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Effect of ozone–tea polyphenols as a drinking water disinfection process on antibiotic resistance genes

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ABSTRACT

In recent years, the wide spread of antibiotic resistance genes (ARGs) has brought tremendous risk to the biological safety of drinking water. With the increasing demand for drinking water quality, ARGs have been regarded as a new pollutant that may cause serious public health problems. A large number of studies have shown that the disinfection process of drinking water treatment plants can remove ARGs. However, the effects of traditional disinfection methods on ARGs have their own disadvantages. Tea polyphenols have attracted more and more researchers' attention as a green, efficient and non-disinfection by-products disinfectant. The effect of the ozone–tea polyphenols disinfection process on ARGs in filtered effluent of waterworks was analyzed by using metagenomic sequencing. The result shows that the ozone–tea polyphenols disinfection process is suitable for specific raw water containing more tetracycline, sulfonamide, β-lactam, and aminoglycoside resistance genes, and the removal rate of total resistance genes in water is higher than the traditional disinfection process. The effect of the ozone–tea polyphenols disinfection process on ARGs is to reduce the transfer of ARGs by destroying ARGs molecules and inhibiting the proliferation of ARGs host cells. As an assistant disinfectant, tea polyphenols have significance for the ability to remove ARGs during traditional disinfection.

Key words: antibiotic resistance genes, disinfection, ozone, tea polyphenols

HIGHLIGHTS

- Tea polyphenols are a green, natural disinfectant with broad-spectrum bacterial suppression and the ability to disinfect continuously.
- β-lactams, tetracyclines, and aminoglycoside resistance genes can be efficiently removed using an ozone–tea polyphenols disinfection technique.
- For water sources containing certain ARGs, an ozone–tea polyphenols disinfection process can be applied, ensuring water quality safety.

1. INTRODUCTION

Antibiotic resistance has become a serious global concern due to misuse and abuse of antibiotics (Bakkeren *et al.* 2020). Antibiotic-resistant bacteria (ARB) develop resistance through expressing antibiotic-resistant genes (ARGs). Vertical gene transfer (VGT) and horizontal gene transfer (HGT) allow ARGs to exist and propagate (Nadimpalli *et al.* 2020). ARB numbers skyrocket as ARGs spread and fluctuate. Antibiotics are ineffective in treating diseases caused by pathogenic ARB. Each year, more than 700,000 individuals die as a result of these diseases (Cassini *et al.* 2020). Antibiotics have been released into the environment as a result of clinical treatment, livestock and poultry breeding, medication production, and other human activities (Padhye *et al.* 2014). ARGs have been found in a variety of aquatic settings (Gao *et al.* 2018). ARG pollution has been found in varying degrees in several basins, water sources, pipe networks, and even household faucets in China (Jiang *et al.* 2013). Inadequate upstream sewage treatment has resulted in ARG contamination of several drinking water reservoirs (Dong *et al.* 2020). ARG pollution of water sources has presented a significant danger to the safety of drinking water and human health. Studies have shown that sulfonamide and tetracycline resistance genes pose the greatest threat to human

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beings. Despite the fact that ARGs are not included in the detection range of drinking water quality standards, scientists have been studying them as a novel pollutant that could pose major public health issues (Pruden *et al.* 2012). A large number of studies have shown that the disinfection process of drinking water has a certain degree of removal effect on ARGs in water.

In a drinking water treatment system, the disinfection process is critical. Some pathogenic bacteria found in water, such as *Vibrio cholerae*, *Cryptosporidium*, *Shigella*, and *Escherichia coli*, can cause significant harm to human health (Oyane *et al.* 2005; Gelover *et al.* 2006). The disinfection process is critical for preventing infectious diseases and preventing the spread of microorganisms in water. Because of its effective disinfection and inexpensive cost, chlorination disinfection is frequently employed (Son *et al.* 2005). However, the reduction effect of chlorine on the ARGs is not obvious and they have the possibility of promoting its spread. Gomez-Alvarez *et al.* (2012) have shown that the contents of tetracycline and β-lactam resistance genes in drinking water after free chlorine treatment are high. By increasing cell membrane permeability, chlorination can aid in the conjugation and transfer of resistance genes (Sharma *et al.* 2016). Many carcinogenic and mutagenic disinfection by-products (DBPs) can be produced by chlorine-based disinfectants, such as trihalomethanes (THMs), haloacetic acids (HAAs), and others. These chemicals not only endanger human health, but can also cause harmful bacteria to manufacture ARGs (Lv *et al.* 2014). As a result, several other disinfection technologies, such as ozone and UV radiation, have gradually evolved into alternate drinking water disinfection schemes. Ozone primarily affects bacterial cell membranes, generating increased permeability and efflux of bacterial intracellular substances, ultimately leading to bacterial death. Furthermore, ozone has the ability to oxidize critical enzymes in bacterial cells, rendering them inert and eliminating bacterial cellular function, resulting in the bacteria's death. Ozone can also kill bacteria by destroying their genetic material, causing them to die and preventing them from proliferating. Although ozone has a clear removal effect on ARGs, it has less of an effect on ARGs like tetracycline and sulfonamide resistance genes (Hu *et al.* 2018). At the same time, because ozone has no disinfectant persistence, it is impossible to prevent the spread of ARGs across pipe networks. Due to the limitations of material type and economy in water, the UV dose cannot guarantee complete inactivation of ARGs. The problem of photoactivation and dark repair of bacteria also improves the growth risk of ARGs in pipe networks since UV has no continuous disinfection ability (Zhang *et al.* 2020).

