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Comparison of Selective Media for Primary Isolation of *Aeromonas* Species from Human and Animal Feces

SUMIT MISHRA, G. BALAKRISH NAIR,†* RUPAK K. BHADRA, S. N. SIKDER, AND S. C. PAL

National Institute of Cholera and Enteric Diseases, Beliaghata, Calcutta, India

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Five selective media were evaluated for their effectiveness in the primary isolation of *Aeromonas* spp. from human patients with acute diarrhea and from healthy domestic animals. Sheep blood agar with 30 mg of ampicillin per liter (ASBA30) yielded a significantly higher percentage of positive specimens as compared with the four other media. The effective combination of two selective media with which 98% of all isolates were detected and with which all of the three human-associated *Aeromonas* spp. could be isolated was ASBA30–DNase-toluidine blue agar. ASBA30 was the most sensitive medium since it permitted more growth of *Aeromonas* colonies and effectively suppressed competing microflora. We recommend the use of ASBA30–DNase-toluidine blue agar for investigations in which an attempt is made to assess the significance of *Aeromonas* spp. in the etiology of human diarrhea.

Species of the genus *Aeromonas* are rather controversial inclusions to the growing list of causal agents of human d iarrhea. While some investigators are skeptical about their diarrheic potential, others are convinced that these organisms play a significant role in a variety of clinical infections, including gastroenteritis (2, 3). The increasing number of reports (1, 6) in recent years implicating *Aeromonas* spp. as the etiologic agent of acute diarrhea, however, suggests that these organisms are more important than is currently appreciated.

In the past it was believed that a selective medium may not be necessary for the isolation of *Aeromonas* spp. from human feces and that routine enteric media like MacConkey agar or salmonella-shigella agar would suffice to isolate these organisms. In more recent investigations, however, it has been shown conclusively that the carbohydrates that are present in enteric agars have some inhibitory effect on *Aeromonas* spp., presumably because of by-products of carbohydrate metabolism (8, 10, 15). It is now increasingly clear that a selective plating medium must be used to obtain a realistic estimate of the importance of *Aeromonas* spp. as a causative agent of human diarrhea.

A variety of selective media have been described in the recent past, but these have not been sufficiently evaluated. With this in mind, this study was initiated to compare the performance of various selective agars for the primary isolation of *Aeromonas* spp. from human and animal feces.

MATERIALS AND METHODS

In this study, five selective agars were evaluated. The names and the abbreviations of the media and the inhibitor(s) incorporated into each medium to make it selective are listed in Table 1. Essentially all the media were prepared according to the original descriptions, except for BBG which was a modification (10) of the original inositol-brilliant green-bile salts agar (16). XDCA and BBG were poured into plates after the agars were heated at 100°C, while the other three media were autoclaved and tempered to 50°C before the desired concentration of ampicillin and 5% defibrinated sheep blood (in the two blood-supplemented agars) was added and subsequently distributed into plates.

Between August and December 1986, a total of 143 stool specimens from hospitalized patients with acute diarrhea were received in Cary and Blair transport medium and were screened for *Aeromonas* spp. and other enteric pathogens. During the same period, 24 fecal samples that were collected from various healthy domestic animals in connection with a community study on campylobacters were also included in this study. The fecal samples or swabs were cultured in parallel on each of the five selective media, and the plates were incubated at 37°C and read at intervals of 24 h for 72 h before they were discarded.

The basis of recognition of *Aeromonas* colonies on the five media is given in Table 1. Colonies that were suggestive of *Aeromonas* spp. were picked and tested for oxidase on filter paper saturated with 1% tetramethyl-*p*-phenylenediamine dihydrochloride. XDCA plates were, however, flooded with the oxidase reagent since the non-xylose-fermenting presumptive *Aeromonas* colonies were too small to be picked. Care was taken to pick up oxidase-positive colonies from XDCA plates immediately (within 10 to 15 s after the lavender discoloration was observed) because of the known inhibitory effects of the oxidase reagent. All oxidase-positive colonies were inoculated into a multitest medium (7) which was modified as slants. Isolates which exhibited an alkaline slant-acid butt reaction were presumptively identified as *Aeromonas* spp. The final confirmation was accomplished with the API 20E system (Analytab Products International, S. A., Vercieu, France) and by determining resistance to 150 μg of the vibriostatic agent 0/129 (Sigma Chemical Co., St. Louis, Mo.) per ml. A representative number of 47 *Aeromonas* isolates that were recovered on the selective media used in this study were further identified by using the scheme described by Popoff (13).

Data on the plating media were analyzed statistically by the test described by McNemar (9) for more than two related samples.

RESULTS

Of the 167 human and animal stool specimens tested on the five selective media for the isolation of *Aeromonas* spp.,

* Corresponding author.
† Present address: Department of Infectious Diseases, National Children's Medical Research Center, 3-35-31 Taishido, Setagaya-ku, Tokyo 154, Japan.

