A REVIEW OF THE OCCURRENCE AND SIGNIFICANCE OF AEROMONADS WITH PARTICULAR REFERENCE TO POTABLE WATER DISTRIBUTION SYSTEMS

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A REVIEW OF THE OCCURRENCE AND SIGNIFICANCE OF AEROMONADS WITH PARTICULAR REFERENCE TO POTABLE WATER DISTRIBUTION SYSTEMS

R A Pitchers

SUMMARY

The objective of this study is to review the literature concerning the occurrence and significance of aeromonads in potable water distribution systems. Aeromonads have been frequently recovered from distribution systems where their occurrence has been considered as a measure of regrowth potential. Also, attention has focused on the group because of their potential threat to public health.

The conclusions derived from the literature review are set out as follows:

1) The classification of the motile aeromonad group has not been satisfactorily defined. In most cases classification to genus level would be appropriate.

2) The majority of selective media for recovery of aeromonads do not perform entirely satisfactorily.

3) Identification of isolates would be better achieved by using a series of specific biochemical tests in preference to the commercially available API system.

4) In natural waters the numbers of aeromonads exhibit seasonal variations which could be linked to temperature and selective utilisation of algal and macrophyte components.

5) In potable waters aeromonads respond to an interaction between a number of factors including chlorine concentration, temperature and residence time.

6) The link between the presence of aeromonads in drinking water and the outbreak of waterborne gastroenteritis has not been established.
1. **INTRODUCTION**

Recently attention has focused on the occurrence of a specific group of bacteria, the aeromonads, in drinking water distribution systems. Typically, aeromonads were considered as components of the heterotrophic bacterial population that frequently represented a nuisance when they interfered with the enumeration of coliform bacteria. The occurrence of aeromonads has been suggested to indicate the potential for regrowth in distribution. Also, it has been suggested that aeromonads should be considered as potential pathogenic bacteria.

Therefore, a review of the literature was conducted to investigate the current status concerning the significance of aeromonads in distribution. An examination of the current taxonomic status of the genus was undertaken (Section 2). On a practical basis consideration was given to the available methods for isolation and identification of aeromonads (Section 3). To identify what factors affect aeromonads the review considered their growth in both natural waters (Section 4.1) and potable waters (Section 4.2). Also, the involvement of aeromonads as potential pathogens was examined (Section 5).
2. TAXONOMY OF AEROMONADS

The genus *Aeromonas* was first proposed by Kuyver and Van Neil (1936) to accommodate those microorganisms, similar to enteric bacteria, which were associated with freshwater environments and motile forms having a polar flagella. The current definition of the genus also includes the characteristics of being; Gram negative, facultative anaerobic bacteria which are oxidase and catalase positive and breakdown carbohydrates fermentatively (Krieg and Holt 1984). At present the genus is designated to the family *Vibrionaceae*. However, molecular genetic evidence, based on 5S and 16S ribosomal ribonucleic acid sequence analysis and DNA hybridisation patterns has indicated that aeromonads were sufficiently different from other members of the family to warrant the creation of a separate family, the *Aeromonadaceae* (Colwell et al 1986).

Within the genus a clear distinction exists between the psychrophilic non-motile and the mesophilic motile aeromonads (Krieg and Holt 1984). The former group are clustered around the single species *Aeromonas salmonicida*. This group has an optimum growth temperature of 22-25 °C with a minimum and maximum growth temperature of 5 °C and 35 °C respectively. An additional non-motile species *A. media* has recently been described (Allen et al 1983). The classification of the mesophilic motile aeromonads to species level has not yet been equivocally established. This group have an optimum growth temperature of 28 °C with minimum and maximum growth temperatures of 5 °C and 38-41 °C. It is this group which are of the most important in the context of this review.