As a green, natural and efficient drinking water disinfectant, tea polyphenols have received more and more attention in recent years. The disinfection mechanism of tea polyphenols is mainly as follows: first, tea polyphenols can disrupt the cell wall membrane and make the cell morphology change. When polyphenols are used to treat bacteria, they interfere with the integrity of the cell wall membrane, causing changes in the cellular morphology of the bacteria (Yi *et al.* 2013). When the bacterial cell membrane is disrupted, the barrier function of the bacteria is destroyed, the bacterial enzyme system is disrupted, and the normal physiological function of the bacteria is affected by the leakage of the cell contents. Second, tea polyphenols can impede the synthesis and expression of proteins inside bacteria. Polyphenols can reversibly bind to proteins through various non-covalent interactions, such as hydrogen bonding, ionic bonding, hydrophobic interactions, and van der Waals forces (Prigent *et al.* 2007). Third, tea polyphenols can limit normal cellular metabolism. Tea polyphenols can inhibit cellular enzyme systems leading to bacterial death. The enzyme system was found to be affected by catechins in studies on the secondary structure of collagenase (Madhan *et al.* 2007). Fourth, tea polyphenols can damage DNA structure. Tea polyphenols can act directly on DNA and affect the normal growth and reproduction of bacteria by inactivating reverse transcriptase. In summary, tea polyphenols are persistent in disinfection and do not produce DBPs. However, due to the large dosage during disinfection, our research group studied its application as an assistant disinfectant for ozone disinfection. The ozone–tea polyphenols disinfection process itself is innovative and can achieve continuous disinfection of drinking water without generating DBPs. The removal of ARGs by this disinfection method has not been studied. The optimal dosage of tea polyphenols as the only disinfectant is 200 mg/L. Although the growth of bacteria can be well controlled at this time, the chromaticity at this dosage is more than 15 degrees, which does not meet the standards for drinking water quality (GB5749-2006). The ozone–tea polyphenols disinfection process can bridge the tea polyphenols alone under the role of color is not up to standard drawbacks. The optimal conditions for the ozone–tea polyphenols disinfection process were obtained through a 3-level full factorial design: ozone dosage = 2.5 mg/L, ozone contact time = 25 min, and tea polyphenols dosage = 20 mg/L (Feng *et al.* 2020). The dose of 20 mg/L tea polyphenols can ensure the chromaticity standard and improve the disinfection economy to achieve the sustainable and effective killing of bacteria. The combined disinfection process achieved good disinfection effect. However, the removal effect of ARGs remains to be studied. Further exploration is needed into whether tea polyphenols can compensate for the drawbacks of ozone in the removal of ARGs. If the ozone–tea polyphenols disinfection process has better ARGs removal effect, it will improve the biological safety of drinking water and bring great changes to the traditional drinking water treatment process. In this experiment, the metagenomic sequencing method was used to analyze the abundance and characteristics of ARGs in water and to explore the effect of the combined disinfection process on various ARGs, so as to provide strong scientific support for the optimization of the traditional ARGs removal process.

2. MATERIALS AND METHODS

2.1. Test material

(1) Tea polyphenols: green tea extract, AR98.0%. Made in Anhui Red Star Pharmaceutical Co., Ltd, China.

(2) Raw water: The water was taken from a waterworks Aquazure Type V filter effluent. The process and water intake location of the waterworks are shown in Figure 1, and the water quality indexes are shown in Table 1.

