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# Distribution of *Aeromonas* phenospecies and genospecies among strains isolated from water, foods or from human clinical samples

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## SUMMARY

A total of 332 *Aeromonas* spp. originating from drinking water ($n = 75$), fresh water ($n = 57$), chicken and ground beef (107), human faecal samples in association with travelling ($n = 49$), human faecal samples not associated with travelling ($n = 38$), and six strains from human blood cultures were studied by phenotypic methods and by using analysis of ribopatterns as a molecular method for the identification of the 13 known hybridization groups (HGs). Also included were the reference strains of each HG. *A. hydrophila* HG 1, *A. caviae* HG 4 and *A. veronii* biotype *sobria* HG 8/10 were the most important genospecies identified in human faecal samples. *A. hydrophila* HG 2 and *A. media* HG 5B predominated in drinking water and *A. hydrophila* HG 2 and HG 3, *A. media* HG 5A and HG 5B predominated in fresh water. In drinking water only one isolate was *A. hydrophila* HG 1 and two isolates were *A. caviae* HG 4. Clinically important *Aeromonas* spp. HG 1 (*A. hydrophila*), HG 4 (*A. caviae*) and HG 8/10 (*A. veronii* biotype *sobria*) were common in chicken and ground beef. In contrast to the drinking water samples, HG 5A was common in chicken and ground beef samples. Atypical, unidentified isolates were most often found in fresh water samples (12/57 strains). Although water has been suspected of being an important source of human *aeromonas* infections, clinically important HGs were found to be in the minority among *Aeromonas* spp. identified in drinking water or fresh water. The distribution of *Aeromonas* spp. HGs among drinking water, chicken and ground beef samples was also different, suggesting that contamination of meat or chicken may not originate from water.

## INTRODUCTION

Mesophilic *Aeromonas* spp. are common organisms in the environment, especially in water and sewage [1, 2], and also occur in untreated and treated drinking water, raw beef, pork, lamb, fish, sea-food as well as in fresh produce [3–6]. It has been suggested that foods are contaminated by the water used, for example, to wash carcasses in processing plants or to wash fresh produce during food preparation. Faecal contamination of meat during the slaughtering process is also

possible and studies by Gray and colleagues [7] indicate that the faecal carriage rate in pigs and cows is about 6–8%. Most motile *Aeromonas* spp. are psychrotrophic and thus they will grow at refrigeration temperatures [8].

The role of mesophilic aeromonads as pathogens of cold-blooded animals has been recognized [9]. Mesophilic aeromonads are also suspected of being human pathogens capable of causing infections ranging from septicaemia to gastroenteritis [10]. In studies on the aetiology of human diarrhoea in developed countries *Aeromonas* spp. have been isolated in 1–3% of faecal samples collected from patients [10, 11]. Drinking water and food are the suspected vehicles (1, 6, 12].

Recently, ten species have been identified phenotypically: *A. hydrophila*, *A. caviae* (*A. punctata*), *A. media*, *A. eucrenophila*, *A. sobria*, *A. jandaei*, *A. veronii*, *A. schubertii*, *A. trota* and *A. allosaccharophila* [10, 13]. Genetic methods differentiate 13 genetic species (hybridization groups, HGs). Phenospecies *A. hydrophila* includes HG 1 (*A. hydrophila*), HG 2 (unnamed) and HG 3 (*A. salmonicida*). Phenospecies *A. caviae* includes HG 4 (*A. caviae*), HGs 5A and 5B (*A. media*) and HG 6 (*A. eucrenophila*). Phenospecies *A. sobria* includes HG 7 (*A. sobria*) and HG 8/10 (*A. veronii* biotype *sobria*). Phenospecies *A. veronii* includes HG 8/10 (*A. veronii* biotype *veronii*) and HG 11 (unnamed). Phenotypically *A. jandaei* (HG 9) and *A. trota* (HG 13) resemble *A. sobria*. *A. trota* is ampicillin sensitive thus differing from other known aeromonads [10]. Identification of the three commonly accepted species (*A. hydrophila*, *A. caviae*, *A. sobria*) is usually made in a clinical laboratory. The differentiation of HGs within a phenospecies requires methods which are not in common use, or genetic methods [14–16].

