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Research progress on the injury mechanism and detection method of disinfectant-injured *Escherichia coli* in the drinking water system

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ABSTRACT

Sublethally injured bacteria can still develop into normal bacteria under favorable growth conditions, and their pathogenicity poses a great threat to human health. In the drinking water system, some bacteria cause sublethal injury under the action of disinfectants, that is, disinfectant-injured bacteria. Hence, the detection of disinfectant-injured bacteria and the elucidation of injury mechanisms are of great significance for ensuring the microbial safety of drinking water systems. This article takes the indicator bacteria *Escherichia coli* as the research object, reviews and summarizes the sublethal injury conditions, damage mechanism, and detection methods of disinfectant-injured bacteria in drinking water, and puts forward a prospect for the future research directions of drinking water disinfection and disinfectant-injured bacteria.

Key words: detection, disinfectant, *Escherichia coli*, injured bacteria, mechanism

HIGHLIGHTS

- Sublethally injured bacteria are formed during the disinfection process.
- Introduce the detection methods of disinfectant-injured bacteria.
- The injury mechanisms of chlorine- and UV-injured bacteria are described.
- It is recommended for an in-depth study of injury mechanisms, minimizing the potential harm of disinfectant-injured bacteria.

1. INTRODUCTION

Many bacteria are living in a sublethal environment; therefore, a large number of sublethally injured bacteria (including pathogenic or opportunistic bacteria) are formed. The sublethal state means that the bacterial cells are injured but not killed when they are in an unfavorable environment. Under appropriate growth conditions, the injured bacteria may resume activity, while the pathogenic bacteria may restore pathogenicity. Sublethal injury in bacteria can be induced by exposure to metals, pH, freezing, biological factors, disinfectants, etc. Bacterial injury has been shown to be associated with increased metal concentrations in some natural rivers. Domek *et al.* (1984) collected and analyzed water samples and found that sublethally injured bacteria in drinking water were significantly correlated with temperature, pH, Cu, and total organic carbon. Other heavy metals, such as Cu, Pb, and Cd, could injure *Escherichia coli* (*E. coli*) in drinking water samples. Wortman & Bissonnette (1985) demonstrated that *E. coli* might get injured under acidic conditions. Musarrat & Ahmad (1988) found that bacteria exhibited a high level of mutagenesis in an alkaline environment, thereby alleviating the injury. Collectively, these studies have shown that a variety of influencing factors in the environment can cause injury to bacteria, thus forming sublethally injured bacteria.

A variety of intestinal microorganisms are pathogenic to the human body, which can be excreted in the form of feces and directly or indirectly contaminated the water supply (Leclerc *et al.* 2002; Ashbolt 2004). It can effectively remove pathogenic microorganisms and reduce the incidence of waterborne diseases by sterilizing drinking water and protecting

water sources. Disinfection process is an important part of ensuring the microbiological safety of drinking water, and common disinfection processes include chlorine disinfection, ultraviolet disinfection, and ozone disinfection. However, some bacteria are able to resist disinfectants. Several studies (McFeters 1990; Tandon *et al.* 2007) have shown that, after exposure to different disinfectants or different dosages of the same disinfectant, some bacteria left have not been killed and remain in a sublethal state, but their physiological functions are defective. These disinfectant-injured bacteria can still reproduce under favorable growth conditions and even regain their pathogenic ability (McFeters & Lechevallier 2000; Bolster *et al.* 2005) or cooperate with other pathogens in the environment (Guo *et al.* 2015; Jin *et al.* 2020), thus posing a great threat to human health (Figure 1). As an indicator microorganism, *E. coli* can be used to characterize the microbial contamination of water sources. In this article, we mainly summarize and analyze the injury of bacteria under different disinfection conditions, the common detection methods of *E. coli* and the difference in the detection effect of injured bacteria, and the injury mechanism of *E. coli* in response to chlorine and ultraviolet disinfection, in order to give a more comprehensive introduction to the disinfectant-injured *E. coli*. Since the existence of disinfectant-injured bacteria will increase the biological risk of drinking water, it is necessary to pay attention to reducing the generation of disinfectant-injured bacteria during the water treatment.

