Heterotrophic Plate Counts and Drinking-water Safety

The Significance of HPCs for Water Quality and Human Health

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Foreword

This monograph examines the appropriate role of the heterotrophic plate count (HPC) measurement in drinking-water quality management. It was developed from a two-day workshop attended by a group of microbiology and public health experts, including those with regulatory and medical expertise, convened by the World Health Organization and NSF International (WHO Collaborating Centre for Drinking Water Safety and Treatment) in Geneva, Switzerland, on 25–26 April 2002. The workshop followed immediately after the International Symposium on HPC Bacteria in Drinking Water — Public Health Implications?, developed by the same organizations. The Session Chairs and other selected participants in the symposium assembled in the workshop to address the issues that led to the formulation of the symposium and to provide a consensus report and conclusions to assist public health officials to interpret the information provided by HPC measurements.

The issues that were addressed include:

- the relationship between HPC in drinking-water systems (including those derived from in-line treatment systems, dispensers and bottled water) and health risks for the general public;

- the role of HPC as an indirect indicator or index for pathogens of concern in drinking-water;
- the role of HPC in assessing the efficacy and proper functioning of water treatment and supply/distribution processes; and
- the relationship between HPC and the aesthetic acceptability of drinking-water.

This report deals with safe water supply extending from source to consumer, including plumbed-in devices, domestic and building environments, and water supplied in bottles or packages. The different ways in which drinking-water may be used in the home are considered, and specific concerns in higher-risk settings and populations at increased risk are addressed.

The Expert Meeting, supported by the papers in this monograph, addressed that debate as to the need, utility or quantitative basis for health-based standards or guidelines relating to HPC-measured growth in drinking-water and provided their consensus conclusions in the Meeting Report (chapter 1). Each chapter was originally the lead presentation in each session of the symposium. Each was revised in light of the other presented papers, the debates and discussions, and the Expert Meeting deliberations to reflect the scientific information that was presented and the collective experiences of the members. This report is the product of the Expert Meeting; its chapters were peer reviewed by members of the expert group and the editors.

We hope this document provides useful information and perspective on the utility and the limitations of HPC data in the management and operation of piped water systems, as well as other means of providing drinking-water to the public.

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List of acronyms and abbreviations

AFLP	amplified fragment length polymorphism
AIDS	acquired immunodeficiency syndrome
ANSI	American National Standards Institute
AOC	assimilable organic carbon
AP-PCR	arbitrarily primed polymerase chain reaction
ARDRA	amplified ribosomal DNA restriction analysis
ATP	adenosine triphosphate
BDOC	biodegradable dissolved organic carbon
BFR	biofilm formation rate
BOM	biodegradable organic matter
BOX-PCR	high-stringency PCR assay targeting regions within various bacterial genomes and bordered by invertedly repeated elements
bp	base pair
BPP	biomass production potential
cDNA	complementary or copy DNA
cfu	colony-forming units
CI	confidence interval
CTC	cyanoditolyl tetrazolium chloride

DGGE	denaturing gradient gel electrophoresis
DNA	deoxyribonucleic acid
DOC	dissolved organic carbon
EC	European Community
EEC	European Economic Community
EPA	Environmental Protection Agency
EU	European Union
FISH	fluorescence *in situ* hybridization
GAC	granular activated carbon
HIV	human immunodeficiency virus
HPC	heterotrophic plate count
ICU	intensive care unit
IF	immunofluorescence
Ig	immunoglobulin
IMS	immunomagnetic separation
INT	iodonitrotetrazolium
ISO	International Organization for Standardization
LMW	low molecular weight
MAC	*Mycobacterium avium* complex
MDOD	mean dissolved oxygen difference
MLEE	multilocus enzyme electrophoresis
MLST	multilocus sequence typing
MRD	Modified Robbins Device
mRNA	messenger RNA
OR	odds ratio
PAI	pathogenicity island
PCR	polymerase chain reaction
PET	polyethylene terephthalate
PFGE	pulsed field gel electrophoresis
PNA	peptide nucleic acid
POE	point-of-entry
POU	point-of-use
PVC	polyvinyl chloride
QMRA	quantitative microbial risk assessment
RAPD	random amplified polymorphic DNA
rDNA	ribosomal DNA
RFLP	restriction fragment length polymorphism
RNA	ribonucleic acid
rRNA	ribosomal RNA
SIV	simian immunodeficiency virus
SPC	standard plate count

SSCP	single-strand conformation polymorphism
TDC	total direct count
TGGE	temperature gradient gel electrophoresis
THM	trihalomethane
tRNA	transfer RNA
VBNC	viable but non-culturable
VTEC	verocytotoxigenic *Escherichia coli*
WHO	World Health Organization
WSP	water safety plan

Robert Koch

In 1883 Robert Koch published an article entitled: *About Detection Methods for Microorganisms in Water*. In that historic paper that marked the introduction of the application of microbial indicators for surveillance of water hygiene, Koch described for the first time the methodology for HPC measurement in water, and showed its value as a measure of water treatment technology performance. The editors of this state-of-the-art review of the role and health significance of HPC wish to recognize the contributions of Robert Koch to water microbiology on this 120th anniversary of his original publication on the topic.

1

Expert consensus

Expert Meeting Group Report

1.1 DEFINITIONS AND SCOPE

1.1.1 Drinking-water

WHO considers that "drinking-water" should be "suitable for human consumption and for all usual domestic purposes including personal hygiene." Diverse regulatory agencies adopt similar definitions. Drinking-water should therefore be suitable for consumption, washing/showering and domestic food preparation. In human health terms, exposure to water and its constituents can occur through ingestion, contact and aerosol inhalation.

Drinking-waters should be safe for lifetime use, taking account of differing sensitivities that occur across life stages, but all are not necessarily suitable for individuals suffering from certain specific immunocompromising disorders.

Piped drinking-water supplies typically involve source abstraction, treatment and distribution. The latter may include ancillary devices at domestic or institutional levels, such as softeners, activated carbon treatment, vending

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machines, dispensers, etc. Drinking-waters also include those obtained from non-piped sources, such as from springs and community wells, in bottles and as ice.

The control of faecal contamination in drinking-water systems and sources, where it occurs, is of primary importance. Faecal-specific indicator bacteria such as *E. coli* are the parameters of first importance in monitoring faecal pollution.

1.1.2 Heterotrophic plate count

Heterotrophs are broadly defined as microorganisms that require organic carbon for growth. They include bacteria, yeasts and moulds. A variety of simple culture-based tests that are intended to recover a wide range of microorganisms from water are collectively referred to as "heterotrophic plate count" or "HPC test" procedures. Accordingly, the terms "heterotroph" and "HPC" are not synonymous.

There is no universal "HPC measurement." Although standardized methods have been formalized, HPC test methods involve a wide variety of test conditions that lead to a wide range of quantitative and qualitative results. Temperatures employed range from around 20 °C to 40 °C, incubation times from a few hours to seven days or a few weeks, and nutrient conditions from low to high. The test itself does not specify the organisms that are detected.

Only a small proportion of the metabolically active microorganisms present in a water sample may grow and be detected under any given set of HPC test conditions, and the population recovered will differ significantly according to the method used. The actual organisms recovered in HPC testing can also vary widely between locations, between seasons and between consecutive samples at a single location.

Microorganisms recovered through HPC tests generally include those that are part of the natural (typically non-hazardous) microbiota of water; in some instances, they may also include organisms derived from diverse pollutant sources.

1.1.3 Microbial growth in water

Microorganisms will normally grow in water and on surfaces in contact with water as biofilms. Growth following drinking-water treatment is normally referred to as "regrowth." Growth is typically reflected in higher HPC values measured in water samples. Elevated HPC levels occur especially in stagnant parts of piped distribution systems, in domestic plumbing, in bottled water and in plumbed-in devices, such as softeners, carbon filters and vending machines.

The principal determinants of regrowth are temperature, availability of nutrients and lack of residual disinfectant. Nutrients may derive from the water body and/or materials in contact with water.

1.1.4 Use of HPC in water management

HPC testing has a long history of use in water microbiology. At the end of the 19th century, HPC tests were employed as indicators of the proper functioning of processes (and of sand filtration in particular) and thereby as indirect indicators of water safety. Use as a safety indicator declined with the adoption of specific faecal indicator bacteria during the 20th century.

HPC measurements nevertheless continue to figure in water regulations or guidelines in many countries. HPC measurements are used:

- to indicate the effectiveness of water treatment processes, thus as an indirect indication of pathogen removal;
- as a measure of numbers of regrowth organisms that may or may not have sanitary significance; and
- as a measure of possible interference with coliform measurements in lactose-based culture methods. This application is of declining value, as lactose-based culture media are being replaced by alternative methods that are lactose-free.

1.2 USES IN PIPED WATER SUPPLIES

1.2.1 Water safety plans

There is an increasing trend towards application of a comprehensive "water safety plan" (WSP) approach to drinking-water supply safety management. This approach is applicable throughout the water supply, from catchment to consumer.

It has been proposed that the WSP approach be included in the next (third) edition of WHO *Guidelines for Drinking-water Quality* and that this would entail five components:

(1) health-based targets based upon public health protection and disease prevention;
(2) system assessment to determine whether the water supply chain (up to the point of consumption) as a whole can deliver water of a quality that meets the defined targets;

(3) monitoring of the steps in the supply chain that are of particular importance in securing safe drinking-water;
(4) management plans documenting the system assessment and monitoring and describing action to be undertaken from normal conditions to extreme events, including documentation and communication; and
(5) systematic independent surveillance that verifies that the above are operating properly.

Piped water systems of large buildings may incur greater growth than encountered elsewhere (because of storage tanks, extensive internal distribution networks and temperature-related growth). The principal health concerns in these networks are cross-connections and growth of *Legionella* bacteria, which are not detected by the HPC test procedures. General water safety is ensured by maintenance protocols, regular cleaning, temperature management and maintenance of a disinfectant residual. For these reasons, authorities responsible for building safety should provide advice and require specific water management safety plans.

1.2.2 Water quality targets

There is no evidence, either from epidemiological studies or from correlation with occurrence of waterborne pathogens, that HPC values alone directly relate to health risk. They are therefore unsuitable for public health target setting or as sole justification for issuing "boil water" advisories. Abrupt increases in HPC levels might sometimes concurrently be associated with faecal contamination; tests for *E. coli* or other faecal-specific indicators and other information are essential for determining whether a health risk exists.

1.2.3 Validation and verification

Experience suggests that HPC monitoring can be used in drinking-water supplies along with other information for validation[1] and verification[2] of

[1] *Validation* is an investigative activity to identify the effectiveness of a control measure. It is typically an intensive activity when a system is initially constructed or rehabilitated. It provides information on reliably achievable quality improvement or maintenance to be used in system assessment in preference to assumed values and also the operational criteria required to ensure that the control measure contributes to effective control of hazards.

[2] In addition to operational monitoring of the performance of the individual components of a supply system, it is necessary to undertake final *verification* for

treatment process performance and other applications. These may include the following:

- HPC measurements can be used to monitor the performance of filtration or disinfection processes.
- In piped distribution systems, HPC measurements are assumed to respond primarily to (and therefore provide a general indication of) distribution system conditions. These arise from stagnation, loss of residual disinfectant, high levels of assimilable organic carbon in the water, higher water temperature and availability of particular nutrients. In systems treated by chloramination or that contain ammonia in source waters, measurement of a variety of parameters, including HPC, but especially including nitrate and nitrite (which are regulated for health protection), can sometimes indicate the possible onset of nitrification.
- HPC values are also used in verification (and by some authorities also for validation) of efficacy of cleaning in diverse applications, including beverage vending machines, food processing and preparation facilities and medical devices. These applications of HPC have not been considered in this review.

1.2.4 Aesthetic quality

Drinking-water must be aesthetically acceptable as well as safe. Aesthetic acceptability is directly relevant to health, since rejection of safe, but unacceptable (undesirable), water may lead users to consume acceptable but potentially unsafe alternative waters. HPC testing may be used in investigating aesthetic quality, and it is used by some authorities as a marker for some of the underlying causes of some aesthetic problems.

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reassurance that the system as a whole is operating safely. Verification may be undertaken by the supplier or by an independent authority or a combination of these, depending on the administrative regime in a given country. It can include testing for faecal indicator organisms, pathogens and hazardous chemicals.

1.3 USES IN NON-PIPED AND OTHER WATER SUPPLIES

1.3.1 Bottled water

Bottled ("packaged") water is considered drinking-water under some regulatory schemes and as a food in others. Some authorities distinguish between natural mineral water and other bottled waters. The WHO *Guidelines for Drinking-water Quality* are referred to directly in international norms (Codex Alimentarius Commission) and are considered applicable to bottled waters.

Bottled waters represent a specific growth situation for microbial flora. Bottled waters may derive from "pristine" sources ("natural mineral water") or from processed waters. They may contain or have added carbon dioxide that will restrict growth potential, but typically no long-lasting disinfectant residual is present. The finished product will often be exposed to elevated (ambient) temperatures over a period of days to weeks before consumption.

Microorganisms naturally occurring in water are a normal part of the microbiota of bottled waters that meet appropriate safety norms. Levels of HPC recovered from bottled water post-distribution may therefore sometimes be significantly higher than those found in municipal water supplies in distribution.

Microbial safety for bottled waters is best pursued by a WSP approach (as summarized in section 1.2.1). *Pseudomonas aeruginosa* and HPC values are used by some as process management indicators in bottled water production and not as health risk indicators.

1.3.2 Plumbed-in devices

Bacterial growth occurs in plumbed-in domestic water devices (including water softeners, carbon filters, etc.) and plumbed-in commercial devices, such as beverage vending machines. HPC values in water samples typically increase in such devices. Increases of HPC (due to growth) in these devices therefore do not indicate the existence of a health risk, as long as the entry water meets acceptable microbial water quality norms (e.g., WHO *Guidelines for Drinking-water Quality*). Appropriate maintenance of these devices is required for aesthetic reasons (see section 1.2.4) — for example, per manufacturers' recommendations. Plumbed-in devices in health care facilities are considered in section 1.4.5.

1.3.3 Conveyances

Water systems on conveyances such as ships and aircraft present specific challenges to water safety management. These include both physical characteristics (extensive complex piping in confined space, physical

movement) as well as organizational issues, such as multiple responsible parties in different locations and at different stages of delivery.

In general, the potential roles for HPC in water safety management in conveyances are similar to those elsewhere (see section 1.2.1). HPC measurements alone are unsuitable for use in independent surveillance by, for example, port health authorities where series results are unavailable; faecal indicator bacteria measurements are essential in this role. This issue is dealt with in the WHO *Guide to Ship Sanitation* and *Guide to Hygiene and Sanitation in Aviation*, which are currently in revision.

When drinking-water is stored in tanks in conveyances, microbial growth invariably occurs. If HPC testing is conducted, the counts measured will often exceed those normally found in piped distribution systems. Obtaining a high count by the HPC test may indicate the need to examine procedures for taking on water, maintenance of the system and disinfection.

1.3.4 Other water exposure media

Swimming pools and spas are outside the scope of this report. They are dealt with in the WHO *Guidelines for Safe Recreational Water Environments*. The role of HPC in humidifiers and air cooling systems is also outside the scope of this report.