Only certain *Aeromonas* species are usually isolated from faecal samples of patients with diarrhoea: *A. hydrophila* (HG 1), *A. caviae* (HG 4), *A. veronii* biotype *sobria* (HG 8/10) and *A. veronii* biotype *veronii* (HG 8/10). In a few cases, *A. media* (HG 5), *A. schubertii* (HG 12) and *A. trota* (HG 13) have also been associated with diarrhoea [10].

The distribution of *Aeromonas* spp. in environmental and clinical samples has been compared at the genospecies level in only a few studies [12, 15]. In the present study *Aeromonas* spp. isolated from fresh water, drinking water, foods of animal origin and human clinical samples were identified to the genospecies level by both phenotypic and genotypic methods. The phenospecies and genospecies distribution among environmental and clinical isolates were compared in order to find which environmental sources are potentially important in the epidemiology of human gastrointestinal *aeromonas* infection.

## MATERIALS AND METHODS

### *Bacterial strains*

A total of 332 of *Aeromonas* spp. strains were from 48 wells and 2 drinking water distribution systems ($n = 75$), 23 fresh water ($n = 57$), 68 chicken and beef meat samples ($n = 107$). Faecal strains from Finnish adult subjects with ($n = 49$) and without any travelling history abroad ($n = 38$) and isolates from human blood cultures ($n = 6$) formed a comparison group [17]. Seventy-seven of the human faecal strains were from patients with diarrhoea. All food and water samples were

collected in Finland during the years 1992–3. Food samples were enriched in tryptic soy broth (Difco) containing 30 μg per ml ampicillin and cultivated after 24 h incubation at 30 °C on the ADA (ampicillin dextrin agar) medium [18]. Water samples (100, 10 and 1 ml) were membrane filtrated on the ADA. Three to five typical yellow colonies selected from ADA medium were cultivated on blood agar. Only oxidase positive colonies with different colonial morphology and haemolysis were chosen for further characterization. Only one isolate representing a species was selected from one sample. Several food or water samples had at least two different *Aeromonas* species. Human clinical isolates were detected on the Aeromonas selective medium (Difco). Included were also the reference strains of all known genetic species of *Aeromonas* spp., except *A. trota* HG 13, obtained from Centers for Disease Control and Prevention, Atlanta, USA and Department of Medical Microbiology, University of Zurich, Switzerland.

The strains were preserved at −70 °C in the Protect micro-organism storage system (LabM, Bury, UK) or in skimmed milk.

*Identification to the phenospecies level*

The strains were first identified to the phenospecies level by using the methods recommended by Popoff [19], Altwegg and colleagues [15] and Carnahan and colleagues [20]. The biochemical tests shown by Altwegg and colleagues [15], Kämpfer and Altwegg [14] and Abbott and colleagues [16] to be useful for identification of the hybridization groups of *A. hydrophila* (HG 1, HG 2, HG 3) and *A. caviae* (HG 4, HG 5A, HG 5B, HG 6) were used. These tests included utilization of DL-lactate, citrate, acetate or urocanic acid as a sole source of carbon, haemolysis, acid production from sorbitol, salicin, sucrose and D-rhamnose (*A. hydrophila*). *A. caviae* strains were tested for utilization of DL-lactate, citrate or acetate as a sole source of carbon. Included were also the cephalothin sensitivity test (30 μg) and the elastase test [16, 20]. Certain strains were also tested for ampicillin sensitivity. Both conventional media and commercial tests API 20E, API 20NE and ID 32GN (bioMerieux, sa Marcy l'Etoile, France) were used. Incubation times for carbon source utilization tests, sugar fermentation tests and the elastase test were 7 days. All tests were incubated at 30 °C.

