SHORT ARTICLES

PRIL-XYLOSE-AMPICILLIN AGAR, A NEW SELECTIVE MEDIUM FOR THE ISOLATION OF AEROMONAS HYDROPHILA

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Aeromonas Hydrophila is a potentially pathogenic micro-organism and has been isolated from cases of gastroenteritis (Rosner, 1964), urinary-tract infections (von Graevenitz and Mensch, 1968), osteomyelitis (Lopez, Queseda and Saied, 1968), septicaemia (Pearson, Mitchell and Hughes, 1972), meningitis (Qadri et al., 1976), and wound infections (Hanson et al., 1977). It has many features in common with members of the Enterobacteriaceae, especially Escherichia coli and Klebsiella species and its presence in samples of faeces may therefore be overlooked (von Graevenitz and Zinterhofer, 1970). A property that distinguishes Aeromonas spp. from the Enterobacteriaceae is the oxidase reaction; this is positive in the former. The other oxidase-positive micro-organisms that may be found in stools are members of the genera Pseudomonas, Vibrio, and Plesiomonas. Aeromonas spp. can be readily distinguished from the other oxidase-positive organisms by tests for the presence of arginine dihydrolase, the oxidation or fermentation of glucose, and the presence of lysine or ornithine decarboxylase (Schubert, 1974). A. hydrophila can metabolise glucose by respiratory and by fermentative pathways; it splits arginine but does not decarboxylate either lysine or ornithine.

A. hydrophila does not require enriched media and can be isolated on the media used for Enterobacteriaceae, for example Salmonella-Shigella Agar (Difco) or MacConkey's agar. On these media the colonies of Aeromonas cannot be distinguished by inspection from those of some E. coli. On blood agar most of the Aeromonas cultures can be recognised because they form greyish colonies surrounded by a zone of haemolysis. Unfortunately, when stool specimens are seeded on blood agar, the spreading of Proteus often hinders the examination of single colonies.

A special medium for the isolation of Aeromonas was proposed by von Graevenitz and Zinterhofer (1970). It is based on the production of deoxyribonuclease by Aeromonas, but it should be noted that this enzyme is also found in Serratia, Enterobacter liquefaciens, and some strains of Proteus, Providencia, and Pseudomonas.

MATERIALS AND METHODS

The new medium (PXA agar) proposed for isolation of Aeromonas from stool specimens consists of nutrient agar containing xylose 1% (w/v), phenol red 25 mg/litre as indicator, ampicillin 30 mg/litre, and Pril 0.02% (w/v).

Ampicillin was added (von Graevenitz and Zinterhofer, 1970) to eliminate most of the enterobacteria. A. hydrophila, unlike most members of the Enterobacteriaceae does not ferment xylose. "Pril" is a quaternary ammonium detergent consisting of a mixture of primary alkyl sulphate, alkyl-benzyl sulphonate, and salts (Böhme Fettchemie, GmbH, Düsseldorf). This preparation was recommended by Döll (1956) for inhibition of swarming of Proteus.

Evaluation of the medium was by the following methods: (a) seeding representatives of all the genera of Enterobacteriaceae from broth cultures on to PXA agar; (b) parallel
seeding of different faecal samples and of *Aeromonas* cultures, serially diluted, on PXA agar and on nutrient agar; (c) determining the minimal number of *Aeromonas* organisms that could be detected on PXA agar when seeded from faecal suspensions mixed with serially diluted cultures of *Aeromonas*; (d) examination of stool samples from patients with diarrhoea.

To facilitate the estimation of the number of *Aeromonas* on isolation plates, a direct oxidase test was used: a 0.2% (w/v) solution of tetramethyl-p-phenylenediamine hydrochloride in distilled water was poured on the plate and after 1–2 min. oxidase-positive colonies were subcultured and later identified by biochemical tests.

**RESULTS**

**Experimental**

Stock strains belonging to all the major groups of Enterobacteriaceae and the genus *Vibrio* were seeded by spreading from nutrient-broth cultures to PXA agar. Of 180 cultures of Enterobacteriaceae, 102 (57%) developed single colonies on this medium but most of the cultures that showed growth fermented xylose and could therefore be distinguished from *Aeromonas*. Only 18 cultures (10%) did not ferment xylose and could thus be confused with *Aeromonas*. These cultures belonged to the following taxa: *E. coli* (2 of 24 tested), *Shigella sonnei* (1 of 18), *Salmonella* (4 of 35), *Enterobacter agglomerans* (1 of 2), *Serratia marcescens* (1 of 4), *Proteus vulgaris* (1 of 10), *Proteus rettgeri* (2 of 5), *Proteus morganii* (4 of 6), and *Providencia* (2 of 3). Of 20 cultures of the genus *Vibrio*, only four developed growth on the PXA agar, and all were xylose-positive.

Faecal samples from eight persons, among them four with diarrhoea, were serially diluted to 10⁻¹⁰ and from all dilutions, 0.2-ml samples were seeded in parallel on PXA agar and on nutrient agar. After incubation for 24 h at 37°C, the numbers of aerobic micro-organisms that developed on these two media were determined. On the non-selective nutrient agar, counts ranged from 2 x 10⁶/g to 8 x 10⁷/g. The numbers on PXA agar were from 5 x 10²/g to 1.1 x 10⁵/g.

Ten different strains of *Aeromonas* were cultivated in nutrient broth and the culture was diluted as above and seeded on PXA medium and nutrient-agar plates. No significant difference was found between the number of *Aeromonas* that developed on those two media (P > 0.1).

Portions of 0.9 ml of a 1 in 10 dilution of faeces in saline were distributed in tubes, and to each tube a 0.1-ml sample from a series of tenfold dilutions of a broth culture of *Aeromonas* was added. After thorough mixing, one loopful, about 0.01 ml, from each tube was streaked on PXA agar. The test was repeated with five different stool samples. *A. hydrophila* was detected in all experiments in almost all the tubes in which c. 100 or more *Aeromonas* cells were present in 1 ml of the mixture.

**Clinical samples**

From patients with diarrhoea in the Bikur Cholim Hospital, 100 stool specimens were examined in parallel on the usual media for isolation of pathogenic enterobacteria and on PXA agar. *A. hydrophila* was isolated as a predominant culture in seven cases; from one patient it was isolated from faeces that also contained *S. typhimurium*.

**Summary**

A new selective medium containing Pril, xylose, and ampicillin is suggested for the isolation of *Aeromonas hydrophila* from faeces.

**REFERENCES**


