HEALTH SIGNIFICANCE AND OCCURRENCE OF INJURED BACTERIA IN DRINKING WATER

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ABSTRACT

Enteropathogenic and indicator bacteria become injured in drinking water with exposure to sublethal levels of various biological, chemical and physical factors. One manifestation of this injury is the inability to grow and form colonies on selective media containing surfactants. The resulting underestimation of indicator bacteria can lead to a false estimation of water potability. m-T7 medium was developed specifically for the recovery of injured coliforms (both "total" and fecal) in drinking water. The m-T7 method was used to survey operating drinking water treatment and distribution systems for the presence of injured coliforms that were undetected with currently used media. The mean recovery with m-Endo LES medium was less than 1/100 ml while it ranged between 6 and 68/100ml with m-T7 agar. The majority of samples giving positive results with m-T7 medium yielded no detectable coliforms with m-Endo LES agar. Over 95% of the coliform bacteria in these samples were injured. Laboratory experiments were also done to describe the virulence of injured waterborne pathogens. Enteropathogens including Salmonella typhimurium, Yersinia enterocolitica and Shigella spp. required up to 20 times the chlorine levels to produce the same injury in enterotoxigenic Escherichia coli (ETEC) and nonpathogenic coliforms. Similar results were seen with Y. enterocolitica exposed to copper. The recovery of ETEC was followed by delayed enterotoxin production, both in vitro and in the gut of experimental animals. This indicates that injured waterborne enteropathogenic bacteria can be virulent.

KEYWORDS

Water; indicator bacteria; coliforms; enteric pathogens; injury; virulence; recovery.

INTRODUCTION

The term allochthonous refers to bacteria that are transient and are not long-term occupants of an ecosystem. This concept accurately describes the status of most coliforms and the enteric pathogenic bacteria within aquatic systems since they reproduce in the gut and are not well adapted to chemical and physical conditions in water. As a result, it should not be surprising that these bacteria can become physiologically injured with aquatic exposure. The most prominent stressful influences in potable and
wastewater systems include disinfectants (Camper and McFeters, 1979; McFeters and Camper, 1983) and metals (Domek, 1984). A number of other stressors such as biological interactions (LeChevallier et al., 1985) have also been described.

One manifestation of injury within waterborne bacteria is an increasing sensitivity to the surface-active selective ingredients used in media to detect indicator bacteria in water (Bissonnette et al., 1975). Injured bacteria are therefore incapable of growth when accepted media are used for the determination of water potability (Bissonnette et al., 1977; LeChevallier et al., 1982; McFeters et al., 1982; LeChevallier et al., 1984 & 1985). Consequently, the use of presently employed media to enumerate indicator bacteria in water can lead to an underestimation of the actual number of viable bacteria that are present. Such data give an overly optimistic estimate of the water quality (McFeters et al., 1986) which could lead to a serious underestimation of the accompanying health threat.

ENUMERATION OF INJURED BACTERIA IN WATER

Injured bacteria from water are capable of recovering tolerance for surface-active chemicals and normal growth following incubation under suitable conditions (Bissonnette, 1977; LeChevallier and McFeters, 1984 & 1985). This observation suggests a need for improved techniques to detect injured bacteria.

The earliest efforts to devise media and methods for the enumeration of injured waterborne bacteria concentrated on fecal coliforms. Section 920 in Standard Methods for the Examination of Water and Wastewater, (APHA, 1985) describes these analytical approaches. More recently, a medium (m-T7) was formulated specifically for the detection of injured coliforms in drinking water (LeChevallier et al., 1982). In field studies m-T7 recovered 43% more verified coliforms from drinking water samples than m-Endo agar. m-T7 was later found to be superior to accepted media for the enumeration of fecal coliforms from chlorinated wastewater effluents (LeChevallier et al., 1984). Over threefold greater fecal coliform recoveries were seen with m-T7 than m-FC when disinfected wastewater effluents were tested. This application of m-T7 provided optimal enumerations when an eight hour incubation at 37°C was followed by 12 hours at 44.5°C. These findings indicate that m-T7 is an effective medium for the enumeration of injured indicator bacteria and its commercial availability (Difco) make it readily available. A number of laboratory variables including diluent composition, temperature and time of exposure, membrane filter surface pore morphology (McFeters et al., 1982) and the addition of chelators (Domek et al., 1984) can also influence the recovery of injured bacteria.

OCCURRENCE OF INJURED INDICATOR BACTERIA IN DRINKING WATER

The results of surveys to detect injured coliforms in drinking water with m-T7 medium have been published recently (McFeters et al., 1986; LeChevallier and McFeters, 1985). Although some site specific differences have been seen, the percentages of injured coliforms observed in the water of participating systems ranged from 43 to 100%. Samples analyzed included water collected immediately after conventional treatment of drinking water, during the backwash cycle, at various points in the distribution system and one week after the break and subsequent repair of a distribution main. The average counts of indicator bacteria detected using m-Endo LES was < 1.0 confirmed coliform/100 ml while m-T7 yielded 5.7 to 67.5 confirmed coliforms/100 ml. In addition, the majority of the samples giving positive results on m-T7 agar produced no detectable coliform counts on m-Endo LES agar. These findings indicate that injured bacteria can comprise the majority of coliforms found in some operating drinking water systems and that these bacteria are largely undetected when accepted analytical methods are used.
Health significance

INJURY AND VIRULENCE OF ENTEROPATHOGENIC BACTERIA IN WATER

Until very recently there has been a paucity of published information describing the injury and virulence of enteropathogenic bacteria in water since most reports have concentrated on comparative bacterial die-off kinetics. It is logical that these bacteria are influenced by stressors in water in ways that resemble the indicator bacteria since many waterborne enteric pathogens are likewise Enterobacteriaceae.