*Identification to the genospecies level*

Ribopatterns of chromosomal DNA were used for the identification of the genetic species (hybridization groups, HGs) of phenotypically identified strains as recommended by Martinetti Lucchini and Altwegg [21]. Chromosomal DNA was isolated by using the guanidium isothiocyanate method [22] with the exception that phenol-chloroform (50:50) was used for DNA extraction instead of chloroform. DNA (5 μg) was digested with *Sma* I ribonuclease (Boehringer Mannheim GmbH Mannheim, Germany). Restriction fragments were electrophoresed in 1·0% agarose gels and transferred by vacuum transfer (Pharmacia) blotting to a nylon membrane (Boehringer Mannheim). 16S and 23S rRNA from *Escherichia coli* (Boehringer Mannheim) was used to prepare the digoxigenin-labelled cDNA probe by reverse transcriptase [23]. *Sma* I fragments with a molecular weight of less than about 4 kb were used for identifying an HG [21]. A reference strain for each HG was included in the ribotyping.

Table 1. *Certain biochemical characteristics of* A. hydrophila (*HG 1, HG 2, HG 3; hybridization group*), A. caviae (*HG 4, HG 5A, HG 5B) and* A. sobria (*HG 7, HG 8/10)*

| | *A. hydrophila* | | | *A. caviae* | | | *A. sobria* | |
|---|---|---|---|---|---|---|---|---|
| Characteristic | HG 1 (47) | HG 2 (52) | HG 3 (37) | HG 4 (39) | HG 5A (23) | HG 5B (22) | HG 7 (14) | HG 8/10 (68) |
| Aesculin | + | + | + | + | + | + | − | − |
| Voges-Proskauer | + | + | + | − | − | − | + | + |
| LDC | + | + | + | − | − | − | + | + |
| Gas (glucose) | + | + | + | − | − | − | + | + |
| Acid from | | | | | | | | |
| Arabinose | + | + | + | + | + | + | − | − |
| Mannitol | + | + | + | + | + | + | + | + |
| Sucrose | + | + | + | + | + | + | + | + |
| Salicin | + | + | + | + | + (90)* | + (90)* | − | − |
| Rhamnose | − | + | − | − | − | − | − | − |
| D-sorbitol | − | − (70)* | + | − | − | − | − | − |
| Utilization of | | | | | | | | |
| Acetate | + | + | + | + | + | + | ND | ND |
| Citrate | − (85)* | − | + (90)* | + | − (80)* | − | ND | ND |
| Lactate | + | − | − | + | − | + | ND | ND |
| Urocanic acid | − | + | + | ND | ND | ND | ND | ND |
| Elastase | + | − (47) | + | ND | ND | ND | ND | ND |
| Cephalothin | R | R | R | R | R | R | S | S |
| $T_{max}$ (°C) (mean − SD) | 41·0 (0·54) | 38·0 (0·63) | 38·5 (0·74) | 40·6 (0·84) | 38·2 (0·65) | 38·2 (0·65) | 35·4 (1·75) | 40·6 (0·85) |

* Per cent of strains.

Table 2. *Biochemical characteristics and hybridization groups of some biochemically 'atypical'* Aeromonas *spp. isolated from human faecal samples*

| Phenotypic name (lane no. in Fig. 2) | Certain characteristics | Hybridization group [21] (HG) |
|---|---|---|
| *A. veronii* biotype *veronii*-like (1) | Esk+; arab−; ceph S; elast−; ODC−; ADH− $T_{max}$ 43·5 °C | 8/10 |
| *A. hydrophila* (2) | Esk+ (weak); arab+; V−P−; elastase−; ceph R; $T_{max}$ 41·6 °C | 8/10 |
| *A. hydrophila* (3) | Esk+; arab−; V−P+; ceph R; elast+; $T_{max}$ 41·1 °C | 1 |
| *A. hydrophila* (4) | Esk+; arab−; V−P+; ceph R; elast−; $T_{max}$ 39·8 °C | 1 |
| *A. hydrophila* (5) | Esk+; arab−; ceph R; elast+; $T_{max}$ 41·6 °C | 1 |
| *A. hydrophila* (6) | Esk+; arab−; ceph R; elast+; $T_{max}$ 42·0 °C | 1 |
| *A. hydrophila* (7) | Esk+; arab+; sorb+; ceph R; elast+; $T_{max}$ 40·0 °C | 1 |
| ? (8) | Esk+ (slow); arab−; sorb+; ceph S; elast−; $T_{max}$ 43 °C | 8/10 |
| *A. trota*? (9) | Esk−; arab−; sal−; sakk−; lys−; ceph R; amp S; $T_{max}$ 42·5 °C | 13 |