TABLE 1. Media evaluated for selective isolation of *Aeromonas* spp. from human and animal feces

Medium (abbreviation)	Inhibitor	Basis of recognition	Reference
Brilliant green-bile salts agar (BBG)	Brilliant green, bile salts	Colorless colonies	10
Xylose-desoxycholate-citrate agar (XDCA)	Sodium desoxycholate, citrate	Non-xylose fermenter	17
Ampicillin-sheep blood agar (ASBA10)	Ampicillin (10 mg/liter)	Hemolysis	5
Ampicillin-sheep blood agar (ASBA30)	Ampicillin (30 mg/liter)	Hemolysis	4
DNase-toluidine blue-ampicillin agar (DNTA)	Ampicillin (10 mg/liter)	Halo of decolorization	18

47 (28.1%) were positive on one or more of the media (Table 2). ASBA30 yielded the highest percentage of positive specimens from both human and animal samples. The recovery rate of *Aeromonas* spp. on BBG was the poorest. The percentage of positive specimens from animals was more than twice as high as that from humans. The rate of isolation on ASBA30 was significantly higher than that on BBG, XDCA, ASBA10 ($P < 0.001$), and DNTA ($P < 0.01$). Rates of isolation on BBG versus XDCA and on ASBA10 versus DNTA were not significantly different.

The sensitivities of the five media separately or in combination are shown in Tables 3 and 4, respectively. Only 10 (21.3%) of the isolates were recovered on all five media on primary isolation. ASBA30 was the most sensitive medium, followed by DNTA and ASBA10. Of the 44 isolates obtained on ASBA30, 7 (15.9%) grew only on this medium (Table 3). Two isolates were recovered only on DNTA, while one isolate was detected only on ASBA10. The effective combination of two media with which 98% of all isolates were detected was ASBA30-DNTA (Table 4).

Based on a semiquantitative estimate of the number of colonies that grew on each medium, it was apparent that ASBA30 was the most sensitive since it permitted more growth of *Aeromonas* colonies compared with the other selective media (Table 5). Although we did not quantitate the contaminants, ASBA30 was more inhibitory to competing fecal flora as compared with the other media.

Among the five media evaluated in this study, BBG and XDCA were the least inhibitory to other fecal flora, and frequently, presumptive *Aeromonas* colonies were overgrown by competing microorganisms. This was especially evident on BBG, on which *Vibrio cholerae* was isolated as a pure culture on several occasions. In addition, on both these selective agars, *Aeromonas* spp. could not be readily differentiated from the background microflora. Additional problems on XDCA were that the *Aeromonas* colonies were far too small, making it difficult to pick up the organism; and for some unknown reason, the medium tended to be soft, in spite of the incorporation of 2.5% agar.

Suppression of coliforms and other members of the family *Enterobacteriaceae* was evident, to a certain extent, on ASBA10 and DNTA. The higher concentration of ampicillin in ASBA30, however, significantly reduced competing microflora without affecting the size, shape, or zone of hemolysis of the *Aeromonas* colonies. Apart from the lower recovery rates, the other disadvantage in DNTA was that plates had to be incubated longer (48 h) for the *Aeromonas* colonies to appear and become characteristic, while in ASBA10 the hemolytic colonies tended to be restricted to the primary or secondary streak areas, and were, more often than not, completely obliterated by competing microflora.

Of the 47 representative *Aeromonas* isolates obtained in this study, 34 (72.4%) were identified as *A. hydrophila*, 8 (17.0%) as *A. caviae*, and 5 (10.6%) as *A. sobria*. *A. hydrophila* was recovered from all the media evaluated in this study. The recovery of *A. caviae* was restricted to XDCA, ASBA30, and DNTA, while *A. sobria* was recovered from ASBA10, ASBA30, and DNTA.

DISCUSSION

The selection of a reliable medium for the primary isolation of *Aeromonas* spp. is an essential requirement for a proper understanding of the clinical and epidemiological significance of the organism. According to Kay et al. (8), the low levels of isolation of *Aeromonas* spp. presently reported in most clinical laboratories may not be a true reflection of the prevalence or medical significance of this organism. Indeed, higher isolation rates have been documented from laboratories which have taken specific measures to isolate the organism (5, 11, 18).

