

Isolation and Identification of Aeromonas Species from Human Stools

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Abstract

One thousand and three diarrhoeal stool samples were processed in our laboratory during the period 1996/1997 for the presence of enteric pathogens especially *Aeromonas* spp., which has emerged as a new agent causing diarrhoea. Ampicillin sheep blood agar was found to be the best medium for the isolation of *Aeromonas* spp. from stool specimens. Enteric pathogens were found in 200 (20%) stools, of which *Aeromonas* spp. was the second commonest pathogen isolated amounting to 21% of isolates. This study clearly indicates that *Aeromonas* spp. must be looked for in every diarrhoea! stool samples, specially in children below 10 years of age. Isolation and identification is cost effective and easy, if the given protocol is observed (JPMA 47:305, 1997).

Introduction

The *Aeromonas*, an aquatic bacterium usually found in fresh waters e.g., rivers, lakes etc. is a gram negative straight rod with rounded ends and not a normal flora of the intestine. *Aeromonas* spp. have been established as an important gastrointestinal enteric pathogen¹⁻⁴. The low level of isolation presently reported in most clinical laboratories may not be a true reflection of prevalence of the pathogen especially in our country where diarrhoeal illnesses are common. This is supported by the fact that isolation rate of *Aeromonas* spp. frequently increases in laboratories which have taken specific measures to isolate it. Isolation of *Aeromonas* spp. from stool samples requires the use of culture media which fulfill two criteria: an inhibiting effect on the growth of saprophytes, and a discriminating capacity which allows it to be recognised among other species which are capable of growth on the medium. A variety of media are available in the market, the efficacy of which are not well established. The confirmation of *Aeromonas* species is usually based on the production of oxidase, inhibition of growth in 6.5% NaCl and on TCBS and being resistant to Ampicillin (except for few strains)⁵. However identification upto species level is costly and a cumbersome affair.

The purpose of this study was to develop a cost effective method for the isolation and identification of *Aeromonas* from stool specimen for a routine clinical laboratory. In all, seven media were evaluated to test the efficacy which included four routine culture media, MacConkey's agar (MAC), Thioglycollate bile salt sucrose agar (TCBS) and Xylose lysine desoxycholate agar (XLD) and four special media, Ampicillin sheep blood agar with 30mg/litre of ampicillin (ASBA30), *Aeromonas* agar (Aer), Cefusulodin Irgasan and Novobiocin agar (CIN) and Glutamate starch phenol red agar (GSP).

Material and Methods

One thousand three stool samples were collected from patients with complaint of diarrhoea submitted to the clinical laboratory, during the year 1996-1997. Diarrhoea was defined as 3 or more loose stools per day. The stool specimens were provided by patients both in the outdoor clinic and admitted in the wards of the hospital.

Stool samples were plated within one hour of collection, directly onto MacConkeys agar No.3 (Oxoid-CM 115), XLD (Oxoid-CM469), ASBA30 (Mueller-hinton agar-Oxoid-CM337 +5% Sheep blood +

Ampicillin selective supplement SR136)⁶⁻⁸. CIN agar (Oxoid- CM653 + Yersinia selective supplement - Oxoid, SR 109) Aeromonas agar(Oxoid-CM833), TCBS agar (Oxoid-CM333) and GSP agar(Merck-10230). The inoculated plates were incubated for 24-48 hours at 36°C + 1°C aerobically. Each plate was examined after 24 hours and 48 hours. Oxidase test was performed from blood agar plate by pouring oxidase reagent on the plate. Oxidase positive colonies were picked up within 10-12 seconds after the appearance of purple colour. All Oxidase positive colonies were isolated and reactions were set up which included , Triple sugar iron agar (TSI), Urea, Indole, H₂S, motility, Xylose, Inositol, Mannitol, Dulcitol and 6.5% NaCl. The results were confirmed by API 10S (Analytab Products International, S.A., Vercieu, France).

Statistical Analysis: Data on plating media were analyzed statistically by McNemar's test⁹

Results

The basis of recognition of *Aeromonas* spp. on different media is given in Table I.

Table I. Basis of recognition of aeromonas species on different media.

Medium	Basis of recognition
1. XLD	Pink colonies
2. ASBA 30	Greyish haemolytic or non-haemolytic colonies
3. GSP	Yellow colonies
4. CIN	Pink opaque colonies
5. MacConkeys agar	Lactose fermenter (pink) or Non-lactose fermenter colonies (colourless)
6. TCBS	Usually no growth
7. Aeromonas agar	Dark green opaque colonies with dark centers

Beta-hemolysis was observed only in 36% of isolates on ASBA30. Forty-one cases of *Aeromonas* were isolated, of which 75% were lactose fermenters and 25% were non-lactose fermenters as seen on MacConkeys agar.

