THE MICROBIOLOGICAL QUALITY OF WATER

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The health significance of heterotrophic bacteria in drinking water

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Tap water is not sterile; it contains organisms which grow in water distribution systems or inside taps and their fittings. The absence of known pathogenic bacteria is assured by the absence of the indicator organisms but concerns have been raised in the past few years that drinking water fulfilling the standards laid down in the EC Directive ECC 80/778 may still cause disease. These concerns have arisen from several sources: work by Pierre Payment; the fact that a cause has been identified in only half of all suspected waterborne outbreaks of disease; reports have suggested that heterotrophic bacteria possessing single pathogenic mechanisms such as haemolysin may cause disease; reports of heterotrophic organisms causing water contact diseases in hospitals. These concerns led to a reappraisal of the pathogenic potential of heteretrophic bacteria, by carrying out an extensive literature search and review commissioned by the UK Water Research Company. This research identified many papers showing an association between drinking water and heterotrophic bacteria but only very few reports of suspected waterborne disease associated with the heterotrophs. The organisms demonstrating potential to cause disease were species of *Aeromonas* and *Yersinia*, but typing of organisms identified in patients and isolated from the water revealed very few similarities.

The potential of *Aeromonas* and *Yersinia* to cause waterborne disease is thought to be very low and the Communicable Disease Surveillance Centre database of laboratory infections due to these two genera of organisms was analysed to produce population-related incidences for each health region in England and Wales. Additionally a laboratory questionnaire revealed different levels of ascertainment of these two organisms in different laboratories of the Public Health Laboratory Service.

Introduction

Tap water is not sterile. It does not contain pathogens but often contains organisms known as heterotrophic bacteria which are normally detected in low numbers by the heterotrophic plate count carried out at incubation temperatures of 22 and 37°C. These heterotrophic bacteria are not identified and the guideline values are 100 at 22°C and 10 at 37°C (EEC 1980). The safety of drinking water, i.e. the absence of pathogenic bacteria, is guaranteed by the protection of source water, the water treatment process, and final chlorination. Because it is difficult to measure low numbers of pathogens, the measurement of the indicator organisms in drinking water – coliforms, *Escherichia coli*, faecal streptococci, and sulphite-reducing clostridia – is carried out in a regular, routine sampling programme. The regulatory values for these organisms are zero per 100 ml. If present these organisms indicate that a pollution incident has occurred and there is a potential for pathogenic organisms to be present; appropriate actions to protect the public health can then be taken. The indicator organisms have fulfilled this function satisfactorily in that events and incidents where low numbers of indicator organisms have been found have not led to outbreaks of disease (DoE 1995). However, concerns have been raised about heterotrophic bacteria and whether their ingestion could explain some episodes of

gastrointestinal illness. Occurrences and outbreaks of human gastrointestinal illness possibly associated with water consumption, without any known pathogenic organisms being detected, have led to the suggestion that the heterotrophic bacteria that are present may be the cause of such illnesses. To look for reported associations a contract funded by the UK Water Industry Research Company was carried out by the Public Health Laboratory Service, in which an extensive search and review of all organisms of medical importance that have been associated with water was carried out (Lightfoot *et al.* 1994).

Heterotrophic bacteria

These are defined as prokaryotic organisms that use reduced, preformed organic molecules (usually from other organisms) as carbon sources (Prescott *et al.* 1993). Many of these heterotrophic organisms can grow in water distribution systems where they may colonise the biofilms growing on the insides of pipes and inside the fittings of taps, or may be present on the spouts of taps due to environmental contamination. Although the water leaving the treatment works is generally free from organisms the ability of sublethally damaged organisms to regrow is influenced by the availability of nutrients but may be reduced by residual chlorine levels. The higher temperatures that occur during the summer months occasionally lead to blooms of heterotrophic organisms in some distribution systems. In addition, point-of-use devices such as water filters can become colonised. In a study of 300 reverse-osmosis units installed in households, bacterial counts ranged from 0 to 10^7 colony-forming units (CFU) per ml, with most containing between 10^4 and 10^5 CFU per ml (Payment 1989). The identified heterotrophs belonged to the genera *Pseudomonas, Alcaligenes, Acinetobacter, Flavobacterium* and *Chromobacterium.*

Although the presence of heterotrophs is a widespread occurrence, no recognised outbreaks of gastrointestinal illness associated with heterotrophic bacteria in drinking water have been reported in the UK.

