Aeromonads are still regarded as unusual enteric pathogens. Even though their pathogenic role has not been established (Hanninen et al., 1995), unambiguous convincing evidence suggests that some aeromonads do cause gastroenteritis (Gluskin et al., 1992; Albert et al., 1999). Watery diarrhoea might be accompanied by symptoms such as abdominal pain, fever, and nausea or vomiting; blood in faeces can also appear in serious cases. Aeromonads cause nonresolvable, intermittent diarrhoea that can persist for several months or even years after the initial infection (Janda & Abbott, 1998). Isolation of aeromonads from human faeces samples is difficult and its success depends on the culture method performed. A valid judgement as to how many aeromonads are involved in diarrhoeal disease is only possible when an appropriate selective medium is used.

Aeromonads, Gram-negative facultatively anaerobic oxidase-positive glucose-fermenting rods, are ubiquitous waterborne organisms occurring in both fresh and saline waters and in soil. Aeromonads generally grow on various agars used for screening of enteric pathogens. For their isolation from human faeces, two media have been recommended: cefsulodin-Irgasan-novobiocin agar (CIN), primarily a selective medium for Yersinia enterocolitica, and ampicillin-blood agar (ABA) (Abbott, 2003). ABA has been recommended as a selective medium owing to the beta-haemolytic activity of the majority of clinically relevant Aeromonas species. Because of an increasing ampicillin resistance in other members of the Enterobacteriaceae, it is becoming difficult to screen with oxidase each beta-haemolytic colony that appears. Therefore we decided that ABA is not a suitable medium for the routine laboratory. The next selective medium described for the isolation of Aeromonas spp. is Aeromonas agar (AA; LAB M). AA contains the selective agents brilliant green and Irgasan, which also enable growth of aeromonads susceptible to ampicillin (http://www.lab-m.com/).

In a 2-year survey from 2003 to 2005, we compared the value of CIN and AA in the isolation of aeromonads. During the first year, routine faeces samples from acute gastroenteritis cases were processed on the following enteric differential media: deoxycholate-citrate agar (DC), MacConkey agar (MC) and CIN. In the second year, the samples were also cultured on AA (Table 1). All media were incubated aerobically at 37 °C for 18–24 h. Colonies that were typical for aeromonads and that grew on one of the agars mentioned above were cultured on nonselective medium (such as blood agar) and examined for oxidase (OXI-strip; Pliva-Lachema Diagnostika); this was demonstrated that the advantage of AA is usually false negative also. It was demonstrated that the advantage of AA is the possibility of confirming the presence of presumptive Aeromonas by performing an oxidase test directly on a colony grown on the agar. Even the lactose-positive variants of aeromonads, often evaluated as E. coli on

<table>
<thead>
<tr>
<th>Selective media used</th>
<th>No. of faecal samples</th>
<th>No. of aeromonads isolated (%)</th>
<th>No. of aeromonads isolated with enteric pathogens (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>DC, MC, CIN</td>
<td>20 430</td>
<td>79 (0-4 %)</td>
<td>62 (78-5 %)</td>
</tr>
<tr>
<td>DC, MC, CIN, AA</td>
<td>22 112</td>
<td>195 (0-9 %)</td>
<td>138 (70-8 %)</td>
</tr>
</tbody>
</table>

*In one sample, Yersinia enterocolitica was also isolated.
†In one sample, Salmonella sp. was also isolated.

In our laboratory, faeces samples are inoculated on MC, DC and CIN to screen for common enteric pathogens such as Salmonella spp., Shigella spp., enteropathogenic/enterotoxigenic Escherichia coli or Y. enterocolitica. None of these media are useful for Aeromonas isolation, although aeromonads are able to grow on all of them. On MC or DC the morphology of Aeromonas colonies is similar to that of the non-pathogens which are commonly present in faecal samples and so aeromonads could not be distinguished from them. In addition, the lactose-positive variants of Aeromonas colonies which grew on MC or DC appear falsely oxidase-negative. On CIN, aeromonads are morphologically indistinguishable from Y. enterocolitica or mannitol-positive Citrobacter strains and the oxidase test is usually false negative also. It was demonstrated that the advantage of AA is the possibility of confirming the presence of presumptive Aeromonas by performing an oxidase test directly on a colony grown on the agar. Even the lactose-positive variants of aeromonads, often evaluated as E. coli on
common enteric differential media, gave a positive oxidase reaction. Moreover, citrobacters appeared on AA as pink colonies and thus were clearly distinguishable from aeromonads. Pseudomonads also grew on AA. They could be differentiated from aeromonads by demonstration of oxidative but not fermentative metabolism (e.g. after inoculating into Hugh and Leifson’s O/F medium).

Inclusion of AA in the study more than doubled the frequency of isolation of aeromonads and thus it was considered to be the most efficient selective medium (Table 1). AA is commercially available as a ready-to-use medium that is suitable for routine laboratories. Therefore it was decided to include AA in standard cultivation processing of faeces samples. Compared to other enteric pathogens, *Aeromonas* spp. were the third most frequent bacteria isolated from patients with acute gastroenteritis (following campylobacters and salmonellas). *Aeromonas* spp. were isolated in approximately 70% of diarrhoea cases without confirmation of any other enteric pathogen (Table 1). In these cases we consider that *Aeromonas* spp. were the cause of the diarrhoea. Faecal specimens were plated on AA directly without enrichment culture, because strains isolated only after enrichment are believed to be not associated with acute diarrhoea (Robinson et al., 1986). We therefore also suppose that all the above-mentioned cases were clinically relevant. Until now, it has appeared that different species and different clones of aeromonads are associated with severe diarrhoea cases (Sinha et al., 2004). Routine use of AA as an additional medium for screening of faecal specimens could elucidate the role of each individual *Aeromonas* species.

**Acknowledgements**

This work was supported by IGA of the Ministry of Health of the Czech Republic, ID code NR/8011-2.

**Anna Andělová, Iva Porazilová and Eva Krejčí**

Institute of Public Health, Centre for Microbiology, Immunology and Parasitology, Partyzánské nám. 7, 70200 Ostrava, Czech Republic

**Correspondence:** Eva Krejčí (eva.krejci@zuova.cz)