2. EXISTENCE AND THREAT OF DISINFECTANT-INJURED BACTERIA

The widespread application of disinfection technology in drinking water systems has greatly reduced the incidence of waterborne diseases, but the threat posed by microorganisms should not be underestimated (Craun *et al.* 2010). The removal and monitoring of microorganisms can avoid disease outbreaks caused by the presence of pathogens in the water. Previous reports demonstrated that the proportion of injured coliforms fluctuated between 70 and 100% in the test samples of the chlorine disinfection drinking water distribution system (Córdoba *et al.* 2010), while those in water samples from filtered backflow of drinking water and damaged municipal pipelines accounted for up to 90% (Bissonnette *et al.* 1975; Means *et al.* 1981). Ray (1979) believed that the permeability barrier of injured cells is impaired, which makes them more susceptible to structural injury caused by various selective compounds. In addition to this injury, metabolic injury can also be induced by metabolism-related functional components. Studies have shown that pathogenic chlorine-injured *enterotoxigenic E. coli* can be repaired *in vitro* or in the intestine of mammals and the potential toxicity was retained (Walsh & Bissonnette 1983; Singh & McFeters 1987). However, the use of specific selective media is not able to detect these injured flora, leading to an underestimation of their existence (Li *et al.* 2017). They can revive in favorable environments (e.g., water pipe networks), develop resistance, and undergo reproduction, thus posing a hazard to drinking water safety.

As a subgroup of sublethally damaged organisms, disinfectant-injured bacteria belong to viable but nonculturable (VBNC) microorganisms under suitable environmental stresses, and they will fail to form colonies under unfavorable

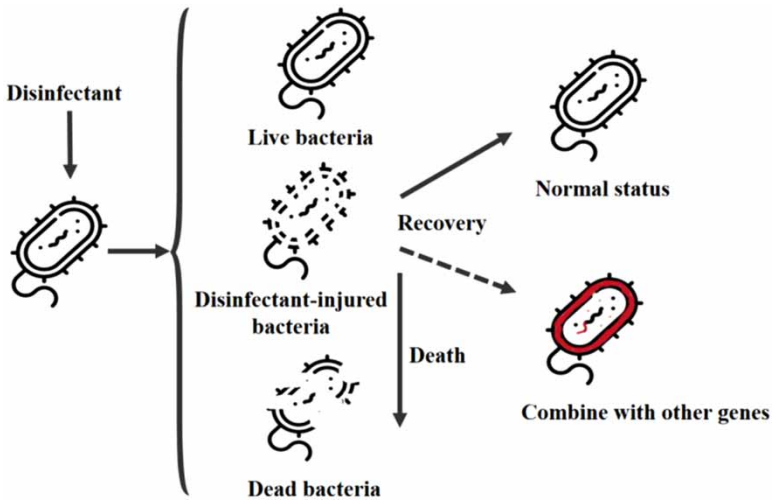

Figure 1 | Schematic diagram of the changes in bacterial physiological responses during disinfection.