1.4 HEALTH ASPECTS

1.4.1 Exposure

Exposure to general HPC microbiota is far greater through foodstuffs than through drinking-water. Levels of exposure regarded as acceptable from foods are much greater than those regarded as acceptable from drinking-water. Limited data are available with which to characterize exposure to specific microorganisms through these two routes. Exposure to HPC microbiota also occurs through air and other environmental sources.

1.4.2 Epidemiology

Some epidemiological studies have been conducted into the relationship between HPC exposures from drinking-water and human health effects. Other studies relevant to this issue include case-studies, especially in clinical situations, and compromised animal challenge studies using heterotrophic bacteria obtained from drinking-water distribution systems. The available body

of evidence supports the conclusion that, in the absence of faecal contamination, there is no direct relationship between HPC values in ingested water and human health effects in the population at large. This conclusion is also supported indirectly by evidence from exposures to HPC in foodstuffs, where there is no evidence for a health effects link in the absence of pathogen contamination.

There are a small number of studies that have examined possible links between HPC and non-intestinal outcomes in general populations. The conclusions of these studies do not support a relatonship.

1.4.3 Health effects — specific organisms

Information on the association of specific HPC microbiota with health effects may be derived from epidemiological studies, including outbreak investigations, or from risk assessments.

Bacteria typically described as "opportunistic pathogens" that may be recovered among HPC microbiota include strains of *Pseudomonas aeruginosa*, *Acinetobacter* spp., *Aeromonas* spp., *Klebsiella pneumoniae*, etc. There is no evidence of an association of any of these with gastrointestinal infection through the waterborne route among the general population.

There are opportunistic pathogens that may regrow in water but that are not detected in HPC measurements, including strains of *Legionella* and non-tuberculous mycobacteria. The public health significance of inhalation exposure to some legionellae has been demonstrated.

There is no evidence that HPC levels *per se*, as measured by established procedures, have a direct relationship to the likely presence of, or act as indices for the numbers or presence of, regrowth organisms such as legionellae, *P. aeruginosa* and non-tuberculous mycobacteria.

1.4.4 Populations at increased risk (including sensitivity through life stages)

Specific strains of microbial species that may be a part of HPC microbiota can cause infection in certain vulnerable people (e.g., the immunocompromised and those with indwelling urinary catheters, intravenous catheters, continuous ambulatory peritoneal dialysis, etc.). Most infections due to these organisms are from non-water sources (endogenous microbiota, cross-infection from other persons in health care wards or the general environment). However, there have been a number of outbreaks reported where the investigations may implicate the water supply. The implication for infections of immunocompromised patients in the general community is unclear.

There are increasing numbers of persons who are immunocompromised to various degrees and types living in communities, including some patients discharged to "home care." Normal "drinking-water" is not always suitable for all such individuals for all uses (e.g., wound irrigation). This relates to water safety in general and not to growth or HPC organisms in particular. Advice should be provided by public health authorities to at-risk groups in general and by practitioners responsible for individuals discharged to home care.

Where the drinking-water supply meets international norms such as the WHO *Guidelines for Drinking-water Quality*, only those people with severe changes from normal, as determined by their physicians or medical agencies (e.g., an absolute neutrophil count <500/µl), are considered immunosuppressed to the extent that they may require specially processed drinking-water.

1.4.5 Health care facilities

Health care facilities include hospitals, health centres, dialysis facilities and dental facilities. These facilities represent a general area of concern for infection control because of the potentially increased susceptibility of the associated population and their risk of infection from organisms growing in their environment.

Health care facilities should have WSPs as part of their infection control strategy. Such plans may be generic (e.g., applicable to health centres in general) or specific when applied to a larger built environment (e.g., many hospitals and nursing homes). Such plans should address microbial growth in addition to control of external contamination by *Pseudomonas aeruginosa* and *Legionella* and should include ancillary equipment such as shower heads and medical devices, such as dialysis units and dental water dispensing equipment, that involve patient contact.

1.5 OUTSTANDING QUESTIONS AND RESEARCH

The state of the evidence indicates that any further research on HPC in general should focus on its use for process management and control applications as described in section 1.2 and is not a high priority for public health protection.

Because of ongoing interest, further research in this area is likely to occur. It may usefully focus on:

- specific heterotrophic organisms of potential concern for human health, along with developments of future molecular techniques that may provide additional public health information;

- the immunocompromised (especially infection control in health care facilities and susceptible persons in the public at large);
- non-ingestion exposures (including aerosol exposure and hypersensitivity reactions) and roles of amoebae in biofilms;
- *Pseudomonas aeruginosa*, which are common in the environment and are occasionally found in drinking-water — they are sometimes associated with wound and other infections in high-risk populations;
- additional research on conditions and routes of exposure and control methods (when appropriate); and
- susceptible populations of relevance to exposure from drinking-water.

The potential role of heterotrophic bacteria in preventing or reducing colonization of water system components by organisms of human health concern also merits further research.

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2

Public health aspects of the role of HPC — an introduction

M. Exner, V. Vacata and J. Gebel

2.1 INTRODUCTION

The question of the public health implications of bacterial counts in water has been raised ever since the introduction of plate counts for assessing water quality by Robert Koch in 1883. In order to answer this question, we must consider historical, current and future developments in our understanding of bacterial counts for the purposes of hygienic assessment of drinking-water quality. This chapter deals with important historical aspects of this issue and recent developments in the field of heterotrophic plate counts (HPC), viewed from a sociodemographic perspective.

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2.2 HISTORICAL ASPECTS

In 1883, at the XI German Congress of Physicians in Berlin, Koch introduced a report entitled "New methods for the detection of microorganisms in soil, air and water." The report came at a time when the bacterial origin of cholera was not yet known. The introduced method consisted of adding 1 ml of water to a nutritive gel, cultivating the gel at 22 °C for 48 h and counting the number of colonies formed. In his report, Koch compared the bacterial growth stemming from tap water, well water and river water. He showed that the procedure could be used for checking the performance of point-of-use filtration systems in households and demonstrated, using growth of pigmented and/or colourless colonies, that a faulty/clogged filter could drastically worsen the quality of water.

With respect to the health risks linked to the presence of microorganisms in water, Koch warned of the danger of false conclusions based on colony counts alone. Until that time, there had been no laboratory methods that would allow the detection of bacteria, and Koch saw no evidence of the presence of pathogens among the variety of waterborne bacteria. He assumed that the level of pathogens in water was low relative to the levels of other bacteria. In his noted work "Water filtration and cholera," published in 1893, he used the case of the Altona waterworks, which drew water from the highly contaminated Elbe River and processed it by slow sand filtration, to document how lowering the level of waterborne bacteria to below 100 colonies/ml could prevent an outbreak of cholera and/or typhoid fever epidemics. When this level rose above 1000 colonies/ml due to insufficient/faulty filtration of the contaminated water, an outbreak of cholera resulted.

The colony count was soon recognized as an excellent indicator of filtration performance. Systematic observations after 1893 showed that sand filtration could prevent outbreaks of waterborne diseases if 1 ml of water was not allowed to contain more than 100 bacteria that could grow on nutritive medium at 20 °C for 48 h and that could be counted under the magnifying glass. In Germany, the limit of 100 bacteria/ml eventually became the standard for slow sand filtration as well as for other filtration and sterilization methods (Gärtner 1915). However, this prescribed limit deals with the general bacterial population only and does not apply to pathogenic bacteria, such as *Salmonella typhi* and *Vibrio cholerae*.

The limit of 100 bacteria/ml was the first epidemiologically based limit for filtered water from infected sources that allowed the control of waterborne gastrointestinal infections, such as cholera, typhoid fever and shigellosis.

It is important to note that the limit of 100 colony-forming units (cfu)/ml is not directly correlated to potential health risk. Rather, it reflects the efficiency of

the filtration process; in this sense, it is only indirectly correlated to the lowering of the risk of infection, particularly for gastrointestinal infections that are acquired by ingestion.

The value of the parameter was related to the epidemic diseases then relevant to Europe, i.e., cholera and typhoid fever, which, in contrast to the later-discovered waterborne pathogens, such as enterohaemorrhagic *Escherichia coli* or *Cryptosporidium*, have a high infectious dose. A more direct correlation between the level of waterborne pathogens and health risk was made possible only after the introduction of faecal load-indicative bacteria, such as *E. coli* and faecal streptococci.

Colony count alone does not allow one to draw any conclusions concerning the risk of infection. Rather, it is a yardstick for:

- the efficacy of filtration processes, such as slow sand filtration and point-of-use filtration;
- the efficiency of disinfection;
- bacterial levels in areas with an increased contamination potential; and
- biostability of household plumbing systems.

The colony count, first determined for incubation temperatures of 20 °C and later also for 37 °C (which allows the inclusion of human pathogens), always has the function of a surrogate parameter comparable to that of turbidity and/or particle count. This tradition is the basis of the German regulation requiring that the colony count be determined after a 48-h growth at 20 °C and/or 37 °C, as well as of the binding limit value of 100 cfu/ml in water taken from the tap of the household end user. Both of these requirements are part of the German Drinking Water Regulation.

2.3 RECENT DEVELOPMENTS

Three factors have influenced recent developments in the field of colony counting for the purposes of hygienic assessment of drinking-water quality:

- the improved nutritive composition of agars, which supports growth of a broader spectrum of waterborne bacteria;
- the discovery in the late 1960s of biofilms, in which most of the microorganisms present in water distribution systems persist and which determine the level of free-floating microorganisms in water; and
- new procedures that allow identification of a wider spectrum of waterborne bacteria.

2.3.1 New procedure for determining colony counts

Only a small fraction (approximately 0.01%) of waterborne microorganisms are thought to belong to the group of culturable heterotrophic bacteria, and approximately 1% of the viable bacteria are not culturable. Using newer detection methods (Reasoner and Geldreich 1985), it is possible to significantly increase the proportion of pigmented and non-pigmented bacteria that can be cultured from drinking-water. The use of media with low nutrient levels (e.g., R2A), which are better suited to the needs of water microflora, allows an increase in the proportion of waterborne microorganisms that can be determined by the cultivation method. A disadvantage of the method is the longer cultivation time (5–7 days at 28 °C), which reduces its value as a parameter for the measurement of the efficiency of processes.

2.3.2 Biofilms

Biofilms represent a specific form of bacterial colonization of water distribution systems. These specific forms determine the biostability of the microbial communities, their persistence and the release of planktonic cell microorganisms into the running water. The biofilms interact with waterborne pathogens and affect their persistence (LeChevallier and McFeters 1985). The persistence of these pathogens is considerably increased if they form a new biofilm or colonize an existing one. The biofilms thus represent bioreactors within water distribution systems, in which the resistance of the microorganisms to disinfection is significantly increased. The potential for biofilm formation and growth is particularly high in narrow-gauge household plumbing. The colony count is directly correlated with the water volume that flows through these end-of-line systems.

2.3.3 Risks from bacteria detected in water

A number of studies have yielded virtually the same characteristic spectrum of heterotrophic bacterial strains. The predominant species in this spectrum are *Acinetobacter* spp., *Aeromonas* spp., *Alcaligenes* spp., *Comamonas* spp., *Enterobacter* spp., *Flavobacterium* spp., *Klebsiella* spp., *Moraxella* spp., *Pseudomonas* spp., *Sphingomonas* spp., *Stenotrophomonas* spp., atypical *Mycobacterium* spp., *Bacillus* spp. and *Nocardia* (Burlingame *et al.* 1986; LeChevallier *et al.* 1987; Payment *et al.* 1988; Payment 1989; Reasoner *et al.* 1989; Manaia *et al.* 1990; Edberg *et al.* 1997; Rusin *et al.* 1997; Norton and LeChevallier 2000).

Studies by Norton and LeChevallier (2000) showed characteristic changes in bacterial populations through potable water treatment and distribution. Therefore, it appears to be necessary to ensure that water treatment and distribution do not cause a shift in the composition of the bacterial population that would favour opportunistic pathogens.

The species predominant in households, particularly in warm-water distribution systems, are legionellas and *Pseudomonas aeruginosa*. The occurrence of infectious fungal strains, such as *Fusarium* spp. and *Aspergillus* spp., in household systems has been reported only recently (Anaissie *et al.* 2001).

There is no clear-cut evidence that heterotrophic bacteria as such pose a public health risk, particularly when they are ingested by healthy people via drinking-water (Rusin *et al.* 1997; Colford *et al.* 2002).

A risk assessment performed by Rusin *et al.* (1997) on animals as well as on human volunteers yielded the oral doses of different microorganisms that are necessary to cause an infection: *Pseudomonas aeruginosa*, 10^8–10^9 cfu; *Aeromonas hydrophila*, $>10^{10}$ cfu; *Mycobacterium avium*, 10^4–10^7 cfu; and *Xanthomonas maltophilia*, 10^6–10^9 cfu.

The risk characterization by Rusin *et al.* (1997) showed that "risks of infection from oral ingestion ranged from a low of 7.3×10^{-9} (7.3/billion) for low exposures to *Aeromonas* to higher risks [of 9×10^{-2} (9/100)] predicted at higher levels of exposure to *Pseudomonas* … This higher risk was only predicted for individuals on antibiotics. Overall, the evidence suggests that specific members of HPC bacteria found in drinking water may be causative agents of both hospital- and community-acquired infections. However, the case numbers may be very low and risks represent levels generally less than 1/10,000 for a single exposure to the bacterial agent."

The number of cases of pulmonary diseases associated with *Mycobacterium avium* is rapidly increasing (Rusin *et al.* 1997) and in some areas is approaching the incidence of *Mycobacterium tuberculosis*. The epidemiological significance of this waterborne opportunistic pathogen is still not clearly defined (Anaissie *et al.* 2001). [Editors' note: Because of the wide interest in the potential health significance of some non-tuberculous mycobacteria in water, including *Mycobacterium avium* complex (MAC), this is the theme of a separate book in the same series as this volume.]

These risk assessments are primarily based on potential infection by ingestion; the risk is considerably higher for persons undergoing antibiotic therapy or immunodeficient persons (LeChevallier and McFeters 1985; LeChevallier *et al.* 1987; Reasoner *et al.* 1989; Rusin *et al.* 1997; Norton and LeChevallier 2000; Anaissie *et al.* 2001). These risk assessments are not

applicable to dermal or inhalation exposure or to persons with invasive devices, such as indwelling urinary catheters or intravenous catheters.

Furthermore, the operation and cleaning of devices, particularly medical devices using drinking-water, need to be taken into account. Contamination of these devices with waterborne heterotrophic microorganisms can lead to multiplication of microorganisms (regrowth) in the devices, leading to a significant risk of infection (e.g., by inhalation or by endoscopes). The cooling water of dental units, which is sprayed into the patient's mouth, is also often heavily contaminated with *Pseudomonas aeruginosa*.

Under such conditions, even low concentrations of certain heterotrophic microorganisms, particularly for people on antibiotics, with immunosuppression or with invasive devices, can be sufficient to cause serious infectious complications. These specific circumstances call for a more detailed assessment.

2.4 ALTERED BASIC CONDITIONS

The altered sociodemographic situation in western societies is reflected in the substance and scope of national regulations concerning drinking-water hygiene.

