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Growth of *Aeromonas* spp. on Butzler *Campylobacter* Selective Agar and Evaluation of the Agar for the Primary Isolation of *Aeromonas* spp. from Clinical Specimens

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The fortuitous finding that *Aeromonas* spp. grew well on Butzler *Campylobacter* selective medium (BCSA) in a microaerobic atmosphere at 42°C prompted us to evaluate the performance of BCSA for selective isolation of aeromonads in comparison with ampicillin (30 μg/ml) sheep blood agar (ASBA30). Overall recovery rates of aeromonads from 563 stool samples from patients with acute diarrhea were higher on ASBA30 (70.4%) than on BCSA (56.3%); however, 21 (29.5%) grew only on BCSA. The three human-associated *Aeromonas* spp. could be recovered on BCSA and ASBA30. We recommend the use of BCSA to laboratories reluctant to include a specific selective medium for aeromonads.

The clinical significance of *Aeromonas* spp., especially as causal agents of human diarrhea, is no longer a debatable issue. As a consequence, there has been recently an increased interest in refining the selective medium for the optimal isolation of aeromonads from clinical (6, 7, 16) and environmental (11, 12) sources. Investigations in this direction have categorically revealed that the isolation of *Aeromonas* spp. has proportionally increased in studies where specific selective media have been employed (6). It is also clear that using routine enteric agar like MacConkey agar or salmonella-shigella agar for the primary isolation of aeromonads would lead to a gross underestimation of the actual incidence.

To determine the bacterial etiology of diarrhea, stool samples submitted to our laboratory are screened on a variety of selective media. Following this protocol enabled us to discover that several of the large grey hemolytic colonies which we frequently encountered on Butzler *Campylobacter* selective agar (BCSA) were, in fact, *Aeromonas* spp. It also became evident that aeromonads grew excellently in the optimum conditions required for the growth of thermophilic campylobacters which include a microaerobic environment (5% oxygen), an increased carbon dioxide concentration (10%), and an incubation temperature of 42°C. This fortuitous finding prompted us to evaluate the efficacy of BCSA in comparison with that of ampicillin (30 μg/ml) sheep blood agar (ASBA30), currently the most efficient selective medium for aeromonads (8), for the primary isolation of *Aeromonas* spp. from clinical specimens. Our rationale was that, if BCSA supported the growth of *Aeromonas* spp., then we could exploit its excellent inhibitory potential, especially for coliforms, fungi, gram-positive organisms, and other members of *Enterobacteriaceae* (4), and use this medium for the selective isolation of aeromonads along with thermophilic campylobacters.

This study was performed between March 1987 and February 1988 on stool samples obtained from 563 patients seeking medical attention for diarrhea at the Infectious Diseases Hospital and B. C. Roy Children's Hospital, Calcutta. Fecal specimens from the patients were received either as rectal swabs transported in Cary and Blair medium or as liquid stools. The samples were plated simultaneously on BCSA and ASBA30. The composition of BCSA is a modified version of the original description (2, 3) and contained Mueller-Hinton agar (Difco Laboratories, Detroit, Mich.) supplemented with 10% defibrinated sheep blood and Butzler formulation of antimicrobial agents (freeze-dried supplement SR 85; Oxoid Ltd., London, England). ASBA30 was prepared as described earlier (8). Samples seeded in ASBA30 were incubated at 37°C, while those seeded in BCSA were incubated microaerophically (Heraeus B 5061 EK/O_2 AUTO ZERO CO_2 incubator) at 42°C. Plates were examined at 24-h intervals for hemolytic and nonhemolytic *Aeromonas*-like colonies and were discarded at 72 h if found negative. On both media, *Aeromonas* colonies were distinguished by their hemolytic zones and also by their distinct dull white coloration. Nonhemolytic, dull white colonies were also picked.

Presumptive *Aeromonas* colonies were picked and tested for oxidase on filter paper saturated with 1% tetramethyl-*p*-phenylenediamine dihydrochloride. All oxidase-positive colonies were inoculated into a multitest medium (5) which was modified as slants. Isolates which exhibited an alkaline slant-acid butt were further characterized by using the API 20E system (Analytab Products International, S.A., Vercieu, France) and by determining resistance to 150 μg of the vibriostatic agent O/129 (Sigma Chemical Co., St. Louis, Mo.) per ml. All the isolates recovered in this study were speciated according to the method of Popoff (13).