Concerns about heterotrophs

The possible role of heterotrophs in human disease has arisen in recent years and has been stimulated by several factors: reports of nosocomial infections; reports of identification of potential pathogenic factors in heterotrophs; reports suggesting associations between the presence of heterotrophs in water and illness in the consumers; failure to identify any recognised pathogens in waterborne outbreaks; reports suggesting that reverse-osmosis filters reduce levels of gastroenteritis.

Many different types of heterotrophic bacteria occur in hospital distribution systems and counts may increase because of stagnation caused by the many "dead legs" that result from previously modified systems. All wet areas in wards such as sluices, showers and baths become colonised with Gram negative bacteria such as *Pseudomonas, Klebsiella, Citrobacter* and *Acinetobacter.* These areas also provide ecological niches for highly resistant organisms which can be transmitted to patients and cause infection problems. The heterotrophic bacteria in the water distribution systems have not caused infection in patients by ingestion, but in clinical areas where water is used to provide humidification in incubators and ventilators the use of tap water has led to respiratory tract colonisation and infections. These problems have been largely eliminated by control of infection policies. It has been recognised that tap water is not sterile and should not be used in situations where organisms from tap water or taps may initiate infection. Instead the policies recommend the use of sterilised water in all situations that could pose a risk to patients. Any harmful effects of heterotrophic bacteria are therefore eliminated. Patients are, however, encouraged to drink tap water, as the heterotrophs present pose no risk unless the patient is significantly immunocompromised

Pathogenic bacteria produce a variety of virulence factors; e.g. adherence factors, so that the organisms can attach to intestinal cells, enzymes including haemolysin that facilitate cell invasion, the production of exotoxins and several other factors that produce immunomodulation. The successful pathogen will possess a whole range of these factors but some are critical; an example is *Vibrio cholerae* with and without the cholera toxin gene, the former producing cholera and the latter being avirulent. It is important to appreciate that the possession of a single virulence factor by an organism not normally considered to be pathogenic may not be significant. The assessment of virulence should therefore include detection systems for a whole range of virulence factors. There are no simple tests available and although haemolysis on blood agar by heterotrophs (Payment *et al.* 1993) and cytopathic effects of Y1 choleral cell overlays (Lye & Dufour 1991) have been put forward as assessment methods they will not indicate which organisms are potential human pathogens.

There have been reports suggesting associations between the presence of heterotrophs in water in the distribution system and illness in the consumers of that water. In a study reported from Egypt, nine out of ten samples analysed from the district of Cairo were positive for *Aeromonas* strains, of which 56% were reported to be enterotoxigenic (Ghanen *et al.* 1993). *Aeromonas* was isolated from diarrhoeic and non-diarrhoeic faeces of children. Typing of the isolates was not performed. There have been two other reports of *Aeromonas* colonisation of distribution systems (Moyer *et al.* 1992; Havelaar *et al.* 1992). In the latter, sophisticated typing systems did not reveal any correlation between isolates made from drinking water and those made from patients. *Enterobacter clocae* was implicated as a cause of human infection in New Haven County, Connecticut, but the typing of the isolates demonstrated differences between the isolates from the distribution system and clinical isolates from a hospital served by the distribution system (Edberg *et al.* 1994).

There are a number of outbreaks of waterborne infection or disease in which no known agent was identified. Of 502 outbreaks in the USA between 1971 and 1985 there were 251 outbreaks of gastroenteritis affecting 61,478 people in which no microbiological cause was identified (Craun 1988). For the years 1991 and 1992 there were 34 outbreaks associated with drinking water and in 23 (68%) there was no identified aetiological agent (Moore *et al.* 1994).

A study in Canada has also increased concerns. A randomised intervention trial showed that people in 229 households which had domestic reverse-osmosis filters installed had significantly fewer gastrointestinal symptoms than people in 307 households without such filtration over a 15-month period, implying that filterable microbiological or chemical agents in water can cause significant levels of gastrointestinal infection in a population (Payment *et al.* 1991). The water concerned met current microbiological standards for indicator organisms.

Many heterotrophs grow in water distribution systems and these reports have raised concerns that should be answered.