Table II. Relevant biochemical properties of *Aeromonas* species.

Characteristic	<i>A. Hydrophilia</i>	<i>A.caviae</i>	<i>A.veronii</i>	<i>A.veronii</i> biovar sobira	<i>A. jandaei</i> biovar veronii	<i>A. schubertii</i>	<i>A. trota</i>
Screening reactions							
Oxidase	+	+	+	+	+	+	+
Growth on:							
TCBS	-	-	-	-	-	-	-
6.5% NaCl broth	-	-	-	-	-	-	-
Phenotypic properties							
Indole	+	+	+	+	+	-	+
Glucose (gas)	+	-	+	+	+	-	+
Inositol	-	-	-	-	-	-	-
Mannitol	+	+	+	+	+	-	+
Hydrogen sulphide	-	-	-	-	-	-	-
Motility	+	+	+	+	+	+	+
D-Xylose	-	-	-	-	-	-	-
Urease	-	-	-	-	-	-	-
Dulcitol	-	-	-	-	-	-	-

Table II summarises the optimum biochemical tests for the identification and confirmation of *Aeromonas* species. The screening reactions were found to be reliable and reproducible. The ideal and cost effective tests were positive reaction with Oxidase, inhibition of growth in 6.5% NaCl and TCBS, no H₂S production, motile, no pigment production on nutrient agar. negative reactions with urease, xylose, inositol, dulcitol and fermentation of mannitol.

Table III. Isolation rates of *aeromonas* spp. from human stool samples on different media.

No. of specimens tested	No positive	%	No. (%) positive on following medium:		
			Media	No.	%
1003	41	4.0	1. XLD	03	0.3
			2. MAC	35	3.5
			3. ASBA	41	4.0
			4. CIN	15	1.5
			5. TCBS	01	0.1
			6. AER	05	0.5
			7. GSP	35	3.5

Table III shows the sensitivities of the seven media separately. The rate of isolation on ASBA (4%), GSP (3.5%), MAC (3.5%) was significantly higher than that on XLD (0.3%), CIN (1.5%), TCBS

(0.1%) and *Aeromonas* agar(0.5%)($P < 0.001$). Sensitivity of MAC and GSP was 78% as compared to ASBA. Recovery was poorest from XLD. All *Aeromonas* which grew on different media other than ASBA, also grew on ASBA. The effective combination of two media with which maximum number of all isolates were detected was ASBA30, MacConkey and GSP agar.

Discussion

The study illustrated that the isolation of *Aeromonas* spp. was not difficult and certainly not very costly. However, while choosing a selective media containing an antibiotic, the high bacterial resistance in this region should be kept in view. We found Ampicillin blood agar with 30mg/l Ampicillin the most convenient, productive and cost-effective media for the isolation of *Aeromonas*. It was observed that haemolysis and oxidase test could be done without any interference. ASBA30 was not inhibitory to competing faecal flora as mentioned by some workers¹⁰.

As certain *Aeromonas* spp. are ampicillin sensitive, they may be missed on ASBA30. To isolate these, an additional media, i.e., MacConkey agar, or GSP should be used¹⁰. *Yersinia* and *Aeromonas* spp. can be isolated on a single medium CIN agar when incubated at 25°C, though for the latter it is not as sensitive as ASBA30. The growth of *Aeromonas* spp. can be increased if modified CIN with 4 mg/i of Cefsulodin instead of 15mg/i is used. This is because higher concentrations of Cefsulodin inhibits normal flora of gut and *Aeromonas* as well¹¹. GSP was a good medium for the primary isolation of ampicillin sensitive strains. It gave prominent yellow coloured colonies surrounded by a yellow zone. Colonies were easy to find among the growth of other normal flora of the gut. These colonies were then isolated on a nutrient agar plate for oxidase testing because this important test should not be performed on a media with sugar content to avoid false results. TCBS does not help the growth of *Aeromonas* spp. at all. XLD agar and *Aeromonas* agar were also not so productive as compared to ASBA, GSP and MAC. Alkaline peptone water enrichment technique was not applied in this study because of its doubtful significance in isolation of *Aeromonas* spp. from clinical cases. It has been found that the use of enrichment culture frequently detected *Aeromonas* spp. in asymptomatic individuals¹².