conditions (Li *et al.* 2017). Disinfectant-injured bacteria are physiologically unhealthy and suffer from reversible injury as a consequence of partial or inappropriate disinfection, and the characteristics of disinfection injury are functionally similar to the VBNC state as bacteria in each of these respective conditions are not considered dead but rather undetectable with selective growth media (Bolster *et al.* 2005). But the injured bacteria can be repaired and detected on the non-selective nutrient media. Preez *et al.* (1995) found that chlorination to concentrations of 0.5 and 1 mg/L resulted in three log reductions in injured and non-injured coliform counts within 1 min. In addition, chlorination caused extensive bacterial injury, and the existence of injured bacteria was found in the test water samples except one. Jin *et al.* (2020) showed that chlorine disinfectant-injured bacteria exhibited enhanced cell membrane permeability and strong oxidative stress responses. More notably, chlorine disinfection can promote the horizontal transfer of antibiotic resistance genes (ARGs) within or between cells, which confers a potential risk for the spread of antibiotic-resistant bacteria in drinking water. Abuse of antibiotics induces the production of ARGs in animals, and it accelerates the spread of resistance genes between bacteria in the environment. Studies have shown that human exposure to antibiotic-resistant bacteria and ARGs in aquatic environments may pose an additional health risk (Amarasiri *et al.* 2020). Khan *et al.* (2016) found that chlorine-resistant bacteria may have transformed from non-antibiotic-resistant bacteria to antibiotic-resistant bacteria, possibly due to the co-selection phenomenon of bacterial resistance toward disinfection and antibiotics in drinking water. Hou *et al.* (2019) showed that the injury to cells was dose-dependent and exhibited two phases: an initial phase with a faster reaction rate and a second phase with a slower one, and the injury rate of bacteria increased with dose in the initial phase. After exposure to 2, 4, or 8 mg/L sodium hypochlorite for 20 min, 25, 49, or 56% of *Pseudomonas aeruginosa* (*P. aeruginosa*) were injured, respectively. Moreover, exposure to a half-lethal dose of chlorine-injured bacteria could promote antibiotic resistance, because the overexpression of MexEF-OprN efflux pump increased the drug resistance of chlorine-injured bacteria (Hou *et al.* 2019). MexEF-OprN efflux pump is encoded by the MexEF-OprN gene cluster, which belongs to the resistance–nodulation–cell division (RND) among the currently discovered types of bacterial drug-resistant pumps and plays an important role in the pathogenicity of *P. aeruginosa* (Liang *et al.* 2016). In another study, Hou *et al.* (2017) found that drug-resistant bacteria accounted for 51.72% among 58 injured non-fastidious bacteria isolated from drinking water, and 80% of them had obvious multi-drug resistance. Besides, Izumi *et al.* (2016) showed that chlorine-injured bacterial cells accounted for 69–77% in diluted electrolyzed water containing 2 mg/L available chlorine. When agricultural water was mixed with electrolyzed water, the injury rate of coliforms was about 75%, and the bactericidal products diluted to the recommended concentration could also cause injury to the coliforms in agricultural water. Fricker & Eldred (2014) compared five culture media with 122 drinking water samples and found that drinking water contained low levels of chlorine-injured *Enterococcus*. Additionally, their ability to recover was also verified in Slanetz and Bartley's medium. Watters *et al.* (1989) showed that exposure to monochloramine for 10 min could also induce a high level (>90%) of sublethal injury in intestinal bacteria.

In addition to chlorine and chlorine-related products, other disinfection methods, such as ultraviolet (UV) light, ozone, and catechins, also injured bacteria but do not cause them to die. UV irradiation is widely used as a disinfection method for drinking water treatment by damaging the structure and function of DNA in bacterial cells. However, the magnitude of its effects may vary considerably, as microorganisms can recover through their own injury repair mechanism even after long-term exposure to UV light (Kollu & Örmeci 2015). Guo *et al.* (2015) have shown that the increased number of bacteria in the effluent after UV disinfection is due to injury repair; that is, sublethal or VBNC strains are formed during UV irradiation. Zhang *et al.* (2015) have also proved that UV rays can induce *E. coli* and *P. aeruginosa* to enter the VBNC state. Under this circumstance, they still retain their pathogenicity, and the recovery ability of *E. coli* is stronger than that of *P. aeruginosa*. Hou (2018) found that the bacterial injury rate gradually increased with the increase of UV radiation dose, and the injury rate reached about 70% when the radiation dose was 11 mJ/cm^2. Moreover, Li *et al.* (2016) found that the disinfection effects of UV irradiation on *E. coli* can be extended to biologically active molecules such as proteins, nucleic acids, and carbohydrates. It is worth noting that UV-injured bacteria have competent properties, in which they are able to undergo a natural transformation and promote the horizontal transfer of ARGs. At the same time, Li (2016) also investigated the effects of ozone treatment on sublethal injury in *E. coli* and found that the multi-tube fermentation method performed better on the detection of ozone-injured bacteria compared to membrane filtration and enzyme–substrate methods. Ozone has been proven to cause a kind of reversible injury to *E. coli*, which is manifested as the loss of ability to grow in m-FC medium, and this effect is most noticeable