The previous classification of the motile aeromonads was proposed by Schubert (1967a, 1967b and 1968) in which the group was divided into *A. hydrophila* with three sub-species and *A. punctata* with two sub-species. A subsequent revision of the genus was undertaken by Popoff and Veron (1976). Extensive biochemical analysis produced three distinct patterns and the motile group was divided into *A. hydrophila* subsp. *hydrophila*, *A. hydrophila* biovar *anaerogenes* and a new species *A. sobria* was proposed. In subsequent studies, comparison of DNA hybridisation patterns, indicated that *A. hydrophila* biovar *anaerogenes* represented a separate species and was renamed as *A. caviae* (Popoff et al
3. ISOLATION AND IDENTIFICATION

3.1 Isolation media

Aeromonads are not fastidious in their nutritional requirements and will readily grow on a wide range of nutrient agars. Also, aeromonads can grow on selective media used for the isolation of coliform bacteria, where they produce colonies that are similar in appearance to them because they share a common fermentative metabolism. This is a particular problem on selective media for coliforms containing lactose and leads to the possibility of aeromonads causing false positive counts (Neilson 1978 and Edge and Finch 1986). However, the ability to ferment lactose appears not to be species specific but is variable within each species. Janda (1985) determined the percentage lactose fermentation within each of the species as *A. hydrophila* (34%), *A. sobria* (11%) and *A. caviae* (68%). However, LeChevallier *et al* (1982) failed to detect any lactose fermenting strains of *A. sobria* in a drinking water distribution system.

Numerous selective media have been developed specifically for the isolation of aeromonads in mixed microbial communities from a variety of locations including aquatic, clinical and food environments (see Joseph *et al* 1988). Many of the selective media developed for clinical and food applications have also been evaluated for the recovery of aeromonads from environmental sources (Millership and Chattopadhyay 1985, Havelaar *et al* 1987, Arcos *et al* 1988, Gray and Stickler 1989 and Knochel 1989). Varying degrees of success were reported and probably not only reflected the inherent ability of each medium to recover aeromonads satisfactorily but also their ability to suppress the background flora present at each location.

However, media have been developed especially for isolation of aeromonads from aquatic environments (Table 3.1). In the majority of these media selectivity is achieved by the specificity of aeromonads to metabolise fermentatively; starch, its intermediate oligosaccharide - dextrin, or its principal component disaccharide - maltose. A variety of sugar alcohols (inositol, mannitol and
Table 3.1. Selective media used to recover aeromonads from aquatic environments

