APPLIED AND ENVIRONMENTAL MICROBIOLOGY, Sept. 1978, p. 536-538
0099-2240/78/0036-0536$02.00/0

Vol. 36, No. 3
Printed in U.S.A.

Primary *Klebsiella* Identification with MacConkey-Inositol-Carbenicillin Agar†

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Received for publication 12 June 1978

MacConkey-inositol-carbenicillin agar has successfully been used as a primary selective medium for *Klebsiella* enumeration. With pure cultures, nearly 100% recovery of *Klebsiella* was observed by membrane filtration. With environmental samples using membrane filtration, 95% of typical pink- to red-colored colonies were verified as *Klebsiella*, as opposed to only 1% of yellow background colonies. Recovery of *Klebsiella* on MacConkey-inositol-carbenicillin agar was as good or better than on mEndo agar LES (Difco Laboratories). Recovery and percent colony confirmation with MacConkey-inositol-carbenicillin agar were greater than for other proposed *Klebsiella* selective media.

It is always desirable to have a selective/differential medium specific for the rapid detection and efficient recovery of an opportunistic pathogen such as *Klebsiella*. Much effort has been devoted in recent years to the isolation and enumeration of *Klebsiella* in clinical, industrial, and natural environments. Such studies have demonstrated that *Klebsiella* indistinguishable from clinical isolates are also associated with various botanical environments including drinking water supplies, pulp and textile mill effluents, sawdust bedding, fresh vegetables, etc. (1, 7-9).

A large portion of our effort has been devoted to the tedious task of picking total coliform colonies from media and determining concentrations of *Klebsiella* by their subsequent identification in routine testing procedures. Although selective/differential media for *Klebsiella* have been recently devised, without exception they have proven either inconvenient or unsatisfactory in our hands.

In this study, MacConkey-inositol-carbenicillin agar (MCIC) was devised and tested for use by membrane filtration as a selective medium for primary *Klebsiella* enumeration. Inositol was used as the substrate because it is fermented by virtually all *Klebsiella* cultures (5) and has already been used in selective *Klebsiella* media (4, 6). Carbenicillin was added as the primary inhibitor in MCIC because environmental and clinical *Klebsiella* have high resistance to this antibiotic (7), unlike other *Enterobacteriaceae*, particularly *Enterobacter aerogenes* (A. P. Dufour and L. B. Lupo, Abstr. Annu. Meet. Am. Soc. Microbiol. 1977, Q5, p. 262). After studies with MCIC were in progress, it was found that Thom (10) had used a similar medium for *Klebsiella* detection in feces; Thom, however, used a higher level of carbenicillin than we used in MCIC medium.

MCIC was prepared by suspending 40 g of MacConkey agar base (Difco Laboratories, Detroit, Mich.) and 10 g of inositol (*myo*-inositol, Sigma Chemical Co., St. Louis, Mo.) in 1 liter of distilled water. After autoclaving the mixture at 121°C for 15 min, it was cooled to 50°C in a water bath. For each liter of medium, 0.05 g of carbenicillin (Geopen, Roerig-Pfizer, Inc., New York, N.Y.) was added (dissolved in 5 ml of sterile distilled water), resulting in a final concentration of 50 μg/ml. After mixing well, the medium was poured into sterile petri plates. Since carbenicillin potency decreased during storage, the plates were kept at 4°C and were used within 72 h.

Klebsiella cultures appeared as pink- to red-colored colonies on the agar surface within 24 h of incubation at 35°C, indicating inositol fermentation. All *Klebsiella* test cultures were obtained from a variety of human clinical, animal, and environmental sources (1, 9). Cultures were divided into three groups (8): (i) fecal coliform-positive *K. pneumoniae*, (ii) fecal coliform-negative *K. pneumoniae*, and (iii) indole-positive *K. oxytoca*. Other organisms tested were isolated at Oregon State University, Corvallis.

Additional *Klebsiella* media used for comparison studies were double methyl violet agar (DV; 2, 3), acriflavine-violet red bile agar (D. Y. C.

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Fung, Abstr. Annu. Meet. Am. Soc. Microbiol. 1976, P2, p. 187), and *Klebsiella* membrane filtration (mK) agar (Dufour and Lupo, Abstr. Annu. Meet. Am. Soc. Microbiol. 1977, Q5, p. 262).

As measured by surface culture growth after streaking, all types of *Klebsiella* grew equally well on MCIC, MacConkey agar base and inositol (without the primary inhibitor, carbenicillin), and the nonselective standard plate count agar (Difco). Although most *Klebsiella* grew on MacConkey-inositol with 100 μg of carbenicillin per ml (as used by Thom [10]), growth was poorer than with the drug at 50 μg/ml. For this reason, the lower carbenicillin concentration was used throughout the remaining experiments.

Recovery using membrane filtration techniques was determined by filtering approximately 30 cells of 18-h *Klebsiella* nutrient broth cultures. The percent culture recovery for all types of *Klebsiella* tested was determined by dividing the number of colonies appearing on MCIC, mK, DV, and acriflavine-violet red bile agar media by the colony count on standard plate count agar (Table 1). Pure cultures of *Enterobacter aerogenes*, *Enterobacter agglomerans*, *Escherichia coli*, and *Serratia marcescens* had 0% recovery by membrane filtration on all media. The nearly 100% recovery for all three types of *Klebsiella* on MCIC was significantly greater than percent recovery for any of the other media. mK recovery, averaging 36% for 18 *Klebsiella* cultures, was lower than the 75% recovery reported for five cultures (Dufour and Lupo, Abstr. Annu. Meet. Am. Soc. Microbiol. 1977, Q5, p. 262). No percent pure culture recovery results have been reported for DV or acriflavine-violet red bile agar.