Of the 563 stool samples from patients with acute diarrhea tested on the two selective media for isolation of *Aeromonas* spp., 71 (12.6%) were positive on either one or both of the media. The overall recovery rate of aeromonads on ASBA30 was higher than that on BCSA (Table 1). The sensitivities of the two media separately or combined are indicated in Table 1. Only 19 (26.7%) of the isolates were detected on both media on primary isolation. A higher percentage of isolates were recovered on ASBA30 than on BCSA; however, 21 (29.5%) isolates grew only on BCSA. Retrospectively, we also subcultured the *Aeromonas* isolates on the selective medium on which they were not initially isolated and found that none of the media were inhibitory for any of the 71 *Aeromonas* cultures isolated in this study and that there were no remarkable differences in the number of *Aeromonas* colonies on subcultures on the two media. These qualitative

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TABLE 1. Results of the use of two selective media for primary isolation of *Aeromonas* species from human sources

No. (%) of specimens	Isolation of *Aeromonas* spp. from[a]:		No. of:		
	ASBA30	BCSA	*A. hydrophila*	*A. sobria*	*A. caviae*
19 (26.7)	+	+	8	6	5
31 (43.7)	+	−	10	14	7
21 (29.6)	−	+	12	7	2

[a] +, Positive isolation; −, negative isolation.

results indicate that the concentration or type of the antibiotic used in either medium or the higher incubation temperature (42°C) used for isolation of campylobacters was not inhibitory to any of the *Aeromonas* strains.

It was not clear why some *Aeromonas* strains grew primarily only on either ASBA30 or on BCSA while their subcultures grew on the opposite medium. To us, it appeared that this was due to an interaction between the density of *Aeromonas* spp. present in the fecal sample and the ability of the medium to inhibit competing microflora. It was evident that in fecal samples where the number of aeromonads was presumably low (as in cases with polymicrobial infection), the number of colonies on BCSA was much greater, compared with that on ASBA30, and this appeared to be related to the ability of BCSA to effectively suppress competing microflora. Although we did not quantify the growth of other competing fecal floras, it was readily apparent that BCSA effectively suppressed normal stool flora while ASBA30 was not so effective in inhibiting other fecal floras which, on occasion, obliterated the *Aeromonas* colonies when only a few were present.

All three *Aeromonas* spp. generally associated with human disease were recovered on both selective media (Table 1). However, the percentage of isolation of *Aeromonas hydrophila* on BCSA was slightly higher than that on ASBA30, while the percentage of recovery of *Aeromonas sobria* and *Aeromonas caviae* was much higher on ASBA30 than on BCSA. A possible reason for the lower recovery rates of *A. sobria* on BCSA could relate to the concentrations of cefazolin and colistin (at 1 mg/ml each) present in BCSA, which may inhibit this species (10).

Another interesting observation was that the size and zone of hemolysis of the *Aeromonas* colonies were much larger on BCSA than on ASBA30. This may be related to the atmosphere of incubation. A CO_2 concentration of 10% has been demonstrated to markedly enhance the production of toxin by *Vibrio cholerae* and enterotoxigenic *Escherichia coli* (15). Similarly, it has been shown that 20% CO_2 enhanced the hemolysin production of *Staphylococcus aureus* by 40-fold, and this was speculated to be due to the increase in intracellular acidity (1).

The ability of BCSA to support growth of all the human-associated *Aeromonas* spp. is indeed interesting, even though its recovery potential is not as good as that of ASBA30. This unique ability should be exploited in routine diagnostic laboratories having an infrastructure for isolation of *Campylobacter* spp. and reluctant to add an *Aeromonas* spp.-specific selective medium for want of time or materials. However, it must be mentioned that the antimicrobial formulation of Skirrow or Blaser for selective isolation of campylobacters would not be suitable for isolation of aeromonads, as has already been documented (9), since most of the *Aeromonas* strains are susceptible to trimethoprim and polymyxin B (10, 14).

On the basis of a previous comprehensive evaluation of selective media for primary isolation of aeromonads from human and animal feces (8), we had recommended the use of two selective media, ASBA30 and DNase-toluidine blue-ampicillin agar for optimal recoveries. On the basis of the present findings, however, we suggest that DNase-toluidine blue-ampicillin agar should be replaced by BCSA. This would permit the recovery of ampicillin-susceptible strains of *Aeromonas* spp., which apparently exist in nature (14), on BCSA and would allow the simultaneous screening of thermophilic campylobacters and aeromonads. We are currently making efforts to improve the recovery rates of aeromonads in BCSA without affecting the recovery rates of campylobacters.

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