<table>
<thead>
<tr>
<th>Isolation agar</th>
<th>Selective agent</th>
<th>Inhibitory agent (a)</th>
<th>Indicator</th>
<th>Colony characteristics</th>
<th>Incubation conditions</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rimler-schotts agar</td>
<td>lysine</td>
<td>citrate</td>
<td>BT3</td>
<td>yellow</td>
<td>35 °C 24 hours</td>
<td>Schotts and Kimler 1973</td>
</tr>
<tr>
<td></td>
<td>ornithine</td>
<td>novobiocin (5)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>maltose</td>
<td>deoxycholate</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MaConkey agar</td>
<td>trehalose</td>
<td>crystal violet</td>
<td>PR</td>
<td>yellow</td>
<td>35 °C 24 hours</td>
<td>Kaper et al 1981</td>
</tr>
<tr>
<td>(Trehalose substituted</td>
<td>bile salts</td>
<td>yellow</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>for lactose</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Peptone-beef extract</td>
<td>glycogen</td>
<td>sodium dodecyl</td>
<td>BTB</td>
<td>yellow with yellow halo</td>
<td>25-30 °C 18-24 hours</td>
<td>McCoy and Filcher 1974</td>
</tr>
<tr>
<td>glycogen agar</td>
<td></td>
<td>sulphate</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>mA agar</td>
<td>trehalose</td>
<td>ampicillin (20)</td>
<td>BTB</td>
<td>yellow on trehalose</td>
<td>35 °C 20 hours</td>
<td>Rippey and Cabelli 1979</td>
</tr>
<tr>
<td></td>
<td>mannitol</td>
<td>deoxycholate</td>
<td></td>
<td>and yellow on mannitol</td>
<td>2 hours</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>ethanol</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ampicillin-dextrin agar</td>
<td>dextrin</td>
<td>ampicillin (10)</td>
<td>BTB</td>
<td>yellow</td>
<td>30 °C 18 hours</td>
<td>Havelaar et al 1987</td>
</tr>
<tr>
<td></td>
<td></td>
<td>deoxycholate</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>GSP agar</td>
<td>starch</td>
<td>penicillin</td>
<td>PR</td>
<td>yellow</td>
<td>25 °C 18 hours</td>
<td>Von Kleiwein 1969</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(10 000 iu)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SGAF agar</td>
<td>starch</td>
<td>penicillin</td>
<td>PR</td>
<td>yellow</td>
<td>28 °C 18 hours</td>
<td>Hueget and Ribas 1990</td>
</tr>
<tr>
<td></td>
<td>glucose</td>
<td>ampicillin (20)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Isolation agar</td>
<td>Selective agent</td>
<td>Inhibitory agent (a)</td>
<td>Indicator</td>
<td>Colony characteristics</td>
<td>Incubation conditions</td>
<td>Reference</td>
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</tr>
<tr>
<td>MIX agar</td>
<td>xylose</td>
<td>ampicillin (20)</td>
<td>BTB</td>
<td>blue-green</td>
<td>30 °C 18 hours 35 °C 24 hours (b)</td>
<td>Curliffe and Adcock 1989</td>
</tr>
<tr>
<td></td>
<td>inositol</td>
<td>bile salts citrate</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dextrin-fuschin</td>
<td>dextrin</td>
<td>none</td>
<td>F</td>
<td>red</td>
<td>30 °C 24 hours</td>
<td>Schubert 1939</td>
</tr>
<tr>
<td>Sulphite agar</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Aeromonas agar</td>
<td>lysine arginine</td>
<td>ampicillin (5)</td>
<td>BTB</td>
<td>dark green-opaque with dark centre</td>
<td>30-35 °C 24 hours</td>
<td>Oxoid Manual 1951</td>
</tr>
<tr>
<td></td>
<td>incositol lactose sorbitol xylose</td>
<td>TB</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Key

BTB - bromothymol blue, PR - phenol red, TB - thymol blue, F - fuschin

Notes

(a) The figure quoted in parentheses indicates an antibiotic concentration in mg/l.
(b) With this technique the conditions refer to anaerobic incubation, in an atmosphere of nitrogen, followed by aerobic incubation.
sorbitol), a disaccharide sugar (trehalose), pentose sugar (xylose) and the animal polysaccharide equivalent to starch, glycogen, have also been used.

The source of carbohydrate used in the medium can have a significant effect on expression of colony characteristics. Havelaar and Vonk (1988) evaluated a range of dextrins, obtained from different manufacturers, to prepare ampicillin dextrin agar (ADA). It was found that the rate of fermentation varied between sources of dextrin and only those products supplied by Merck and Difco were suitable. As different methods are used to manufacture dextrin its composition and quality will vary between suppliers (Cowan 1974).

To enhance the selectivity of the isolation media antibiotics are additionally incorporated to suppress the background flora. To specifically inhibit the growth of the Enterobacteriaceae, ampicillin is commonly used at a range of concentrations from 5-30 mg/l (Table 3.1). This would be particularly important where dextrin is used as the selective carbohydrate as bacteria from the genera Klebsiella and Enterobacter are also capable of fermenting this substrate (Schubert 1987). Alternatively, the use of penicillin-G has been described (Von Kielwein 1969). However, it has been found that this antibiotic was not sufficiently selective for aeromonads and the efficacy of the medium was further improved by the incorporation of ampicillin (Huget and Ribas 1990).