2.4.1 The scope of national drinking-water regulations

In order to protect health, we need to know the quality of water consumed/used, which may be very different from both that "supplied" to the consumer (i.e., entering the house) and that "manufactured" by the supplier. Current national regulations usually do not cover the whole distribution system to the consumer's tap. The WHO *Guidelines for Drinking-water Quality* (WHO 1993, 1998) provide the framework and the "scientific point of departure" for the European Community (EC) and national drinking-water directives. These Guidelines already form the basis for EC Council Directive 98/83 EC as well as the forthcoming German Drinking Water Regulation, which set rules for water quality intended for human use. Such water is presumed to be used for drinking, for preparation of food and drinks, for washing and body care, and for cleansing of objects that come into contact with the human body. It is not allowed to contain pathogens at concentrations that could endanger human health. The stringent regulations must be met all the way to the consumer's tap. In this context, there is now a need to answer the question regarding to what extent HPC can affect distribution system materials, household point-of-use or point-of-entry water treatment equipment, bottled water, water vending machines, beverages and water coolers.

2.4.2 Regulations for water quality in high-risk areas

There has been a dramatic increase in infections caused by microorganisms, including certain heterotrophic microorganisms that are found in water (Huang *et al.* 2002). Particular attention will have to be paid to securing the necessary water quality in high-risk areas, such as hospitals and places where immunosuppressed patients are treated. In these areas, HPC is used by some authorities for indicating the risk of the presence of opportunistic pathogens (Hargreaves *et al.* 2001). These are also the areas in which the HPC-determinable pathogens (*Pseudomonas* spp., *Burkholderia*, *Stenotrophomonas*) pose a high risk of infection for vulnerable persons.

2.4.3 Changes in sociodemographic conditions

The number of immunosuppressed and catheterized patients continues to increase. There is an even more dramatic growth in domestic ambulatory care. Hospitals are no longer the only places in which patients are treated with antibiotics or invasive catheterization.

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3

The history and use of HPC in drinking-water quality management

P. Payment, D.P. Sartory and D.J. Reasoner

3.1 INTRODUCTION

As civilizations developed, it became evident that water, especially good quality water, was necessary for their advancement. For centuries, good water was defined as water that was clear, pleasant to the taste and not malodorous. Good food had similar requirements. However, both contaminated water and food were still the causes of countless deaths. Outbreaks of cholera and typhoid occurred for centuries, but the role of water in these outbreaks was not demonstrated until 1849–1854. John Snow identified water as the source of a cholera outbreak in London and became the father of modern epidemiology. Even at the time of Snow, smell, appearance, taste and chemical analysis were the only analytical tools that the water analysts had to determine the wholesomeness of drinking-water. Too often they were wrong, and outbreaks were frequent.

 Heterotrophic Plate Counts and Drinking-water Safety. Edited by J. Bartram, J. Cotruvo, M. Exner, C. Fricker, A. Glasmacher. Published by IWA Publishing, London, UK. ISBN: 1 84339 025 6.

By the end of the 19th century, with the development of bacteriology, culture media and the gelatin plate, it became possible to obtain what appeared to be quite accurate counts of germs by counting the number of colonies developing on these plates within a defined set of conditions. The simplicity of the method was such that it was rapidly put to use by the 19th-century sanitarians. Air, water, soil, food, humans and animals were all studied to determine where and how germs lived, as they were apparently responsible for a wide variety of waterborne and foodborne diseases.

We are now in the early 21st century, and, reading the accounts of these 19th-century sanitarians, there is a striking resemblance between our so-called modern problems and the problems they had to resolve. The questions they raised are the same ones that we are discussing now. In terms of water quality, it is quite fascinating to observe that the orders of magnitude of the numerical values used to define good quality water have remained the same. While much has been written on the subject of water bacteriology, the books of Hamlyn (1990) and Prescott and Winslow (1904) provide a magnificent view of early water bacteriology.

3.2 GERMS AND DISEASE: FROM DISCOVERY TO CULTIVATION

Counting microbes is an exercise that has been taking place since the advent of the microscope. Through his simple single-lens microscope, Antoni van Leeuwenhoek in 1673 was probably the first to see microbes. Others followed, but the poor resolution of their lenses did not offer a very precise view of the bacterial world. By the 1830s, quality achromatic objectives had been developed and microscopes were being made that opened a new world to the eyes of the bacteriologists.

In England, John Snow demonstrated that water played a significant role in cholera outbreaks. In 1854, another severe epidemic of cholera occurred in London. Through painstaking documentation of cholera cases and correlation of the comparative incidence of cholera among subscribers to the city's two water companies, Snow showed that cholera occurred much more frequently in customers of the water company that drew its water from the lower Thames, which was contaminated with London sewage, than in customers of the other company, which obtained its water from the upper Thames.

By 1861, Louis Pasteur had disproved the spontaneous generation theory, and he later demonstrated the link between germs and disease. Robert Koch described a mechanism whereby a disease such as cholera was spread: it was

excreted in faeces, was transported to water and then infected those who subsequently drank the water. A similar mode of transmission was later described for typhoid fever, and subsequent interest in the role of water in the transmission of disease was thus initially focused on these two infections.

In 1872, Ferdinand Cohn developed a bacterial culture medium containing ammonium salts and yeast ash complemented with various sugars; this medium provided the bacteriologist with a tool to test the growth requirements of bacteria. In 1881, Koch published a paper in which he described the gelatin plate method — a revolution in bacteriology — for growing pure cultures of bacteria. Obtaining pure cultures was now easier, and the enumeration of germs was possible. In 1882, the use of agar instead of gelatin was introduced, and in 1887, Richard Julius Petri invented the petri dish.

The birth of microbiology in 19th-century Europe was the basis for water and food microbiology and the first step in understanding the role of water and food as vehicles for the transmission of disease (Beck 2000). Growing germs was not an easy task, but bacteriologists were discovering the basic nutrients that these germs needed to grow. They now had the tools to study water and food. These methods were rapidly adopted by sanitarians from all countries on both sides of the Atlantic.

3.3 KOCH: ASSESSING FILTER EFFICIENCY AND SETTING LIMITS

Water filtration had been introduced in 1804 in Scotland as a means of producing better quality water for a clothes-washing industry. Water from the River Cart was passed through trenches filled with stones before being passed through a ring-shaped settling chamber. The water was clear and contained less suspended solids, thereby not soiling the clothes. Because the process produced more water than needed, the surplus was sold to the town inhabitants. The product was of good quality, and others rapidly followed this lead. The first sand filters were developed by James Simpson in England in the 1820s.

By the end of the century, it was common to have filtered water, and the protective effect of this filtration was dramatically demonstrated in 1892 in Germany. The Elbe River, near Hamburg, was contaminated by sewage from a cholera-stricken refugee camp. Hamburg experienced an outbreak that killed over 7000 people, while the city of Altona, using the same water but filtered, experienced only a few cases unrelated to the water. Koch investigated this outbreak and exchanged information with water analysts all over Europe. He suggested that filtering was better than not filtering, that careful management of filters was better than poor management, that even careful management could

not protect the public absolutely, and finally that "when all was said and done, he, personally, would rather not drink this filtered water at all. Yet one had to live with uncertainty, to trust something less than rigorous demonstration, and be satisfied with estimates of risk."

Overall, the rudimentary bacteriological analysis of all types of water during these catastrophic outbreaks led the early sanitarians to better quality source water and water treatment and thereby a reduction in waterborne outbreaks. It also pointed out the value of water treatment to protect public health. Koch proposed a limit of 100 cfu/ml as the objective to protect public health. This value was proposed to assess the "purity" of source water and, hence, its usability as a source of drinking-water. It was also proposed as a means to assess water filtration efficiency in order to produce safe drinking-water from "impure" sources. It was only later that the same value was also used to evaluate the efficiency of the disinfection of drinking-water by chlorine and other means. For several years, Koch had also been analysing waters and counting colonies that grew in agar at "blood heat," thinking that these organisms would likely include pathogens.

The value proposed by Koch has remained unchanged until today and has apparently remained aimed at the protection of public health by a more or less direct evaluation of source water and treatment. Since the discovery of water bacteria and their relation to disease, the United Kingdom and the USA approached the plate count with two different philosophies, as described in sections 3.4 and 3.5 below.

3.4 WATER MICROBIOLOGY: THE UNITED KINGDOM EXAMPLE

3.4.1 Early water microbiology

Although Robert Koch had demonstrated the use of solid media for culturing bacteria in London in 1881, it was only in 1884 that British water analysts and sanitarians began to take interest in it and *The Lancet* published a lengthy description of the plate culturing method, noting that "the numbers and nature of the organisms present in a sample of water may be estimated and ascertained" using this technique. The book of Hamlyn (1990) presents an account of British efforts to understand water quality and control waterborne diseases; it has been an inspiration for this section.

The simplicity of the technique was its greatest problem, as it tempted those with little or no bacteriological training to try the process. Many recognized that the method required skills in order to obtain accurate results, but the British

sanitarians realized that bacteriological examination ought to be carried out on a widespread basis for the examination of water supplies and for ascertaining the relative value of domestic filters.

One of the early advocates of bacteriology was Percy Frankland (1858–1946), who worked with his father at the School of Mines, where he was an assistant in the water laboratory. After learning of the plate culturing method at an exhibition in London, he visited Koch's laboratory to master this new method. Frankland used the method from 1885 onward to measure the numbers of bacteria in water and evaluate the efficiency of filtration. He observed what is now well established: filters are effective for the removal of bacteria, they lose their efficiency with time, and smaller filters become clogged and support bacterial growth. In his words, this was an "exceedingly beautiful and ingenious test for ascertaining the number of individual organisms present in a given water," with "little value" for distinguishing bacterial types (quoted in Hamlyn 1990). Overall, more and more people agreed that plate cultures showed the value of filtration in removing microorganisms.

Many questions on these methods were also raised at the time; surprisingly, they are still familiar even to modern water bacteriologists.

Was gelatin-peptone the best medium? What was the sensitivity of the medium for waterborne pathogens? Comparison of media and their ability to support pathogens became a familiar exercise. Lower counts on nutrient-rich media and upon incubation at "blood temperature" were observed, as was the poor growth of pathogens on nutrient-poor media. By the late 1890s, most analysts would insist that use of several media was necessary if one was to speak confidently on the bacterial content of a water.

What was the relationship between the bacteria in the water and the bacterial counts and species growing on the plates? Having observed the bacteria under the microscope and recorded different counts of bacteria on different culture media, scientists realized that the number of colonies that grew on the plate could not be regarded as the true total number of bacteria in the water.

What do the bacterial counts indicate? To the British, it became rapidly evident that these determinations indicated what would be the probable fate of pathogens gaining access to the water supply and their potential to reach the consumers. A method of treatment reducing the largest proportion of organisms of all kinds would also be the most likely to reduce pathogens should they be present.

Interpretation of the data was becoming controversial: some questioned the bacterial counts, since microbial populations would rapidly increase in suitable conditions. Koch had suggested a standard of 100 colonies/ml as the limit of acceptability, but what would be the risk of drinking that "acceptable" water if

after a week in a container it contained 10 000 colonies/ml? The same question is raised today.

The use of plate counts became widespread, and an incubation temperature of 18–22 °C became the norm, with daily examination of plates for up to five days (Horrocks 1901). Additional counts of bacteria after incubating a second set of plates for 40–48 h at 36–38 °C were recommended in 1904 (Royal Institute of Public Health 1904), as these bacteria were considered more likely to represent those that could grow in the human body and, therefore, could be indicative of faecal contamination, although it was recognized that many other naturally occurring bacteria were also capable of growing at this temperature (Savage 1906). During this time, counts at 18–22 °C or 20–22 °C were typically conducted using nutrient gelatin plates, and those at 37 °C were conducted using nutrient agar plates (Royal Institute of Public Health 1904; Savage 1906).

3.4.2 Early use of heterotrophic plate counts (HPC)

Formal guidance on the bacteriological examination of water supplies and the interpretation of results was first published by the United Kingdom Ministry of Health in 1934 (Anonymous 1934) as what was to become universally known as "Report 71." The recommended method involved dispensing 1-ml aliquots of water, mixing with nutrient agar and incubation of one set of plates at 20–22 °C for three days and another set at 37 °C for two days, which, apart from a change of medium, has continued to today and is widely used throughout the world. The number of bacteria enumerated at 20–22 °C was said to give "some indication of (1) the amount of food substance available for bacterial nutrition and (2) the amount of soil, dust and other extraneous material that had gained access to the water," whereas the count at 37 °C "affords more information as to dangerous pollution," as "the organisms developing at this temperature are chiefly those of soil, sewage, or intestinal origin, and their number, therefore, may be used as an index of the degree of purity of the water" (Anonymous 1934). The report also stated that the colony count of a single sample had comparatively little significance and that "it is difficult to state limits which, if exceeded, involve unfavourable comment on the hygienic quality of the water." The ratio of the count at 22 °C to that at 37 °C was said to be helpful in explaining sudden fluctuations, with high ratios being associated with bacteria of clean soil or water saprophyte origin and, therefore, of "small significance" (Anonymous 1934). This approach was reaffirmed in the second edition of Report 71, published five years later (Anonymous 1939).

3.4.3 Guidance on the use of HPC

Experience gained over the next 17 years, however, led to a change of emphasis in the third edition of Report 71 (Anonymous 1956; Society for Water Treatment and Examination 1956), which stated that "although plate counts at 22 °C and 37 °C reflect by an increase in the numbers, particularly at the higher temperature, the access of faecal pollution, they are not now usually employed for this purpose." Their principal use was now one of a more general detection of "any form of contamination," maintaining their role as indicators and not a health parameter in their own right. The report presented a review of the agar plate count (written by E. Windle Taylor, then Director of Water Examination of the Metropolitan Water Board, London), which discussed the wide variability of numbers of bacteria from differing water types and sources and technical aspects of the method, concluding that "high plate counts at either temperature, even if confirmed, do not necessarily indicate that a water is a danger to health." They were, however, "undesirable since the presence of large numbers of bacteria in water may cause food spoilage." The key value of plate counts was their use in assessing the efficacy of water treatment processes, providing an "estimate of the general hygienic quality of a water" (particularly with regard to food production), and "a rising plate count may give the earliest sign of pollution" (e.g., in wells) (Anonymous 1956). This interpretation of the value of plate counts was reiterated in the fourth and fifth editions of Report 71 (Anonymous 1969, 1982), which also stated that "colony counts are not essential for assessing the safety of domestic water supplies." The fourth edition also introduced yeast extract agar as the medium of choice for the enumeration of colony counts and confirmed an incubation time of only 24 h for counts at 37 °C, introduced in the 1956 third edition. The 1982 fifth edition also noted that "organisms which grow best at 37 °C usually grow less readily in water and are more likely to have gained access from external sources" and that "a sudden increase ... would call for immediate investigation since it might be an early sign of more specific or serious pollution" (Anonymous 1982). All reference to the use of HPC to potentially indicate faecal contamination had been dropped.