## RESULTS

### *Identification to the phenospecies level*

The characteristics most useful for identification of the strains are shown in Table 1. In drinking water, *A. hydrophila* and *A. caviae* were the predominating species, comprising 54·6% and 32·4% of the identified isolates, respectively. Nine unnamed aesculin negative isolates had characteristics most closely resembling *A. sobria* (12%). In fresh water, almost half of the 57 strains (45·6%) were *A. hydrophila* and 17·5% were *A. caviae*. Only three typical *A. sobria* isolates (5·2%) and one *A. jandaei* isolate were identified. Unnamed aesculin negative (15 isolates) and aesculin positive (2 isolates) aeromonads were common (29·8%).

Among the 107 chicken and ground beef strains, all three common *Aeromonas* species, *A. hydrophila* (43%), *A. caviae* (24%) and *A. sobria* (29%) were found.

In human faecal samples collected from people who had travelled, *A. sobria* was more common (42·9% of 49 isolates) than in samples from people without any travelling history (26·3% of 38 isolates) (Table 2). In contrast, *A. caviae* was most common species (44·7% of 38 strains) in samples from patients without any known history of travelling outside the Nordic countries. *A. hydrophila* was less common in both types of samples (Table 3) [17].

### *Phenotypic and molecular identification of HGs (hybridization groups)*

Ribopattern analysis was used as a molecular method for identification of an HG. Ribopatterns of some typical strains of *A. hydrophila* (HG 1, HG 2, HG 3), *A. caviae* (HG 4, HG 5A, HG 5B, HG 6) and *A. sobria* (HG 7, HG 8/10) are shown in Fig. 1*a–c*, respectively.

Ribotyping allotted typical strains phenotypically identified as *A. hydrophila* into HG 1, HG 2 or HG 3, those identified as *A. caviae* into HG 4, 5A, 5B or 6, and those identified as *A. sobria* into HG 7 or HG 8/10. Carbon source utilization tests

Table 3. *Distribution of* Aeromonas *phenospecies and genospecies (HG; hybridization group) among strains isolated from environmental and clinical samples*

| Source of isolation (no of isolates) | *Aeromonas* phenospecies/genospecies | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | *A. hydrophila* | | | *A. caviae* | | | | *A. sobria* | | *A. veronii* | *Aeromonas* sp. |
| | HG 1 | HG 2 | HG 3 | HG 4 | HG 5A | HG 5B | HG 6 | HG 7 | HG 8/10 | HG 8/10 | |
| Drinking water (75) | 1 | 38 | 2 | 2 | 3 | 15 | 5 | 6 | — | — | 3 |
| Fresh water (57) | — | 10 | 16 | — | 4 | 5 | 1 | 5 | 3 | — | 12 unknown (1 (HG 9) |
| Chicken and ground beef meat (107) | 26 | 4 | 17 | 10 | 16 | — | — | 3 | 28 | — | 3 unknown |
| Human faecal samples (in association with travelling) (49) | 9 | — | 1 | 10 | — | 1 | — | — | 21 | 5 | 2 (HG 13) |
| Human faecal samples (without any travelling history) (38) | 9 | — | — | 17 | — | — | — | — | 10 | 2 | — |
| Human isolates from blood cultures (6) | 2 | — | — | 1 | — | — | — | — | 2 | 1 | — |

