APPLIED MICROBIOLOGY, Apr. 1968, p. 577-578
Copyright © 1968 American Society for Microbiology
Vol. 16, No. 4
Printed in U.S.A.

A New Plating Medium for the Isolation of Enteric Pathogens

I. Hektoen Enteric Agar

SYLVIA KING AND WILLIAM I. METZGER

Department of Microbiology, Cook County Hospital, and The Hektoen Institute for Medical Research, Chicago, Illinois 60612

Received for publication 26 January 1968

A new agar plating medium for the isolation of enteric pathogens is described. This medium contains greater quantities of peptone to offset the inhibitory effects of bile salts. Additional carbohydrates and larger quantities of those previously used have also been incorporated into the medium to differentiate pathogens from some of the slow lactose fermenters. The use of an indicator system never used before in a differential plating medium opens new possibilities for improvement over traditional and newer selective media. Known strains from the various genera of *Enterobacteriaceae* have been tested for colonial differentiation as well as for possible inhibitory effects. The results show the medium to be highly selective for the shigellae as well as for other enteric pathogens.

It is a common occurrence in diagnostic laboratories that certain strains of shigellae can be isolated readily on plating media such as E M B Agar but not on more selective media such as S S Agar. Enteric media in current use are either too inhibitory for many of the shigellae or not sufficiently inhibitory for normal intestinal flora. In addition, differentiation of colonies on current enteric media is not good enough to avoid the subculturing of many nonpathogens which do not ferment lactose. Such work is time-consuming and wasteful, especially in large laboratories receiving many specimens daily.

The present investigation was undertaken with the following objectives: (i) to develop a medium which would grow shigellae as readily as other pathogens while inhibiting normal intestinal flora, and (ii) to facilitate presumptive colonial recognition of enteric pathogens in such a medium with some degree of confidence. The medium which has been prepared meets these criteria and has been named Hektoen Enteric Agar (HE) after the institute where the study was conducted.

MATERIALS AND METHODS

This investigation was based upon attempts to overcome inhibition of shigellae by enriching the medium with extra amounts of carbohydrates and peptones (S. King and W. I. Metzger, Bacteriol. Proc., p. 77, 1967) and by use of an indicator system of minimal toxicity (E. Neter and A. Brody, Intern. Congr. Microbiol., 1950).

The HE Agar we prepared was composed of lactose (12 g), sucrose (12 g), salicin (2 g), bile salts (15 g), sodium chloride (5 g), proteose peptone (12 g), beef extract (3 g), agar (14 g), and distilled water (1,000 ml). To these components, we added 16 ml of 0.4% bromothymol blue and 20 ml of Andrades' indicator, and the *p*H of the medium was adjusted to 7.5. The medium was boiled until the components were completely dissolved; then 20 ml of a solution containing sodium thiosulfate (34 g), ferric ammonium citrate (4 g), and distilled water (100 ml), and 20 ml of a solution containing sodium deoxycholate (10 g) and distilled water (100 ml) were added. The *p*H was readjusted to 7.5.

Sucrose and salicin were incorporated into the medium to eliminate from consideration organisms that might ferment these sugars rapidly. Colonies belonging to *Parocolobactrum arizonae* were recognized as nonfermenters.

We have used the indicator system of bromothymol blue and Andrades' for many years in semisolid carbohydrate fermentation media with little or no apparent toxicity. For this reason, it was incorporated into the present medium.

An H_2S indicator was added in the same amount as that employed by Taylor (1).

The inhibitor system of bile salts and sodium deoxycholate in the amounts listed was formulated after many plating trials with known enteric organisms. Both a bile salts complex and individual components of bile salts were investigated, and the former was found to be more efficient than any single com-

TABLE 1. *Growth of microorganisms in the presence of bile salts and sodium deoxycholate (0.2%)*

Percentage of bile salts	Microorganisms					
	Escherichia coli	*Citrobacter*	*Proteus mirabilis*	*Paracolobactrum arizonae*	*Salmonella*	*Shigella*
0.5	+++	+++	++	+++	+++	+++
1.0	++	+++	+	+++	+++	+++
1.5	−	+	−	++	+++	+++
2.0	−	−	−	++	+++	++
3.0	−	−	−	−	+++	+

TABLE 2. *Colony characteristics of Enterobacteriaceae on HE agar*

Organisms	Description
Shigella, Providencia	Green colonies, moist raised
Salmonella, Paracolobactrum, Proteus	Blue-green to blue colonies with or without black centers
Pseudomonas	Green or brownish colonies, flat, irregular
Coliform	Salmon-colored colonies

ponent. However, a final concentration of 1.5% bile salts and 0.2% sodium deoxycholate was found to be optimal for the inhibition of many nonpathogens while relatively noninhibitory for enteric pathogens.

RESULTS AND DISCUSSION

Table 1 shows the relative degrees of growth of various enteric organisms in increasing concentrations of bile salts, each containing 0.2% sodium deoxycholate. At a concentration of 1.5% bile salts, *Escherichia coli* and *Proteus mirabilis* were completely inhibited and the *Citrobacter* group was almost completely inhibited. Several strains of each organism listed in the table were tested, as were many additional species of *Salmonella*. Findings within genera were comparable. It is to be expected that strain variation will occur as related to the degree of inhibition by any system. For example, we recently isolated a strain of *S. typhimurium* from stool that grew on E M B Agar but was completely inhibited on S S and HE Agars.

Table 2 shows the colonial characteristics of enteric organisms on HE Agar. Lactose fermenters are readily distinguishable from organisms which do not ferment lactose. In addition, the shigellae assume a different color than do many of the other organisms which do not ferment lactose. Of course, biochemical and serological confirmatory tests are necessary.

The addition of sucrose and salicin to S S Agar was reported by Neter and Brody (Intern. Congr. Microbiol., 1950). However, these investigators felt that pathogenic paracolons might be lost on such a medium. None of the *P. arizonae* tested by us have demonstrated fermentation on our medium.

Good growth of shigellae on HE Agar (S. King and W. I. Metzger, Bacteriol. Proc., p. 77, 1967) may be due to (i) growth promotion by added amounts of carbohydrates and peptones, (ii) lessened toxicity of the indicator system, (iii) lessened toxicity of the inhibitor system, or (iv) a combination of the above. When bile salts from various sources and from several lots from the same source were tested, we found considerable variation in the extent of inhibition of given organisms. We selected a smaller quantity of bile salts (Case Laboratories, Inc., Chicago, Ill.) than that which originally had given us optimal results. Perhaps inhibition of certain strains of enteric organisms on a given medium is related not only to strain variation but also to differences in the bile salts complex.

The HE Agar medium described is characterized by good growth of enteric pathogens, including shigellae, inhibition of many nonpathogens, and good colonial differentiation of major groups. The primary differences between this medium and existing media are (i) increased amounts of fermentable carbohydrates and peptones, and (ii) the indicator system. It is suggested that these improvements overcome the inhibitory effects of bile salts and sodium deoxycholate on less hardy intestinal pathogens, especially shigellae. We believe this medium will be helpful in diagnostic laboratories.

LITERATURE CITED

1. TAYLOR, W. I. 1965. Isolation of shigellae. I. Xylose lysine agars; new media for isolation of enteric pathogens. Am. J. Clin. Pathol. **44**:471–475.