3.4.4 Interpretation of HPC levels

Significant strides in the understanding of microbial behaviour, particularly with regard to heterotrophic bacterial populations, in water supplies during the 1980s and 1990s were reflected in the sixth edition of Report 71, published in 1994 (Standing Committee of Analysts 1994). The three key areas where plate counts were of value, outlined in the 1956 third edition, remained, but multiplication of bacteria within distribution systems due to available nutrients (assimilable

organic carbon) in the water or fixtures and fittings and the growth of biofilms and their potential role in taste and odour problems were also recognized (interestingly, a relationship between available nutrients and bacterial growth had been alluded to in the 1934 and 1939 editions of Report 71, but not since). The report stated that "in practice, changes in the pattern of colony counts of samples from a given water supply are usually more significant than the actual numerical count of any particular sample" and that "the counts themselves have little direct health significance." The report recognized that some potentially opportunistic pathogens (e.g., *Pseudomonas aeruginosa* and *Aeromonas* sp.) may be part of the colony count population, and "their appearance in large numbers in water indicates that conditions in the distribution system have become suitable for growth as opposed to survival of these organisms." However, it concluded that without evidence of faecal contamination, "elevated colony counts should not be viewed with concern in terms of the health of the population as a whole." Regular enumeration of colony counts from a distribution system did, however, provide useful data with which to assess any long-term trends in the general microbial quality of drinking-water.

This interpretation of the use of colony counts is retained in the seventh edition of the guidance (Standing Committee of Analysts 2002a, 2002b), prepared with regard to the new United Kingdom legislation (Anonymous 2000) arising from the 1998 European Union (EU) Directive (European Union 1998). The guidance re-emphasizes that "it is not the absolute numbers of colony count bacteria enumerated from a supply that are of importance, but whether there are significant changes or long-term trends in those numbers." Although the requirement to enumerate colony counts at 37 °C is no longer stipulated in the EU Directive, it has been retained in the United Kingdom legislation and is still considered to be of some value, "in that it can provide an early indication of a significant deterioration in quality before coliform bacteria or other indicator bacteria are detected (for example, due to ingress into a distribution system)" (Standing Committee of Analysts 2002a).

This edition also reintroduced the option of incubating 37 °C plates for up to 48 h (Standing Committee of Analysts 2002b), as had been the norm prior to 1956, and is also in agreement with the International Organization for Standardization (ISO) standard ISO 6222:1999 (ISO 1999), stipulated by the 1998 EU Directive (European Union 1998) as the method to be used. The lower incubation range in the ISO standard is 22 °C ± 2 °C, which is a wider range than the 20–22 °C historically used in the United Kingdom and recommended by the United Kingdom guidance (Standing Committee of Analysts 2002b).

When the United Kingdom adopted the first EU Directive on drinking-water (European Union 1980), the guideline values for plate counts (10/ml at 37 °C

and 100/ml at 22 °C) were not formally included. Instead, the regulations stated that there should be "no significant increase over that normally observed" (Anonymous 1989a). Guidance from the regulators (Anonymous 1989b) stated that "continuous review is needed of colony counts" and that further investigation should be taken if "there is a sudden and unexpected increase in a colony count, particularly the 37 °C count, compared with that normally found" or "there is a significant trend of increasing colony counts in the supply over a period of a few years." Both the current EU Directive (European Union 1998) and United Kingdom regulations (Anonymous 2000) do not set numerical standards or guideline values for colony counts, which are defined as indicator parameters, but state that there should be "no abnormal change." This is in keeping with the approach that colony counts are an operational tool for the management of water quality in distribution systems. It does, however, beg the question as to what an "abnormal change" is. There is currently no official guidance on this in the United Kingdom (or Europe), and, consequently, there are several approaches that have been adopted by water suppliers.

Many suppliers employ simple numerical values for an indication of an abnormal change in counts from regulatory samples; some have based these values on the guideline values of the first EU Directive (European Union 1980), whereas others have adopted higher values (e.g., >10, >20, >50, >100, >200, >300, >500, >1000 cfu/ml at 22 °C or 37 °C). These values generally serve as triggers to review previous data and make an assessment of any significance of the increase. Some have established arbitrary levels of increase ranging from 0.5 log to >2.3 log increases over previous results. This has the advantage that it automatically takes into account the natural rise and fall in heterotrophic bacterial populations that occur during the seasons. A few suppliers have adopted a statistical approach (several others indicated that they were also investigating a statistical approach), based upon a comparison with mean counts. The time base of the data for which mean counts are calculated can vary, depending upon the seasonal variation in the counts and the frequency of analysis, with some covering the previous few weeks and others a period of a year or more (e.g., 20 times a three-year mean, >3 standard deviations from previous six results, >1.5 times a 12-month rolling mean or the >98th percentile of rolling annual mean).

3.4.5 Current use of HPC in the United Kingdom

The principal use of the data gathered from regulatory monitoring is to monitor trends or deterioration (in terms of rising counts) in quality, and some suppliers have targeted trend monitoring with data from service reservoirs. Other uses of the data are chlorine management, modelling of microbial populations,

performance assessment of treatment works, assessment for planned maintenance of infrastructure (e.g., cleaning of service reservoirs) and secondary indicators of quality following isolation of coliforms or other primary indicators. Most suppliers have regular review periods, typically monthly, half-yearly or annually, some undertaking reviews on both a regular basis and by an unusual result. Most of these reviews are undertaken on an informal basis, but several have a formal programme, some linked in with their quality assurance procedures (e.g., ISO 9002 — ISO 1994).

Undertaking plate counts as part of a suite of analyses when responding to claims of ill health is the most widespread use, with most suppliers doing counts at both 37 °C and 22 °C, but a few only at 37 °C. The rationale is that plate counts may indicate a significant event within the distribution system, not that HPC bacteria may be related to ill health. Plate counts are also widely used when investigating complaints of off-tastes or odours, as changes in HPC populations may indicate proliferation of biofilms, which can be associated with microbially mediated generation of some organoleptic compounds (Standing Committee of Analysts 1998). Operational plate counts are also commonly used as part of acceptance criteria for new mains prior to being put into supply and in assessing water quality following mains rehabilitation work.

The use of counts of heterotrophic bacteria has, therefore, a long history in the United Kingdom. The count at 22 °C has been used as a general indicator of water quality since 1885. The count at 37 °C was originally introduced with the belief that it could indicate potential faecal contamination, but this was soon disregarded, although it is still used for operational management in the United Kingdom, despite being dropped in the EU Directive.

Coliform bacteria are also no longer regarded as indicators of faecal contamination, but are of use as indicators of general microbial quality. This acknowledges that some coliform bacteria may be part of the natural bacterial flora in water and proliferate in biofilms. Coliforms are also considered useful for monitoring treatment processes and assessing the disinfection of new or repaired mains (Standing Committee of Analysts 2002a).

3.5 THE AMERICAN PERSPECTIVE ON THE PLATE COUNT

3.5.1 Early water bacteriology in the USA

It did not take long for these “new methods” to cross the Atlantic, and by 1904, the first edition of *Elements of Bacteriology with Special Reference to Sanitary Water Analysis* (Prescott and Winslow 1904) contained most of what is today

considered modern bacteriology. The principles of this book, re-edited until 1946 (sixth edition), are still pertinent to the discussions that we have today. A similar book, written by William G. Savage, entitled *The Bacteriological Examination of Water-Supplies* and published in 1906 in London, presents the British story and the state of knowledge in England at that time.

In the preface to the first edition of their book, Prescott and Winslow (1904) summarize the context:

> Bacteriology has long since ceased to be a subject of interest and importance to the medical profession merely, but has become intimately connected with the work of the chemist, the biologist, and the engineer. To the sanitary engineer and the public hygienist a knowledge of bacteriology is indispensable.
>
> In the swift development of this science during the last ten years perhaps no branch of bacteriology has made more notable progress than that which relates to the sanitary examination of water. After a brief period of extravagant anticipation, and an equally unreasonable era of neglect and suspicion, the methods of the practical water bacteriologist have gradually made their way, until it is recognized that, on account of their delicacy, their directness, and their certainty, these methods now furnish the final criterion of the sanitary condition of a potable water.
>
> The treatment of the subject in the many treatises on General Bacteriology and Medical Bacteriology is neither special enough nor full enough for modern needs. The classic work of Grace and Percy Frankland is now ten years old; and even Horrocks' valuable "Bacteriological Examination of Water" requires to be supplemented by an account of the developments in quantitative analysis which have taken place on this side of the Atlantic.

The plate count had been applied to a variety of waters, and what were considered "normal values" were being confirmed. Prescott and Winslow (1904: pp. 8, 9, 10) wrote:

> With regard to what may be considered normal values for rain we have no very satisfactory figures. Those obtained by Miquel (Miquel, 1886) during the period 1883-1886, showing that rain contains on the average 4.3 bacteria per c.c. in the country (Montsouris) and 19 per c.c. in Paris, are probably lower than would be yielded by the present methods of examination ... In the larger streams several conditions combine to make the bacterial number lower ... A good river-water under favorable conditions should thus contain only a few hundred bacteria ... The student will find numerous analyses of natural waters in Frankland's classic work (Frankland, 1894). He notes, for example, that the Lake of Lucerne contained 8 to 51 bacteria per c.c., Loch Katrine 74, and the Loch of Lintralthen an average of 170. The water of

> Lake Champlain examined by one of us (S.C.P.) in 1896 contained on an average 82 bacteria per c.c. at a point more than two miles out from the city of Burlington ...

Many observers had believed that groundwaters were nearly free from bacteria, because often no colonies appeared on plates counted after the usual incubation period of two days. Longer periods of incubation yielded higher counts, occasionally in very large numbers, and the multiplication of bacteria in the samples after collection or bottling had been observed. The conclusion was that all water types contained bacteria and that one needed to find the correct medium to grow these organisms. However, for the sanitary bacteriologist, the limits were different (Prescott and Winslow 1904: pp. 19–20):

> That the customary methods for determining the number of bacteria do not reveal the total bacterial content, but only a very small fraction of it, becomes apparent when we consider the large number of organisms, nitrifying bacteria, cellulose-fermenting bacteria, strict anaerobes, etc., which refuse to grow, or grow only very slowly in ordinary culture media, and which, therefore, escape our notice.
>
> ... the numbers obtained by the ordinary procedure were only from 5 to 50 per cent of those obtained by the use of Heyden's Niahrstoff agar. For practical sanitary purposes, however, our methods are fairly satisfactory. Within limits, it is of no great importance that one method allows the growth of more bacteria than another.
>
> When we are using the quantitative analysis as a measure of sewage pollution only two things are essential. First, media should be of standard composition, so that results obtained at different times and by different observers may be comparable ... Secondly, it is desirable that the section of the total bacterial flora which we obtain should be thoroughly representative of that portion of it in which we are most interested — the group of the quickly growing, rich-food-loving sewage forms. In this respect our meat gelatin-peptone appears to be unrivalled ... To emphasize this difference with constancy is all that we require of a method for practical work.

The conditions of sample conservation had also been investigated and had shown that there "is first a slight reduction in the number present, lasting perhaps for six hours, followed by the great increase noted by earlier observers. It is probable that there is a constant increase of the typical water bacilli, overbalanced at first by a reduction in other forms, for which this is an unsuitable environment." These results made it obvious that samples must be examined shortly after collection and that they must be kept cool during their storage. At this time, the recommendation was that "It is, therefore, necessary to adhere strictly to the recommendations of the A.P.H.A. Committee that the interval between sampling and examination should not exceed twelve hours in

the case of relatively pure waters, six hours in the case of relatively impure waters, and one hour in the case of sewage."

The incubation period was, as it is still today, the subject of much discussion. American and German bacteriologists counted the number of colonies after 48 h, while the French were using longer incubation periods and obtaining higher counts. The Americans considered that the longer incubation period was in fact obscuring the difference between good and bad waters, because the fast-growing bacteria were associated with sewage originating from the human intestine. Whatever the conditions of the test, Prescott and Winslow (1904: p. 35) considered the interpretation of this simple test as a complex process:

> The information furnished by quantitative bacteriology as to the antecedents of a water is in the nature of circumstantial evidence and requires judicial interpretation. No absolute standards of purity can be established which shall rigidly separate the good from the bad. In this respect the terms "test" and "analysis" so universally used are in a sense inappropriate. Some scientific problems are so simple that they can be definitely settled by a test. The tensile strength of a given steel bar, for example, is a property which can be absolutely determined. In sanitary water analysis, however, the factors involved are so complex and the evidence necessarily so indirect that the process of reasoning much more resembles a doctor's diagnosis than an engineering test.

On either side of the Atlantic, classes of water were being defined. In France, as early as 1891, Miquel classified waters as follows: "water with less than 10 bacteria per c.c. was 'excessively pure,' with 10 to 100 bacteria, 'very pure,' with 100 to 1000 bacteria, 'pure,' with 1000 to 10 000 bacteria, 'mediocre,' with 10 000 to 100 000 bacteria, 'impure,' and with over 100 000 bacteria, 'very impure.'" In Germany, water containing fewer than 100 bacteria was presumably from a deep source and uncontaminated by surface drainage; one with 500 bacteria was open to suspicion; and one with over 1000 bacteria was presumably contaminated by sewage or surface drainage (Sternberg 1892).

By 1904, it was also clear that organisms growing at body temperature and those fermenting lactose were not numerous in normal waters, with total counts rarely exceeding 50/ml. However, when polluted waters were examined, counts of acid producers on "litmus-lactose-agar" plates were likely to run into hundreds. The method, therefore, was considered "one of the most useful at the disposal of the bacteriologist. It yields results within twenty-four hours, and the conclusions to be drawn from it are definite and clear" (Prescott and Winslow 1904).

The Americans did not consider the plate count as part of their water regulations until recently.

3.5.2 Measuring HPC microorganisms in the USA

In the USA, bacteriological methods for the analysis of water were proposed by the American Public Health Association in collaboration with the American Water Works Association in the first edition of what was to become known as "Standard Methods" (APHA 1905). From its first edition in 1905 until its 20th edition in 1998 (APHA *et al.* 1998), the methods have been modified on several occasions. The basic plate count on nutrient gelatin at 20 °C for 48 h was used for several years and was later modified to include agar as the solidifying agent and a shorter incubation period of 24 h, which remained the main method until the 1980s. Because food microbiologists using the plate count had standardized the method at 35 °C for water, food and dairy products, this became the recommended temperature of incubation in the 10th edition (APHA *et al.* 1955). By 1985, several variations were in use (i.e., pour plate, spread plate and membrane filtration), and the plate count was referred to as the "heterotrophic plate count" or HPC.

By the end of the 1980s, American bacteriologists had developed culture media that could detect a higher proportion of heterotrophic bacteria (Reasoner and Geldreich 1985). The media were developed to maximize bacterial recoveries; they yielded higher counts when incubated for 5–7 days at 20 °C or 28 °C and permitted the examination of larger sample volumes by membrane filtration methods. Because of the limited inclusion of fewer nutrients at higher concentration, these media detect higher numbers of fewer different species of the diverse heterotrophic bacterial population.

By the mid-1980s, the Americans, who had no standard for the plate count at the time, had several groups review the "plate count" and its implications. The bacterial plate count for analysing water had been used in combination with the coliform test for a number of years and appeared in 1914 as a US drinking-water standard with a limit of 100 cfu/ml. As experience accumulated with the total coliform test and plate count test, the fact emerged that the latter provided unreliable information on the presence of bacterial pathogens in drinking-water. For this reason, the test was not included in the succeeding US Public Health Standards of 1925 and thereafter. While there was no requirement for plate counts as a drinking-water standard even in the 1970s, the US Environmental Protection Agency (EPA) stated its belief that "the standard plate count is a valid indicator of bacteriological quantity of drinking water, and recommends that it be used in appropriate cases in conjunction with the coliform tests as an operational tool" (US EPA 1975). At the same time, the National Academy of Sciences (1977) stated that "the Standard Plate Count is a valuable procedure for evaluating the bacterial quality of drinking water."