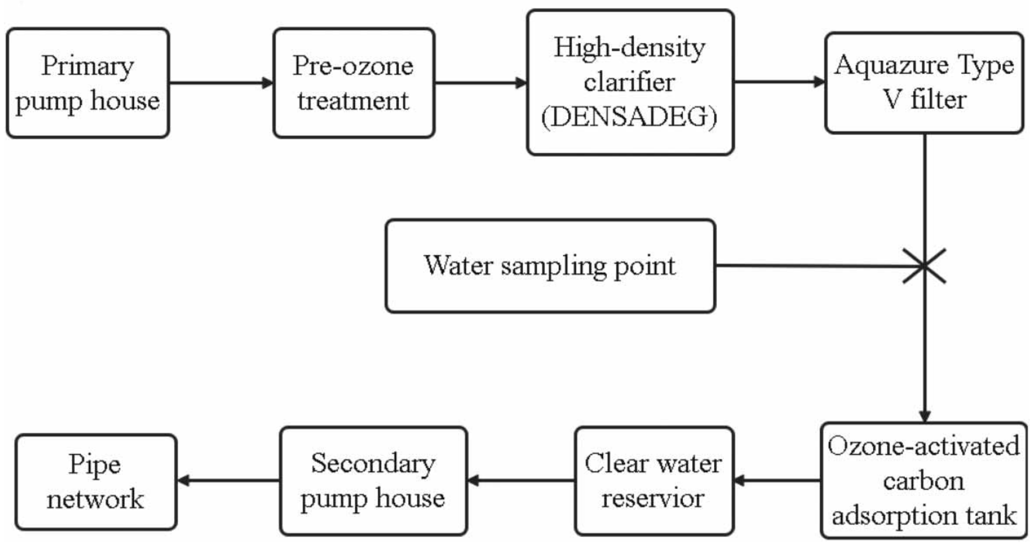


Figure 1 | Waterworks process and water intake location.

Table 1 | Raw water quality

Parameter	Values	Instruments	Methods
pH	7.5–7.8	FiveGo - Single Channel Portable pH Meter	Direct Reading
Turbidity (NTU)	0.1	HACH-2100AN Turbidity Meter	
Chroma (degree)	1–2	PFXi-995 High Precision Automatic Colorimeter	
Total number of bacteria (CFU/mL)	565	Plate Counting	Plate Counting
NH_4^+-N (mg/L)	0.05–0.06	HANNA-HI83208 Multifunctional Water Quality Analyzer	Salicylic acid method
TP (mg/L)	0.60–0.70	HANNA-HI83208 Multifunctional Water Quality Analyzer	Digestive-Ascorbic Acid Method
TOC (mg/L)	0.82–0.98	Shimadzu TOC-L Organic Carbon Meter	High-temperature catalytic combustion method
		E.Z.N.A™ Mag-Bind Soil DNA Kit	Splitting method

2.2. Test device and operating conditions

The ordinary ozone generator was used to prepare ozone. The gas production of the ozone generator was adjusted to the medium level (3 g/h). The effective contact volume of the water sample was 10 L. The gas flow meter was used to control the ventilation volume. The ozone concentration was adjusted by the time of ozone in the water sample, and the tail gas treatment device was set up. The ozone contact device is shown in Figure 2.

2.3. Water quality and analysis

The total number of bacteria was determined according to the plate count method in Standard Examination Methods for Drinking Water-Microbiological Parameters (GB/T 5750.12-2006). The experiment was carried out in a sterile environment. The mediums were inoculated and cultured in a biochemical incubator at 37 °C for 48 h.

According to the ozone generator for water and wastewater treatment (CJ/T 322-2010), the ozone concentration in the water sample is detected by the iodometric method and retested with the ozone concentration detector. The temperature of the experimental procedure was 25 °C, and the pH was 7.5–7.8 as shown in Table 1.

The iodometric method is used to determine the ozone concentration in water. Ozone can oxidize the iodine ion in potassium iodide, so that it is generated into free iodine, which has color. Sodium thiosulfate and free iodine react to generate sodium iodide, so that the solution in the reaction is discolored. A starch solution that can react with free iodine to develop color is used as an indicator during the test.

$$O_3 + 2KI + H_2O \rightarrow O_2 + I_2 + 2KOH$$

$$I_2 + 2Na_2S_2O_3 \rightarrow 2NaI + Na_2S_4O_6$$

(1) 20% potassium iodide solution: weigh 20.0 g of potassium iodide in a beaker with a balance, dissolve it with boiled and cooled water and transfer it to a 100 mL brown volumetric flask to fix the volume, store it for one day and then use it again.
(2) Dilute sulfuric acid: concentrated sulfuric acid and distilled water are configured in the ratio of 1:5 to dilute sulfuric acid.
(3) 0.01 mol/L sodium thiosulfate standard solution: weigh 0.248 g of sodium thiosulfate and add it to the boiled distilled water, then transfer it to a 100 mL volumetric flask and shake well.
(4) 1% starch indicator: weigh 1 g of starch and add it to 80 mL of distilled water, boil it, cool it and then transfer it to a 100 mL volumetric flask to fix the volume to 100 mL, place it for 24 h and then take the supernatant.
(5) Use a measuring cylinder to measure 20 mL of 20% potassium iodide solution in a 500 mL conical flask, add 200 mL of the ozone solution to be measured, then use a pipette to draw 5 mL of dilute sulfuric acid solution, shake well, and place it in an area protected from light for 5 min.
(6) Perform a titration using a 0.01 mol/L sodium thiosulfate pair and record the volume of sodium thiosulfate solution consumed.

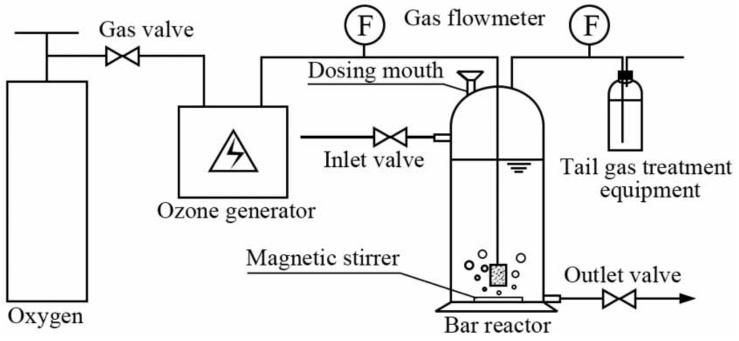


Figure 2 | Ozone contact device schematic.

