Selective Medium for Isolation of *Klebsiella pneumoniae*

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Selective media for *Klebsiella pneumoniae* have been important in studies of hospital-acquired infections. On an agar medium which included ornithine, raffinose, and Koser citrate, *K. pneumoniae* strains grew as yellow mucoid colonies at 24 h and there was some increase in colony size at 48 h. Other members of *Enterobacteriaceae* were inhibited or produced small pink colonies on this same medium. *Pseudomonas*, *Providencia*, *Acinetobacter*, and *Proteus* species did not grow or showed very poor growth. The growth and appearance of these bacteria were not influenced by pH changes over a pH range of 5.2 to 6.4. Of 368 swabs of body sites cultured on MacConkey agar and on the test medium, 121 *K. pneumoniae* isolates on MacConkey agar and the same number on the test medium resulted. There were no discrepancies between the two media. Upon direct plating of stool, however, more *K. pneumoniae* colonies were isolated on the test medium than on MacConkey agar. Colonies on the test medium were more readily selected and identified than the colonies on MacConkey agar. There was also no inhibition of *K. pneumoniae* growth on the test medium compared with blood agar medium. This medium may be useful for the selective isolation of *K. pneumoniae*.

In epidemiological studies of nosocomial infection, selective media have been used to isolate particular species of bacteria from mixed cultures. A number of different selective media have been used to isolate *Klebsiella pneumoniae*, which has been a significant pathogen in hospital-acquired infections (4). On these media, however, it has been difficult to differentiate *K. pneumoniae* from *Enterobacter* spp. in particular. We have developed a selective medium for the isolation of *K. pneumoniae* to examine the distribution of *K. pneumoniae* in patients and in the environment.

MATERIALS AND METHODS

Media. The following medium was prepared containing per liter of distilled water: agar (BBL Microbiology Systems, Cockeysville, Md.), 20.0 g; Koser citrate (Difco Laboratories, Detroit, Mich.), 5.7 g; phenol red (5% phenol red in 50% EtOH), 10.0 ml; L-ornithine (Spectrum Chemical Manufacturing Corp., Redondo Beach, Calif.), 10.0 g; and raffinose (BBL), 7.0 g. The pH was adjusted to 5.6 with 1 N HCl, and the medium was autoclaved at 121°C for 15 min and cooled before being poured into petri plates. (This is a modification of a medium suggested to us by S. D. Kominos [personal communication].) MacConkey agar plates and blood agar plates were obtained from Clinical Standards Laboratory, Carson, Calif.

Organisms. *K. pneumoniae*, *Acinetobacter anitratus*, *Proteus* spp., *Enterobacter* spp., and *Pseudomo-*

nas sp. were clinical isolates from the Microbiology Laboratory at Rancho Los Amigos Hospital. The identity of these isolates was confirmed by standard techniques (1).

Appearance of colonies. Overnight broth cultures in brain heart infusion broth (Difco) were diluted at 1:10⁻³ with saline, and 0.01 ml was spread with a calibrated loop onto the surface of the test medium and MacConkey agar. The plates were observed at 24, 48, and 72 h, and the size and color of colonies on both media were recorded.

Effect of media on bacterial survival. Growth of *K. pneumoniae* 1260, 1302, and 1390 were compared on 5% sheep blood agar (blood agar), MacConkey agar (Clinical Standards Laboratories), and the test medium. Serial, 10-fold dilutions of overnight brain heart infusion broth cultures were prepared in saline. A calibrated loop (0.01 ml) was used to inoculate duplicate plates of the three media with each dilution. The colonies were counted after 48 h of incubation at 37°C.

Growth over a range of pH values. The pH of the test medium was adjusted with 1 N HCl before the addition of agar. The pH was not affected by autoclaving. Overnight broth cultures of *K. pneumoniae* (five strains), *Enterobacter* (four strains), *Escherichia coli* (one strain), and *A. anitratus* (one strain) were prepared and streaked onto plates of each pH value. The size and color of colonies were compared after incubation at 37°C for 48 h.

Clinical isolation of *K. pneumoniae* on test medium and MacConkey agar. A total of 368 swabs obtained from rectal, perineal, and urethral areas were placed in Koser citrate broth (Difco) and incubated

for 18 to 24 h at 37°C. The broth cultures were then streaked onto MacConkey agar and the test medium and compared for efficacy in the isolation of *K. pneumoniae*.

The effectiveness of the test medium in the isolation of *K. pneumoniae* from 24-h stool specimens was also examined. One gram of stool was placed in a tube containing 9 ml of saline and sterile glass beads. The contents of the tube were blended in a Vortex mixer until a homogeneous suspension resulted. Serial 10-fold dilutions in saline were made, and 0.01 ml was spread with a calibrated loop onto duplicate plates of blood agar, MacConkey agar, and the test medium. The plates were incubated at 37°C, and colony counts were made after incubation for 48 h.

K. pneumoniae isolates were confirmed by routine biochemical procedures.

RESULTS

Table 1 shows the comparison of the growth of six strains of *K. pneumoniae* and 24 isolates of other gram-negative bacilli on the test medium and on MacConkey agar. All six strains of *K. pneumoniae* showed abundant growth, large colonies, and a golden yellow color. The colonies were larger at 48 h.