at a culture temperature of 44.5 °C (Finch *et al.* 1987). Epigallocatechin gallate (EGCG) is a class of catechins isolated from tea, which is the main component of tea polyphenols with antibacterial and antiviral effects (Cui *et al.* 2012). Liu (2020) explored the oxidative injury of EGCG to *E. coli* in the presence of Ca^{2+} and found that EGCG exerted certain inhibitory effects on the antioxidant enzymes of *E. coli* and ultimately affected their antioxidant defense systems. In addition, Ca^{2+} may activate the efflux pump and cause *E. coli* to develop resistance to EGCG, thereby forming sublethally injured bacteria (Liu 2020).

In summary, different disinfection methods can cause varying degrees of injury to bacteria under different environments and disinfectant doses. Both the inactivation and injury of bacteria showed a certain dose-dependent that the injury rate and the lethality rate gradually increased with the increase of the disinfectant doses. The above studies have shown that chlorine-injured bacteria were formed in the doses range of 0.5–8 mg/L. Although UV and ozone have high oxidizing properties, they have still shown a high injury rate under the action of sub-dose disinfection. These disinfectant-injured bacteria are able to reverse or repair the injury in a suitable environment, which should be considered as part of the microbiological safety of drinking water.

3. COMMON DETECTION METHODS OF *E. COLI* AND DETECTION EFFECT ON DISINFECTANT-INJURED BACTERIA

The recovery and reproduction of disinfectant-injured bacteria can become an implicit microbiological risk. Thus, it is necessary to systemically screen and accurately test the microbial characteristics of drinking water supplies. As an indicator microorganism, *E. coli* is a very important detection tool for assessing the safety of drinking water supplies. The currently available methods for detecting *E. coli* in drinking water are the membrane filtration (MF) method, multi-tube fermentation (MTF) method, and enzyme substrate (EST) method (Table 1). However, there are few reports on the detection methods of disinfectant-injured bacteria, and the common detection methods for *E. coli* show the difference in the detection rate of injured *E. coli*. As a result, the assessment of microbial safety in drinking water may be biased. The number of injured bacteria is usually calculated based on the difference between the counts of selective medium and non-selective medium as shown in formula (1). That is, the colonies are regarded as injured bacteria that cannot grow on selective medium, but can grow on non-selective medium without inhibitors. The injury rate and the detection rate of injured bacteria by different methods are

Table 1 | Methods for the detection of *E. coli* in drinking water

Detection method	Principle	Performance characteristics
Membrane filtration	The water sample is filtered with a membrane filter to retain bacteria and then cultured on a selective medium. The detection of *E. coli* can be achieved by counting every single colony	MF method is widely used for the detection of microorganisms in drinking water due to its relatively simple operation, but its detection rate of injured bacteria is relatively low or sometimes undetectable (Rompré *et al.* 2002; Liu *et al.* 2015)
Multi-tube fermentation	*E. coli* can ferment lactose to produce acid and gas. The existence of coliform bacteria can be determined through the primary fermentation test, plate separation, and secondary fermentation test	MTF method lacks precision in both qualitative and quantitative aspects and often takes a long time. It is better than the MF method when testing turbid or colored water. The MTF method can effectively detect chlorine-injured bacteria (Rompré *et al.* 2002; Liu *et al.* 2015)
Enzyme substrate	The specific enzymes present in microorganisms can be used to detect *E. coli*. The detection or counting method can be performed in a single medium without time-consuming separation processes	Detection of *E. coli* can be carried out by an immobilized enzyme substrate method, which has the advantages of rapid and high specificity. However, it performs poorly in the detection of disinfectant-injured bacteria, because the method has limited ability to promote cell repair (Li *et al.* 2017)

calculated as shown in Equations (2) and (3).

