, and 10 (18%) strains of anaerobic streptococci were isolated only on GBA. Other grampositive organisms 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

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

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	7
Staphylococci	4		205	209
Proteus sp.	3		50	53
Pseudomonas sp.			45	45
Providence group		10 (77%)	3	13
Escherichia coli			78	78
Klebsiella-Enterobacter	3		44	47
"Coliforms" [a]			76	76
Others[b]	6	11 (16%)	51	68

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

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

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 streptococcal isolates (Table 1). The streptococci grown only on GBA were not Lancefield grouped. About 25% of the beta-hemolytic streptococci isolated on the routine media during the period were Lancefield group A and almost 50% were Lancefield group B. There is 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. The latter workers concluded that kanamycin would be a preferable selectant in a medium designed for the isolation of *Bacteroides*. Even when using GBA as a general-purpose selective medium, however, the *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-

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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