(7) Calculate the ozone concentration in the water sample to be measured according to the formula:

$$C(O_3)(\text{mg/L}) = A_{\text{Na}} \times B \times 24,000/V_0$$

where $C(O_3)$ is the concentration of ozone to be measured, mg/L; A_{Na} is the volume consumed by the volume of sodium thiosulfate, mL; B is the concentration of sodium thiosulfate solution in the test, 0.01 mol/L; V_0 is the volume of the sample taken, mL.

Adjust the ozone generator gas production to medium level (3 g/h). The effective contact volume of the water sample in the test was 10 L, and the optimal concentration of 2.5 mg/L was finally determined when the ozone was passed for 65 s.

Using Shimadzu TOC-L organic carbon meter, the water sample is measured by a high-temperature catalytic combustion method, and the water sample is sent through a high-temperature combustion tube (680 °C), the organic matter in the water is burned and converted to carbon dioxide under the catalyst, and the carbon content is measured by a non-dispersive infrared absorption carbon dioxide analyzer, which is the total carbon (TC). Then, the water sample was sent into the reaction tube at room temperature, and the inorganic carbon in the water was converted to carbon dioxide under acidification conditions ($\text{pH} \approx 4$), and the result was measured as total inorganic carbon (IC). TOC is the difference between TC and IC.

Since the number of microorganisms in the test water sample was small, it was necessary to enrich the test sample and then extract DNA. A sand core filtration device and a 0.22 μm microfiltration membrane were used to filter 100 L samples and intercept microorganisms. DNA was extracted from the filtered microfiltration membrane by kit in time. DNA concentrations were measured using the E.Z.N.A™ Mag-Bind Soil DNA Kit. The extracted DNA was subjected to high-throughput sequencing on Illumina Hiseq. Relatively effective data was obtained through data evaluation and quality control, and then assembled. The splice results were predicted by Prodigal. Genes with lengths equal to or more than 100 bp were selected and translated into amino acid sequences for gene prediction. The abundance information of genes in each sample was calculated after deduplication. Metagenomic sequencing and bioinformatics analysis were commissioned to Sangon Biotech Co., Ltd (Shanghai).

3. RESULTS AND DISCUSSION

3.1. Effect on total ARGs

A total of 220 ARGs were detected in raw water samples, including 29 vancomycin resistance genes, 23 tetracycline resistance genes, and 18 β-lactam resistance genes, which were the most diverse ARGs detected. The 20 ARGs with the largest number are shown in Figure 3, among which MacB was the most. It shows that the effect of the conventional coagulation sedimentation process on ARGs is not obvious, and ARGs need to be further removed in the subsequent disinfection process (Zhang 2013).

The water samples treated by the ozone–tea polyphenols disinfection process were sequenced by metagenome sequencing to study the effect of ozone–tea polyphenols disinfection on total ARGs. The reduction of the number of major ARGs in water samples treated by the ozone–tea polyphenols disinfection process is shown in Figure 4. The results indicate that the ozone–tea polyphenols disinfection process can effectively reduce the number and types of ARGs. The process has a certain removal effect on the 20 largest ARGs in raw water. The best effect was CeoA, and the removal rate was 76.5%. The worst effect was VanRA, and the removal rate was only 35%. The average removal rate of the 20 largest ARGs was 56.5%. This indicates that the ozone–tea polyphenols disinfection process can effectively reduce the content of ARGs in drinking water, and can be used for specific raw water containing more CeoA resistance genes.

3.2. Effect on different types of ARGs

The effect of the ozone–tea polyphenols disinfection process on ARGs types is shown in Figure 5. It can be seen from the figure that the process can effectively reduce the types of tetracycline, aminoglycoside, fluoroquinolone, cephalosporin, β-lactam, trimethoprim, and chloramphenicol resistance genes.

Vancomycin, tetracycline, sulfonamide, β-lactam, efflux pump, and aminoglycoside resistance genes in water samples with high concentrations were studied. The concentrations of various ARGs in water samples treated by the ozone–tea polyphenols disinfection process were studied to explore the effect of the ozone–tea polyphenols disinfection process on different types of ARGs, as shown in Figure 6.