$$\text{Number of injured bacteria} = \text{Number of bacteria in TSYA medium} - \text{Number of bacteria in m} - \text{Endo medium} \quad (1)$$

$$\text{Injury rate} = \frac{\text{Number of bacteria in TSYA medium} - \text{Number of bacteria in m} - \text{Endo medium}}{\text{Number of bacteria in TSYA medium}} \times 100\% \quad (2)$$

$$\text{Detection rate} = \frac{\text{Number of bacteria detected by different methods}}{\text{Number of bacteria in TSYA medium} - \text{Number of bacteria in m} - \text{Endo medium}} \times 100\% \quad (3)$$

Some scholars have conducted experimental comparisons on the detection of disinfectant-injured bacteria using the above-mentioned methods. Rompré *et al.* (2002) found that the MF method may not be able to detect injured *E. coli*, because the commonly used selective medium m-Endo lacks repairing components and contains substances that inhibit the growth of injured bacteria (e.g., sodium deoxycholate and Tergitol 7). Liu *et al.* (2015) showed that the injury rate of *E. coli* was between 30 and 100% at different times by chlorine disinfection. The improved non-selective medium is used as the standard to compare the detection effects of chlorine-injured *E. coli* in the samples disinfected for 5 and 20 min, and the results are shown in Table 2. The findings indicate that MF and EST methods are not suitable for detecting injured *E. coli* due to low or undetectable levels, but the MTF method can repair the injury and detect all chlorine-injured bacteria (Liu *et al.* 2015). Li (2016) also used an improved non-selective medium as the standard to compare the detection effects of ozone-injured *E. coli*, as shown in Table 3. In comparison, the MTF method exhibited higher sensitivity in detecting sublethally injured bacteria. However, the repair of sublethal injury by MTF is related to time, in which a prolonged period of culture time can improve the accuracy of the detection results (Li 2016). Another study (Li *et al.* 2017) has also shown that the MTF method possesses a better detection effect on sublethally injured *E. coli,* as it involves a tryptone-containing medium that can promote the recovery of a sublethal injury. In summary, with *E. coli* as a typical microorganism, the MTF method exhibited a higher detection rate

Table 2 | The detection rate of chlorine-injured *E. coli* by various methods (Liu *et al.* 2015)

			Detection rate of injured *E. coli* (%)			
Sample	**Disinfection time (min)**	**Concentration of injured bacteria ($\times 10^7$ CFU/mL)**	**Repair culture**	**MF**	**MTF**	**EST**
1	5	9.0	100	0[a]	100	0.04 ± 0.01[a]
	20	1.4	100	0[a]	100	0[a]
2	5	19.7	100	0[a]	100	47.50 ± 0.45[a]
	20	4.0	100	0[a]	100	0[a]
3	5	8.3	100	0[a]	100	0[a]
	20	0.8	100	0[a]	100	0[a]
4	5	2.7	100	0[a]	100	0.09 ± 0.02[a]
	20	0.8	100	0[a]	100	0[a]

Note: [a]Compared with the repair culture method, $P<0.05$.

Table 3 | The detection rate of ozone-injured *E. coli* by various methods (Li 2016)

		Detection rate of injured *E. coli* (%)		
Sample	**Injury rate (%)**	**MF**	**MTF**	**EST**
1	73.72	0	100	0
2	92.10	0	100	3
3	96.22	0	98.19	0.71
4	97.67	0	55.95	4.64
5	100	0	21.74	0

of disinfectant-injured bacteria compared to MF and EST methods. It is suitable for the detection of sublethally injured bacteria, but it takes a long time and may not be appropriate for rapid testing.

In addition, several molecular methods such as immunoreactivity assay, polymerase chain reaction (PCR), and *in situ* hybridization (FISH) techniques have also been proposed for the detection of coliform bacteria (Ngwa *et al.* 2013; El-Sayed *et al.* 2019; Rompré *et al.* 2002). Quantitative PCR (qPCR) has been proven to be highly sensitive and specific in detecting waterborne pathogens and can be used to estimate the level of contamination due to the detection of damaged or destroyed bacterial cells and their contents (El-Sayed *et al.* 2019). Flow cytometry (FCM) is gradually used for various water quality testing and evaluation due to its multi-parameter measurement, high efficiency, rapidity, etc. Kong *et al.* (2015) have shown that FCM able to analyze cell membrane integrity, DNA damage, and enzyme activity, which has a great effect on the detection of VBNC. Al-Qadiri *et al.* (2008a) found that Fourier transform infrared (FT-IR) spectroscopy can be used to determine the presence of VBNC pathogenic bacteria as well as sublethal injury that are underestimated or not recognized by conventional microbial techniques. Another study showed that FT-IR spectroscopy can also be applied to determine the presence and quantity of injury pathogens in food (Al-Qadiri *et al.* 2008b).