However, these media have not been found to be entirely selective for aeromonads and allowed the growth of other organisms, of which pseudomonads predominated. This has led to modifications in the isolation technique and incubation conditions to improve recovery of aeromonads. A frequently adopted method is incubation under anaerobic conditions as aeromonads are facultatively anaerobic and can grow in the absence of oxygen. The background flora, in comparison, tends to be composed of aerobic non-fermenting bacteria which will not grow under these conditions. Millership and Chattopadhyay (1985) reported that incubation under totally anaerobic conditions gave improved recoveries of aeromonads. However, Havelaar et al (1987) found that when aeromonads were incubated in the absence of oxygen, their ability to ferment the carbohydrate was reduced and therefore colonies did not develop satisfactorily. As a consequence of the impaired ability of aeromonads to ferment under total anaerobic conditions, Cunliffe and Adcock (1989) used a combination of both
anaerobic and aerobic incubation. Schubert (1987) created sufficiently anaerobic conditions by overlaying the isolation plate with molten medium which inhibited growth of the aerobic background flora.

Additionally, because the genera *Aeromonas* and *Vibrio* are closely related the majority of media selective for aeromonads will also allow the growth of vibrios. This is usually considered to be a particular problem in estuarine and marine environments and involves principally the group *F. vibrios* (Nakano *et al* 1981 and Kaper *et al* 1981). However, this could also be a problem in other aquatic environments as the distribution of vibrios is not confined to saline waters since freshwaters are known to support a vibrio population (Lee 1990). Growth of vibrios can be prevented by incorporating a specific antibiotic into the selective medium, usually referred to as vibriostatic agent O/129. Palumbo *et al* (1984), Havelaar *et al* (1987) and Arcos *et al* (1989) have all reported the addition of this agent at a concentration of 50 µg/l successfully inhibited the growth of vibrios. An alternative approach was taken by Huget and Ribas (1990) who recommended that a medium without sodium chloride would prevent growth of vibrios, since all vibrios, with the exception of *V. cholerae*, required it for optimal growth.

### 3.2 Identification systems

Unfortunately, to confirm the identity of presumptive aeromonad isolates, additional tests must be applied after selection and purification because most media do permit the growth of other bacteria. Aeromonads can be identified by determination of their response to a series of biochemical test reactions. Such schemes are based on the reactions proposed by Popoff and are given in the ninth edition of Bergey’s Manual of Determinative Bacteriology (Kreig and Holt 1984). Cunliffe and Adcock (1989) and Monfort and Baleux (1990) have both proposed schemes for the identification of aeromonads to species level. Although the use of a relatively large number of tests would allow more reliable identification this approach would not lend itself to routine applications where numerous isolates required screening.
To meet these requirements a system was developed in which the number of
differential tests had been reduced to the minimum level to still permit
speciation (Medema, personal communication). The differentiation of
presumptive isolates to species level is based upon four common biochemical
test reactions. To confirm identity to genus level aeromonads were positive
for the oxidase reaction and possessed a fermentative metabolism in Hugh and
Leifson's medium. Two additional tests were used to determine speciation
(Table 3.2).

Table 3.2. Classification of aeromonads to species level

<table>
<thead>
<tr>
<th>Aeromonad species</th>
<th>Biochemical test reaction</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Gas from glucose</td>
<td>Esculin hydrolysis</td>
</tr>
<tr>
<td>A. hydrophila</td>
<td>positive</td>
<td>positive</td>
</tr>
<tr>
<td>A. sobria</td>
<td>positive</td>
<td>negative</td>
</tr>
<tr>
<td>A. caviae</td>
<td>negative</td>
<td>positive</td>
</tr>
<tr>
<td>unknown</td>
<td>negative</td>
<td>negative</td>
</tr>
</tbody>
</table>

Also, commercial systems have been used to identify aeromonads. Most commonly
used are either the API 20 E (LeChevallier et al 1982, Burke et al 1984 and
Gray and Stickler 1988) or the API 20 NE (Arco et al 1988 and Huget and Ribas
1990). The API 20 E primarily recognises bacteria from the family
Enterobacteriaceae but does include A. hydrophila. The API 20 NE is designed
specifically for non-enteric bacteria and recognises all three current species
in the motile aeromonad group. However, an evaluation of both these systems
found they were unable to identify aeromonads satisfactorily (McGarey and Wrona
1989). Both systems misidentified A. caviae as a species of Vibrio.
Additionally, the API 20 NE had variable identification rates for A. hydrophila
and A. sobria of 40% and 60% respectively. With the API 20 E all isolates of
A. hydrophila were correctly identified.