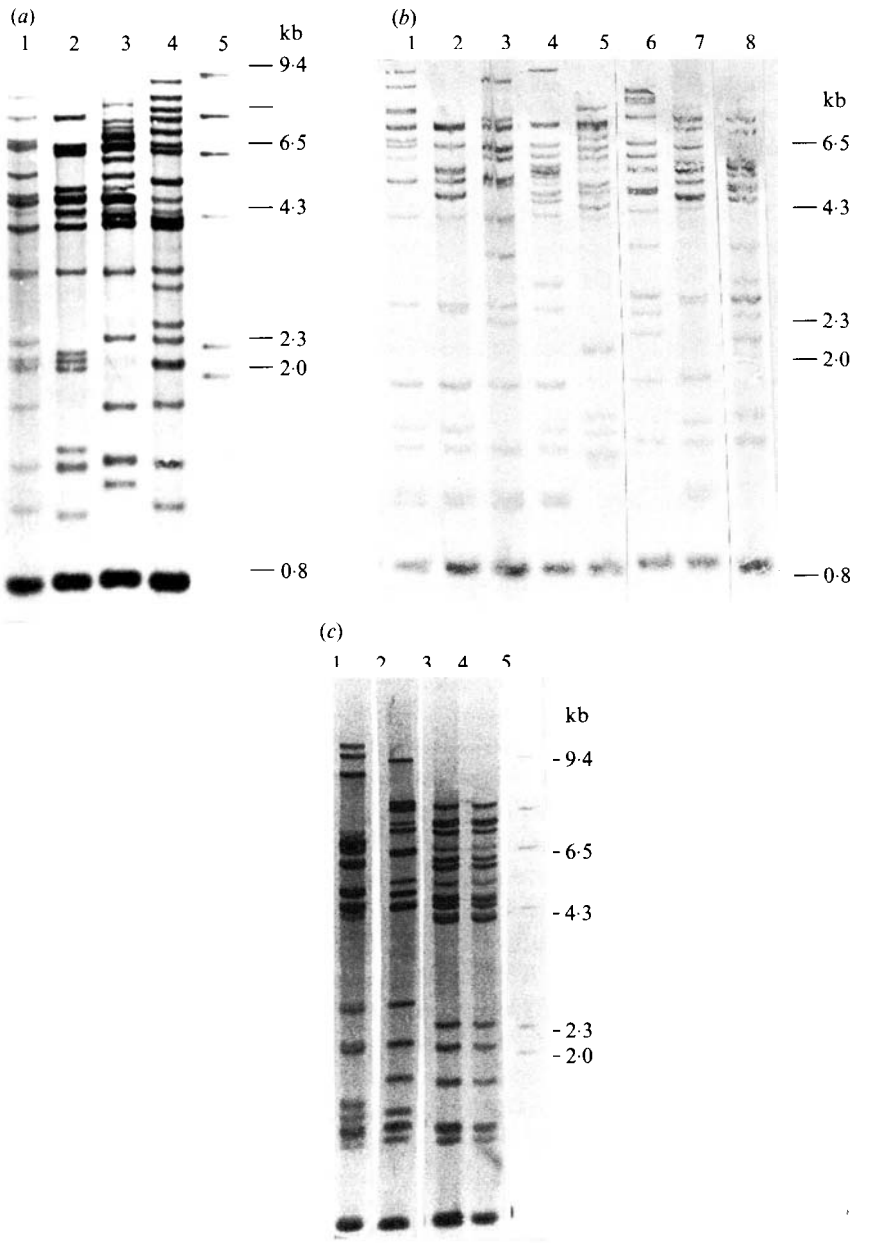


Fig. 1. A. Ribosomal RNA gene patterns for *Sma* I genomic DNA digests of *A. hydrophila* HG 1 (lanes 1 and 2), HG 2 (lane 3) and HG 3 (lane 4). B. Ribosomal RNA gene patterns for *Sma* I digests of *A. caviae* HG 4 (lane 5), *A. media* HG 5A (lanes 1, 2, 4 and 7), *A. media* HG 5B (lanes 3 and 6) and *A. eucrenophila* HG 6 (lane 8). C. Ribosomal RNA gene patterns for *Sma* I genomic DNA digests of *A. sobria* HG 7 (lanes 1 and 2) and *A. veronii* biotype *sobria* (lanes 3 and 4). Molecular weight markers (kb) are shown on the left side of the Figure. Banding patterns of molecular weight about 0·8–4 kb were used for the identification of an HG [21].