4. RESEARCH PROGRESS ON THE INJURY MECHANISM OF BACTERIA IN DIFFERENT DISINFECTION PROCESSES

It has been reported that the content of sublethally injured *E. coli* is relatively high in drinking water. The injured bacteria generated after the disinfection process have the potential to recover under favorable growth conditions and become an important component for controlling the microbial safety of water supplies. By clarifying the formation law, nature, and mechanism of bacterial injury caused by different disinfection processes, we can accurately identify the potential hazards of disinfectant-injured bacteria. At present, chlorine disinfection, UV disinfection, and ozone disinfection, which are commonly used in water treatment, can produce sublethal damage bacteria. However, most of the existing studies have focused on the mechanisms of chlorine-injured and UV-injured bacteria.

4.1. The injury mechanism of *E. coli* in response to chlorine disinfection

Chlorine disinfection is widely used in drinking water disinfection due to its high sterilization efficiency, long shelf life, and good economical values. However, sublethally injured bacteria may be generated during chlorination of water supplies (Preez *et al.* 1995; Yang & Zhang 2016). In the process of chlorine-mediated injury, the physiological activities that are dependent upon membrane functions including respiration and nutrient transport are impaired in the chlorine-mediated injury process (McFeters & Lechevallier 2000). Al-Qadiri *et al.* (2008a) used FT-IR spectroscopy to detect the injury caused by chlorine to bacteria in water, and the results showed that chlorine altered the spectral characteristics of the bacterial ester functional groups such as lipids, structural proteins, and nucleic acids, and the injured bacterial cells exhibited apparent denaturation between 1,800 and 1,300 cm^{-1}. Jin *et al.* (2020) found through experiments that the permeability of cell membrane in disinfectant-injured bacteria was increased during NaClO exposure, suggesting a strong oxidative stress response of bacteria against disinfection. Therefore, the level of reactive oxygen species (ROS) detected in the injured *E. coli* was significantly increased, which mainly include $^{\cdot}O_2^-$, $^{\cdot}H_2O_2$, and $^{\cdot}OH$. Studies have shown that intracellularly produced ROS exert bactericidal action by causing DNA damage, protein denaturation, and lipid peroxide production (Dwyer *et al.* 2007; Van Acker & Coenye 2017). To protect bacteria from the destruction of ROS, the corresponding cellular antioxidant system is activated, including superoxide dismutase, catalase, and glutathione peroxidase enzymes, which are the main antioxidant enzymes in cells and maintain the normal state of the body by removing $^{\cdot}O_2^-$, H_2O_2, and $^{\cdot}OH$ (Wang *et al.* 2016). These results all indicated that after exposure to NaClO, a strong oxidative stress reaction occurred in *E. coli*, which caused its oxidative injury and formed disinfectant-injured bacteria (Jin *et al.* 2020). Studies have shown that oxidative stress can lead to the peroxidation of lipids on bacterial cell membranes, which will destroy the structural integrity of the cell membrane and affect the related functions of the membrane (Farr & Kogoma 1991; Ibrahim *et al.* 2000). Tandon *et al.* (2007) found that chlorine-injured *E. coli* and *Enterococcus faecalis* become sensitive to ROS, giving higher counts under ROS-neutralized enumeration conditions than under conventional aerobic conditions.

