

Comparison of Blood Agar, Ampicillin Blood Agar, MacConkey-Ampicillin-Tween Agar, and Modified Cefsulodin-Irgasan-Novobiocin Agar for Isolation of *Aeromonas* spp. from Stool Specimens

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The performance of four media for the isolation of *Aeromonas* strains from stool specimens, the importance of ampicillin-susceptible *Aeromonas* strains in the selection of culture media, and the usefulness of beta-hemolysis in screening blood-containing media for *Aeromonas* strains were evaluated in two phases. In the first phase, 36 of 1,672 stool specimens yielded *Aeromonas* isolates. Ninety-seven percent of the isolates were detected on blood agar containing 20 µg of ampicillin per ml (ABA), and 47% were detected on MacConkey agar containing 100 µg of ampicillin per ml and 1% Tween 80. In the second phase of the study, 43 of 1,924 stool specimens yielded *Aeromonas* isolates. Fifty-one percent of the isolates were detected on blood agar and on modified cefsulodin-Irgasan-novobiocin agar, and 84% were detected on ABA. The combination of ABA and modified cefsulodin-Irgasan-novobiocin agar provided 100% recovery of the *Aeromonas* isolates encountered. All of the *Aeromonas* isolates detected on blood agar were also detected on ABA, and 89% of the *Aeromonas* isolates detected on these media were beta-hemolytic. These results suggest that ABA is superior to the other media evaluated for the isolation of *Aeromonas* strains from stool specimens, but optimal recovery of the organism may require the use of more than one medium. The results also suggest that the occurrence of ampicillin-susceptible strains is not a limitation on the use of ABA, but at least 10% of *Aeromonas* isolates will be missed if beta-hemolysis is used to screen ABA plates for these organisms.

Aeromonas hydrophila group species have increasingly been implicated as causes of gastroenteritis (1, 4), and many laboratories are now routinely culturing stool specimens for these organisms. Ampicillin-containing blood agar (ABA) has been the most widely used medium for the isolation of *Aeromonas* strains from stool specimens, but a variety of other media have also been suggested for the isolation of *Aeromonas* strains (2, 3, 5, 6). The purpose of the present study was to compare blood agar (BA), (ABA), MacConkey agar containing ampicillin and Tween 80 (MAT), and modified cefsulodin-Irgasan-novobiocin agar (CIN) for the recovery of *Aeromonas* strains from stool specimens. An additional purpose of this study was to evaluate the use of beta-hemolysis for screening BA cultures for *Aeromonas* strains and to evaluate the importance of ampicillin-susceptible *Aeromonas* strains in the selection of stool culture media. The results indicate that ABA is superior to the other media for the isolation of *Aeromonas* strains. In addition, 89% of the *Aeromonas* strains isolated in this study were beta-hemolytic, and no strains that were inhibited by the ampicillin content of ABA were encountered.

MATERIALS AND METHODS

***Aeromonas* culture media.** We purchased 5% sheep blood in blood agar base 2 (BA) and 5% sheep blood plus 20 µg of ampicillin per ml in blood agar base 2 (ABA) from a

commercial supplier (Prepared Media Laboratories, Tualatin, Oreg.). MacConkey agar containing 100 µg of ampicillin per ml and 1% Tween 80 (MAT) was made locally according to a formula provided by S. Giercke (personal communication), except that MacConkey agar containing crystal violet was used. Modified CIN (2), containing 4 µg of cefsulodin (*Yersinia* selective agar; Difco Laboratories, Detroit, Mich.) per ml, was also made locally. Lots of each medium were tested for the ability to support growth and demonstrate beta-hemolysis or Tween precipitation with a known strain of *A. hydrophila*.

Stool specimens and study design. The Enteric Section of the British Columbia Provincial Health Laboratories receives stool specimens from physician's offices and hospitals throughout the province, and these specimens were used in this study. The specimens were received in Cary-Blair transport medium and were cultured on media routinely used for the detection of enteric pathogens and on the media under evaluation.

