review

Microbiological standards for water and their relationship to health risk

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Summary: *Maintenance of the microbiological quality of water has been used as an important means of preventing waterborne disease throughout the twentieth century. The commonest microbiological tests done on water are for coliforms and* Escherichia coli *(or faecal coliform). This paper reviews the legislative and other guidance for microbial standards in drinking and bathing waters and considers evidence for the relationship between the microbiological quality of water and risk to human health. In the past measures of the microbiological quality of water correlated well with risks of acquiring gastrointestinal disease. More recent work suggests that gastrointestinal disease is more strongly associated with the presence of enterococci than of* E. coli. *New diseases such as cryptosporidiosis have been shown to cause outbreaks of waterborne disease when levels of conventional microbiological parameters are satisfactory. In response to this, and because of failure of prosecution in one outbreak, the United Kingdon (UK) Government has introduced new legislation that requires water providers to perform a risk assessment on their water treatment facilities and to implement continuous monitoring for cryptosporidium. A new European directive on drinking water has been introduced and legislation on cryptosporidium in drinking water has been proposed in the UK.*

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Introduction

The microbiological examination of water is used worldwide to monitor and control the quality and safety of various types of water. These include potable waters (water intended for drinking or use in food preparation), treated recreational waters (swimming pools, spa pools, and hydrotherapy pools), and untreated waters used for recreational purposes such as sea, river, and lake water.

Microbiological examination of water samples is usually undertaken to ensure that the water is safe to drink or bathe in. Many potential pathogens could be associated with water; it is thus impractical to screen samples for all possible pathogens. Instead, various indicator organisms have been used as surrogate markers of risk. Most waterborne disease is related to faecal pollution of water sources[1], therefore water microbiology is largely based on the need to identify indicators of faecal pollution such as coliforms and *E. coli*, but the use of enterococci and *Clostridium perfringens* is increasing. In addition the less specific term ‘faecal coliforms’ (which includes species of *Klebsiella*, *Enterobacter*, and *Citrobacter*) is used in recreational water testing because the examination of large numbers of colonies to identify *E. coli* is labour intensive.

Various standard and guideline values have been introduced over the years. Many of these have become legally enforceable, while others have been recommended by appropriate bodies and trade associations. This paper describes the standards that are currently law in the United Kingdom (UK), and guidelines that represent the best advice currently available.

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Water intended for drinking

All water intended for human consumption in the UK is governed by legislation based on European Union (EU) council directives (table 1)[2,3]. With the exception of natural mineral waters all waters are covered by directive 80/778/EEC[2], replaced by directive 98/83/EEC[3] that came into force in December 1998 and has

TABLE 1 **Microbiological guidelines and standards for drinking water**

Type of water	Parameters	Permitted concentration or value	Guide level	Imperative level
Mains water[9] (continuous sampling recommended)	coliforms / *E. coli* Cryptosporidium oocysts*	0/100mL		 < 10/100L
Private supplies[10] Classes A to E and 1 to 4	coliforms / *E. coli* colony counts at 22˚C and 37˚C	0/100mL	No significant increase over normal levels	
Class F	coliforms / *E. coli*		No organism detrimental to public health	
Natural mineral waters sampled any time up to sale[14]	coliforms/*E. coli* enterococci *Pseudomonas aeruginosa* sulphite reducing clostridia parasites/pathogens	0/250mL 0/250mL 0/250mL 0/50mL Absent		
within 12 h of bottling[14]	As above plus colony count 22˚C/72h colony count 37˚C/48h	 100/mL 20/mL		
Drinking water in containers at any time[14]	coliforms/*E.coli* enterococci *Pseudomonas aeruginosa* sulphite reducing clostridia colony counts at 22˚C and 37˚C	0/100mL 0/100mL 0/100mL 0/20mL Should show no appreciable increase after bottling		
within 12 h of bottling[14]	As above plus colony count 22˚C / 72h colony count 37˚C / 48h presumptive coliforms	 100/mL 20/mL 0/100mL		
Vending machines[7]	*E. coli* *Pseudomonas aeruginosa* colony count 22˚C / 72h colony count 37˚C / 24h	0/100mL 0/100mL ≤10 000/mL ≤1 000/mL†		
Meat premises[18,19] routine sampling	coliforms / *E. coli* colony count 22˚C / 72h colony count 37˚C / 48h	0/100mL ≤100/mL ≤10/mL		
if coliforms found:	resample as for routine plus test for: enterococci sulphite reducing clostridia	 0/100mL 0/20mL		

* applies to treatment works shown by risk assessment to be potentially contaminated
† provided that colony counts are no more than 10 times greater than those in water entering the machine

to be enacted into member state law within two years. The introduction of new legislation in the UK directed specifically at cryptosporidium[4] has created the precedent of using the detection of a pathogen, rather than an indicator organism, as an indicator of potable water quality.