Heterotrophs of concern

Although many genera and species of heterotrophic bacteria have been isolated from water and have been found to colonise distribution systems, no outbreaks of associated human disease have been conclusively reported. Suspicions have been raised about several organisms such as *Klebsiella* spp. and *Citrobacter* spp., but their frequent isolation and lack of involvement in human gastrointestinal disease make them very unlikely candidates. There are concerns about the potential for *Aeromonas* spp. and *Yersinia enterocolytica* to cause diarrhoeal disease.

Species of *Aeromonas* are ubiquitous in the environment and commonly occur in soil, marine (Kaper *et al.* 1981) and freshwater habitats (Rhodes & Kator 1994). Marine recreational waters pose a potential source of human infection. In a study in southern Italy, many of the isolated

strains produced several virulence factors and all isolates produced cytotoxin and haemolysin. Three isolates produced enterotoxin and all isolates bound to human intestinal cells in varying degrees (Krovacek *et al.* 1994). A survey of chlorinated water in which 286 samples were taken from taps and storage tanks in nine London and Essex boroughs, and nine local hospitals, revealed the presence of *Aeromonas hydrophila* in 25% of samples during the summer months and in 7% during the winter months (Millership & Chattopadhyay 1985).

Aeromonas spp. have been isolated from supplies of drinking water throughout the world and are able to grow in drinking water. Their growth is associated with the accumulation of biofilm on internal surfaces and is influenced by temperature, the availability of organic matter and the degree of stagnation. Biofilm can accumulate in the presence of 0.8 mg chlorine per litre.

In Swedish water distribution systems, sampling demonstrated counts of up to 3 x 10^4 CFU per 100 ml in raw water and up to 750 CFU per 100 ml in tap water samples (Stenstrom 1995). The significance of *Aeromonas* in drinking water is not fully understood. It is recognised that on occasions the ingestion of *Aeromonas* spp. may lead to diarrhoeal disease and this is associated with an enterotoxin (Janda & Duffey 1988). There are numerous reports of *Aeromonas* isolates from patients with diarrhoea but also reports of *Aeromonas* strains that produce a heat-labile cytotoxin, have enterotoxin activity (Ljungh *et al.* 1977; Turnbull *et al.* 1984) and the possession of other pathogenic characteristics. It is suggested that when all are present in a strain, enteric infection may be caused. Human volunteer challenge trials using five enteropathogenic strains of *Aeromonas hydrophila* demonstrated diarrhoea in only 2 of 57 persons with administered doses ranging from 10^4 to 10^5 CFU (Morgan *et al.* 1985). A number of factors such as age, immune competence, previously developed immunity, exposure and infective dose of the organisms, as well as the possession of virulence factors, could affect the ability of *Aeromonas* to establish overt infection.

The absence of defined outbreaks and the low levels of infectivity in human volunteer experiments suggests that people have a relatively high degree of resistance to infection with *Aeromonas*.

The significance of *Aeromonas* in drinking water in the Netherlands has been reviewed (Van de Kooij *et al.* 1988), and the health authorities in the Netherlands have defined maximum values for *Aeromonas* present in drinking water: i.e. 200 CFU per 100 ml in water distribution systems and 20 CFU per 100 ml in water leaving the production plant. However, there have not been any outbreaks of disease in the UK even though blooms of *Aeromonas* occur in some distribution systems during the summer months.

Yersinia is a genus of heterotrophic bacteria with eleven recognised species, some of which cause disease in man, and both pathogenic and non-pathogenic strains of *Yersinia* have been found in surface water and unchlorinated drinking water (Lassen 1972; Caprioli *et al.* 1978; Cafferkey *et al.* 1993). The source of the organisms is the environment or non-human hosts such as wild animals and birds. However, only certain serotypes of *Yersinia enterocolytica* that occur in the environment are considered to be pathogenic for humans. This depends on the possession of virulence factors associated with pathogenesis of infection. Serotypes 0:3, 0:4,32, 0:5,27, 0:6,30, 0:6,31, 0:8, 0:9 and 0:21 are thought to be pathogenic for humans and cause diarrhoea and mesenteric adenitis, a disease that often mimics appendicitis. Other serotypes have been isolated from patients with infection but their role is uncertain. The most common serotype of *Yersinia enterocolytica* associated with human infection is serotype 0:3. The significance of *Yersinia* in patients with diarrhoea is uncertain, however; on occasions it can cause mesenteric adenitis and reactive arthiritis with an antibody response and is clearly pathogenic. On other occasions most isolates from patients with mild diarrhoea do not contain the full set of virulence markers found in isolates from systemic infections.