Water and raw sewage samples were also tested for *Klebsiella* recovery on MCIC by using membrane filtration techniques. Any pink- to red-colored colonies, regardless of size, were considered as presumptive *Klebsiella*. All background growth was yellow. The specificity of MCIC for *Klebsiella* detection was demonstrated by the fact that 95% of all presumptive colonies were verified as *Klebsiella* (Table 2). The only other organism appearing as a "typical" colony was *Enterobacter aerogenes*. Only 1% of the background growth colonies were *Klebsiella*. Both indole-positive (*K. oxytoca*) and indole-negative *Klebsiella* were detected on MCIC.

Equal or greater numbers of *Klebsiella* per 100 ml were detected in environmental samples (Table 3) by using MCIC, as compared to num-

TABLE 1. *Comparative recovery of Klebsiella by membrane filtration on selective media*[a]

Culture	Mean % culture recovery with:[b]			
	MCIC	mK[c]	DV	AF
FC-positive *K. pneumoniae*	97 (7/7)	37 (3/5)	0 (0/3)	63 (6/6)
Range	84–111	0–83	0	34–79
FC-negative *K. pneumoniae*	98 (7/7)	34 (3/7)	1 (1/3)	32 (2/3)
Range	93–107	0–72	0–2	0–75
I-positive *K. pneumoniae*	98 (7/7)	37 (3/6)	9 (2/3)	57 (9/9)
Range	89–123	0–83	0–18	25–68
Mean	98 (21/21)	36 (9/18)	3 (3/9)	55 (17/18)

[a] FC, Fecal coliform; I, indole; AF, acriflavine-violet red bile agar.

[b] Percent recovery calculated as number of colonies on selective agar per number of colonies on standard plate count agar. Numbers in parentheses represent number of cultures growing on each medium per total number of cultures tested.

[c] Counts recorded after 48 h of incubation.

TABLE 2. *Verification of colony type on MCIC medium*

Sampling location	No. of samples	Colonies			
		Typical (pink-red)		Atypical (yellow)	
		No. tested	% *Klebsiella*[a]	No. tested	% *Klebsiella*[a]
Oak Creek	23	160	93	109	2
Coyote Creek	4	20	100	10	0
Well water	4	6	100	5	0
Raw sewage	2	27	100	48	0
Total	33	217	95	172	1

[a] Values indicate percent verified as *Klebsiella* by Simmons citrate, indole, motility, lysine and ornithine decarboxylase, and urease tests.

TABLE 3. *Comparative environmental detection of Klebsiella on selective media*

Sample type	Sampling time	No. of samples	*Klebsiella* detected/100 ml on:[ab]				Total coliforms/100 ml[a] (mEndo agar LES)
			MCIC	mK[c]	DV	mEndo agar LES	
Raw sewage	1	1	2.5×10^6 (100)	6.0×10^5 (100)	1.0×10^5 (10)	$<1.0 \times 10^7$	2.0×10^7
Raw sewage	2	1	7.0×10^5 (100)	2.5×10^5 (84)	7.0×10^4 (14)	7.0×10^5	4.0×10^6
Stream	1	1	4.0×10^1 (93)	9 (89)	6 (0)	1.0×10^1	3.6×10^2
Stream	2	3	4.5×10^1 (97)	NT	NT	3.7×10^1	1.7×10^2
Stream	3	7	6.0×10^1 (98)	NT	NT	5.3×10^1	5.2×10^3

[a] Values indicate average results for each sampling time.

[b] Percent verification for each *Klebsiella* selective medium reported in parentheses. Colonies on all media were verified as *Klebsiella* by Simmons citrate, indole, motility, lysine and ornithine decarboxylase, and urease tests. NT, Not tested.

[c] Values indicate counts recorded after 48 h of incubation.

bers of *Klebsiella* appearing as total coliforms on mEndo agar LES (Difco). In spite of heavy background growth at times, 93 to 100% of all typical colonies were confirmed as *Klebsiella*. *Klebsiella* detected by MCIC represented from 1 to 26% of the total coliforms present in the environmental samples. In laboratory studies, 100% of added *Klebsiella* were detected by both membrane filtration and streak plate techniques, representing <0.00001% of the total coliforms originally present. Studies using DV and mK media resulted in not only lower *Klebsiella* recovery than those using MCIC, but also in lower percent verification, particularly with DV.

MCIC has therefore been found to be a highly specific medium for primary *Klebsiella* identification. The level of carbenicillin used was apparently sufficient to inhibit growth of *Enterobacter aerogenes* in both pure culture and environmental samples. Presumptive *Klebsiella* colonies can easily be counted, even if there is background growth on membrane filter surfaces. This medium had advantages over other proposed selective *Klebsiella* media in having a higher percent recovery, a higher percent confirmation of typical colonies, and an incubation time of only 24 h. Based on the results of these experiments and those of Thom (10), MCIC could routinely be used for *Klebsiella* enumeration in both environmental and clinical samples.

This work was supported by U.S. Environmental Protection Agency research grant R-8044 56 and by National Science Foundation research grant BMS 75-13799. S. T. Bagley was an N. L. Tarter Research Fellow at Oregon State University for part of the research period.

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