Numerical values were more difficult to define. In 1989, the US EPA addressed the issue in one of its rules and set the level to 500 cfu/ml at 35 °C, as a non-health-related secondary standard, mainly for considerations relating to interference with the coliform test. Both the "Surface Water Treatment Rule" (US EPA 1989a) and the "Coliform Rule" (US EPA 1989b) contained requirements for monitoring the HPC, as a high HPC is associated with false-negative coliform tests when lactose-based media are employed and as HPC is a surrogate indicator for chlorine residuals in distribution systems. The method chosen for measuring HPC was left to the water utility, but the numerical objective was the same.

According to Reasoner (1990), HPC is a useful tool for 1) monitoring the efficiency of the water treatment process, including disinfection; 2) obtaining supplemental information on HPC levels that may interfere with coliform detection in water samples collected for regulatory compliance monitoring; 3) assessing changes in finished water quality during distribution and storage and in distribution system cleanliness; 4) assessing microbial growth on materials used in the construction of potable water treatment and distribution systems; 5) measuring bacterial regrowth or aftergrowth potential in treated drinking-water; and 6) monitoring bacterial population changes following treatment modifications, such as a change in the type of disinfectant used.

3.5.3 Interference with the total coliform assay

Documents prepared by the US EPA by the mid-1980s show that the Americans were mainly focusing on the interference of high plate counts with the coliform assay and the presence of opportunistic pathogens in the bacterial population defined by the plate count (US EPA 1984).

Reasoner and Geldreich (1985), who were the developers of the new culture media for HPC, presented the various uses of the HPC: evaluation of the treatment process(es), primarily disinfection; evaluation of the levels of HPC that may interfere with coliform compliance; evaluation of the quality of finished treated drinking-water and of distribution system cleanliness; and evaluation of the potential for biofilm formation.

The Americans relied mainly on total coliform and thermotolerant (faecal) coliform assays to assess their water quality, and the preferred methodology was membrane filtration. Setting total coliforms as the key method to all water analysis, they integrated the HPC, not for its operational value, but mainly to limit the interference with total coliform enumeration. Investigations had suggested that high HPC densities (i.e., over 500/ml as enumerated on standard plate count [SPC] media) could substantially interfere with membrane filtration tests that were lactose-based, but that this phenomenon may not occur

consistently. Overcrowding on membrane filter plates appeared to be a major reason for atypical coliform colonies. In addition to interference with coliform analysis methodology, large numbers of SPC bacteria were also suggested to reduce coliform levels during sample transit and storage. Geldreich *et al*. (1978) collected 613 samples from flushes of dead-end water mains in Cincinnati, Ohio. Data analysis demonstrated a correlation between excess SPC densities and desensitization of the membrane filtration method. They concluded that the method was less efficient when SPC densities exceed 500–1000 cfu/ml.

As these studies indicate, American water bacteriologists were essentially working with data suggesting the presence of interfering factors in some waters; whether these were intrinsic factors of a physicochemical nature (organic and inorganic precipitates) or related to a predominance of certain bacteria types has not been fully explained.

However, general guidelines were formulated. Values of less than 100 cfu/ml were considered achievable for all systems. Values from 100 to 500 cfu/ml, anticipated during seasonal increases or at certain locations in the system (dead end, low residual), would suggest a need for flushing. Values greater than 500 cfu/ml would suggest poor microbial quality. The last category was not defined in terms of action to be taken. In other cases, 5- to 10-fold increases over normal levels were set as a guideline to prompt an investigation (US EPA 1984).

3.6 OPPORTUNISTIC PATHOGENS AND HEALTH EFFECTS

On this theme appear the most controversial discussions of the last part of the 20th century. Using various media designed specifically for this task, it is possible to grow various pathogens, such as *Legionella*, *Mycobacterium*, *Escherichia coli*, *Campylobacter* and many other species, from water samples. While none would dispute the fact that most, if not all, bacterial pathogens are "heterotrophic bacteria," many equated the plate count with these pathogens. The following citation is a typical mixed-message example of what can be found in texts of the period: "Many members of the SPC population have longer survival times than fecal contaminants in water, and many (e.g., *Mycobacterium*, *Bacillus*, and *Clostridium*) are more resistant to disinfectant than fecal pathogens."

Some bacteria counted in the HPC are certainly more resistant to disinfection; *Bacillus* spores have been described as a good indicator of treatment efficiency. Mycobacteria are very slow growers, are very difficult to grow and would not be counted on an HPC plate. Clostridia are strict anaerobes

and therefore would not be found in the population growing on the plate count media and would not be "members of the SPC."

The list of colony-forming bacteria on HPC media and identified in water is long and illustrates the diversity of the environment: *Acinetobacter*, *Actinomycetes*, *Alcaligenes*, *Arthrobacter*, *Corynebacterium*, *Micrococcus*, *Moraxella*, *Pseudomonas*, *Aeromonas*, *Citrobacter*, *Enterobacter*, *Yersinia*, *Hafnia*, *Klebsiella*, *Serratia*, etc. (Payment 1999). The same bacteria are found often in large numbers in food products.

While there have been several studies of the bacterial species found in water, the identification of bacterial isolates from the environment has always been impaired by a poor database. It is highly probable that many of the isolate identifications reported in the literature over the years are incorrect. Comparisons of various available identification systems have shown that the same isolate will be identified differently according to the database used. In the 1980s, many identifications were made employing clinical systems for which the database was not appropriate for environmental strains. Molecular methods have changed our views of the "species," and we should at least question many of the bacterial identifications in the literature. Some may be correct to the genus level and a few to the species level, but none can define the pathogenicity of these bacteria, as we will see further.

From the 1980s until now, many researchers in the water industry have equated the genus or species names of the bacterial isolates found in the plate count to those of isolates implicated in clinical disease. Few water bacteriologists were involved in clinical microbiology, and the isolates named were equated to pathogens and disease. Few pondered the true complexity of pathogenicity: among the myriad of *E. coli* strains that can be found in water, only a few are pathogenic. In a clinical setting, it is only through the identification process down the serological pattern that clinicians can identify the true pathogen and the relationship to disease in a particular environment. Finding *E. coli* in urine has a different significance than finding it in stools. Isolating a strain of *Campylobacter* or *Salmonella* in stools does not necessarily mean that it is the cause of disease (de Wit *et al*. 2000).

For some true pathogenic strains (i.e., strains that had been isolated from diseased individuals and shown to cause disease according to Koch's principles), oral infective dose data were available. As many of the isolates from water samples had the same identification (genus, species), most water microbiologists took the quantum leap: their isolates could also be pathogens, and even bacteria implicated very rarely in clinical disease became foes.

An EPA-supported study compared influent and effluent SPC densities for 25 point-of-use devices and generally found about a log or more increase in the effluent. It was concluded that there was a risk to immunocompromised

individuals: "Among the opportunistic pathogens which grow on these filters are *Pseudomonas aeruginosa* and *Flavobacterium* species. The proliferation of these pathogens may pose a health risk to compromised individuals consuming the effluent water" (Calderon and Mood 1988, 1991).

Many scientists went further, and statements such as the following were common: "A positive relationship between SPC densities and waterborne disease outbreaks has been reported in a few cases, but published data are sparse" (US EPA 1984: p. 33) or

> There are cases on record where a change in the SPC density has signaled the imminence of a waterborne outbreak. In 1926, for example, Hanover, Germany, experienced flooding of wells by highly contaminated river water. A substantial increase in SPC numbers was not initially accompanied by positive coliform counts. Hanover experienced 40,000 cases of gastroenteritis followed by an outbreak of typhoid fever (Muller, 1977). Muller (1977) also reported that similar observations occurred at Pforzheim in 1919 and at Gelsenkirchen in 1889. [US EPA 1984: pp. 48–49]

Those were sufficient reasons to jump to the conclusion that the correlation was universal. It failed to acknowledge that HPC numbers were often high in the absence of any overt disease and that one could not demonstrate a correlation. The epidemiological value of the anecdotal evidence is poor, but the statement influenced a large number of water specialists. In fact, it fell in the same category as coliforms and *E. coli*: both are used as indicators of treatment of faecal contamination, but, to many plant operators, they are disease-causing organisms.

The conclusion to most of the debates and of committees formed to study the risk is summarized by this statement: "While there is no conclusive evidence to date that opportunistic pathogens have caused disease via the waterborne route, there is strong supportive evidence this is true. Since virtually everyone in the U.S. is exposed to SPC bacteria whenever they consume or otherwise use potable water, including the compromised population, this is an area of concern" (US EPA 1984: pp. 59–60).

This statement, in its simplicity, fails to acknowledge the major source of exposure to HPC bacteria: food. As is shown in section 3.8 below, the HPC rapidly found its way in the food industry, where it has become a tool to study food degradation. The food industry faced the same problem and came up with a very different solution. Many food products could contain more than 1 000 000 cfu/ml before they began to deteriorate to a point where they were spoiled. This

was defined by the food industry not in terms of public health but in terms of food quality.

Several studies were also concerned with the presence of virulence factors in HPC bacteria (Lye and Dufour 1991; Payment *et al*. 1994; Edberg *et al*. 1997; Drinking Water Inspectorate 1998). They recognized that there were bacteria in drinking-water that contained recognized virulence factors, but that they were in small numbers and that only animal studies or epidemiological evidence could demonstrate the significance of these bacterial strains. Recent studies in immunocompromised animal models determined the true meaning of these virulence factors detected *in vitro*, and these studies have shown that none of the HPC bacteria isolated from drinking-water and expressing various virulence factors were pathogenic for immunocompromised mice (Stelma *et al*. 2002).

3.7 HEALTH EFFECTS: EPIDEMIOLOGICAL STUDY

Epidemiology again became a tool to answer the questions raised and the potential risks. The immunocompromised population had been growing rapidly with the spread of HIV/AIDS, and, with the advances in medicine, there was now an increasing number of transplant patients artificially immunosuppressed by drugs.

Because of lack of faith in tap water quality, a large number of households were using various point-of-use devices based on activated charcoal to remove chemical contaminants from water. It did not take long to show that these filters supported bacterial growth and that the effluent often contained more bacteria than the incoming water (Geldreich *et al*. 1985). Heterotrophic bacteria were using the accumulated organics in the activated charcoal filter matrix to proliferate. These could be the source of opportunistic pathogens, or the filters themselves might support the growth of incoming bacterial pathogens.

The first epidemiological studies on possible health effects were conducted in the USA by Calderon and Mood (1988, 1991) on a large number of households using various point-of-use or point-of-entry devices based on granular activated charcoal. High HPC levels were observed, but there were no apparent health effects demonstrated.

A prospective epidemiological study on the health effects of tap water was conducted in Canada. It included 600 families, 300 of which had been provided with reverse osmosis units to remove contaminants from their tap water (Payment *et al*. 1991a). The installation of the device had a protective effect for gastrointestinal disease transmitted by tap water: the individuals in the filter group experienced 35% fewer gastrointestinal episodes that those in the unprotected group. HPC counts at 20 °C and 35 °C had been obtained from the reverse osmosis units on several occasions, and it was thus possible to correlate

the level of disease in the family with the HPC counts at 37 °C (Payment *et al*. 1991b). The apparent association was driven by a few outliers in the data set and probably gave this result apparent, but unlikely, statistical significance.

In a second study, the same group (Payment *et al*. 1997) used bottled water as a means of testing the health effects of drinking-water. Highly purified bottled water and tap water (from a water filtration plant) were given to two groups of families; a third group consumed tap water, and a fourth group consumed water from a tap equipped with a bleeder valve that continuously purged the system, thereby preventing stagnation and regrowth of heterotrophic organisms. The results confirmed that tap water was a significant source of gastrointestinal disease in the population (17–40%). While the bottled purified (reverse osmosis filtered and ozonated) water remained relatively free of bacteria, the water collected at the water treatment plant supported an active HPC growth within a few days, as would be observed in the distribution system upon stagnation. The HPC population grew from 2 to 30 000 cfu/ml (25 °C) and from 0 to 985 cfu/ml (37 °C) in a week, with extremes at 1 400 000 cfu/ml (25 °C) and 895 000 cfu/ml (37 °C). The individuals who had consumed water with high bacterial counts had reported less illnesses than those consuming tap water. They had the same level of illness as those consuming pure bottled water with very few bacteria. The group of families consuming water from a tap equipped with bleeder valves had a level of gastrointestinal illnesses slightly higher than those in the tap water group. This indicated that regrowth of bacteria in drinking-water was not the source of the observed illnesses.

These studies all suggested that high bacterial counts from bacteria developing in tap water or bottled water were not contributing to an increase of gastrointestinal illnesses in a normal population (i.e., a population composed of individuals of all ages and normally healthy).

3.8 HPC BACTERIA IN FOOD

Historically, the bacterial plate count occupies a strong position as an analytical tool for determining the microbial quality of a variety of raw and processed food products, such as meats, dairy products and canned foods. It was among the first of the definitive scientific methods employed for quality control in such products, and its use continues today as the major tool for their bacteriological examination.

The European Economic Community (EEC) directives for various food products would appear totally unacceptable to most water bacteriologists; however, this is what we eat everyday. A few examples from various EEC directives or the United Kingdom guidelines (PHLS Advisory Committee for

Food and Dairy Products 2000) are presented in Table 3.1 and illustrate the order-of-magnitude difference between the two worlds.

Table 3.1. EEC directives and United Kingdom guidelines for the microbial quality of food products

Product	Microorganisms	Maximum value
1) EEC directives		
Egg products (Directive 89/437/EEC)	Aerobic mesophilic bacteria Enterobacteriaceae	100 000 cfu/g or ml 100 cfu/g or ml
Pasteurized drinking milk (Directive 92/46/EEC)	Plate count at 21 °C	50 000 cfu/g
Minced meat (Directive 94/65/EEC)	Aerobic mesophilic bacteria *E. coli* (non-pathogenic)	5 000 000 cfu/g 500 cfu/g
Frozen milk-based products (Directive 92/46/EEC)	Coliforms Plate count	100 cfu/g 50 000 cfu/g
2) United Kingdom guidelines		
Pork pies, sausage roll, raw pickled fish, mousse	Aerobic colony count 30 °C, 48 h	<10 000 cfu/g
Ice cream, pizza, cakes and pastries (without dairy cream), mayonnaise, cooked vegetables	Aerobic colony count 30 °C, 48 h	<100 000 cfu/g
Sliced beef and poultry, seafood meals, cakes and pastries (with dairy cream), dried fruit, coleslaw	Aerobic colony count 30 °C, 48 h	<1 000 000 cfu/g
Sliced ham, smoked fish, prepared mixed salads, sandwiches and filled rolls	Aerobic colony count 30 °C, 48 h	<10 000 000 cfu/g

A survey conducted in 1999 in Australia provides an interesting perspective on self-serve salad bars (West Australia State Health Laboratory Service 1999):

> The median SPC value was 185,000 cfu/g. Forty-six (63.9%) samples had an SPC less than 1,000,000 cfu/g, nineteen (26.4%) had an SPC between 1,000,000 and 10,000,000 cfu/g, seven samples had an SPC greater than 10,000,000 cfu/g. There were no samples with an SPC greater than 100,000,000 cfu/g.