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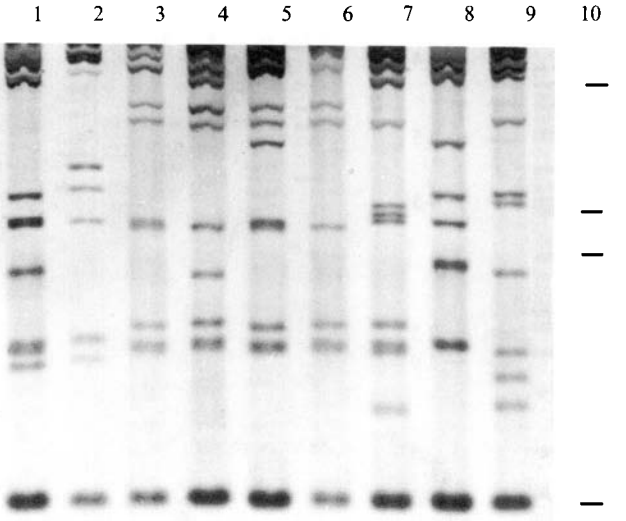


Fig. 2. Ribotyping patterns of certain biochemically atypical *Aeromonas* sp. Chromosomal DNA was digested with *Sma* I. Bands of molecular weight (about 0·8–4 kb) were used for the identification of an HG [21]. Phenotypic characteristics of the strains are presented in Table 2. Lane 1, HG 8/10; lane 2, HG 8/10; lane 3, HG 1; lane 4, HG 1; lane 5, HG 1; lane 6, HG 1; lane 7, HG 1; lane 8, HG 8/10; lane 9, HG 13 and lane 10 molecular weight markers in kb.

(Table 1) were useful in the differentiation of HG 1 from HG 2 and HG 3, and HG 4 from HG 5A and 5B. All HG 3 strains were sorbitol positive, but 15 out of 52 HG 2 isolates were also sorbitol positive. Rhamnose positive strains were most common in HG 2. Ribotyping was useful in the differentiation of HG 4, HG 5A, HG 5B and HG 6. As described [27], determination of maximum growth temperature was of value in the differentiation of HG 1 from HG 2 and HG 3, HG 4 from HGs 5A, 5B and 6 and HG 8/10 from HG 7.

Hybridization groups of environmental isolates with atypical biochemical characteristics were not always identified by using ribotyping patterns. These isolates originated from fresh water (12 isolates), drinking water (3 isolates) and chicken and ground beef meat (3 isolates). Unnamed isolates from environmental sources were aesculin negative (16/18) in most cases and they did not produce acid from arabinose, salicin or sucrose.

Biochemically atypical human isolates resembling *A. hydrophila*, but with certain atypical characteristics were identified either as HG 1 (*A. hydrophila*) or HG 8/10 (*A. veronii* biotype *veronii*) (Table 2, Fig. 2). All *A. veronii* biotype *veronii*-like (HG 8/10) strains, which were only isolated from clinical samples, were ADH positive and ODC negative, although amino acids were tested with API 20E, in Möller's decarboxylation medium and in Fay and Barry medium as recommended by Altwegg and colleagues [24]. Three of those eight isolates were arabinose positive. Ribotyping pattern of one strain is presented in Figure 2, lane 1. Elastase test and cephalothin sensitivity proved to be of value in differentiating *A. hydrophila* from *A. veronii* biotype *veronii*. Ribotyping confirmed that two ampicillin sensitive isolates resembling *A. sobria* belonged to HG 13 (Table 3, Fig. 2, lane 9).

*Distribution of Aeromonas genospecies among environmental and clinical isolates*

The distribution of different HGs among environmental and clinical specimens was compared (Table 3). Differences were seen in the occurrence of the HGs in various samples. The most common HGs identified were *A. hydrophila* HG 2 and *A. caviae* HG 5B in drinking water, *A. hydrophila* HG 2 and HG 3 in fresh water and *A. hydrophila* HG 1, HG 3, *A. caviae* HG 4, HG 5A and HG 8/10 (*A. veronii* biotype *sobria*) in chicken and ground beef. One isolate was identified as a member of HG 9 (*A. jandaei*). Certain HGs, such as HG 6 and HG 7 occurred only in water samples.