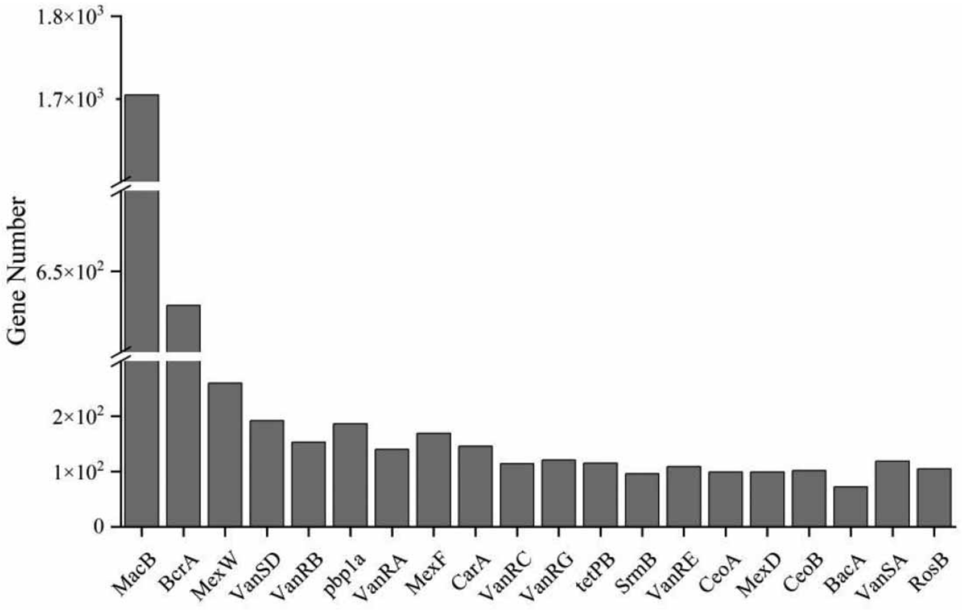


Figure 3 | Major ARGs and their quantity in raw water samples.

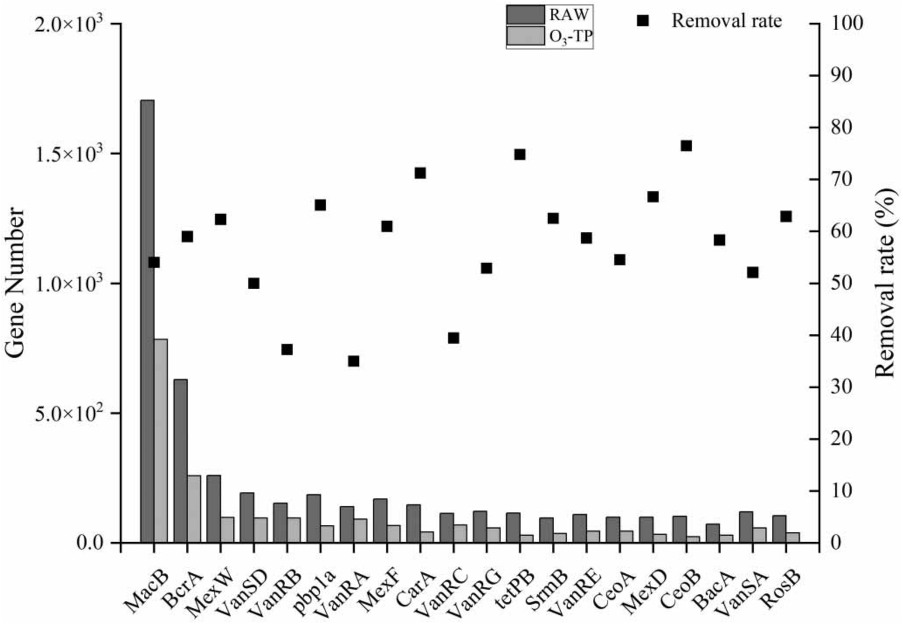


Figure 4 | Reduction of the number of major ARGs in water samples treated by the ozone–tea polyphenols disinfection process.

The results show that the ozone–tea polyphenols disinfection process has different effects on different types of ARGs. It has a good effect on some ARGs and can effectively inhibit their growth. However, it has no obvious effect on some ARGs and has the risk of promoting the ARGs spread.

Figure 6(a) and 6(b) shows that the ozone-polyphenol disinfection process had an obvious reduction effect on 15 of the 20 tetracycline resistance genes detected in water samples with high concentrations, and the effect on 11 of them was more obvious, with the removal rate above 50%. Only tetC, tetO, tet41, tetQ, tetA showed the phenomenon of promoting transfer