The same is true in the USA, as the following citation from a Massachusetts requirement for frozen desserts illustrates (Massachusetts Department of Public Health 1999):

The bacteriological limits for frozen desserts set forth in 105 CMR 561.009 are 10 coliform colonies per gram and 50,000 standard plate count (SPC) per gram. It is the responsibility of local boards of health to enforce monthly testing and reporting requirements for frozen dessert establishments, as well as to take appropriate actions when bacteriological violations have been found ...

Q. Does a standard plate count (SPC) slightly above the standard of 50,000 colonies per gram present a public health concern?

A. The limit of 50,000 SPC is intended as a guideline. Usually the SPC represents harmless organisms, especially if there are no coliforms associated with the sample. Spoilage organisms usually begin to affect the frozen dessert product in numbers much greater than 50,000. It usually takes counts of 1,000,000 or greater to create spoilage. According to 105 CMR 561.009, exceeding 50,000 once is not considered a violation. When a SPC is only slightly high, i.e., 150,000, consider the company's track record.

3.9 MANDATORY OR GUIDELINE HPC VALUES IN THE 1970S AND 1980S

After the initial impetus, bacteriological tests became a simple routine measurement for the control of water treatment; in many countries, the plate count was not defined by mandatory values. Most regulatory texts simply stated that the absence of pathogens was expected and that control was to be achieved using coliform bacteria.

Apart from semantics and terminology, what was meant by the "total count of bacteria" in water did not change much, the basic definition being "the number of bacterial colonies produced on an agar plate under defined medium and incubation conditions." Heterotrophic bacteria include all those bacteria that can use organic nutrients for growth. The aquatic environment contains an extremely diverse flora of these organisms. All known primary and secondary bacterial pathogens, whether transient or indigenous, that are spread by the water route are heterotrophic. No single analytical tool can satisfactorily detect and enumerate all heterotrophic bacteria or measure their full range of metabolic activities.

In addition to the term "standard plate count," many designations have been used: "heterotrophic plate count," "total viable count," "total count," "plate count," "total bacterial count," "bacterial count," "water plate count" and "colony count," as well as "aerobic, mesophilic viable bacteria." Some are used in the water industry, some in the food industry, others in biology. The "standard plate count" term was used in the USA until the 15th edition of

Standard Methods (APHA *et al.* 1980) and was changed in the 16th edition (APHA *et al.* 1985). The nomenclature currently refers to the "heterotrophic plate count" as defined in the 20th edition of Standard Methods (APHA *et al.* 1998).

Several countries adopted mandatory values for the colony counts of water (Table 3.2). They are still used in most of these countries (see chapter 12), and they are very similar to the values suggested by Koch at the end of the 19th century. The European Union (1980) did recommend guideline values for total bacterial counts in drinking-water of 10 cfu/ml at 37 °C and 100 cfu/ml at 22 °C. Even if this appears convenient in its simplicity, there are differences in the defined conditions of medium and incubation, as well as other analytical parameters, from one country to another, as illustrated in Table 3.3. These guideline values, however, were dropped in the 1998 directive (European Union 1998).

Even if setting guideline values appears convenient, the impact of these differences on results is not really known. When setting an international level for any type of water (especially in point-of-use devices or bottled waters), these differences could significantly affect any decision made on the basis of the numerical results obtained. Furthermore, the rationale for using a particular value is rarely apparent in the texts supporting the regulations. WHO *Guidelines for Drinking-water Quality* still provide such information (WHO 1996).

3.10 STANDARDS AND GUIDELINES IN THE 1990S

The current standards or guidelines for HPC bacteria in tap water vary slightly between different nations. In general, HPC monitoring is used as a tool to gain information on the water treatment process and the general bacteriological quality of the water leaving the water treatment plant and within the distribution system. Examples of specific guidelines for drinking-water (tap or bottled) from several countries and agencies have been reviewed in chapter 12.

3.11 CONCLUSIONS

The HPC was the basic test that led public health officials and water treatment engineers to improve the quality of drinking-water. The plate count was rapidly replaced in most regulations by coliform testing, which provided a better indication of the sanitary quality of the water. In the early 1900s, the HPC was being used only as a secondary test to further assess treatment efficiency. While several technological developments led to media capable of detecting higher numbers of bacteria, very little was done to assess the variations in the bacterial subpopulations isolated on these different media at different temperatures.

Table 3.2. Some mandatory colony count values in Europe in 1977 (adapted from Muller 1977)

	Application	Mandatory value (counts/ml)	Temperature (°C)
Poland	Public supply	100	20
		25	37
	Well water	100	37
		500	20
Yugoslavia	Treated water	10	37
	Underground (raw)	100	37
	Surface (raw)	300	37
Romania	Public water supply (>70 000 consumers)	20	??
	Other water supplies	100–300	??
Switzerland	Raw water	100	??
	Raw water (distributed)	300	??
	Immediately after treatment	20	??
	Distribution system	300	??
Netherlands	Tap water	100	20
Sweden	Tap water	100	20
Germany (GDR)	Tap water	100	20
Spain	Good quality water	50–65	37
	Tolerable water	100	37
France		No guide	
United Kingdom		No guide	
USA		No guide	

The guideline values proposed by Koch at the end of the 19th century are very similar to those set by today's regulations in many countries. Various rationales have been proposed to justify the choice of specific guideline values: a few considered possible health effects, some considered attainable values, others found that HPC interfered with other tests, some found it useful for various tasks, many simply followed suggested guidelines.

The concerns relating to the presence of opportunistic pathogens within the bacterial population detected in the plate count have essentially been put to rest by several studies. Recent literature suggests that direct health effects are improbable, especially when compared with the extremely high plate counts that have been considered acceptable in food products. The historical background in the food industry provides ample evidence that these bacteria are mostly

Table 3.3. Example of the diversity of methods for the determination of plate count in drinking-water as set by water regulations in various countries during the 1980s (modified from NATO 1984)

		Canada	Netherlands	Norway	FRG	Sweden	France	UK
Procedure		Pour plate	Pour plate	Pour plate	Pour plate	Pour plate	Pour plate	Pour plate
Samples	ml/plate	As required	1	1	1	1	1	1
	Replicate	2	2	2	2	2	2/dilution	1–2/dilution
	Dilution	As required	As required	As required	As required	As required	As required	As required
	Diluent	Phosphate-buffered distilled water	0.1% peptone water	0.9% NaCl	Sterile tap water	Phosphate-buffered distilled water	Distilled water or Ringer's solution ¼×	Ringer's solution ¼×
Media	Medium	Tryptone glucose yeast extract agar	Tryptone glucose yeast extract agar	Tryptone glucose yeast extract agar	Meat extract peptone agar	Meat extract peptone agar	Yeast extract agar	Yeast extract agar
	Sterilize	15 min, 121 °C	15 min, 121 °C	15 min, 121 °C	20 min, 120 °C	20 min, 120 °C	20 min, 118 °C	20 min, 115 °C
	Incubation	48 h 35°C	48 h, 37 °C 72 h, 22 °C	72 h, 20 °C	44 h, 20 °C	48 h, 22 °C	24 h, 37 °C 72 h, 20–22 °C	24 h, 37 °C 72 h, 20–22 °C
Counting	Aids used	Quebec colony	Automatic colony	Hand lens	Hand lens (8×)	Hand lens	Hand lens	Hand lens

harmless, non-pathogenic organisms. That they can cause disease in extreme conditions remains possible (e.g., cuts, surgery, immunosuppression, etc.): many microorganisms given an opportunity to enter the human body can cause great harm. This is not the case when they are ingested.

In 2002, after more than 125 years, the case for setting HPC levels in drinking-water still remains an open question in the minds of many. This brief review of the HPC in history suggests that the main cause for concern has been the focus of water bacteriologists on the sanitary consequences of the HPC. Early bacteriologists had rapidly determined that in the absence of faecal contamination, the role of the HPC was not as an indicator of public health risk. Food bacteriologists, faced with the same problem, also accepted that HPC bacteria were mainly nuisance organisms, and they set guidelines that are orders of magnitude higher than those for drinking-water. Therefore, the future use of HPC in water testing appears to be mainly as a validation and verification test, with no direct relationship to public health.

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4

The presence of bacteria in water after regrowth

C.R. Fricker

4.1 INTRODUCTION

The processes used for producing potable water are not intended to produce bacteria-free water; rather, they are concerned with removing microorganisms that are a potential health threat and making water aesthetically pleasing. The types of processes used to treat water appear to have relatively little effect on the types of bacteria that can pass through the treatment process, although disinfection with oxidizing disinfectants will tend to remove vegetative bacteria, whereas bacterial spores may well be unaffected. It is well understood that there are many factors that affect an organism's susceptibility to chlorine disinfection, and one of the major factors appears to be the degree of metabolic activity. Organisms that have grown in low-nutrient systems or have been "starved" tend to be considerably more resistant to disinfection than those grown under laboratory conditions in nutrient-rich media. Thus, caution must be applied in

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interpreting studies of the growth and survival of bacteria in drinking-water, particularly where disinfection has been employed.

4.2 DETECTION OF BACTERIA IN WATER

Natural waters contain a myriad of different bacterial species, many of which have not been cultured, much less identified. The number of organisms present varies considerably between different water types, and it is generally accepted that sewage-polluted surface waters contain greater numbers of bacteria than do unpolluted waters. However, this may not in fact be the case. The methods used widely in water microbiology tend to favour the detection of mesophilic bacteria that are able to grow on nutrient-rich media. This severely limits the number and range of bacteria detected. Thus, sewage-polluted water contains large numbers of Enterobacteriaceae, which are readily cultured using the techniques normally used in water microbiology laboratories. However, other waters may contain bacteria that do not grow readily on nutrient-rich media and thus may not be detected. The introduction of a new medium (R2A) by Reasoner and Geldreich (1985) that had a lower nutrient content than most media employed in water microbiology demonstrated that many new bacteria could be detected in some water types, especially when the incubation period was extended. Furthermore, using a fluorescein diacetate as a marker of bacterial viability, Reynolds and Fricker (1999) demonstrated that in some water samples, only a tiny proportion of bacteria were detected using culture techniques that included the use of yeast extract agar and R2A medium. While in some samples only 1% of bacteria were detected by culture, in other samples in excess of 50% of bacteria were detected. Thus, it is extremely difficult to predict the true microbiological load of any water sample using culture alone.

Many other viability markers have also been used to demonstrate the presence of bacteria in water, with varying degrees of success. Different groups of these markers work on different principles, including the presence of certain enzymes, an intact respiratory chain or membrane permeability. While there have been a small number of studies comparing the performance of these different markers, they have been extremely difficult to interpret. The reason is that there is currently no “gold standard” to determine whether a cell is viable, other than demonstrating cell division. Other studies have used molecular techniques to detect bacteria in water. Studies initially used nucleic acid probes to the 16S ribosomal RNA (rRNA) present in bacterial cells. Such probes can be designed to be extremely specific for a given bacterial species (Prescott and Fricker 1999) or much broader groups of bacteria. Probes can even be constructed to detect all prokaryotic organisms (Amann and Ludwig 2000). However, while the number of rRNA molecules decreases after cell death, use

of 16S rRNA probes may still detect cells that are no longer capable of reproduction. An alternative approach has been to utilize nucleic acid probes to messenger RNA (mRNA) (Fricker 2000) for detection of "viable" organisms, utilizing the premise that mRNA has an extremely short half-life and will disappear to non-detectable levels shortly after cell death. However, studies with *Escherichia coli* demonstrated that *E. coli*-specific mRNA could be detected 20 days after cells had been "killed" by chlorine disinfection. Thus, the determination of the number of viable cells present within a particular sample is dependent on the definition of "viability" that is used. Again, there are many definitions of "viability," but, with the exception of demonstrable cell division, none is universally accepted. Because of the extreme variation in the numbers of bacteria present in any given water sample, depending on the test procedure used, it is difficult and often impossible to compare findings from different studies. In general, most workers use some culture procedure to describe the numbers of bacteria present, but with the caveat that any culture procedure will detect only a (small) fraction of the total bacteria present.

4.3 THE "VIABLE BUT NON-CULTURABLE" STATE

There has been much discussion about the existence of the viable but non-culturable (VBNC) state in bacteria, but it is generally accepted that some bacteria may respond to adverse conditions by entering a phase whereby they are able to metabolize and survive but are unable to produce colonies on artificial media on which they would normally grow. While starvation or low-nutrient conditions may trigger this response, other factors, such as pH, salinity and other unknown conditions, may also be involved. The existence of VBNC cells has been described for a wide variety of organisms, including vibrios, campylobacters, aeromonads, legionellas and members of the Enterobacteriaceae. In some experiments, it has been demonstrated that these VBNC cells are able to infect suitable animals when introduced experimentally. However, at present, without resorting to *in vivo* experiments, it is impossible to determine if cells that are present in a sample but unable to grow on culture media are able to reproduce and thus be of concern to human health.

4.4 MICROBIOLOGICAL REGROWTH

The growth of bacteria in water distribution systems and water treatment devices has been recognized for many years. Such growth is affected by many different factors, including the types of bacteria present in water released from a water treatment plant, the temperature, disinfectant concentration, the presence

of sediment in the pipe work, the types and amount of nutrients present and the rate of flow of the water. Many of these factors cannot be controlled, and thus microbial regrowth will continue to be investigated. The organisms involved in microbial regrowth are those that have been released from the water treatment plant or that have been introduced into the distribution system at some point downstream of the water treatment plant. If it is assumed that the water treatment plant is performing adequately, then the numbers of bacterial pathogens released into the water distribution system will be low, and those that are present are likely to be killed during transport in systems where residual disinfectant is present. However, a break in the integrity of the distribution system (e.g., burst water main) can lead to the ingress of contaminated water. Such water may contain organisms that are potentially pathogenic for humans.

Many bacteria that enter the water distribution system are unable to survive or indeed colonize the distribution system (Reasoner *et al*. 1989), but many bacteria, including indicator bacteria such as *Enterobacter*, *Citrobacter* and *Klebsiella*, as well as potentially opportunistic pathogens such as *Aeromonas, Pseudomonas*, *Flavobacterium* and *Acinetobacter*, are often found in colonized water distribution systems.

4.5 MICROBIAL PATHOGENS IN WATER

As our knowledge of clinical microbiology increases and epidemiological surveillance improves, the range of microorganisms that have been shown to cause waterborne outbreaks of disease has grown. No longer are the classical waterborne pathogens *Vibrio cholerae* and *Salmonella typhi* the most frequently detected cause of waterborne outbreaks of disease (although they still account for substantial illness in many developing countries). Newly described infections, such as those caused by *Cryptosporidium*, now account for many of the disease outbreaks linked to water. However, the number of "sporadic" infections attributable to water is unknown. Many of the pathogens associated with water are also transmitted by food, and thus it is difficult to determine the source of most sporadic infections. The number of different types of bacteria that have the potential to cause disease in human beings and have been isolated from water is large, and yet the incidence of infection in human beings is often extremely low, even in areas where the water distribution system is continually colonized.

Unless there has been a breakdown in the water treatment process (usually a failure of disinfection) or a large ingress of contaminated water, then the occurrence of "traditional" bacterial enteric pathogens, such as *Salmonella*, *Shigella*, *Vibrio* and *Campylobacter*, is rarely, if ever, seen. However, a wide variety of "opportunistic pathogens," such as *Aeromonas*, *Pseudomonas* and

some species of *Mycobacterium*, are commonly found. The significance of their presence in water supplies in the etiology of human disease, however, is not well defined.