Surveillance of *Aeromonas* and *Yersinia* infections

Through its network of Public Health Laboratories and the Communicable Disease Surveillance Centre (CDSC), the surveillance of many infections important for public health are carried out. All Public Health Laboratories and many hospital laboratories routinely report isolates of *Aeromonas* and *Yersinia* to CDSC and this system of laboratory reporting gives an indication of the incidence of human infections due to *Aeromonas* and *Yersinia*. But when a patient develops diarrhoea several factors may preclude reporting: the patient may not consult a doctor; the doctor may not request a specimen; the patient may not submit a specimen; the laboratory may not actively look for *Aeromonas* and *Yersinia* in all specimens submitted.

In a separate UK Water Industry Research Company contract (Lightfoot *et al.* 1995) the Public Health Laboratory Service analysed the database relating to the reports of *Aeromonas* and *Yersinia* for 1989 to 1994, and related them to the population figures of the National Health Service regions (Figs 1 and 2). In addition a questionnaire was used to determine the level of ascertainment in each of the Public Health Laboratories and this probably explains the major variations encountered (Table 1).

Analysis of the database revealed that in the 5-year period between 1991 and 1994 there were 2,718 isolates of *Aeromonas* spp. and 2,006 isolates of *Yersinia* spp. These numbers are low compared with recognised causes of gastroenteritis. The reported isolations were also analysed to determine geographical variations and revealed a wide variation. This is probably explained by the results of the laboratory questionnaire which revealed different levels of ascertainment. A startling increase in reports of *Yersinia* in the Wessex region is explained by the high level of ascertainment in a laboratory where every faeces is examined for *Yersinia*, using a highly sensitive enrichment culture method. In this particular laboratory, and also in those laboratories (4 in each case) that examined all faeces submitted for *Aeromonas* and *Yersinia* using enrichment techniques, the numbers were still low compared with the recognised causes of gastroenteritis.

Table 1. *Responses of UK Public Health Laboratories to a questionnaire on the examination of faecal stools for the presence of* Aeromonas *and* Yersinia *in 1991 to 1994.*

Criteria	*Aeromonas*	*Yersinia*
Laboratories responding	50	50
Special interest	4	4
Examining all faecal specimens for organism	4	5
Use of special criteria to initiate investigation	43	43
Use of direct culture	21	42
Use of enrichment culture	6	21

Conclusions

There is no evidence to suggest that heterotrophic bacteria in drinking water are producing outbreaks of human gastrointestinal disease. Concerns have been raised particularly about *Aeromonas* and *Yersinia*, and reports of outbreaks have been made outside the UK. Where *Aeromonas* has been identified in drinking water, parallel investigations in the human population ingesting this water have shown that, following the use of sophisticated typing systems, there is no correlation between strains isolated from humans and strains present in the drinking water. There is no doubt that humans are intermittently exposed to *Aeromonas*, in the environment and in food as well as in drinking water. Certain distribution systems are known to have *Aeromonas* blooms in the summer months but no increased incidence of *Aeromonas*