The *Enterobacter* spp., which are sometimes difficult to distinguish from *K. pneumoniae* on MacConkey agar, grew on the test medium. However, the growth was not as luxuriant as that of *K. pneumoniae*, the colony size was significantly smaller, and the colony color was different from that of *K. pneumoniae*. The only other gram-negative rod showing a yellow colony color was one strain of *A. anitratus*, which showed a colony size and color similar to those of *K. pneumoniae*. In repeated experiments, however, this strain of *A. anitratus* showed variable growth and a pink colony color. *Pseudomonas*, *Providencia*, and *Proteus* species tested showed poor growth on the test medium. None of the *Proteus* spp. showed swarming on the test medium or on MacConkey agar.

Comparison of growth at various pH values. The growth of the bacteria was examined at pH 5.2 to 6.4. *K. pneumoniae* strains showed little variation in growth (i.e., colony size and color) from pH 5.2 to 6.4 after incubation for 24 h. At pH 6.4, the colony size of *K. pneumoniae* was smaller, but the color remained fairly constant. After incubation for 48 h, the colonies showed little variation in size or color from pH 5.2 to 5.9. From pH 6.0 to 6.4, the colony size remained large, but colony color tended to range from orange to salmon pink.

Change in pH seemed to have little effect on the growth or color of other gram-negative rods tested on the medium.

Effect of media on survival of *K. pneumoniae*. Colony counts of *K. pneumoniae* grown on blood agar, MacConkey agar, and the test medium inoculated with serial 10-fold dilutions of 18- to 24-h broth cultures of *K. pneumoniae* showed some differences in growth. Results from a 10^{-5} dilution of the overnight culture inoculated onto blood agar, MacConkey agar, and the test medium are shown in Table 2. The number of colonies given for each medium is the mean of duplicate plates. The number of colonies seen on the test medium was similar to the number seen on blood agar. Approximately half as many colonies were seen on MacConkey agar.

Clinical specimen growth on the test medium and MacConkey agar. The effectiveness of the test medium in isolating *K. pneumoniae* from mixed cultures grown from patient swabs was shown to be equal to that of the MacConkey agar. Of 368 swabs from rectal, perineal, and urethral areas, 121 were found to contain *K. pneumoniae* colonies on both MacConkey agar and the test medium. Of 24 stool cultures on MacConkey agar, 7 showed growth of *K. pneumoniae*, and 10 of 24 showed growth of *K. pneumoniae* on the test medium. In neither of these

TABLE 1. Known isolates on Klebsiella medium and MacConkey agar

Organism	No. of strains	Color		Size of colony relative to MacConkey agar
		24 h	48 h	
<i>K. pneumoniae</i>	6	Yellow	Yellow	Same
<i>Pseudomonas aeruginosa</i>	3	Pink	Pink	Small
<i>Proteus mirabilis</i>	3	Colorless	Colorless	Pinpoint
<i>Proteus rettgeri</i>	2	Pink	Pink	Small
<i>Proteus stuartii</i>	1	Pink	Pink	Small
<i>Acinetobacter anitratus</i>	6	Colorless to pink	Pink ^a	Small
<i>Enterobacter agglomerans</i>	2	Colorless to pink	Pink to red	Small
<i>Enterobacter cloacae</i>	1	Pink	Orange	Small
<i>Escherichia coli</i>	3	Colorless to pink	Colorless to pink	Same
<i>Enterobacter aerogenes</i>	3	Colorless to pink	Colorless to pink	Variable

^a One of six strains had large yellow colonies.

TABLE 2. *Survival of K. pneumoniae on different media*

<i>K. pneumoniae</i> strain no.	No. of bacteria recovered		
	Blood agar ^a	MacConkey agar	Test medium
1260	139 (100) ^b	74 (53)	129 (93)
1302	131 (100)	58 (44)	129 (98)
1390	152 (100)	57 (38)	120 (79)

^a Growth on blood agar was considered to be 100%.

^b Numbers within parentheses indicate percentages.

clinical studies were there isolates of species other than *K. pneumoniae* that had the characteristics of the *K. pneumoniae* colonies on the test medium.

DISCUSSION

In epidemiological studies of *K. pneumoniae* colonization of patients and the environment, microorganisms present in low numbers may be overlooked unless selective media are used. A number of selective media for *K. pneumoniae* have been described previously (2, 3, 5, 6). Citrate medium has been the most frequently used selective medium, but it has not been possible to distinguish *Enterobacter* spp. from *K. pneumoniae* on this medium.

The test medium containing raffinose and ornithine that we have examined may prove to be a helpful tool in the isolation of *K. pneumoniae* from mixed cultures. The characteristic golden color of *K. pneumoniae* caused by its utilization of the raffinose in the medium appears to be a reliable indicator. It is difficult to differentiate *K. pneumoniae* from the *Enterobacter* spp. on MacConkey agar. *K. pneumoniae* is predominantly raffinose positive and ornithine negative. Members of the genus *Enterobacter*, however, are predominantly ornithine positive and show

the red color of the basic reaction. One of six strains of *A. anitratus* did produce yellow colonies on a single occasion, but this was not replicated in further experiments. This result was unexplained, particularly since, in our experience, the species does not ferment raffinose.

When dilutions of an overnight culture of three strains of *K. pneumoniae* were plated on MacConkey agar and on the test medium, there was significantly less growth on MacConkey agar. There were no differences between the MacConkey agar and the test medium in the isolation of *K. pneumoniae* from swabs cultured overnight in citrate broth. The numbers of organisms in an overnight broth culture were adequate to be recovered on both types of media. The direct plating of stool specimens, however, showed a better recovery of *K. pneumoniae* on the test medium. In three instances, *K. pneumoniae* was isolated on the test medium but was not recovered from MacConkey agar. Since the test medium contains no antibiotic or inhibitory agents, even small numbers of *K. pneumoniae* could be expected to grow on it.

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