4.5.1 *Aeromonas* spp.

The significance of these environmentally ubiquitous organisms in the etiology of human gastrointestinal disease remains unclear, despite intensive investigation (WHO 2002). While certain species, mainly *A. caviae*, *A. hydrophila* and *A. veronii* subspecies *sobria*, have been isolated from patients (particularly infants) with diarrhoea (Sow *et al*. 1977; Chatterjee *et al*. 1989; Krovacek *et al*. 1989; Ashiru *et al*. 1993), these organisms are also found frequently in the faeces of subjects who are asymptomatic (C.R. Fricker and R.W.A. Park, unpublished observations).

There is no doubt that *Aeromonas* can frequently be detected in a variety of waters, and several investigators have described its detection in potable water (LeChevallier *et al*. 1980, 1982; Clark *et al.* 1982; Millership and Chattopadhyay 1985; Havelaar *et al*. 1990) at levels of up to 1900 cfu/ml. However, even with modern tools for discrimination between bacterial isolates, it is difficult to determine if the types of organisms present in drinking-water are the same as those found in patients with diarrhoea. Havelaar and colleagues (Havelaar *et al*. 1992) used serotyping and cell wall fatty acid analysis to attempt to correlate the presence of *Aeromonas* in drinking-water and in patients with diarrhoea. The differences they encountered between the strains from the two sources led them to conclude that the strains isolated from water were essentially different from those isolated from human faeces. Similar studies (Moyer *et al*. 1992; Hanninen 1994; Kirov *et al*. 1994) also failed to confirm a link between the strains found in drinking-water and those isolated from human faeces.

Aeromonas is also frequently found in foods, and many studies have been carried out to determine its incidence. In all published studies, *Aeromonas* was found in a variety of foodstuffs with isolation rates of up to 84% and concentrations of up to 10^5/g, with ready-to-eat foods being frequently contaminated (Fricker and Tompsett 1989; Palumbo *et al*. 1989; Knochel and Jeppesen 1990; Hanninen 1993). In human volunteer experiments, doses of 10^{10} failed to produce diarrhoea, and many subjects did not excrete the organism. However, the pathogenic traits of the inoculum were not clearly defined. The frequent occurrence of these organisms in both food and water, together with the apparent high infectious dose and the lack of any clearly identified foodborne or waterborne outbreaks, suggests that the role of *Aeromonas* in diarrhoeal disease is minimal.

4.5.2 *Pseudomonas*

There have been no reported studies demonstrating a link between consumption of potable water and enteric disease. *P. aeruginosa* is frequently found in drinking-water, where it is considered to be a nuisance organism rather than a pathogen. It has been reported in up to 3% of drinking-water samples at a concentration of up to 2300 cfu/ml (Allen and Geldreich 1975). In human volunteer studies, an oral dose of 10^6 cfu/ml was required to colonize the gut, but none of the volunteers experienced any disease symptoms (Buck and Cooke 1969). In view of the widespread incidence of *P. aeruginosa* in water and foods and the apparent lack of gastrointestinal disease linked to the organism, it would appear that the presence of this organism in potable water does not pose a threat to human health in the population at large through the causation of diarrhoeal disease.

4.5.3 *Mycobacterium avium* complex (MAC)

While the most clinically prevalent member of the genus *Mycobacterium*, *M. tuberculosis*, is not thought to be transmitted by water, *M. avium* complex (MAC) has been shown to be found growing in water systems. MAC has been isolated from many natural water systems (Kazda 1973; George *et al.* 1980; Biondi *et al*. 1982), together with many other species. However, the incidence of MAC in treated drinking-water is much lower, although it can be found more commonly in water systems in large buildings such as hospitals. In a study in Los Angeles, California, USA (Glover *et al*. 1994), MAC was found in 9% of samples taken from private dwellings but in 70% of samples taken from hospitals. Another study (du Moulin *et al*. 1988) suggested that the increased temperature of hospital water supplies favoured the proliferation of MAC.

There is no doubt that MAC causes serious disease in immunocompromised patients and that there is an association between the presence of MAC in hospital water supplies and human disease. However, the role of MAC in causing disease in the general community is much more tenuous. Further work is required to determine the link between MAC and Crohn's disease, although it seems unlikely that infection with MAC is the only predisposing factor. [Editors' note: Because of the wide interest in the potential public health significance of some non-tuberculous mycobacteria in water, including MAC, this is the theme of a separate book in the same series as this volume.]

4.5.4 Other microorganisms

There are many other opportunistic pathogens that can be found in water supplies and that may cause disease in the community. However, the incidence

of infection with these organisms is generally low, certainly with regard to gastrointestinal disease. One other organism perhaps merits mention, *Legionella pneumoniae.* This organism causes a specific type of pneumonia known as Legionnaires' disease. While sporadic cases do occur, the majority of cases are linked to large buildings such as hospitals and hotels and to the presence of the organism in cooling towers. The organism can be found in potable water supplies but predominates within poorly maintained hot water systems, where it is able to out-compete other organisms because of its ability to grow at elevated temperatures. It is not thought to cause gastrointestinal disease. A WHO publication on *Legionella* and the prevention of legionellosis will be published next year (WHO, in revision).

4.6 REGROWTH OF BACTERIA IN POINT-OF-USE AND POINT-OF-ENTRY DEVICES

As mentioned previously, there are many factors that can influence the growth of bacteria in aquatic environments. The use of point-of-use (POU) devices such as carbon filters and water softeners has increased substantially, and there has been considerable debate over the growth of bacteria in such devices and the potential health hazard that such growth presents. There is no doubt that the installation of a POU device presents an opportunity for regrowth of the bacteria present in the influent water. This has been demonstrated in many studies (Brewer and Carmichael 1979; Camper *et al*. 1985; Reasoner *et al*. 1987; Rollinger and Dott 1987). The important question with regard to this regrowth is with respect to the potential role of the bacteria in human disease.

Not surprisingly, the range of bacteria found in POU devices is as extensive as the range of organisms found in potable water, although when the microbiological flora from these devices is examined, the numbers of different species is often small, with one or two organisms predominating (Geldreich *et al*. 1985; Rollinger and Dott 1987). The predominant organism within a given POU device changes with time and reflects the flora of the incoming water as well as the characteristics of the water. When POU devices are used on microbially safe drinking-water supplies, then the growth of organisms should not result in the presence of frank pathogens, although the number of opportunistic pathogens may increase. There remains debate as to whether the growth of these organisms represents a potential threat to human health. Organisms that have been recovered from POU devices include *Aeromonas*, *Pseudomonas*, *Acinetobacter*, *Flavobacterium* and many others. All of these organisms have been suggested as being opportunistic pathogens when present

in drinking-water. However, there is no credible evidence that consumption of water containing these organisms has resulted in human gastrointestinal disease.

Many studies have been conducted on the growth of bacteria on POU devices; in general, all studies have shown that the number of bacteria in the effluent water increases over that found in the influent water. However, the organisms that predominate are those organisms that are adapted to growing in an aquatic environment with low temperatures and low nutrient concentrations. In a study of the colonization of POU devices, Geldreich *et al*. (1985) concluded that "Although some opportunistic pathogens grew in the carbon filters, the concentration of such organisms released in the product water did not approach the 10^6 to 10^{10} cells per dose considered to be infective for an immunocompromised consumer. However, opportunistic organisms not tested in this study may be able to colonize point of use devices and attain concentrations that could be infective." In a later study of the health effects of bacterial regrowth in POU granular activated carbon (GAC) filters, Calderon and Mood (1988) concluded that "Considering the ubiquitous nature of the organisms that colonized the POU-GAC filters, it may well be that these organisms just do not cause infections or disease in man." Despite these studies being completed over a decade ago, no new evidence has been presented that can demonstrate that regrowth of bacteria in municipal water supplies or in POU devices has led to human disease.

4.7 POTENTIAL BENEFICIAL EFFECTS OF REGROWTH

In order to colonize a POU device effectively, bacteria must be able to out-compete other organisms both spatially and with respect to the effective use of available nutrients. Organisms commonly found growing in water systems, either natural or human-made, tend to be those that have a low optimal growth temperature and are adapted to growing in low-nutrient environments. Frank human pathogens do not fulfil these criteria, as their optimum growth temperature is usually around 37 °C and they generally require complex nutrient sources for rapid growth. In studies of the growth of organisms on GAC filters, Camper *et al.* (1985) demonstrated that sterile filters could rapidly become colonized with pathogens such as *Salmonella typhimurium*, *Yersinia enterocolitica* and enterotoxigenic *E. coli*. However, when high levels of these pathogens were introduced into filters that had an established microflora originating from pathogen-free water, the organisms attached at a lower rate and persisted for much shorter periods of time, despite the fact that the levels of pathogens used were much higher than would normally be encountered in potable water, even if a failure of disinfection had occurred. This "protective"

effect was due to the competition of other bacteria that were more adapted to growth in such conditions.

4.8 THE EFFECT OF AUTO-DISINFECTION OF POU DEVICES

Some POU devices (mainly water softeners) have been designed to disinfect the ion exchange resin automatically, typically after a period of 96 h. The concept is that such disinfection will reduce the microbial load on the resin and the consequent release of organisms into the effluent. In studies performed on the microbial colonization of different water softeners (C.R. Fricker, unpublished observations), a water softener with automatic disinfection was compared with a more traditional softener that did not have the facility to disinfect. Initially, municipal tap water was allowed to run through the softeners at a rate similar to that which would be used in a domestic household. Water samples of the effluent were taken at regular intervals over periods of several weeks. Samples were examined for the presence of coliforms and *E. coli* and heterotrophic plate count (HPC). No coliforms or *E. coli* were detected in the influent or effluent water. HPC levels increased over time, and this increase was most noticeable when there was no flow (i.e., overnight); however, when the flow commenced in the morning, the numbers of bacteria fell. Regeneration of the traditional softener caused a further reduction in the levels of HPC detected. Disinfection of the other softener resulted in a steep decline in the numbers of bacteria detected in the effluent to levels similar to those in the incoming water. However, the levels detected climbed quickly after disinfection, reaching levels similar to those seen with the traditional softener.

Both softeners were disinfected and fed with influent water contaminated with 10% sewage (a level of bacteria that would be extremely unlikely to be encountered in a potable supply) and allowed to stand overnight. The softeners were then allowed to function normally, and the levels of *E. coli* present were monitored. Because the traditional softeners regenerate more frequently than the softener with auto-disinfection, the rate of decline in *E. coli* was no different in the two types of softener. The physical action of the flowing water and the more rigorous "washing" of the resin during regeneration removed the contaminating bacteria, such that removal of the *E. coli* occurred within four days. Thus, while disinfection of the resin reduced the bacterial load significantly, overall there was no significant difference in the numbers of bacteria released by the two types of softener.

4.9 CONCLUSIONS

Bacterial regrowth, whether in a municipal distribution system, POU device or bottle of water, reflects the initial flora, the temperature, available nutrients and water characteristics. Regrowth can be managed under some circumstances, but it is almost impossible to prevent the growth of microorganisms that are adapted to the aquatic environment. In all studies published to date, the levels of bacteria that are present in the effluent water of POU devices or municipal distribution systems where regrowth is occurring are far smaller than the levels of bacteria seen in many foodstuffs. The types of bacteria present may be somewhat different, but many of the species present in water are also present in foods, and the levels of bacteria that are permissible in foods are considerably higher than those attained in water, even after passage through a POU device. The essential issue that requires clarification is whether the bacteria that are able to regrow in an aquatic environment represent a significant health threat. Despite extensive studies performed over a considerable period of time, there has not been a single reputable publication that has demonstrated that regrowth in a properly maintained water distribution system, bottle of water or POU device has resulted in human infection.

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5

Bacteria of potential health concern

N.F. Lightfoot

5.1 INTRODUCTION

Organisms detected by the heterotrophic plate count (HPC) are ubiquitous in drinking-water. In order to assess the basis of confidence in existing measurements, particularly of the HPC, we have to re-examine the current role of potential waterborne bacteria in human disease. In this chapter, we therefore consider the following groups of bacteria of concern:

- commensal bacteria;
- recognized pathogens;
- emerging bacteria; and
- bioterrorist threat agents.

The key factor in assessing the utility of the HPC will be whether it will provide a trigger for the successful investigation for these agents.

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This chapter addresses only the bacteria of concern; there are, of course, viruses and protozoa that fall into the same categories. Furthermore, *Cryptosporidium* oocysts and *Giardia* cysts and virus particles are not incorporated into biofilms permanently and of course do not go through growth cycles.

5.2 COMMENSAL BACTERIA

Many bacteria live within our gastrointestinal tract, on our bodies or in the environment with which we come into daily contact without there being any resulting disease. In these situations, the bacteria are non-pathogenic and are called commensal bacteria, which means "eating at the same table." It is recognized, however, that many of these organisms can cause infections such as wound infections or septicaemia if they are introduced into body tissues, particularly if the person is immunocompromised.

Although many genera and species of heterotrophic bacteria have been isolated from water and have been found to colonize 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 enterocolitica* to cause diarrhoeal disease.

5.2.1 *Aeromonas*

Species of *Aeromonas* are ubiquitous in the environment and commonly occur in soil, marine (Kaper *et al*. 1981) and freshwater habitats (Rhodes and 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 and 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 carbon and the degree of

stagnation. Biofilm can accumulate in the presence of chlorine at a concentration of 0.8 mg/litre.

In Swedish water distribution systems, sampling demonstrated counts of up to 300 cfu/100 ml in raw water and up to 750 cfu/100 ml in tap water samples (Kuhn *et al*. 1997). The significance of *Aeromonas* in drinking-water is not fully understood. It is recognized that, on occasions, the ingestion of *Aeromonas* spp. may lead to diarrhoeal disease, and this is associated with an enterotoxin (Janda and 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 possess other pathogenic characteristics. It is suggested that when all are present in a strain, enteric infection may result. 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, immunocompetence, previously developed immunity, exposure and infective doses of the organisms, as well as the possession of virulence factors, could affect the ability of *Aeromonas* to establish overt infection.

In the United Kingdom study of infectious intestinal disease in England, the percentage isolation rates were the same in diarrhoeal cases and in matched controls (Food Standards Agency 2000).

The absence of defined outbreaks and the low levels of infectivity in human volunteer experiments suggest 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 der Kooij 1988), and the health authorities in the Netherlands have defined maximum values for *Aeromonas* present in drinking-water: i.e., 200 cfu/100 ml in water distribution systems and 20 cfu/100 ml in water leaving the production plant. However, there have not been any outbreaks of disease in the United Kingdom, even though blooms of *Aeromonas* occur in some distribution systems during the summer months.

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, 9 out of 10 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* colonization of distribution systems (Havelaar *et al*. 1992; Moyer *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.

Additional information on *Aeromonas* may be found in WHO (2002).

5.2.2 *Yersinia*

Yersinia is a genus of heterotrophic bacteria with 11 recognized species, some of which cause disease in humans, 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 organism is the environment or non-human hosts, such as wild animals and birds. However, only certain serotypes of *Yersinia enterocolitica* 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 O:3, O:4,32, O:5,27, O:6,30, O:6,31, O:8, O:9 and O:21 are thought to be pathogenic for humans and cause diarrhoea or 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 enterocolitica* associated with human infection is serotype O:3. The significance of *Yersinia* in patients with diarrhoea is uncertain, however; on occasion, it can cause mesenteric adenitis and reactive arthritis 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.