Three HGs, HG 1, HG 4 and HG 8/10 clearly predominated in human faecal samples; only 4 of 87 isolates belonged to some other HG (*A. hydrophila* HG 3, *A. media* HG 5B and two *A. trota* HG 13) as mentioned above. Human blood isolates showed an HG distribution similar to that of the faecal isolates.

Isolates with atypical biochemical characteristics and with unidentified genetic species were most often found among fresh water isolates.

## DISCUSSION

Usually, analysis of ribotyping patterns of small chromosomal fragments digested with *Sma* I confirmed that the three phenons identified as *A. hydrophila*, *A. caviae* and *A. sobria* belonged to HGs 1, 2 or 3 (*A. hydrophila*), HGs 4, 5A, 5B or 6 (*A. caviae*) and HG 7 or HG 8/10 (*A. sobria*), respectively. These results support the suggestion of Martinetti, Lucchini and Altwegg [21] that of *Sma* I ribotyping patterns could be used as a tool for identifying the genetic species of *Aeromonas* spp. As shown earlier these small fragments are not always visible or they are faint [12, 21, 25]. The small fragments were detected if at least 5 $\mu$g of chromosomal DNA was digested. The ribopattern produced by the reference strains of each HG with 16S and 23S rDNA as the probe were similar at 0·8–4·0 kb as was shown by Martinetti, Lucchini and Altwegg [21] with their probe, which was plasmid pKK3535 containing the rrnB operon of *Escherichia coli*. Thirty-five clinical strains representing different HGs were tested with both of those probes, and the results were identical (results not shown). This indicates that commercially available 16S and 23S RNA of *E. coli* can also be used as a probe when identifying an HG of *Aeromonas* spp. Although ribotyping was shown to be useful for confirming the identity of certain uncommon *Aeromonas* species e.g. *A. jandaei* (HG 9- and *A. trota* (HG 13), or for confirming the identity of certain less characterized species, such as *A. eucrenophila* (HG 6) and *a. sobria* (HG 7) it was not working in the identification of certain atypical *Aeromonas* spp. isolated, in particular, from fresh water. About 20% of fresh water isolates remained unnamed. They probably may represent some atypical or new HGs.

Lactate, citrate and urocanic acid utilization and D-rhamnose or sorbitol fermentation have proved to be of value in the phenotypic identification of HGs 1, 2 and 3 of *A. hydrophila* [14, 15, 26]. Only one sorbitol positive *A. hydrophila* HG 1 was found. Lactate and citrate utilization and haemolysis on blood agar were useful in the differentiation of HG 4, HG 5A and HG 5B [15]. As shown earlier [28] *A. veronii* (HG 8/10) is easily misidentified as *A. hydrophila*. To our knowledge, it

was the first time sorbitol positive *A. veronii* biotype *veronii* was described [14, 16, 20]. Cephalothin sensitivity testing and elastase production were shown to be useful additional tests in differentiating atypical *A. hydrophila* from *A. veronii* biotype *veronii*. As shown earlier, determination of maximum growth temperature is a useful test in the differentiation of HG 1 from HG 2 and HG 3, HG 4 from HG 5 and HG 6, HG 7 from HG 8/10 [26, 27].

The epidemiology of infections caused by *Aeromonas* spp. is not well known. However, human *Aeromonas* spp. infections are often suspected of being food-borne or water-borne since *Aeromonas* spp. commonly occur in food and water [2, 6, 29, 30]. Only a few studies have compared the distribution of different *Aeromonas* HGs among clinical and environmental sources [12, 15]. It is known that about 85% of human faecal isolates belong to HG 1 (*A. hydrophila*), HG 4 (*A. caviae*), HG 8/10 (*A. veronii* biotype *sobria*). HG 5A, HG 5B, HG 9, HG 12 and HG 13 have also been isolated in a few cases [10, 15]. Also in the present study, *A. hydrophila* HG 1, *A. caviae* HG 4 and *A. veronii* biotype *sobria* HG 8/10 accounted for 80–90% of human isolates. *A. veronii* biotype *veronii* (HG 8/10) was a common *Aeromonas* species associated with travelling. *A. trota* was isolated from faecal samples of Finns who had been in Morocco. *A. trota* strains first described by Carnahan *et al.* [31] had also been isolated from people living in subtropical areas.