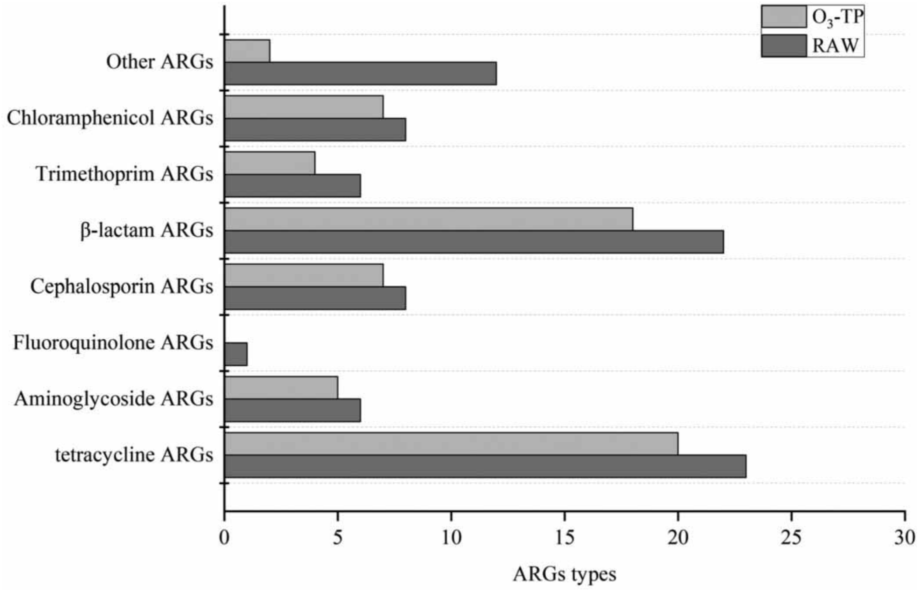


Figure 5 | Effect of the ozone–tea polyphenols disinfection process on ARGs types.

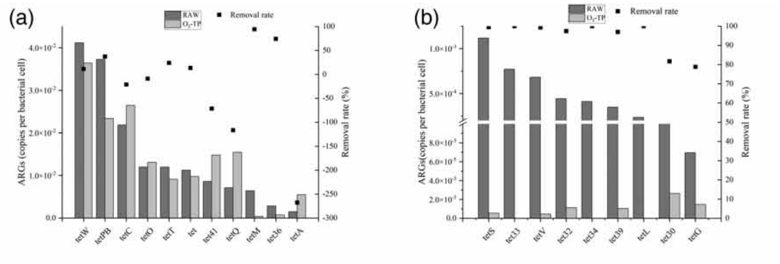
and spread. The overall removal effect of tetracycline resistance genes was 75%. The disinfection process can effectively reduce the threat of tetracycline resistance genes to human health.

Figure 6(c) and 6(d) shows that vancomycin resistance genes were the most abundant resistance genes detected in water samples. After analyzing the 27 vancomycin resistance genes with higher concentrations, it was found that the ozone–tea polyphenols disinfection process had a general removal effect on them. The removal efficiency of five vancomycin-resistant genes (VanT, VanRD, VanB, VanWG, and VanC) was better, and the removal rates were all more than 90%. However, the 18 resistance genes showed the phenomenon of promoting transfer and spread (VanSE, VanD, VanYD, VanXA, VanXB, VanE, VanRA, VanSD, VanRC, VanRB, VanRG, VanSB, VanHB, VanRE, VanSA, VanSG, VanSC, VanHA, and VanTG). Therefore, the ozone–tea polyphenols disinfection process is not suitable for raw water containing more vancomycin resistance genes.

Figure 6(d) and 6(f) shows that the effect of ozone–tea polyphenols disinfection process on efflux pump resistance genes was similar to that of vancomycin. The effect of this process on different types of ARGs was quite different. The removal rate of five efflux pump resistance genes was close to 80% (AdeC, OprJ, MdtH, mdtF, and OprA), but six efflux pump resistance genes were significantly promoted (MexA, MexF, MexH, MexD, MexI, and MexB). The overall effect is to promote the transfer and spread.

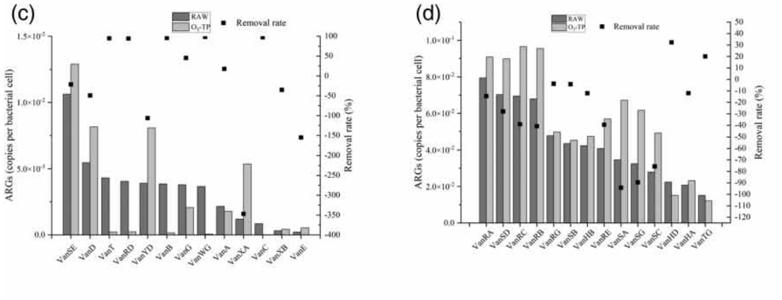
Figure 6(g) and 6(h) shows that the ozone–tea polyphenol disinfection process had a good removal effect on the 11 β-lactam resistance genes with higher concentrations in water samples. Among them, the removal rates of 8 ARGs were more than 90%, and the worst removal rate was more than 50%. This indicates that the ozone–tea polyphenols disinfection process can effectively remove β-lactam resistance genes in water. The removal effect of the ozone–tea polyphenols disinfection process on the penicillin resistance gene is very good. The removal effect of low concentration penicillin resistance genes was better than that of high concentration penicillin resistance genes. Xu *et al.* (2016) found that ARGs would be further enriched in the water distribution system, especially β-lactam resistance genes. Its concentration increased from 1.1×10^4 copies/mL in treated water to 5.1×10^5 copies/mL in tap water. Martínez *et al.* (2015) have found that β-lactam resistance genes had become one of several ARGs with high detection concentration in drinking water sources, and were also recognized as high-risk ARGs. Inhibition and removal of β-lactam resistance genes by the ozone–tea polyphenols disinfection process can greatly reduce the pollution of β-lactam resistance genes to drinking water in water distribution systems and reduce the threat of ARGs to human health.