We recommend the use of two media, ASBA30 and GSP agar for the primary isolation of *aeromonas* spp. because the study clearly indicated that the best medium was ASBA30 with maximum isolation followed by GSP or MAC medium. with an added advantage of growing those *Aeromonas* which are ampicillin sensitive. Although MacConkeys medium was also sensitive enough, but it does not differentiate the colonies of *Aeromonas* from other coliforms and it grows *Aeromonas* both as non-lactose fermenters and lactose fermenters. A large number of biochemical tests have been evaluated for the final identification of *Aeromonas* spp.^{13,14} which have proved to be specific and reproducible. Based on the results of this study, we propose a simple scheme for the identification of suspected *Aeromonas* isolates in a routine laboratory (Figure).

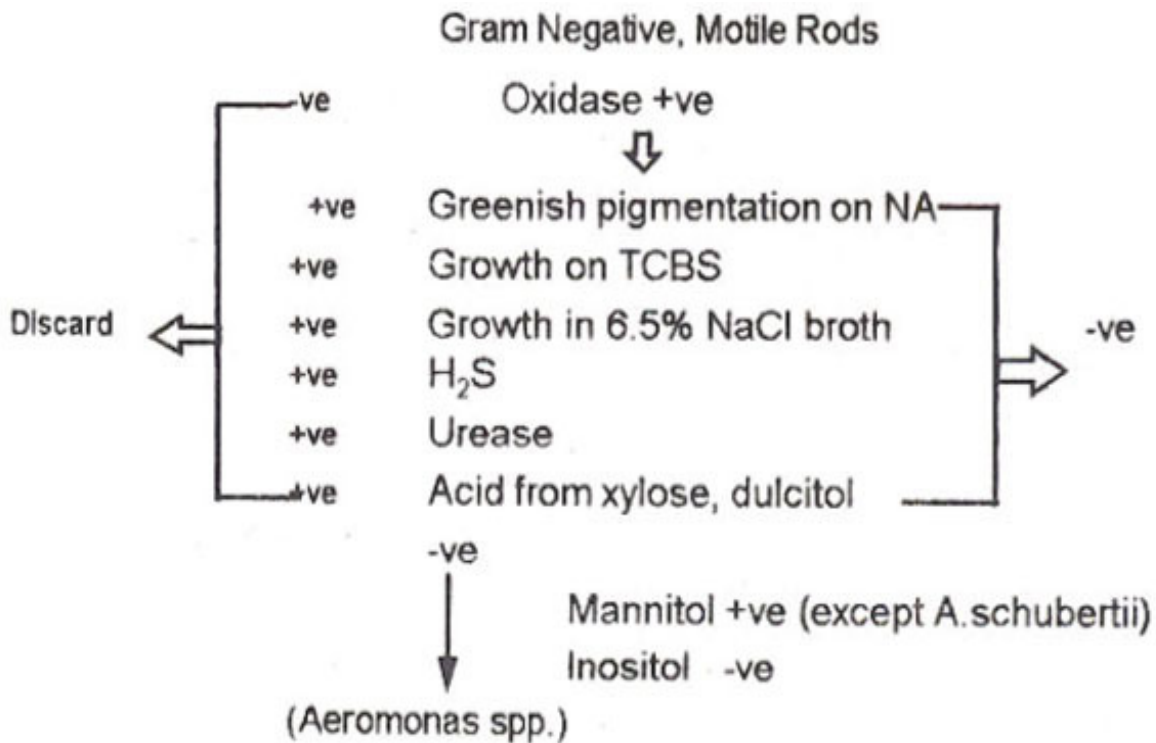


Figure. A simple scheme for the identification of aeromonas spp. +ve = positive; -ve = negative; NA = nutrient agar; TCBS = thioglycollate litrate bile salt sucrose agar; GSP = glutamate sucrose phenol red agar.

The scheme was cost effective since the oxidase test excluded members of Enterobacteriaceae at an early stage of investigation, while growth in 6.5% NaCl broth and greenish pigmentation on nutrient agar eliminated the halophilic vibrios and some Pseudomonads respectively. H₂S production and positive indole reaction also helped in eliminating any Salmonella or vibrios. Some laboratories may still find it costly to use ASBA30 as an additional media, then Aeromonas can easily be picked up from MacConkeys media if the recommended isolation protocol is adhered. In clinical laboratories of our county, the need for isolation and identification of Aeromonas spp. is important. The suggested scheme should prove useful.

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