Some Observations on the Incorporation of Novobiocin into Hektoen Enteric Agar for Improved Salmonella Isolation

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Novobiocin was incorporated into Hektoen enteric agar as a means of suppressing those Proteus and Citrobacter organisms that produce colonies which are often mistaken for Salmonella.

It has already been established by Jeffries (2) and Hargrove et al. (1) that novobiocin is an effective agent for suppression of non-salmonellae in enrichment broths. This preliminary study was conducted to determine whether the selectivity of Hektoen enteric agar (HEA) (Difco Laboratories) for Salmonella could be improved by the incorporation of novobiocin and to determine what concentration of antibiotic would be required to accomplish this.

HEA has been reported as a plating medium for Salmonella isolation by King and Metzger (3). It is routinely used for Salmonella isolation in this laboratory and in various other quality control laboratories. Although selective for Salmonella, HEA suffers the disadvantage of also being selective for certain other non-salmonellae. Some Citrobacter and Proteus species produce colonies that appear similar to salmonellae (black-centered colonies) on HEA and that are sometimes identified as Salmonella.

For testing purposes, pure cultures of salmonellae and related organisms, which are often found in tetrathionate broth enrichments of feed materials, were employed in this study. Cultures were grown in lactose broth for 18 h at 37 C. chilled to stabilize their growth, diluted, and surface plated onto dry HEA plates containing novobiocin at 0, 2, 5, 10, 15, and 20 μg/ml (Upjohn Co.). These cultures included six serotypes of Salmonella, namely S. cubana, S. seftenberg, S. typhimurium, S. urbana, S. montevideo, S. worthington, and representative strains of Citrobacter freundii, C. diversus, Escherichia coli, Proteus mirabilis, and P. morganii. Various unidentified strains of Citrobacter, E. coli, Proteus, and Salmonella, which previously had been isolated from feed materials, chicken skins, frozen eggs, and sliced, prepared apples and identified by their biochemical reactions in differential media, were also employed. Broth cultures were surface plated in quadruplet at two different concentrations. HEA plates were incubated at 37 C for 18 to 20 h prior to counting. A reduction in bacterial colonies was considered an inhibitory result. Table 1 shows the relative degrees of growth of these enteric organisms with increasing concentrations of novobiocin in HEA.

When novobiocin at 5 or 10 μg/ml was incorporated into HEA, the Proteus and Citrobacter varieties employed in this study were inhibited. Two of the 10 Citrobacter and 12 of the 14 Proteus cultures produced black-centered colonies similar to salmonellae. A 50% reduction in Citrobacter growth was observed when novobiocin was added to HEA at 10 μg/ml of agar. No growth was observed with any of the Proteus strains employed in this study at this level. The E. coli varieties used in this study presented no problems, because they produced salmon-colored colonies; however, the growth of most strains tested appeared reduced at these concentrations of antibiotic.

Ten serotypes of Salmonella grew without any apparent inhibitory effects when novobiocin was used at 2, 5, 10, and 15 μg/ml in the HEA. All of these serotypes produced black-centered colonies on HEA. At 20 μg of antibiotic per ml of agar, Salmonella grew, but at a much slower rate and without the production of the typical black-centered colonies.

Novobiocin was stable at concentrations up to 20 μg/ml in HEA. Agar plates containing novobiocin stored for several weeks at 22 C gave
similar inhibitory results to freshly prepared plates with the nonsalmonellae.

A considerable difference was noted in the results obtained from HEA plates with and without novobiocin added when they were streaked with tetrathionate broth enrichments of feed materials. Twenty different Salmonella-positive feed materials were selected for this study. These, bone meal, fish meal, feather meal, and poultry-by-products meal, because of their content of Citrobacter and Proteus organisms, presented large numbers of colonies which could be mistaken for Salmonella on the HEA plates without novobiocin. HEA plates containing the antibiotic at 10 or 15 μg/ml consistently developed fewer Proteus and Citrobacter colonies, thus reducing the need for picking large numbers of presumptive Salmonella colonies for further biochemical characterization to establish the presence of salmonellae.

These findings suggest that the selectivity of HEA for Salmonella can be improved by the addition of novobiocin without adversely affecting Salmonella recovery. These results in HEA substantiate the findings in LICNR broth by Hargrove et al. (1) and in tetrathionate broth by Jeffries (2). The addition of novobiocin to other selective agars for improving their selectivity for Salmonella isolation is suggested.

Since only a relatively small number of salmonellae were tested, it is possible that some may not produce eugenic growth on HEA in the presence of novobiocin and/or their colonial morphology may be altered.

HEA is frequently used by diagnostic laboratories for the isolation of Salmonella and Shigella. Because the growth of Shigella on HEA in the presence of novobiocin has not been studied, this modified medium, for obvious reasons, should not be used for the isolation of enteric pathogens from clinical specimens.

**LITERATURE CITED**

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