Current legislation relies on testing for total coliforms, faecal coliforms (*E. coli*), and total colony counts. The faecal coliform test was developed as a marker of faecal pollution when *Salmonella typhi* was the commonest known cause of waterborne outbreaks[4a]. Typhoid fever's virtual disappearance from the western world is a testament to its success. A French study identified higher rates of illness among inhabitants of villages whose drinking water failed European directive standards than among inhabitants of villages whose drinking water satisfied the standards[5]. The marker most closely associated with illness was the enterococci count, although faecal coliforms were also independently associated with illness. Total coliforms and total counts were not independently associated with illness.

The recent history of waterborne outbreaks in the UK and United States (US) has highlighted the role of pathogens that are less susceptible to chlorination than most bacterial pathogens. In particular, many outbreaks of cryptosporidiosis occur in water supplies that have not failed coliform testing[1]. Several prospective studies have found a small but statistically significant increase in gastrointestinal illness in populations who drink water that apparently complies with the coliform standard[6-8]. Temporal associations have been found between the incidence of cryptosporidiosis or gastrointestinal infections in communities and turbidity of the drinking water[9,10].

Public supplies

Public supplies are provided by water undertakers via mains distribution system and are usually subject to various treatment processes such as coagulation, filtration, and chlorination. The EU council directive of 1980 on water intended for human consumption included microbiological parameters for total coliforms, faecal coliforms, enterococci, sulphite reducing anaerobes, and colony counts at 22˚C and 37˚C[2]. It was enacted into UK law in the *Water Supply (Water Quality) Regulations 1989*[11]. UK legislation regards *E. coli* as synonymous with faecal coliforms and does not give numerical values for colony counts[11]. Baseline colony counts should be established for each supply and any increases should be investigated. The water undertaker is responsible for the collection and analysis of samples taken within the framework of the legislation. Samples are taken from points before supply and randomly from taps in consumers' premises. Permitted concentrations or values (PCV) are given for 55 parameters, most of which are chemical. Water is considered to be unwholesome if one or more PCVs are exceeded although there may be no significant risk to health. Water is considered to be unfit if there is either a significant risk to health or the water is offensive to drink. The definition of unfit is determined by medical judgement or legal precedent and the supply of such water is a criminal offence.

Water supplied to consumers' premises is tested routinely by the water undertaker for coliforms and *E. coli*. Coliforms must not be detected in 95% of samples when more than 50 samples are taken from the same sampling point during a one year period. The detection of *E. coli* in any one sample constitutes an infringement of the regulations.

Local authorities do not sample water supplied to consumers' premises under UK legislation but may become involved in the analysis of samples in connection with monitoring programmes or of samples submitted in connection with complaints of quality or alleged illness. Care should be taken when interpreting results of such samples.

A new European directive for drinking water was recently published[3] and should be incorporated into UK legislation within the next year or so. This directive covers water in containers more explicitly than its predecessor[2]. It proposes that water should be monitored on a check basis and an audit basis. Check monitoring of water intended for human consumption would be performed regularly to provide information on the microbiological and aesthetic quality, the effectiveness of drinking water treatment, and compliance with relevant parametric values. The microbiological parameters of water supplies are coliforms and *E. coli*, standards for which remain unchanged. In addition, if the supply originates from or is influenced by surface water, then *Clostridium perfringens* (including spores) must be counted. The new standard for *C. perfringens* differs from that in the old directive in that the organism must be absent in 100 mL, rather than in 20mL. The exact nature of the testing regimen to be enacted into UK law has not yet been published. In addition to the conventional microbiological parameters, recent UK legislation requires continuous monitoring of 'at risk' water treatment works for cryptosporidial oocysts[4]. All water treatment works in England and Wales have to undergo a risk assessment, and at risk sites must be monitored continuously over a 24 hour (h) period to count cryptosporidial oocysts in 1000L of water. The UK legislation will create a new criminal offence, that of supplying water containing >10 cryptosporidial oocysts / 100L. At least 1000L of water will need to be filtered within each 24h.

Private supplies

About 1% of the UK population obtains water from a private supply, one not managed by a water company. Such private supplies may originate from a well, borehole, or spring and may include no treatment. Private supplies may range in size from a spring that supplies an isolated dwelling to a borehole that supplies a hospital or food manufacturing plant. The quality of water from private supplies must comply with the parameters given in the *Private Water Supplies Regulations 1991*[12]. The local authority is responsible for monitoring private supplies. Although only a small proportion of the population relies on private domestic supplies, outbreaks associated with private supplies are almost as common as outbreaks associated with mains supplies[1]. Furthermore, the organisms that cause outbreaks in private supplies are more varied and include bacterial pathogens that are relatively sensitive to chlorine.

