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Selective Medium for the Isolation of Streptococci from Clinical Specimens

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Incorporating neomycin and nalidixic acid into a blood-agar base resulted in a medium highly selective for beta-hemolytic streptococci under conditions in which detection of streptococcal colonies by conventional means would have been very difficult.

Small numbers of beta-hemolytic streptococci on blood-agar can be difficult or impossible to detect in clinical specimens because of the presence of a myriad of other microorganisms. Neomycin, which has little or no effect on streptococci, can be incorporated into blood-agar to increase the probability of detecting beta-hemolytic streptococci (1). The present investigation is an evaluation of the use of a neomycin-nalidixic acid combination for the preparation of a highly selective medium for streptococci.

A conventional blood-agar plate and a plate containing the antibiotic mixture were streaked with throat swabbings from 208 patients. The blood-agar consisted of 2% Tryptose (Difco), 0.5% NaCl, 1.5% agar, and 6% defibrinated sheep blood (3). A 2-ml amount of a solution containing 3 mg of neomycin sulfate (Sigma Chemical Co.) and 1.5 mg of nalidixic acid (Sterling-Winthrop) was added to 100 ml of melted and tempered blood-agar base for the antibiotic medium. This gave final concentrations of 15 μg of nalidixic acid and 30 μg of neomycin per ml. Both plates were incubated overnight at 37 C under 90% N_2-10% CO_2 and were examined for the presence of streptococci, staphylococci, *Neisseria*, and gram-negative rods.

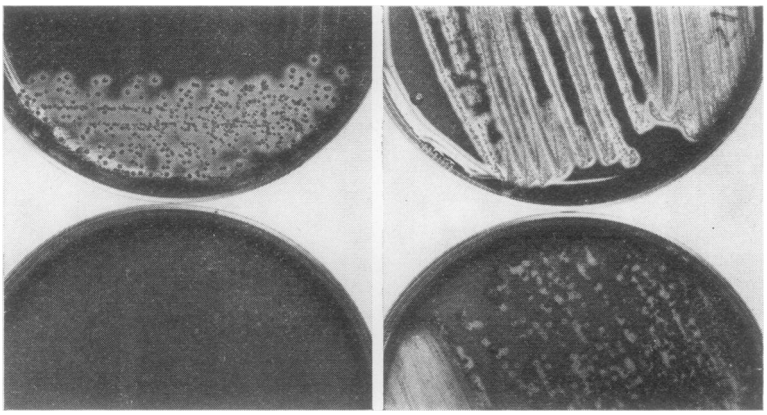

FIG. 1. *Selective medium for detection of streptococci. Plates at top contain conventional blood-agar medium and show uninhibited growth of beta-hemolytic staphylococci on the left and gram-negative rods on the right. Plates at the bottom contain antibiotic medium and show inhibition of staphylococci and gram-negative rods, respectively.*

Eighty-seven (41%) of the plates exhibited beta-hemolytic streptococci on both types of media; 64 of these isolates were identified as group A by fluorescent-antibody techniques (2). Two additional specimens were positive only on the antibiotic medium and both were group A beta-hemolytic streptococci. The presence of neomycin and nalidixic acid did not appear to have any effect on the growth of streptococci or

their hemolytic activity. Staphylococci (present in 80% of the specimens), gram-negative rods (present in 10.5%), and *Neisseria* were completely inhibited by the antibiotics. Figure 1 illustrates the improved visualization of beta-hemolytic streptococcal colonies. In both cases, detection of streptococcal colonies would have been extremely difficult on the conventional medium.

Where throat swabbings on Loeffler's serum slants are transported by mail, overgrowth by gram-negative rods and staphylococci is a serious problem and complicates primary isolation of beta-hemolytic streptococci. The use of the inhibitory medium described has considerably enhanced the laboratory examination of such specimens. When large numbers of specimens are to be examined for streptococci only, the use of this selective medium is recommended.

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