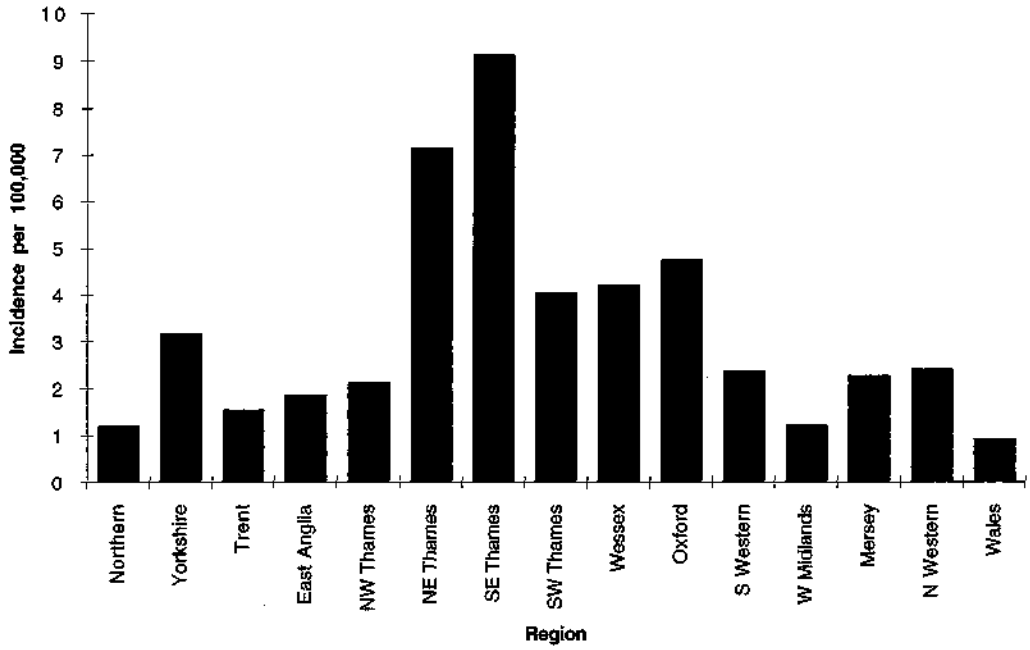

Figure 1. Numbers of reports (per 100,000 heads of population) of *Aeromonas* spp. in 15 regions of the UK National Health Service in the period 1989 to 1993.

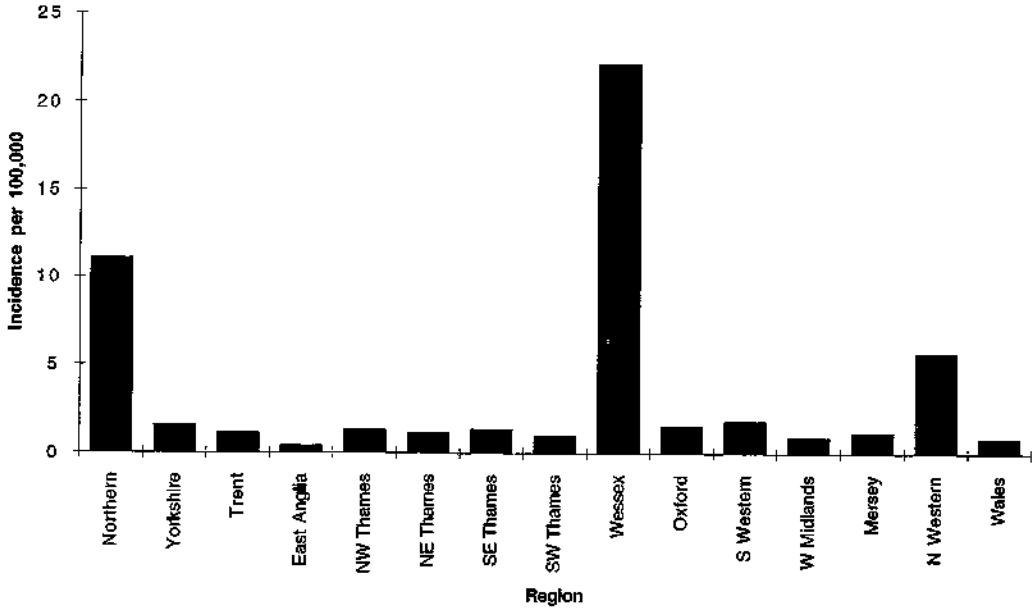

Figure 2. Numbers of reports (per 100,000 heads of population) of *Yersinia enterocolitica* in 15 regions of the UK National Health Service in the period 1989 to 1993.

infection in the population has been observed in these populations. There are two explanations for this: the population may become immune without overt serious illness following repeated low level exposure, or the organisms encountered may not exhibit a sufficient spectrum of pathogenic mechanisms to initiate infections in those exposed. Some pathogenic mechanisms may even be disabled in environmental strains. *Yersinia* spp. are animal pathogens that are widely distributed in the environment and the contamination of waterbodies can occur from human or animal sewage. They can survive and grow in water and have been shown to cause small outbreaks of waterborne infection associated with well water or unchlorinated supplies. They are sensitive to chlorination and should not survive in distribution systems where adequate levels of residual chlorine are maintained.

Therefore there appears to be no major burden of disease due to these organisms. However, it is suggested that surveillance of infections in the human population should be continued with standard levels of ascertainment, and the identification of heterotrophs from distribution systems should be continued, with typing being carried out if *Aeromonas* spp. or *Yersinia* spp. are detected.

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