Private supplies are primarily divided into category 1 supplies (used only for domestic drinking, cooking, and washing) and category 2 supplies (include supplies to nursing homes, hospitals, other institutions, and premises where food and drink are prepared for retail sale). Category 1 supplies are further subdivided into class A to F supplies, depending on the amount of water and number of people supplied. For example, a class A supply will supply more than 5000 people and provide more than 1000m^3 of water each day whereas a class E supply supplies less than 5m^3 each day to fewer than 25 people. A class F supply is a category 1 supply that serves a single dwelling. Similarly, category 2 supplies are subdivided into classes 1 to 4 depending on the amount of water supplied: a class 1 supply provides more than 1000m^3 each day and a class 4 supply provides 2 to 20m^3 a day.

Private water supplies are tested routinely for coliforms and *E. coli*, which must not be detected in 100 mL samples taken from supplies of any category or class apart from category 1 class F. Neither microbiological standards nor sampling frequencies are laid down in the legislation for class F supplies, but all private supplies (including class F) are required not to contain any 'element, organism or substance (other than a parameter)' that 'would be detrimental to public health'. In addition, class A to E and class 1 to 4 supplies should be examined for colony counts at 22˚C and 37˚C at the frequencies given in the relevant regulations[2]. The results of colony counts are interpreted in the same manner as for mains supplies.

The monitoring of private supplies is problematic as water quality can change with the weather, and smaller supplies are monitored infrequently. Some local authorities have large numbers of private supplies in their areas, which generates considerable work.

Drinking water in containers

An increasing amount of water is being sold in bottles and other containers such as water dispensers in offices[13]. With the exception of natural mineral waters, drinking water sold in containers is covered by the same European directives that cover private and public water supplies[2,3]. Microbiological standards for bottled water other than mineral water have their own UK regulations, which were updated in 1999[14]. Spring water comes from a single underground source. Table water comes either from more than one source, which may or may not be an underground supply (which could be a public supply). Both are regulated by the *Drinking Water in Containers Regulations*[14]. The standards for indicator organisms, other than colony counts, apply up to the point of sale. The standard for total colony counts applies only within 12 h of bottling. Under the new regulations water offered for sale in bottles or containers will have to undergo check sampling, which will include testing for coliforms and *E. coli*, *Pseudomonas aeruginosa*, and colony counts at 22˚C and 37˚C. The unit volume analysed for bottled waters will be 250 mL for *E. coli*, enterococci, and *P. aeruginosa.*

Natural mineral water

Natural mineral water is defined by European directive as 'microbiologically wholesome water... originating in an underground water table or deposit and emerging from a spring tapped at one or more natural or borehole exits'[15]. To be classified as natural mineral water, the source has be recognised as such by the appropriate authority, in the UK the local environmental health department[14]. The water must not be treated in any manner to alter the chemical and microbiological composition. The microbiology of bottled natural mineral waters has been reviewed elsewhere[16]. Reports of outbreaks associated with bottled natural mineral water are rare and have been associated with failure to satisfy statutory standards[17]. The microbiological analyses required to comply with the *Natural Mineral Water Regulations* are similar to those required under the water in containers regulations described above, except that sample volumes for most parameters are greater (250 mL)[14]. As above, colony count standards apply to water sampled within 12 h. After 12 h the colony count should be no more than that which results from the normal increase in the bacterial content of the water at source.

Vending machines

The microbiology of vending machines is discussed in detail elsewhere[18]. Very few outbreaks have been traced back to vending machines. The quality of water from vending machines depends on the quality of the water supply and on the design and maintenance of the machine. The water supply to the machine must comply with the standards for water supplied to customers' premises as discussed above. A recurrent issue is the high colony counts found in many drinks vending machines[13,18,18a]. It is to be expected that colony counts in water from vending machines will be higher than in the mains but this does not, in itself, pose a health risk. The Automatic Vending Association of Britain has produced detailed guidelines for the microbial quality of drinks vending machines (table 1)[19]. These guidelines differ from those published some ten years earlier[19a] in that *P. aeruginosa* is no longer included.

Meat handling premises

The quality of private water supplies for abattoirs, meat cutting premises, and cold stores is monitored by local authorities[20,21]. If public supplies are used, water in the relevant supply zone is monitored by the water undertaker on a random or semi-random basis. The operator is responsible for the quality of the water once it enters the premises and the meat hygiene service is responsible through the official veterinary surgeon (OVS) for ensuring compliance with the regulations. Sampling is arranged by the operator or by the OVS and samples may be analysed by public health laboratories.

Samples from premises on a public supply should be tested at least once a year, preferably quarterly or monthly. Samples from premises on private supplies and on public supplies with intermediate storage (water taken from the mains and stored on site before use) should be tested once a month for the parameters shown in table 1. Re-sampling and testing for a wider range of parameters is necessary if coliforms are detected.