Figure 6(i) shows that there were few types of aminoglycoside resistance genes detected in water samples. The ozone–tea polyphenols disinfection process had an obvious removal effect, and the average removal rate was more than 80%. It can effectively reduce transfer and spread of ARGs in drinking water.



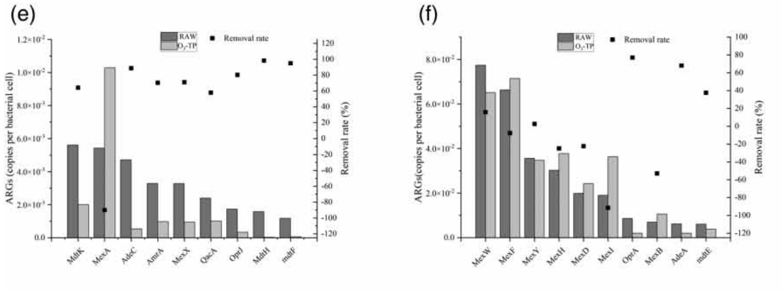
Tetracycline resistance genes I

Tetracycline resistance genes II



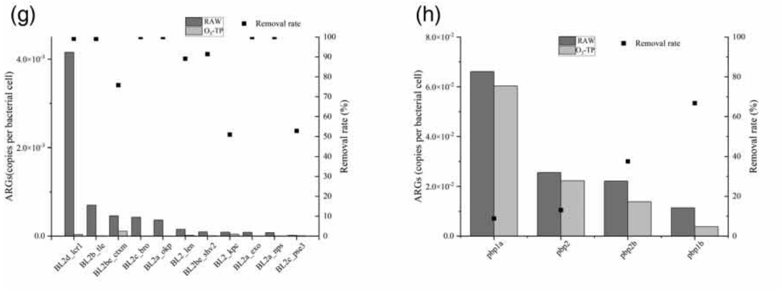
Vancomycin resistance genes I

Vancomycin resistance genes II



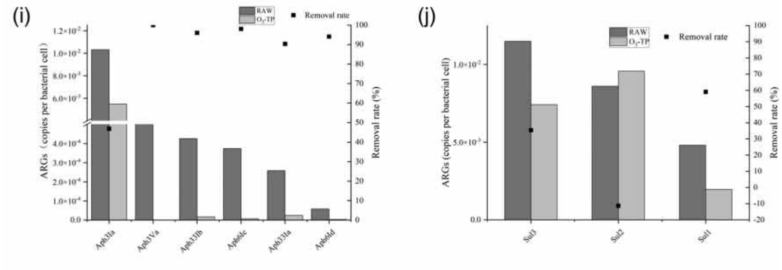
Efflux pump resistance genes I

Efflux pump resistance genes II



β-lactam resistance genes I

β-lactam(penicillin) resistance genes II



Aminoglycoside resistance genes

Sulfonamide resistance genes

Figure 6 | Effect of the ozone–tea polyphenols disinfection process on different types of ARGs.

Figure 6(j) shows that the detection rate of sulfonamide resistance genes in water samples was very high. The reduction of sulfonamide resistance genes can greatly reduce the risk of drinking water pollution. The ozone–tea polyphenols disinfection process has a good effect on sulfonamide resistance genes overall. Especially for sul1, the removal effect reached 60%. However, it did not inhibit sul2 but promoted growth.

3.3. Mechanism of the ozone–tea polyphenols disinfection process on ARGs

When ozone is used as a disinfectant, the reduction effect of ARGs is poor (Zhuang *et al.* 2015). Zheng *et al.* (2017) found that the abundance of vancomycin, β-lactam, and tetracycline resistance genes increased after ozone disinfection, and the removal rate of ARGs did not increase significantly when ozone concentration increased. Ozone can react with cell wall, protein, DNA, lipids, and polysaccharides in bacteria. It was found that the effect of ozone on bacteria was not selective, because the interaction between ozone and bacteria first occurred in the cell wall and other components, followed by bacterial DNA. Therefore, the non-selective characteristics of ozone disinfection restrict the removal effect of ARGs. Although ozone disinfection is efficient in drinking water treatment, it cannot effectively control emerging ARGs pollutants, and there are still potential risks.