Among the five selective media evaluated in this study, ASBA30 was, undoubtedly, the best. Results of an earlier evaluation study (15) revealed that blood agar incorporated

TABLE 2. Isolation of *Aeromonas* spp. from human and animal feces on five selective media

Source	No. of specimens tested	No. (%) positive	No. (%) positive on the following medium:				
			BBG	XDCA	ASBA10	DNTA	ASBA30
Human	143	32 (22.3)	11 (7.7)	12 (8.4)	20 (14.0)	19 (13.3)	30 (20.9)
Poultry fowl	15	10 (66.7)	4 (26.7)	4 (26.7)	7 (46.7)	9 (60.0)	10 (66.7)
Goat	6	2 (23.3)	0	0	0	1 (16.7)	1 (16.7)
Cow	1	1 (100.0)	0	0	0	1 (100.0)	1 (100.0)
Buffalo	2	2 (100.0)	0	1 (50.0)	1 (50.0)	1 (50.0)	2 (100.0)
Total	167	47 (28.1)	15 (9.0)	17 (10.2)	28 (16.8)	31 (18.6)	44 (26.3)

TABLE 3. Results of the use of five selective media for the primary isolation of *Aeromonas* spp. from human and animal sources

No. of specimens	Primary isolation of *Aeromonas* spp. from the following medium:				
	BBG	XDCA	ASBA10	DNTA	ASBA30
10	+	+	+	+	+
2	−	+	+	+	+
1	+	+	−	+	+
1	+	−	+	+	+
1	+	+	+	−	+
8	−	−	+	+	+
1	+	−	+	−	+
1	−	+	−	+	+
1	+	−	−	+	+
5	−	−	−	+	+
4	−	−	+	−	+
2	−	+	−	−	+
7	−	−	−	−	+
2	−	−	−	+	−
1	−	−	+	−	−
120	−	−	−	−	−

with 10 mg of ampicillin per liter was the most satisfactory medium for the isolation of fecal *Aeromonas* spp. when compared with four other media. Similarly, significantly higher levels of *A. hydrophila* were obtained by Kay et al. (8) when ASBA10 was included in the isolation protocol. In both these studies (8, 15) ASBA30 was not used. Results of the present evaluation indicate that the recovery rates of *Aeromonas* spp. can be further enhanced (at least by 1.5 times) if ASBA30 is used.

Perhaps the major drawback of ASBA30 is that the higher concentration of ampicillin will inhibit some of the ampicillin-susceptible *Aeromonas* strains which reportedly exist (14). The MIC for 60 strains belonging to three species of *Aeromonas* (*A. hydrophila*, *A. sobria*, and *A. caviae*) of human origin has, however, been reported to be quite high (12). Rahim et al. (14), in contrast, found that about 50% of the environmental strains of *A. hydrophila* isolated from freshwater fishes in Bangladesh were susceptible to 12.5 μg of ampicillin per ml. Based on the results of the reports described above (12, 14) and on those of the present evaluation of the various media, we recommend the use of two selective media, ASBA30 and DNTA, for the primary isolation of aeromonads. This would permit the recovery of ampicillin-susceptible strains of *Aeromonas* on DNTA which would otherwise be excluded by the high concentration of ampicillin in ASBA30.

TABLE 4. Comparison of individual and combined media isolation rates of *Aeromonas* spp. from 47 culture-positive samples

Medium	No. positive	% total
BBG	15	31.9
XDCA	17	36.1
ASBA10	28	59.5
DNTA	31	65.9
XDCA-DNTA	34	72.3
ASBA10-DNTA	38	80.8
ASBA30	44	93.6
ASBA10-ASBA30	45	95.7
ASBA30-DNTA	46	97.8
ASBA10-DNTA-ASBA30	47	100.0

TABLE 5. Qualitative growth of *Aeromonas* spp. of the 47 positive stool samples on the primary isolation plate of the five selective media

Quantity of growth[a]	No. of *Aeromonas* colonies that grew on the following medium:				
	BBG	XDCA	ASBA10	DNTA	ASBA30
+++	6	11	10	9	30
++	3	5	2	8	9
+	6	1	16	15	5
−	32	30	19	15	3

[a] Symbols: +++, >50 colonies or confluent growth in streak areas; ++, 11 to <50 colonies; +, 1 to 10 colonies; −, no *Aeromonas* spp. recovered.

Another reason for recommending the use of a combination of two media is to ensure recovery of all three human-associated *Aeromonas* spp. since *A. caviae*, which is the nonhemolytic aeromonad, is likely to be missed on ASBA30 if hemolysis is taken as the basis of recognition of an *Aeromonas* colony. In this study we encountered several oxidase-positive, nonhemolytic strains on the ampicillin-blood agars. We are still in the process of identifying these isolates. The important finding, however, is that by using the combination of ASBA30-DNTA all three human-associated *Aeromonas* spp. can be isolated.

The clinical significance of the 32 human isolates recovered in this study will be discussed elsewhere. It must be mentioned, however, that several isolations were made with other enteropathogens, notably the enteropathogenic vibrios; and thus, some criteria must be evolved in the future to assess the role of *Aeromonas* spp. in such cases.

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