When surface water is used for the production of drinking water, the probability of finding aeromonads will be greater than when groundwater is used. Proper treatment and disinfection should adequately deal with these organisms.

From the ecological viewpoint, the assessment of aeromonads in water is not a closed issue. In a further study of the growth-limiting temperature in an oligosaprobic river we found densities of 250 aeromonads/ml. A considerable part of the freshly isolated *A. hydrophila* strains did not grow in a 37 °C water bath and only one strain grew at 41 °C (table 4). *A. sobria*, *A. punctata* and *A. caviae* were even more sensitive to incubation temperatures above 37 °C. On the other hand, 40 strains of *A. hydrophila* from cases of diarrhea (positive ileal loop tests) which were received by courtesy of Dr S. Sanyal from Varanasi, India, grew without exception at 41 °C, and so did two strains of *A. sobria* from clinical specimens received by courtesy of Dr E. Delbeke from the Languedoc region, France[2]. The ability of aeromonads to grow at these temperatures has not yet received proper attention in pathogenicity studies.

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Aeromonas and *Plesiomonas*: Isolation procedures for pathological specimens

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Key words. Aeromonas; Plesiomonas; isolation; CIN agar; *Yersinia enterocolitica.*

Aeromonas as well as *Plesiomonas* have been implicated in a wide variety of infectious diseases[6]. Isolation of these organisms from clinical specimens and from environmental and food samples which may be considered to be sources of human and animal infections is usually performed without difficulty, since both genera can easily be grown on most routinely-used common media, such as blood or MacConkey agar.

Problems may arise when quantitative recovery is required or in cases where large numbers of contaminating microflora are present. Depending on the purpose for which the culture is required, one or several of the following factors will influence the choice of medium: 1) selectivity, i.e. the ability to suppress background flora, 2) ability to differentiate between contaminating flora and the organism looked for on the basis of colonial morphology, and 3) quantitative recovery of the desired organisms.

Aeromonas hydrophila group

A variety of different media have been proposed for special applications (for a summary see von Graevenitz and Bucher[3]). Differential agents used include starch or different sugars (mannitol, trehalose, xylose). If a sugar contained in an agar is fermented, it must be realized that direct oxidase testing cannot be performed on the colonies, since oxidase reactions may become false negative at a pH ≤ 5.1[4]. To increase selectivity the use of ampicillin, novobiocin, sodium deoxycholate, sodium lauryl sulfate, Pril (a quaternary ammonium detergent) and other agents has been described. No single medium has received general acceptance. Very recently a starch-ampicillin agar has been proposed which allows quantitative recovery and ready differentiation from background flora of *Aeromonas* spp. from foods[10].

For stool specimens two systems are widely used. Blood agar containing ampicillin[7] allows direct oxidase testing but exhibits only a low selectivity. In addition, ampicillin-sensitive *Aeromonas* strains have been described[11]. Sensitivity to ampicillin seems to be especially abundant in the Philippines, where in the Kirby-Bauer-test only 57% of isolates were classified as 'resistant' (M. Kilpatrick, personal communication). A more cost-effective alternative is Cefsulodin-Irgasan-Novobiocin (CIN) agar, a medium which allows simultaneous screening for *Aeromonas* spp. as well as *Yersinia* spp.[1]. Two important points have to be considered using CIN agar[1]: 1) the medium should contain only 4 mg/l cefsulodin (e.g. Difco) instead of 15 mg/l (e.g. Oxoid), and 2) incubation should be at 25 °C, since high concentrations of cefsulodin (fig. 1) and high incubation temperatures may inhibit growth of certain *Aeromonas* strains, mainly *A. sobria* (M. Altwegg, unpublished). This medium is very selective (table), with only about 50 % of primary plates showing growth, and less then half of these with suspicious colonies, but it does not allow direct oxidase testing owing to the mannitol that is fermented. A very easy screening procedure including two MIO (motility-indole-ornithine medium, Difco Laboratories,

Selectivity of CIN agar at different Cefsulodin concentrations (% of routine stool specimens)

	Direct inoculation		APW enrichment	
	15 mg/l	4 mg/l	15 mg/l	4 mg/l
No growth	38%	31%	42%	31%
Only coliforms	40%	42%	33%	42%
Suspicious colonies	22%	27%	25%	27%

Detroit, Mich.) tubes (incubated at 22 °C and 37 °C, respectively), and subculture onto blood agar for oxidase testing, allows preliminary identification: *Aeromonas* spp. are ODC negative/Ox$^+$, *Yersinia* are Ox$^-$ and motile at 22 °C but non-motile at 37 °C.