All systems to identify aeromonads require the performance of an oxidase test
on the isolate. It has been shown that aeromonads growing on a medium
containing a carbohydrate yielded a false negative reaction if the test was performed directly on the colony (Hunt et al 1981). The oxidase test reaction was inhibited by acid produced during fermentative metabolism. Thus, before the test was undertaken, it was necessary to first subculture all isolates onto nutrient agar, which does not contain a fermentable carbohydrate substrate.
4. OCCURRENCE OF AEROMONADS IN AQUATIC ENVIRONMENTS

4.1 Occurrence in natural waters

Aeromonads are common aquatic bacteria and have been isolated from diverse environments ranging from freshwater to marine ecosystems. They have been isolated from waters of a high salinity although it has not been established whether they represent a truly indigenous marine population (Abeyta and Weckel 1988). Although, Hazen et al (1978) recovered aeromonads from marine waters with salinities up to 100°/oo, their occurrence was considered to represent survival of the bacterium following input from freshwater sources. Similarly, Nakano et al (1981) found that aeromonads were capable of surviving in marine waters with salinities of 22-33°/oo.

In freshwaters they have been found in waters of varying composition. Hazen et al (1978) isolated A. hydrophila from a diverse selection of waters with temperatures between 4 and 45 °C and over a pH range of 5.2 to 9.8. Additionally, aeromonads are commonly associated with polluted waters through the input of sewage effluent (Araujo et al 1989 and Montfort and Baleux 1989). Schubert (1990) suggested that a ratio of anaerogenic to non-anaerogenic aeromonads could be used as an index of water quality as different species were found in natural waters compared with sewage effluent.

4.1.1 Factors affecting the growth of aeromonads

Numerous studies have been undertaken to identify the factors affecting the growth of aeromonads in natural waters. Hazen et al (1978) surveyed a number of natural freshwaters environments where a good correlation was found between numbers of aeromonads and conductivity of the water. It was suggested that some unmeasured water quality parameter, which varied proportionally with conductivity, was affecting the aeromonad population. Studies by Rippey and Cabelli (1985) indicated that the numbers of aeromonads were related to the trophic status of the water as measured by: dissolved reactive phosphorus, chlorophyll a and Secchi depth. These were parameters of primarily
photoautotrophic (algal) biomass. Growth studies conducted in nutrient unsupplemented, filtered, autoclaved natural waters inoculated with an aeromonad population indicated that A. hydrophila responded directly to exogenous nutrient inputs, particularly levels of phosphorus. It was inferred that although the aeromonad population paralleled the phytoplankton population one was not the result of the other but that both were responding to the same nutrient.

Conversely, Hazen and Esch (1983) suggested that levels of phytoplankton directly influenced the aeromonad population. A distribution pattern was found where the maximum aeromonad population paralleled the seasonal changes in phytoplankton density. The effect of the phytoplankton was to increase the dissolved oxygen concentration, total phosphorus and total organic carbon. Monfort and Baleux (1990) also considered that aeromonads were responding directly to the influence of algae. It was suggested that either the aeromonads were responding to specific nutrients released by the algae or that they were more resistant to bacterial toxins produced by the algae. However, Nakano et al (1981) observed an inverse relationship between chlorophyll a and the aeromonad population in some locations. Their study suggested that the density of the aeromonads was not linked to a single factor but was related to the cumulative effect of several physicochemical parameters.

It has also been observed that aeromonads are associated with decomposing aquatic macrophytes. Hazen (1979) recovered high numbers of aeromonads from waters in which Myriophyllum spicatum (water milfoil) was decaying. The bacteria were able to degrade the macromolecules released from the rotting vegetation.