The study was conducted in two phases. In the first phase, 1,672 specimens were cultured on ABA and MAT for *Aeromonas* isolates and on bismuth sulfite agar, deoxycholate-citrate-lactose-sucrose agar, *Campylobacter* selective agar, Hektoen enteric agar, and standard CIN (15 µg of cefsulodin per ml) for other enteric pathogens. In the second phase of the study, 1,924 specimens were cultured on BA, ABA, and modified CIN (2) for *Aeromonas* isolates and on bismuth sulfite agar, deoxycholate-citrate-lactose-sucrose agar, *Campylobacter* selective agar, and sorbitol-MacConkey agar for other enteric pathogens. Modified CIN was used for the detection of both *Aeromonas* and *Yersinia* strains in this phase of the study.

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TABLE 1. Isolation of *Aeromonas* spp. on four media

Study phase	Total no. of specimens	Total no. (%) positive	No. (%) positive on:			
			BA	ABA	MAT	Modified CIN
1	1,672	36 (2.2)	NT ^a	35 (97)	17 (47)	NT
2	1,924	43 (2.2)	22 (51)	36 (84)	NT	22 (51)

^a NT, Not tested.

Processing of cultures and identification of isolates. In the first phase of the study, beta-hemolytic colonies of gram-negative bacilli growing on ABA and colonies surrounded by a halo on MAT were subcultured to BA and tested for oxidase. In the second phase of the study, beta-hemolytic and nonhemolytic colonies of gram-negative bacilli growing on BA and ABA were tested for oxidase. Colonies growing on modified CIN were subcultured to BA and tested for oxidase. Oxidase testing in both phases of the study was done by the filter paper method (Kovacs reagent). Oxidase-positive isolates were identified by classical biochemical testing according to published criteria (7).

RESULTS

In phase 1 of the study, ABA was compared to MAT for recovery of *Aeromonas* isolates, and more than twice as many isolates were detected on ABA (Table 1). Of 43 *Aeromonas* isolates detected in phase 2 of the study, 51% were detected on BA and CIN, and 84% were detected on ABA.

Twelve specimens yielded the organism on BA and ABA but not on modified CIN, and 10 specimens were positive on all three of the media. Nine specimens were positive only on ABA, and seven specimens were positive only on modified CIN. Another five specimens yielded *Aeromonas* strains on ABA and modified CIN but not on BA. All specimens that were positive on BA were also positive on ABA. However, 14 specimens yielded *Aeromonas* strains on ABA but not on BA. Of the 36 isolates detected on BA and/or ABA, 32 (89%) were beta-hemolytic, and 4 (11%) were nonhemolytic. Six of the specimens positive for *Aeromonas* strains also yielded other enteric pathogens, and *Aeromonas* strains were the only potential pathogens detected in 37 of the 43 positive specimens.

The isolation of *Aeromonas* strains from BA, ABA, and modified CIN in various combinations is presented in Table 2. The use of BA and modified CIN in combination resulted in the detection of 79% of the *Aeromonas* isolates. The combination of BA and ABA gave the same results as ABA used alone. ABA used in combination with modified CIN resulted in the detection of all of the *Aeromonas* isolates encountered in phase 2 of the study. The use of BA in addition to ABA and modified CIN did not add any additional isolates.

DISCUSSION

The results of this study demonstrate that ABA is superior to MAT, BA, and modified CIN for the isolation of *Aeromonas* strains from stool specimens. ABA detected 30 to 50% more *Aeromonas* isolates than did any of the other media. However, modified CIN did detect 16% of the isolates that were not detected on BA or ABA, and the use of ABA plus modified CIN produced the highest recovery of *Aeromonas* isolates in the study. The results of this study

TABLE 2. Comparison of rates of isolation of *Aeromonas* spp. from 43 culture-positive specimens on individual and combined media

Medium or media	No. (%) positive
BA	22 (51)
Modified CIN	22 (51)
BA + modified CIN	34 (79)
ABA	36 (84)
BA + ABA	36 (84)
ABA + modified CIN	43 (100)
BA + ABA + modified CIN	43 (100)

further demonstrate that BA offers no advantages over ABA for the detection of *Aeromonas* isolates. The results also indicate that approximately 10% of *Aeromonas* isolates are nonhemolytic on BA and ABA and that these isolates would not be detected if only beta-hemolytic colonies were tested.