Studies were initiated in our laboratory to investigate the injury and virulence of enteropathogenic bacteria stressed in water. These studies concentrated on the effects of chlorine and copper as stressors since earlier findings indicated that they were among the most important causes of bacterial injury in drinking water (Camper and McFeters, 1979; McFeters and Camper, 1983; Domek et al., 1984). Initial results demonstrated that Yersinia enterocolitica, Salmonella typhimurium and Shigella spp. required significantly higher levels of both chlorine and copper to cause injury (>90%) than coliforms and the enterotoxigenic Escherichia coli (ETEC) strains tested (LeChevallier et al., 1985; Singh et al., 1985). These results are of significance since they revealed that the indicator bacteria are more sensitive to stressors in drinking water than common waterborne pathogens. In addition, studies of Y. enterocolitica revealed that the virulence (LD50) of injured pathogen populations was approximately 20 fold less than uninjured control cells. This finding is of potential importance since it suggests that the pathogenicity of the injured population is not completely lost although it is somewhat impaired.

Experiments were also performed to examine the recovery, growth and enterotoxin synthesis of ETEC following copper-induced injury (Singh and McFeters, 1986). Recovery of desoxycholate tolerance was demonstrated in vitro in both rich (3 hours) and minimal media (6 hours). Growth of the injured cells and the synthesis of enterotoxin were also seen both in vivo and in vitro following an extended lag. Levels of enterotoxin activity approached those of controls after the recovery of injured cells had occurred. These results indicate that the enterotoxigenic potential of ETEC was fully retained and could be expressed following copper-induced injury. This conclusion is supported by the findings of another series of in vitro experiments which demonstrated the revival, growth and pathogenicity of ETEC following both copper and chlorine injury when introduced into the gut of experimental animals (Singh et al., 1986). Collectively, the results of these experiments demonstrate that enteropathogenic bacteria stressed under conditions approximating drinking water retain some virulence determinants and that they are fully expressed after the cells recover from injury. However, the impaired ability of the injured populations for attachment, invasion or colonization of gut epithelial cells could modify the outcome of natural infections.

SIGNIFICANCE OF INJURED BACTERIA IN WATER

It is difficult to reach firm conclusions regarding the significance of injured enteric bacteria in water since the importance of uninjured coliforms and other indicator bacteria is often debated. However, it is possible to discuss this consideration from the perspective of the information presented.

Some dissatisfaction with current concepts of indicator bacteria is based upon instances where coliforms were undetected or found in very low numbers associated with outbreaks of waterborne disease. While it is clear that some such instances might be explained by diseases caused by agents such as viruses that are more persistent than the indicator bacteria, injury might also be a factor. Failure to detect injured coliforms with accepted media might mask the source(s) of the indicator bacteria and confound remedial measures by treatment plant operators and public health authorities in situations such as unexplained "coliform regrowth" occurrences. In these instances the use of media and methods that allow the detection of injured indicator bacteria would provide a greater degree of analytical sensitivity.
than currently accepted procedures. For example, in situations such as those documented in the literature where injury is greater than 90%, the use of m-T7 would result in approximately ten-fold higher coliform enumerations. This would allow the initiation of corrective action of treatment defects earlier or provide useful information in epidemiological investigations of waterborne disease outbreaks. However, it must not be overlooked that reporting higher numbers of indicator bacteria is undesirable from the regulatory standpoint although it is likely that properly designed and operated systems would experience little change in coliform recoveries.

The available information indicates that pathogens, upon aquatic exposure and injury, retain virulence properties that may be expressed and pose a potential health hazard following recovery (LeChevallier et al., 1985; Singh et al., 1985; Singh and McFeters, 1986; Singh et al., 1986). This realization provides the rationale for giving injured but viable enteropathogens the same significance as freshly cultured cells since they can recover and remain a health threat. In addition, it must be remembered that enteropathogenic bacteria are less sensitive to aquatic injury than coliforms. This finding underscores the importance of detecting injured coliforms as a valid indication of potential health hazards from enteropathogenic bacteria in water. Hence, the bacteria that are currently afforded public health significance in water, including coliforms and pathogens, are also meaningful when injured.

CONCLUSIONS
The findings of these studies indicate that 1) injury of indicator and pathogenic bacteria can occur within drinking water systems, 2) methods are available that effectively detect injured coliforms, 3) the vast majority of coliforms within these systems may be undetected because sublethal stress leads to decreased detection on conventional media, 4) pathogenic bacteria, except ETECs, also become injured but at higher levels of stressing agents, and 5) injured pathogens express virulence determinants following recovery. As a result, injured coliforms are of public health significance and their detection affords an added measure of sensitivity to assist in the early detection of treatment deficiencies or contamination within domestic potable water systems.

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