Of the various physical factors affecting the aeromonad population, temperature appears to be significant. Fleirmans et al (1977) found that the highest numbers occurred during the spring. Similarly, studies on the seasonal abundance of A. hydrophila in the Anacostia River (Washington, USA) showed that temperature was the predominant factor controlling the population (Seidler et al 1980). The numbers of aeromonads in the top and bottom waters as well as in the sediment exhibited a seasonal fluctuation. Counts increased during periods when the water temperature was elevated and reached a maximum in waters.
above 20 °C. Further studies on this location by Cavari et al (1981) also showed a seasonal distribution with peaks occurring during the summer months. Experiments conducted in the laboratory showed that aeromonads were unable to survive in waters at 4 °C. At this temperature they were not able to supply the energy requirements to maintain a viable population.

4.2 **Growth in potable water distribution systems**

Aeromonads are frequently isolated from water in distribution systems and have been recovered from both chlorinated and unchlorinated waters. According to van der Kooij (1988), early work in Holland recognised the potential problems of aeromonads in drinking water. The health authorities set water quality standards in which the maximum acceptable concentration of aeromonads in water leaving a treatment works should not exceed 20 CFU/100 ml and in distribution 50 CFU/100 ml. Improved techniques for the detection of aeromonads resulted in an increase of the limit to 200 CFU/100 ml for water in distribution.

In locations where aeromonads have been recovered they constitute only a small fraction of the total microbial population. An investigation by LeChevallier et al (1984) reported that aeromonads contributed approximately 10% of the total bacterial population in chlorinated water. However, in unchlorinated water aeromonads accounted for an increased proportion of 21%. Other studies have generally found this value to be lower. In an extensive survey of the bacterial population in a distribution system aeromonads comprised between 0.9 and 2.4% of the total bacterial population (Clark et al 1982). Also, van der Kooij (1988) found that aeromonads only represented 0.2% of the total microbial population. Similarly, Knochel and Jeppesen (1990) recovered aeromonads at concentrations of between 1-40 CFU/100 ml which represented approximately 10% of the total bacterial population.

4.2.1 **Factors affecting the growth of aeromonads in distribution**

In treated water it appears that an interaction between temperature and chlorine concentration is significant in controlling the aeromonad population. Burke et al (1984a) monitored aeromonad populations in a metropolitan drinking
water supply over one year. When the concentration of chlorine was below 0.3 mg/l a seasonal variation in the population occurred and temperatures above 14.5 °C increased the count. Conversely, temperature had no effect on the growth of aeromonads when the total available chlorine concentration was above 0.3 mg/l.

In other distribution systems the occurrence of aeromonads has only been considered as a function of seasonality. Millership and Chattopadhyay (1985) reported isolation rates of 25% in summer and 7.0% in winter from a chlorinated water supply. Similarly, during a one year survey of a distribution system, aeromonads were more frequently isolated during warmer months when the temperature of the water ranged between 15 to 20 °C (LeChevallier et al 1982). Conversely, Mascher et al (1988) failed to find any seasonal correlation in occurrence of aeromonads with numbers of less than 40/100 ml being recovered throughout the year.

In studies where the occurrence of aeromonads has been examined as a function of location within distribution systems greatest recoveries have occurred at the extremities of networks. Edge and Finch (1986) found that aeromonads were frequently associated with the outer zones of the network. Similarly, work in this laboratory has shown that aeromonads predominate at the end points of distribution systems. In a survey of a distribution system network, aeromonads were only found in the dead ends (Pitchers and Jago, unpublished). It was suggested that residence time was an important factor in that it provided opportunity for proliferation of aeromonad once other conditions for growth were favourable.