The significance of *A. hydrophila* as a cause of gastroenteritis has been well documented in recent studies (1, 4). However, the methods used for the primary isolation of this organism have been controversial, and a number of different media have been suggested for the culturing of *Aeromonas* strains from stool specimens (2, 3, 5, 6). Among these media, BA containing various concentrations of ampicillin has been most often recommended (5, 6). MAT has been suggested as a selective-differential medium for the isolation of *Aeromonas* strains and the differential feature provided by the production of a halo of precipitate around colonies of the organism suggested that MAT might offer advantages as a routine plating medium. However, a comparison of MAT and ABA in the present study revealed that ABA yields twice as many isolates as does MAT. The relatively poor performance of MAT in this comparison may have been due to the high content of ampicillin in the medium.

Although these results suggest that ABA may be the best medium currently available for the isolation of *Aeromonas* strains from stool specimens, the routine use of ABA would require the addition of this medium to the battery of media used for stool cultures. A more cost-effective approach would be to develop a medium that could be used for the detection of *Aeromonas* strains in addition to other enteric pathogens. Modified CIN, containing a reduced concentration of cefsulodin, has been suggested as a medium that could be used for the isolation of both *Aeromonas* and *Yersinia* strains (2). Our results indicated that this medium yielded 33% fewer isolates than did ABA, and modified CIN could not be recommended as a single medium for the isolation of *Aeromonas* strains from stool specimens. However, 16% of the *Aeromonas* isolates encountered in the study were detected only on modified CIN, and the combination of ABA and CIN resulted in the detection of all of the *Aeromonas* isolates encountered in this study. These results confirm the findings of another recent study indicating that ABA should be used in combination with another medium for the optimal detection of *Aeromonas* strains (6). All of the *Aeromonas* isolates encountered in the present study were recovered by using the combination of ABA and modified CIN, but a disadvantage of modified CIN as compared with standard CIN (containing 15 µg of cefsulodin per ml) was that the detection of *Yersinia* strains was more difficult because of heavier growth of normal stool flora organisms on modified CIN.

A cause of concern about the use of ABA has been the possible occurrence of ampicillin-susceptible *Aeromonas*

strains which would not be detected on the medium. In the present study, we compared BA and ABA for the culturing of *Aeromonas* strains from stool specimens. No isolates that grew on BA but not on ABA were encountered. Therefore, no strains that were inhibited by the ampicillin content in ABA used in this study were encountered. However, 33% of the *Aeromonas* isolates were detected on ABA but not on BA, suggesting that the selective effect of the ampicillin in ABA is beneficial for the recovery of *Aeromonas* isolates. These findings indicate that ABA is more effective than BA for the isolation of *Aeromonas* strains from stool specimens and that inhibition of ampicillin-susceptible strains is not a significant limitation on the use of the medium. However, the results of our ABA (20 µg of ampicillin per ml) and MAT (100 µg of ampicillin per ml) comparison suggest that higher concentrations of ampicillin may reduce the yield of *Aeromonas* isolates.

Screening of ABA plates for beta-hemolytic colonies reduces the amount of testing required for the detection of *Aeromonas* isolates on this medium. In the first phase of the present study 10% of the ABA plates had beta-hemolytic colonies, 23% of these colonies were oxidase positive, and 44% of the oxidase-positive colonies were identified as *Aeromonas* isolates. These findings indicated that screening of the plates for beta-hemolytic colonies eliminated 90% of the specimens from further testing. Such screening would improve the efficiency of culturing for *Aeromonas* strains, but nonhemolytic strains of the organism would be missed if only beta-hemolytic colonies were tested. The occurrence of nonhemolytic *Aeromonas* isolates was investigated in the second phase of the present study, and the results indicated that 11% of the isolates were nonhemolytic. These results suggest that approximately 10% of *Aeromonas* isolates would be missed if only beta-hemolytic colonies were tested. Therefore, potential *Aeromonas* colonies on ABA should be tested regardless of their hemolytic reactions.

In summary, the present study suggests that ABA is superior to MAT, BA, and modified CIN for the isolation of *Aeromonas* strains from stool specimens, but the combina-

tion of ABA and modified CIN provides the highest yield of *Aeromonas* isolates. Our results further indicate that the inhibition of ampicillin-susceptible strains of *Aeromonas* on ABA is not a limitation on its use. In addition, our findings suggest that more than 10% of *Aeromonas* isolates are nonhemolytic and will not be detected if only beta-hemolytic colonies are tested. We recommend the use of ABA, screened for oxidase-positive colonies, with or without modified CIN for the routine detection of *Aeromonas* isolates in stool specimens.

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