LETTER

The emergence of *Escherichia coli* resistance to repeated water disinfection

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ABSTRACT

Serious public health consequences could result from pathogen resistance to common disinfectants used in drinking water treatment, especially when potable water is reused. The reuse of potable water with frequent disinfection may encourage the emergence of resistance. This study looked at how *Escherichia coli* developed resistance to repeated monochloramine and ferrate disinfections. After 12 or more treatment rounds, *E. coli* cultures repeatedly exposed to monochloramine developed resistance, whereas repeated ferrate disinfection did not. Initial rounds of disinfection with monochloramine caused cells to become Viable But Nonculturable (VBNC); however, further monochloramine treatments increased culturability, which coincided with a reduction in the percentage of VBNC cells after disinfection. monochloramine. The development of monochloramine resistance was significantly influenced less by the frequency of treatment and more by the total number of disinfection episodes (12 times). The evolved monochloramine-stressed cultures were successfully inactivated (>3-log10) by ferrate in addition to preventing resistance, indicating that the evolutionary adaptations against monochloramine were unsuccessful against ferrate. Ferrate is a promising disinfectant that warrants more investigation due to the absence of resistance to it. The results of this study show that bacterial resistance development can be influenced by the frequency of disinfection as well as the type of disinfectant used. Therefore, ongoing monitoring is required to assess the resistance profile of harmful bacteria in order to evaluate present and future water disinfection strategies, particularly within potable water reuse.

Key Words: *Resistance; Disinfectants; Monochloramine; Nonculturable; Cult urability; Evolutionary*

INTRODUCTION

The mortality and illnesses brought on by waterborne pathogens have dramatically decreased because of advancements in the drinking water treatment sector. Disinfection specifically has improved pathogen clearance, reducing the spread of waterborne infections. However, outbreaks linked to drinking water still happen often in the US. The Centers for Disease Control and Prevention's (CDC) most recent waterborne sickness monitoring data identified 66 outbreaks linked to drinking water, accounting for at least 337 illness cases. Exposures to Legionella and Norovirus infections, respectively, are said to have been the cause of 45% and 30% of outbreaks and illnesses, respectively.

The difficulties associated with treating and disinfecting drinking water are made worse by the developing global water shortages. For instance, the public's awareness of potential health dangers makes the reuse of treated wastewater effluent—which may provide an alternative and sustainable source of drinkable water—challenging. Advanced Water Treatment (AWT), which uses a multi-barrier treatment technique, is applied to reduce potential health hazards and increase public acceptance. However, despite AWT, microbial growth has been seen in water supply systems or reservoirs. Reduced bacterial resistance to disinfection may have Therefore, it is essential to have a better understanding of the variables that affect microbial sensitivity to disinfectants in order to anticipate the efficacy of disinfection, particularly for the reuse of potable water. There are many physical, chemical, and microbiological aspects of source water that can affect how well it disinfects, including temperature, pH, the amount of organic matter, and microbial load. However, it is unknown whether circumstances pertaining to the reuse of potable water, such as regular disinfection, could encourage bacterial resistance to disinfectants. The ability of survivors from disinfected bacterial populations to repopulate and, eventually, acquire resistance to disinfection after repeated disinfections is a crucial question. In order to improve public health assessments of potable water reuse, it is necessary to ascertain the impact of repeated disinfection on the development of bacterial resistance.

Although the emergence of bacterial resistance to antibiotics has been extensively studied in the literature, little is known about the impact of frequent cleaning on bacterial resistance. In bacterial cells, chemicals can cause a variety of defence reactions, including the healing of cell damage, neutralisation of toxic components, restoration of cellular homeostasis, or targeting inactivity through decreased cell growth and metabolism. Collectively, defensive systems

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change the physiological reactions of bacteria at the phenotypic and frequently genetic level, reducing the progeny's vulnerability to chemical exposure. However, there aren't many findings in the literature about the mechanisms underlying bacterial resistance to or tolerance of disinfectants.

Additionally, because chlorine-based disinfectants are so widely used, the majority of studies have concentrated on the development of resistance against them, even if the studies' findings are inconsistent. According to Farkas-Himsley, Escherichia coli developed chlorine-resistant strains when intentionally exposed to high chlorine concentrations in buffered solutions. Following numerous exposure cycles to the disinfectant in rich growth media, Gundlach and Winter found an incremental rise in *E. coli* resistance to hypochlorous acid. It was discovered that the development of resistance was fueled by the constitutive production of OxyR-regulated genes, which improved the cells' response to oxidative stress. Meanwhile, other research has demonstrated that low levels of cyclic Adenosine Monophosphate (cAMP) and the cAMP receptor led to high resistance to hypochlorous acid (3 mM–7 mM) in *E. coli*.

Haas and Morrison, however, demonstrated that *E. coli's* repeated chlorination did not result in highly tolerant organisms. However, the protocol's intermediate sub-culturing phase in rich growth media between each chlorine treatment in this investigation might have changed the bacteria's sensitivity. Similar to this, repeated treatments of *E. coli* cultures with sodium hypochlorite did not alter their resistance to the disinfectant. The development of resistance in response to other disinfectants was the subject of fewer studies. Following 40 exposure cycles in phosphate-buffered saline, a study using fast-acting hydroxyl radicals as the active disinfectant revealed no development of resistance in *E. coli.* As a result, variations in disinfectant action and culture techniques pertinent to source water chemistry may have an impact on the mechanisms that result in the development of bacterial resistance or tolerance.