The quality of the water used for treatment has also been shown to affect the aeromonad population. Edge and Finch (1986) recovered much lower numbers of aeromonads in distribution systems where the water was derived from a groundwater source rather than a surface water source. Studies by van der Kooij and Hijnen (1988) suggested that the nutritional versatility of aeromonads provided them with a selective advantage over the competing heterotrophic population. It was found that an aeromonad strain M800 had a high substrate affinity for mixtures of amino acids and long chain fatty acids. Thus, it was inferred that they were able to compete successfully with other members of the microbial community for these compounds even when present at low
substrate concentrations. Further investigations demonstrated that aeromonads were capable of utilising the plumbing materials used in distribution system pipework (van der Kooij 1988). Soft soap, which is used as a lubricant in pipe joints, was capable of promoting the growth of aeromonads at low substrate concentrations. It was suggested that these organisms were found in distribution system biofilms where they could utilise the biomass components of other microorganisms. Additionally, this could represent a survival mechanism as biofilm inhabiting bacteria have been found to possess a greater resistance to chlorination (LeChevallier et al 1988).
5. SIGNIFICANCE TO HEALTH OF AEROMONADS IN POTABLE WATER

The specific role of aeromonads as causative agents in human disease has not been conclusively established. Primary and secondary wound infections have been reported to arise through injuries sustained in waters with a high aeromonad count (Kaper et al 1981 and Abeyta and Weckell 1988). They have also been isolated from invasive diseases such as septicemia (Janda and Duffey, 1988).

However, by far the most common association in pathogenesis is with gastroenteritis. Aeromonads have been isolated from diarrhoeal stools even when no other recognised enteric pathogen has been recovered, although the isolation rates from infected individuals are typically low (Janda and Duffey 1988). Altwegg (1985) recovered aeromonads from less than 2% of patients with diarrhoeal symptoms. A range of virulence factors that might be involved in pathogenesis have been identified. These include the production of exotoxins, including enterotoxins and hemolysins, cytotoxins and the ability to adhere and invade Hep-2 cells (Thomas et al 1990). However, human challenge experiments with strains possessing one or more of these virulence factors have failed to produce diarrhoeal symptoms in healthy individuals.

As aeromonads possess a widespread occurrence their transmission to humans can be through a number of routes, the two most significant vectors being food and water. Aeromonads have been frequently isolated from a wide range of food products (Palumbo et al 1985, Nishikawa and Kishi 1988 and Knochel and Jeppesen 1990). Kirov et al (1990) reported a strong correlation in the possession of virulence factors between food and clinical aeromonad isolates.

With respect to transmission via drinking water there is no firm evidence to implicate the role of aeromonads as causative agents of gastroenteritis. In a limited epidemiological study Burke et al (1984a) did find a relationship between the abundance of aeromonads in a distribution system and the number of reported outbreaks of diarrhoea. However, this was a non-randomised study and would not include all patients possessing gastroenteritis. Other workers have isolated aeromonads from distribution systems and have considered them as potential pathogens because they have demonstrated that some of these strains
were capable of producing certain virulence factors in *in vitro* assays. LeChevallier et al (1982) recovered only *A. sobria* from 27% of samples in a drinking water network. It was found that over 80% of strains were cytotoxic but that none were enterotoxic. Burke et al (1984a) monitored an unchlorinated domestic water supply and found that 61% and 66% of the aeromonads produced enterotoxin and hemolysin respectively. Mascher et al (1990) isolated aeromonads from a distribution network in which 50% of the strains were capable of producing enterotoxins.

It would appear that differences exist in the distribution of biochemical and virulence factors between aeromonads isolated from environmental and clinical sources. At the biotype level Burke et al (1984c) reported that 58.5% and 39.4% of environmental isolates compared to 15.0% and 6.8% of faecal origin were able to ferment arabinose and salicin respectively. Similarly, there were differences in the distribution of virulence factors between the two groups of isolates. Both sets of isolates demonstrated enterotoxic activity at 91.2% and 70.2% for those of clinical and environmental origin respectively. However, over 70% of drinking water strains exhibited a non-pathogenic haemaglutination pattern compared with 10.0% of faecal origin.
6. DISCUSSION

Before investigations can be undertaken on the occurrence of aeromonads consideration needs to be given to techniques for their isolation and identification. It would appear that a number of factors operate to make this process difficult. In the first instance, classification of the motile aeromonad group has not been fully established and it seems likely that additional species exist. However, until classification of the group has been resolved then isolates should be designated to one of the three currently recognised species.