When tea polyphenols are used as assistant disinfectants of ozone, most tetracycline resistance genes and some vancomycin resistance genes can be effectively controlled, especially for aminoglycoside and β-lactam resistance genes. The ozone–tea polyphenols disinfection process has a good control effect on sul1 and sul3 with increased abundance after ozone disinfection. Song *et al.* (2020) found that the host bacteria of tetracycline resistance genes were mostly Gram-negative bacteria. Tea polyphenols have a strong killing effect on Gram-negative bacteria and can effectively inhibit the spread of tetracycline resistance genes. Most of the host bacteria of β-lactamase ARGs are Gram-positive bacteria, Gram-negative cocci, and Haemophilus, and most of the host bacteria of aminoglycoside ARGs are Gram-negative bacilli, *Pseudomonas* spp. genus, *Pseudomonas* genus, *Mycobacterium tuberculosis* genus, and *Staphylococcus*. Tea polyphenols have a broad spectrum of antibacterial properties. And tea polyphenols remove resistance genes mainly by inhibiting the growth of the corresponding host bacteria. It was found that EGCG and ECG, the monomers of tea polyphenols, had good inhibitory effects on tetracycline efflux pumps. This hindered the role of ARGs and reversed the antibiotic resistance of ARB. Compared with tetracycline resistance genes, the effect of ozone–tea polyphenols disinfection on sulfonamide resistance genes was lower, because the tolerance of sulfonamide resistance genes to ozone treatment was higher than that of tetracycline, which was similar to the results of Zheng *et al.* (2017). Tea polyphenols as an assistant disinfectant can make up for the drawbacks of ozone disinfection.

The direct oxidation of ozone molecules plays an important role in the removal of ARGs. The hydroxyl radicals generated by oxidation can attack the double bonds and the negative electron parts existing in different types of ARGs (Lueddeke *et al.* 2015). It can also break the main chain and side chain of protein to fragment it, but hydroxyl radicals usually react non-selectively with most compounds to reduce the removal rate of ARGs. Our group has done quantitative experiments on hydroxyl radicals before. The ozone–tea polyphenols disinfection process was used to treat the raw water, and the ozone contact time was chosen to be 15 min by controlling the ozone dosage of 0, 1, 2, and 3 mg/L, and the tea polyphenol dosage of 10, 20, 30, 40, 50, 60, 70, 80, 90, and 100 mg/L, and the hydroxyl radicals in the water were detected after 5 min of tea polyphenols dosing. When the ozone dosage was 0 mg/L, the amount of –OH in water increased with the increase of tea polyphenols dosage, indicating that the higher the concentration of tea polyphenols in water, the larger the amount of free –OH in water. When ozone was added to the water, with the increase of tea polyphenols concentration, the amount of –OH in the water appeared to be the lowest value at the tea polyphenols dosage of 30 mg/L, and even lower than the amount of –OH at the ozone dosage of 0 mg/L and tea polyphenols dosage of 30 mg/L, indicating that at 30 mg/L, the amount of –OH could not be free in the water –OH, while consuming the oxygen radicals in the water, making the sterilization effect weaker. The amount of –OH in the water gradually increased as the amount of tea polyphenols increased from 30 to 100 mg/L, the rising trend was smooth, and there was no sudden increase in the amount of –OH, and the larger the amount of ozone was added at the same amount of tea polyphenols, the lower the amount of –OH was lower, which means that the tea polyphenols consumed the oxygen radicals in the water, and the measured –OH was free in the water. When tea polyphenols exist in water, they can free out a part of hydroxyl radicals, which increase with the increase of tea polyphenols concentration. However, when ozone and tea polyphenols coexist, the concentration of hydroxyl radicals will affect the role of tea polyphenols. If hydroxyl radicals produced by ozone are consumed mostly during disinfection, tea polyphenols will help to destroy ARGs. If ozone disinfection is not complete or there are a large amount of residual hydroxyl radicals generated by ozone, the performance of tea polyphenols will be inhibited and cannot play a role. Therefore, the ozone dosage and ozone exposure time of the ozone–tea polyphenols disinfection process can greatly affect the reduction of ARGs.

4. CONCLUSION

(1) The ozone–tea polyphenols disinfection process demonstrated a significant removal effect on 20 ARGs with high water content, such as MacB, with an average removal rate of 56.5%. This method showed a high success rate in removing CeoA resistance genes and can be applied to specific raw water that contains more CeoA resistance genes.

(2) Tetracycline, sulfonamide, β-lactam, and aminoglycoside resistance genes were effectively removed by the ozone–tea polyphenols disinfection process. This may effectively compensate for the drawbacks of ozone disinfection's inadequate removal of tetracycline and sulfonamide resistance genes, as well as manage the enrichment of β-lactam resistance genes in water distribution systems. The risk of ARGs on human health can be greatly reduced if tetracycline and β-lactam resistance genes are efficiently removed. The ozone–tea polyphenols disinfection process was suitable for specific raw water with higher concentrations of tetracycline, sulfonamide, β-lactam, and aminoglycoside resistance genes, whereas raw water with higher concentrations of vancomycin resistance genes required additional control measures to prevent their spread and transfer.

(3) The efficient death of Gram-negative bacteria by the ozone–tea polyphenols disinfection process may be responsible for the efficient elimination of tetracycline resistance genes (the main host cells of tetracycline resistance genes). The effect of the ozone–tea polyphenols disinfection process on ARGs was to reduce the spread of ARGs by damaging the ARG molecules and inhibiting the proliferation of host cells of ARGs. Moreover, the ozone dosage and ozone exposure time in the ozone–tea polyphenols disinfection process may greatly affect the reduction of ARGs.

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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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