The contamination of raw meat and chicken with *Aeromonas* spp. is suspected to originate more often from environmental sources, e.g. from water used for cleaning in processing plants than from faecal contamination during slaughtering process [5, 30]. Analysis of the distribution of different *Aeromonas* HGs in meat and drinking water suggested that meat is most probably contaminated by sources other than water. The predominant HGs in drinking water samples taken from 44 different wells and two water distribution systems were *A. hydrophila* HG 2, *A. caviae* HG 5B and *A. sobria* HG 7, while the HGs predominating in meat and chicken samples were *A. hydrophila* HG 1, *A. caviae* HG 4 and HG 5A and *A. veronii* biotype *sobria* HG 8/10. The source contaminating meat and chicken with *Aeromonas* spp. of HG 1, HG 4 and HG 8/10, is not known, but it may be faecal contamination during slaughtering. Aeromonads are psychrothrophic organisms which grow at refrigeration temperatures [8] and they are known to be spoilage organisms of meat [30]. Whether storage of meat at refrigeration temperatures selects certain HGs as predominating organisms is not known. Although meat and chicken were shown to be contaminated with clinically important HGs, proper heat treatment destroys the organisms [30].

Aeromonads may occur in fresh water in high numbers, up to cfu $10^5$ per 100 ml and in drinking water up to cfu $10^3$ per 100 ml [2]. The distribution profile of different HGs in fresh water and drinking water was similar, suggesting that *A. hydrophila* HG 2 and HG 3, *A. media* HG 5 and *A. sobria* HG 7 are adapted to a water environment. For example, *A. media* (HG 5) and *A. eucrenophila* (HG 6) were originally isolated from fresh water [32, 33]. Drinking water has been suspected of being an important source of human intestinal infections [6, 29, 34]. This study showed that, in most cases the *Aeromonas* HGs occurring in drinking water were not the same as those found in human faecal samples. HG 5B was common in drinking water and this genetic species has also been isolated from human faecal samples [10, 15]. Havelaar and colleagues [1] biotyped and

serotyped and made cluster analysis of cell wall fatty acid methyl esters of *Aeromonas* strains isolated from patients with diarrhoea or from drinking water. Their results also indicated that there was little overall similarity between those two groups. Similarly, Moyer and colleagues [12] found that human clinical strains and the strains isolated from a drinking water distribution system which was a suspected source of infection belonged to totally different HGs. Although the clinically important HGs comprise a minority of the aeromonads in water, they are probably selected and colonize the human gut and may cause diarrhoea.

The detection methods used may also affect the distribution of different HGs detected in different samples. The enrichment procedure used to detect aeromonads in meat and chicken samples may have selective advantage for certain HGs. The membrane filtration method was used for water samples while clinical samples were cultivated directly on the selective medium. The selective medium used for clinical samples contained irgasan and brilliant green as selective substances, and the media used for water and food samples contained ampicillin and bile salts. Certain *Aeromonas* species, such as *A. trota*, which was isolated from two clinical samples are known to be ampicillin sensitive [31]. In a medium containing glucose *A. caviae*, in particular, is known to activate a pathway that produces acetic acid thereby becoming unviable ("suicide phenomenon") [35]. The enrichment medium used in the present study was tryptic soy broth containing glucose. In all samples except human clinical samples, it was rather common for one sample to contain two different *Aeromonas* species.

In conclusion, the majority of *Aeromonas* spp. occurring in water seem to be species adapted to water. Clinically important HGs may be present in drinking water or fresh water as a minority. Chicken and ground beef commonly contain the same *Aeromonas* spp. which occur in human diarrhoeal and non-diarrhoeal faecal samples.

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