5.2.3 *Klebsiella*

A study of *Klebsiella* species isolated from water in Germany identified so-called virulence factors such as pili, serum resistance and siderophore production in isolates from surface waters and compared them with clinical isolates (Padschun *et al*. 2001). Fifty-three per cent of surface water samples were positive for *Klebsiella pneumoniae*. The surface water isolates resembled the clinical isolates in the expression of these virulence factors, and it was suggested that further studies should be carried out to determine the public health implications. *Klebsiella* is ubiquitous in nature and is a commensal organism of the gastrointestinal tract, where it does not cause disease. It may be involved in urinary tract infection, particularly in females, where it is transferred across the perineum to the urethra, and it may be involved in wound infections, particularly following bowel surgery.

5.2.4 *Pseudomonas*

There are many species of *Pseudomonas* that are widespread in the environment and commonly occurring in soil and water. They are capable of growth in low-nutrient situations and can grow in water in distribution systems if they gain access and on materials used in domestic plumbing situations. They may colonize taps and grow on surfaces, such as plastic pipes in drink vending machines. *Pseudomonas aeruginosa* is the most important species for public health considerations, although it does not cause any effects if it is ingested. It is resistant to many antibiotics and can produce serious nosocomial infections if it gains access to the body through wounds or intravenous lines. Hospital control of infection procedures that limit the use of tap water is an effective measure to prevent disease. In the community, *P. aeruginosa* may readily colonize spa pools and lead to wound infections if persons with open wounds or sores use them. Care must also be taken in the care of contact lenses and contact lens solutions to prevent contamination by *P. aeruginosa* on taps, leading to eye infections from water contact.

5.2.5 Virulence factors

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; 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 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; although haemolysis on blood agar by heterotrophs (Payment *et al*. 1993) and cytopathic effects on Y1 and renal cell overlays (Lye and Dufour 1991) have been put forward as assessment methods, they will not indicate which organisms are potential human pathogens.

Virulence factors enable bacteria to survive in hostile environments. The approach to a better understanding of them should be to identify disease states where the organisms are involved through epidemiological studies, investigate the pathogenic mechanisms in detail, examine the host responses and then look at possible transmission routes, appropriate interventions and protection of the public.

5.2.6 Hospital-acquired infection

Many different types of heterotrophic bacteria occur in hospital distribution systems, and counts may increase because of stagnation caused by the many "dead ends" that result from previously modified systems. All wet areas in wards, such as sluices, showers and baths, become colonized 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; however, in clinical areas or in special situations such as home care, where water is used to provide humidification in incubators, nebulizers and ventilators, the use of tap water has led to respiratory tract colonization and infections. These problems have been largely eliminated by policies for infection control. It has been recognized 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 sterilized or boiled 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 heterotrophic bacteria present pose no risk unless the patient is significantly immunocompromised, in which case boiled water is recommended.

5.3 RECOGNIZED WATERBORNE PATHOGENS

Many of the organisms that cause gastroenteritis can be transmitted by the waterborne route when there is faecal contamination from humans or other animals. The human pathogens that can be transmitted orally via drinking-water are listed in Table 5.1 (compiled from data provided by the Communicable Disease Surveillance Centre), together with a summary of their health significance and main properties.

Table 5.1. Orally transmitted waterborne pathogens and their significance in water supplies

Pathogen	Health significance	Persistence in water supplies[1]	Resistance to chlorine[2]	Relative infective dose[3]	Important animal reservoir
Bacteria					
Campylobacter jejuni, *C. coli*	High	Moderate	Low	Moderate	Yes
Pathogenic *Escherichia coli*	High	Moderate	Low	High	Yes

Pathogen	Health significance	Persistence in water supplies[1]	Resistance to chlorine[2]	Relative infective dose[3]	Important animal reservoir
Salmonella typhi	High	Moderate	Low	High[4]	No
Other salmonellae	High	Long	Low	High	Yes
Shigella spp.	High	Short	Low	Moderate	No
Vibrio cholerae	High	Short	Low	High	No
Yersinia enterocolitica	High	Long	Low	High (?)	No
Pseudomonas aeruginosa[5]	Moderate	May multiply	Moderate	High (?)	No
Aeromonas spp.	Moderate	May multiply	Low	High (?)	No
Viruses					
Adenoviruses	High	?	Moderate	Low	No
Enteroviruses	High	Long	Moderate	Low	No
Hepatitis A	High	?	Moderate	Low	No
Enterically transmitted non-A non-B hepatitis, hepatitis E	High	?	?	Low	No
Norwalk virus	High	?	?	Low	No
Rotavirus	High	?	?	Moderate	No (?)
Small round viruses	Moderate	?	?	Low (?)	No
Protozoa					
Entamoeba histolytica	High	Moderate	High	Low	No
Giardia intestinalis	High	Moderate	High	Low	Yes
Cryptosporidium parvum	High	Long	High	Low	Yes
Helminths					
Dracunculus medinensis	High	Moderate	Moderate	Low	Yes

? - not known or uncertain

[1] Detection period for infective stage in water at 20 °C: short, up to one week; moderate, one week to one month; long, over one month.

[2] When the infective stage is freely suspended in water treated at conventional doses and contact times. Resistance moderate, agent may not be completely destroyed.

[3] Dose required to cause infection in 50% of health adult volunteers: may be as little as one infective unit for some viruses.

[4] From experiments with human volunteers.

[5] Main route of infections is by skin contact, but can infect immunosuppressed or cancer patients orally.

The number of recognized outbreaks is low compared with reports of outbreaks due to other routes of transmission. Details of outbreaks of these potential waterborne pathogens in the United Kingdom are given in Table 5.2.

Table 5.2. Outbreaks of bacterial pathogens associated with infectious intestinal disease in the United Kingdom

Causative agent	Incubation period	Duration of symptoms	Laboratory reports, UK, 2001	Waterborne outbreaks, UK, 1991–2000
Campylobacter spp.	2–5 days	4–6 days	56 420	20
E. coli (enteropathogenic)	12–72 h	<2 weeks	n.a.	
E. coli (enterotoxigenic)	12–72 h	3–5 days	n.a.	
E. coli (verocytotoxigenic)	1–6 days	4–6 days	768	6
Salmonellas (non-enteric fever)	12–72 h	<3 weeks	16 465	1
Salmonellas (typhi, paratyphi)	1–3 weeks	10–14 days	17*	0
Shigella spp.	1–7 days	<2 weeks	966*	0
Vibrio cholerae (O1, O139)	2–3 days	<7 days	30*	0
Vibrio spp. (not *V. cholerae* O1, O139)	12–18 h	<7 days	n.a.	0

* Provisional figures.
Source: Communicable Disease Reports, Public Health Laboratory Service.

5.3.1 *Campylobacter*

Campylobacter are the most common cause of human bacterial gastroenteritis in the United Kingdom, with *Campylobacter jejuni* being the predominantly isolated species. They are widespread in the environment and occur very commonly in the intestinal tracts of animals, including birds. Ninety-five per cent of ready-prepared chickens are contaminated with *Campylobacter*, and poultry meat is thought to be an important source of infection. Wild birds also have a high intestinal colonization rate. *Campylobacter* can easily be isolated from surface waters, and a number of outbreaks in the United Kingdom have been associated with private water supplies. An outbreak of gastroenteritis

associated with contamination of a public water supply occurred in Wales in September 2000. Two hundred and eighty-one people out of a population of 1215 served by the supply developed gastroenteritis following an incident of influx of surface water into a holding tank for treated water. Fifteen of the cases were positive for *Campylobacter*, but it was not isolated from the water; indicator organisms were detected.

5.3.2 *Escherichia coli*

Most *E. coli* are not pathogenic and are part of the normal human bowel flora. Some types possess virulence factors and cause gastroenteritis in humans by several different mechanisms. Seven such groups have been defined, of which three may be waterborne (Food Standards Agency 2000):

- Enteropathogenic *E. coli* have been associated with outbreaks in children in nurseries and hospital wards. These strains belong to particular "O" serotypes.
- Enterotoxigenic *E. coli* are a common cause of diarrhoea in travellers. They are identified by the production of a heat-stable toxin and a heat-labile toxin.
- Verocytotoxigenic *E. coli* (VTEC) cause serious diarrhoeal disease, with bloody diarrhoea and painful abdominal cramps. In 10–15% of cases, haemolytic uraemic syndrome develops as a complication, which can result in kidney failure or even death. The most frequent serotype isolated is O157, but other serotypes, such as O139, have been reported. The organism is common in cattle and sheep and other farm animals, in which it behaves as a commensal organism and does not cause any recognized disease. The infectious dose for VTEC is very low, about 10–100 organisms, which explains their potential to cause waterborne outbreaks when animal faeces-contaminated material gains access to water supplies past treatment or where treatment is inadequate.

In North America, there have been two outbreaks of waterborne VTEC gastroenteritis. In August 1999 at the Washington County Fair in New York State, USA, contaminated well water infected over 1000 people and resulted in two deaths (Anonymous 1999). In May 2000, a waterborne outbreak occurred in Ontario, Canada, where 1286 people were infected. Six people died, and 65 patients were admitted to hospital. The source of the contamination was manure

runoff accelerated by high-density farming and flooding, which probably had occurred over two months (Anonymous 2000).

Indicator organism tests will indicate the potential for the presence and survival of pathogenic *E. coli* in water, but it should be remembered that conventional analytical methods may not detect VTEC, as they do not all grow at 44 °C. Limitations in indicator organism detection systems highlight the need for water safety management. Fortunately, these organisms are highly susceptible to water disinfection techniques.

5.3.3 *Salmonella*

The salmonellas cause two distinct types of disease. One group of two species, *Salmonella typhi* and *Salmonella paratyphi*, is the cause of the enteric fevers, typhoid and paratyphoid. The other group, consisting of over 2000 serotypes of what is now considered to be one species, *Salmonella enterica*, causes gastroenteritis. These serovars were previously considered to be separate species and were named after the city or animal from which the organism was initially isolated. Transmission of salmonellas is by the faecal oral route and often involves food and sometimes water. The enteric fever salmonellas are associated only with humans and human disease and remain important causes of waterborne disease worldwide, but nowadays very rarely in developed countries. The gastroenteritis salmonellas are widespread in animals and are often found in poultry, eggs and meat products. Food is the major vehicle of infection, but transmission via water does occur, even though the bacteria survive for only a few hours or days in surface water. Normal water treatment processes are adequate to remove the organism from drinking-water. The organisms are susceptible to chlorine disinfection. The infectious dose for humans for the enteric fever salmonellas is about 10^2–10^3 organisms, whereas the infectious dose for humans for the gastroenteritis salmonellas is about 10^6–10^8 organisms, mainly because of their susceptibility to gastric acid.

The enteric fevers are systemic infections presenting with high fever (40–41 °C), headache, malaise and rigors. Diarrhoea does not usually occur, and patients often experience constipation in early enteric fever.

A massive epidemic of typhoid fever occurred in Tajikistan in 1997, resulting in 8901 cases and 95 deaths (Mermin *et al.* 1999). Investigations revealed inadequate treatment of faecally contaminated water, and tap water samples showed a mean faecal coliform level of 175 cfu/100 ml. The outbreak was controlled after installing coagulation and chlorination at the water treatment plants. Tank water contaminated with *Salmonella enterica* serovar Saintpaul caused an outbreak of 28 cases among 200 workers on a construction site in 1999 (Taylor *et al.* 2000). The contamination was believed to have been caused

by frogs or mice. The HPC would have indicated a developing problem and could have triggered further investigation.

5.3.4 *Shigella*

Species of *Shigella* are the causative organisms of dysentery and are almost entirely human pathogens; no other animal species play a role in maintenance or spread of infection in the community. Occasionally, higher primates become infected by human-to-animal transmission. Shigellas are transmitted by the faecal–oral route and sometimes, because the infectious dose is low, around 10^2 organisms, by person-to-person spread. Patients excrete large numbers of organisms, between 10^5 and 10^8 per gram of faeces. Point source outbreaks associated with infected food handlers are reported from time to time (Jewell *et al*. 1993). Occasionally, waterborne outbreaks occur, although the organism does not survive for more than a few hours or days in surface water, and normal water treatment processes are adequate to remove it from drinking-water.

Shigella sonnei caused a waterborne outbreak of gastroenteritis in Ioannina in Greece in 2000, affecting 288 persons in a community of 2213. The organism was isolated from tap water as well as patients (Alamanos *et al*. 2000).

5.3.5 *Vibrio*

The *Vibrio* genus is composed of over 30 species, of which the most important is *V. cholerae*, the cause of epidemic cholera, a predominantly waterborne infection. The species *V. cholerae* is subdivided into 140 O-serovars, of which the toxin-producing strains are O1 and O139. The epidemiological picture of cholera has changed and now has a wide distribution. The seventh pandemic that began in 1961 was caused by El Tor strains; it appeared in Peru in 1991, having been absent from South America for some considerable time. Within a year, it had spread to all other countries of South and Central America. The O139 strain appeared in Bangladesh in 1992, where it still persists. In 1998, it was isolated from 3.4% of patients with acute secretory diarrhoea admitted to hospital in Calcutta.

The O1 strain continues to occur in about 19.7% of patients (Basu *et al*. 2000). Cholera is a disease of humans, and approximately 5% of patients become carriers. The organism survives well in the environment, and viable but non-culturable organisms have been described (Colwell and Huq 1994). There is quite clearly potential for further epidemic spread.

Other *Vibrio* species, particularly *Vibrio parahaemolyticus*, have been associated with diarrhoea, often through the consumption of raw, contaminated

seafood. Vibrios are removed from raw waters by chlorination and normal water treatment processes.

5.4 EMERGING PATHOGENS

Although many of the established waterborne pathogens have been controlled by sanitation measures and water treatment processes, new diseases continue to be identified, and new discoveries present a better understanding of existing chronic diseases. Many of these discoveries raise questions about possible waterborne transmission. The bacteria that now need to be considered in this developing area are *Helicobacter pylori*, *Mycobacterium* species, *Burkholderia pseudomallei* and *Francisella tularensis*.

5.4.1 *Helicobacter pylori*

Although spiral-shaped organisms have been observed in the stomachs of humans for many years, it was not until 1982 that a *Campylobacter*-like organism was isolated from patients with gastritis and a causative relationship between a new species, *Helicobacter pylori*, and gastric disease realized (Warren and Marshall 1983). *H. pylori* is a pathogen of global proportions and is generally accepted as the cause of most gastric and peptic ulcers. These diseases may lead to gastric adenocarcinoma.

H. pylori occurs worldwide in developing and developed countries. Where low degrees of hygiene and socioeconomic problems exist, infection rates may approach 100%. In developed countries, infection rates are probably between 30 and 60%. The verification code for this document is 137262

Transmission from person to person is not fully understood, mainly because of the difficulty in culturing the organism and identifying it outside the body. Epidemiological studies show the cluster phenomenon of *H. pylori* infection in families. It is suggested that infected mothers may play a key role in transmission within families (Rothenbacher *et al*. 1999).

H. pylori has been identified in faeces, and it is assumed that transmission is therefore oral–oral or faecal–oral.

The organism has not been isolated from the environment or from drinking-water, and waterborne transmission remains a possibility that should be investigated. The epidemiology, however, points to person-to-person transmission in early life.