4.2. The injury mechanism of *E. coli* in response to ultraviolet disinfection

UV disinfection has broad-spectrum bactericidal properties and can kill bacteria, fungi, viruses, and spores, but the duration of disinfection is short (Tran *et al.* 2014). Studies have shown that injured bacteria are formed during UV disinfection, and

bacteria can repair UV-induced DNA damage (Guo *et al.* 2011, 2012), leading to microbial reactivation. Xu *et al.* (2017) conducted research on the injury mechanism of *E. coli* during UV disinfection via cell membrane integrity analysis, adenosine triphosphate detection, DNA and RNA damage and found that the common dosages (<100 mJ/cm^2) of UV disinfection in water treatment caused less injury to the cell membrane of *E. coli* and reduced their total ATP content. When the bacteria are in a sublethal or VBNC state, they still have a certain metabolic activity, which provides support for the resuscitation process. The severity of DNA damage induced by UV disinfection is dependent on the fragment length of bacterial cells, and the injury is more obvious in 16 s rRNA gene fragments. As UV dose increases, RNA injury becomes more serious, DNA damage repair pathway is destroyed, and recovery ability is weakened (Xu *et al.* 2017). The RecA gene in RNA is a key gene of the SOS damage repair mechanism (Jungfer *et al.* 2007). When the UV dose was greater than 50 mJ/cm^2, *E. coli* gradually lost the ability to repair SOS damage, which is beneficial to inhibit the recovery phenomenon (Xu *et al.* 2017). Another study by Xu *et al.* (2018) found that DNA damage repair (RecA), chromosomal replication initiator protein (dnaA), single-stranded DNA binding protein (ssb), and glutamic acid decarboxylase (gadA) gradually lost their functions with the increase of UV dose. Thus, the disappearance of RecA can be used to indicate irreversible damage to microorganisms. Li *et al.* (2016) carried out Raman spectroscopy to detect UV-injured *E. coli* and found that the wavebands representing the structure of a variety of biological macromolecules have intensity and vibration displacement. That is, UV disinfection can manifest varying degrees of injury to different biologically active molecules (e.g., proteins and nucleic acids). The changes in the morphological features of UV-injured bacteria are small, but the permeability of the cell membrane is increased, making it easier for extracellular substances to enter the bacteria. In addition, UV disinfection can induce the antioxidant enzymology system of bacteria after causing injury to bacteria, so the enzyme activity in UV-injured bacteria increased (Li *et al.* 2016).

5. CONCLUSION AND OUTLOOK

1. There are many factors that cause sublethal injury in bacteria, and some of the disinfectant-injured bacteria may be generated during the disinfection of drinking water. It is believed that disinfectant-injured bacteria can recover in the pipe network systems under appropriate conditions, obtain reproduction ability and even pathogenicity, or promote the horizontal transfer of ARGs. Thus, it is of great importance to ensure the microbiological safety of drinking water by mitigating the hidden risk of disinfectant-injured bacteria.
2. Compared with MF and EST methods, the MTF method has a higher detection rate of disinfectant-injured bacteria, but it takes a longer time and is not suitable for real-time detection. Therefore, it is necessary to develop convenient, accurate, and efficient detection methods that are conducive to the microbiological quality assessment of drinking water, in order to minimize the risk of waterborne diseases caused by disinfectant-injured bacteria.
3. The existing bacterial injury mechanism research is not complete, which mainly focuses on the injury regularity and injury nature. Hence, more in-depth studies are needed to elucidate the injury mechanism of bacteria exposed to multiple disinfection approaches, assess the influence of water quality factors (e.g., heavy metal ions) on the formation of disinfectant-injured bacteria, and explore the association between sublethally injured bacteria and increased antibiotic resistance, in order to effectively assure the microbiological safety of drinking water.
4. In the future, on the basis of clarifying the recovery process, influencing factors, and injury mechanism of disinfectant-injured bacteria, it is required to put forward on how to reduce the generation of disinfectant-injured bacteria by controlling the disinfection process parameters (e.g., disinfectant dosage, contact time, and environmental factor control), which in turn can improve the microbial quality of drinking water.

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CONFLICT OF INTEREST

We declare that we have no financial and personal conflicts of interest.

DATA AVAILABILITY STATEMENT

All relevant data are included in the paper or its Supplementary Information.

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