Although aeromonads are easy to recover from aquatic environments the majority of selective media do not give a satisfactory performance. Because of the similarity between aeromonads and vibrios both will be recovered on most isolation media. As vibrios are likely to occur in all waters, possibly including potable water as well, then incorporation of the vibriostatic agent 0/129 into the isolation medium to prevent their growth should be considered for all applications. Also, the majority of selective media are not able as such to prevent the growth of aerobic non-fermenting bacteria which can, through their excessive growth, obscure the the aeromonad colonies. However, this growth can be suppressed by alterations to the incubation conditions to create a more anaerobic environment.

Also, identification is made more difficult by the lack of a suitable confirmation system for use in routine applications where large numbers of isolates require screening. Unless vibrios are prevented from growing on the isolation medium it is essential that any identification system should be able to discriminate aeromonads from vibrios. Again the vibriostatic agent could be employed as an additional diagnostic feature. Use of a series of specific biochemical tests appears to offer the best alternative as the commercial identification system API does not appear to be able to discriminate satisfactorily between aeromonads and vibrios. However, other commercial identification systems do exist although it is not known how well they function.
In natural waters the densities of aeromonads appear to show a seasonal distribution and it has been suggested that the aeromonad population was responding to favourable temperatures. However, it is difficult to establish a clear association with temperature as other factors would also be likely to exhibit a seasonal variation. Indeed, other studies have shown that high numbers of aeromonads corresponded with peaks in phytoplankton populations and also decomposing vegetation. Thus, aeromonads could be selectively responding to specific changes in the composition of the water through selective utilisation of the macromolecular components released from these organisms. In potable water production where a surface derived source water is used nutrients could be passed into distribution to allow for selective growth of aeromonads. To detect any specific response associated with seasonal availability of nutrients it would be useful to determine if a shift occurs in the proportion of aeromonads to the total bacterial population.

The majority of studies on potable water systems have tended to consider the occurrence of aeromonads as a function of one or two parameters of which the effects of chlorine concentration and temperature are most often examined. It would appear that aeromonads are only recovered when the total available chlorine concentration is low. In support of this, laboratory studies have shown that aeromonads have a lower resistance to disinfection than other Gram negative bacteria (Konchel 1990 and Medema et al 1990). When disinfection is inadequate the aeromonad population will respond to such influences as temperature, retention time and nutrient availability. Because of the complexity of distribution systems the numbers of aeromonads present at any one location will be a function of an interaction of all of these parameters if not more.

The most common association between aeromonads and pathogenicity is as causative agents of gastroenteritis although this remains to be conclusively established. Therefore, it is not known if the presence of aeromonads in drinking water will present a threat to public health. However, evidence from laboratory studies has indicated that a proportion of the population of aeromonads from drinking water do possess a range of virulence factors. It would be useful to conduct epidemiological studies to determine if aeromonads are of significance to public health.
7. CONCLUSIONS

1) The classification of the motile aeromonad group has not been satisfactorily defined. In most cases classification to genus level would be appropriate.

2) The selective media for recovery of aeromonads do not perform entirely satisfactorily. Modification of the incubation conditions to create an anaerobic environment will suppress the background flora.

3) Identification of isolates would be better achieved by using a series of specific biochemical tests in preference to the commercially available API system. In routine application the number of tests can be reduced but it is essential to have a diagnostic reaction to discriminate aeromonads from vibrios.

4) In natural waters the numbers of aeromonads exhibit seasonal variations which could be linked to temperature and selective utilisation of algal and macrophyte components.

5) In potable waters aeromonads respond to an interaction between a number of factors including chlorine concentration, temperature and residence time.

6) The link between the presence of aeromonads in drinking water and the outbreak of waterborne gastroenteritis has not been established.
REFERENCES