Isolation frequency from fecal specimens can be increased using alkaline peptone water[7,8] or gram-negative broth[8] as enrichment. Also, cold enrichment (4 °C) in phosphate-buffered saline as recommended for *Yersinia* spp. yields additional isolates (M. Altwegg, unpublished). The isolation procedure used in our laboratory is outlined in figure 2.

Aeromonas salmonicida

The causative agent of furunculosis in fish (salmonids) is commonly isolated on tryptic soy agar. Atypical isolates that may cause an ulcerative form of this disease in a wide variety of fish species are fastidious, slow growing organisms and require media supplemented with blood or hemin[5]. Media should be incubated at 20 °C–25 °C, since *A. salmonicida* does not grow at 37 °C.

Plesiomonas shigelloides

There are no media available that are especially designed to isolate *Plesiomonas* sp. Due to the low incidence of this organism the use of a special medium for fecal specimens may not be cost-effective. Inositol-bile salts-brilliant green (IBB) agar can be used for both *Aeromonas* and *Plesiomonas*[3,9], but direct oxidase testing may not be done for the latter. In this laboratory a suboptimal method (oxidase test with lactose-negative colonies on enteric media used for the isolation of *Salmonella* and *Shigella*) has yielded several *Plesiomonas* isolates without much effort, but strains that readily ferment lactose are missed by this procedure. Quite often fermentation of sugars by *Plesiomonas* strains is delayed on solid media. The fact that about 50% of *Plesiomonas* strains are susceptible to ampicillin[2] precludes the use of media containing this antibiotic. The use of alkaline peptone water for enrichment is controversial[9].

Figure 1. Inhibition of *Aeromonas* spp. on CIN agar at 25 °C at different concentrations of Cefsulodin; ○, *A. caviae*; ⊕, *A. hydrophila*; ●, *A. sobria*.

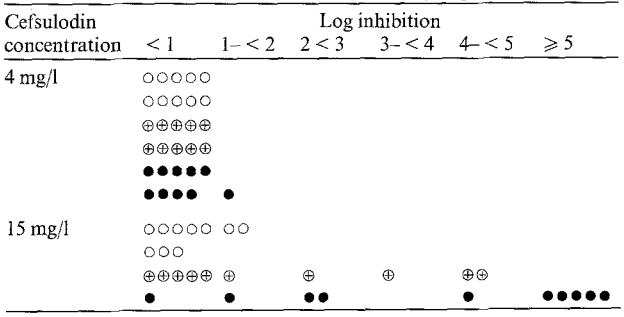
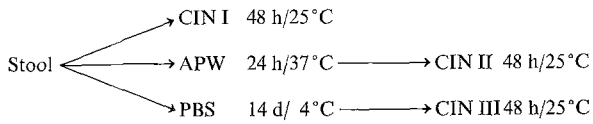


Figure 2. Procedure for the simultaneous isolation of *Yersinia* and *Aeromonas* spp. from stool specimens.



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Identification of *Aeromonas* in the routine laboratory

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Key words. Aeromonas; identification; taxonomy; routine.

Over the last decade there has been a markedly increased interest in the possible role of *Aeromonas* species as a cause of disease, in particular diarrhoea, in man. Unfortunately this research has often been hampered by the confused state of the taxonomy of the genus. The division of *Aeromonas* into the species *A. hydrophila, A. sobria, A. caviae,* and *A. salmonicida* which appears in Bergey's Manual of Systematic Bacteriology[6] is based on a numerical taxonomic study of 68 strains by Popoff and Véron[8] and a subsequent DNA homology study[7]. Some of Popoff and Véron's[8] phenotypic results do not correlate well with other published data. They found all the strains examined to be lysine decarboxylase negative although many others have found this to be a useful test for differentiating species of *Aeromonas*. Despite their astounding preponderance in the aquatic environment and possible role in human disease, there have been remarkably few taxonomic studies of aeromonads in recent years.

For comparison, 48 strains of *Aeromonas* were included in a taxonomic study of *Vibrio fluvialis*[2] but, although groups corresponding to those of Popoff and Véron[8] were found, it was not possible to reliably identify them. Accordingly we embarked on a numerical taxonomic study of *Aeromonas*[3]. The 163 strains studied included a selection of reference strains and isolates from a wide range of environments including human faeces, other clinical material, veterinary specimens, natural waters, seafood, other foods and the domestic and hospital environment. Duplicates of ten strains were included to enable test and operator error to be estimated. Of 195 characters examined for each strain eight were insufficiently reproducible (growth at different pHs,