Recreational waters

As with drinking water, microbiological analysis of recreational water is aimed largely at detecting markers of faecal pollution. Colony counts play a rather greater role as a marker of general water quality, however, and tests for *P. aeruginosa* are often carried out. *P. aeruginosa* is associated with disease, usually otitis externa or folliculitis, in people who have used swimming pools and spa pools[22]. The microbiological quality of water in swimming baths, spa pools, and hydrotherapy pools is not governed directly by legislation, but the pool manager is required, under the *Health and Safety Act 1974,* to ensure the health and safety of employees and others who use the pool[23]. Suggested levels for satisfactory operation and for intervention have been published (table 2)[24-26]. The 'target level' as used in this article is the count that should be obtained under satisfactory conditions of operation and the 'guide level' is the count above which corrective action must be taken.

For swimming pools a count of <10 coliforms per 100 mL is acceptable if the colony count is <10 / mL, *E.coli is* not detected, and the pH and levels of disinfectant are satisfactory[24]. Coliforms should not be detected in consecutive samples. Colony counts of 10 to 100 / mL are acceptable if coliforms are not detected. Investigations must be performed if raised colony counts (>10 / mL) persist.

The treatment system must be checked if the colony count in water from a spa pool exceeds 100 / mL[25]. If coliforms, *E. coli*, or *P. aeruginosa* are detected in a 100 mL sample then the pool must be drained and checked. Pseudomonal folliculitis is a particular problem associated with spa pools and strengthens the need for regular microbiological monitoring.

If the colony count in a hydrotherapy pool approaches 100 / mL then the dominant organisms present on the colony count plate must be identified to determine the presence of staphylococci, *P. aeruginosa*, and *E. coli*[26]. The presence of *P. aeruginosa* in association with a colony count 100 / mL or the presence of *E. coli* indicates poor pool management. For routine purposes it is advisable to test for the presence of *P. aeruginosa* in a 100 mL sample.

Bathing beach water

A bathing beach is defined as a beach where large numbers of people are known to bathe[27]. The regulations apply both to coastal and inland waters. Epidemiological research on the effects on health of swimming at bathing beaches has shown that swimming in bathing beaches carries some risk of illness even when the beach complies with existing legislative standards[1,28-31]. The risk to health increases in proportion to the amount of faecal pollution as measured by indicator organisms (figure 1), but the bacterial indicator most strongly associated with risk to health seems to be the enterococcus count[29-31].

The microbiological quality of water on designated bathing beaches must comply with the standards given in the *Bathing Waters (Classification) Regulations 1991* (table 2)[27]. Faecal coliforms are coliform organisms that grow at 44°C; they are regarded as more specific indicators of faecal contamination than total coliforms, which are counted at 37°C. Samples are taken once every two weeks throughout the bathing season (between May and September in the UK). For compliance 80% of samples should not exceed the guide level and 95% should not exceed the imperative level.

Conclusions

The coliform standard has stood the test of time as the primary indicator for health risk associated with water for drinking and bathing but appears to be beginning to show some weaknesses. The main concern is the apparent rise in the incidence of waterborne pathogens such as cryptosporidium which are relatively resistant

TABLE 2 Microbiological guidelines and standards for bathing waters

Type of water	Recommended frequency of sampling	Parameters	Guide Level	Target level	Imperative level
Swimming baths[24]	Once a month	*E. coli* coliforms *Pseudomonas aeruginosa* (optional) colony count 37°C / 24h	0/100mL ≤10/100mL 0/100mL ≤100/mL	 0/100mL* ≤10/mL	
Spa pools[25]	Once a month	coliforms / *E. coli* *Pseudomonas aeruginosa* colony count 37°C / 24 h	0/100mL 0/100mL <100/mL		
Hydrotherapy pools[26]	Twice a week	coliforms / *E. coli* colony count 37°C / 24h *Pseudomonas aeruginosa* / staphylococci	0/100 mL ≤100/mL See text	 ≤10/mL	
Bathing beach water[27]	Once in 2 weeks†	total coliforms faecal coliforms enterococci salmonella‡ enteroviruses‡	500/100mL 100/100mL 100/100mL		10000/100mL 2000/100mL 0/L 0 PFU/10L

* For swimming baths a coliform count of >0 and <10 is acceptable only if colony count at 37°C / 24 h is <10, no *E. coli* are present, and if a further sample is negative for coliforms.
† For compliance 80% of samples should not exceed the guide level and 95% should not exceed the imperative level.
‡ When there are grounds for suspecting there has been a deterioration in water quality or that the organisms are present.
PFU=plaque forming units

to chlorination and cause outbreaks even when the microbial quality of water is otherwise satisfactory. Another issue is the apparent superiority of enterococci as indicators of health risk in both drinking and bathing waters. Nevertheless, it is unlikely that the coliform standard will be replaced as the primary routine microbiological test of water in the next 20 years.

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