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Review article

Detection and various environmental factors of antibiotic resistance gene horizontal transfer

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ABSTRACT

Bacterial antibiotic resistance in water environments is becoming increasingly severe, and new antibiotic resistant bacteria (ARB) and antibiotic resistance genes (ARGs) have also attracted the attention of researchers. The horizontal transfer of ARGs in water environments is considered one of the main sources of bacterial resistance in the natural environment. Horizontal gene transfer (HGT) mainly includes conjugation, natural transformation, and transduction, and conjugation has been investigated most. Several studies have shown that there are a large number of environmental factors that might affect the horizontal transfer of ARGs in water environments, such as nanomaterials, various oxidants, and light; however, there is still a lack of systematic and comprehensive reviews on the detection and the effects of the influence factors of on ARG horizontal transfer. Therefore, this study introduced three HGT modes, analysed the advantages and disadvantages of current methods for monitoring HGT, and then summarized the influence and mechanism of various factors on ARG horizontal transfer, and the possible reasons for the different effects caused by similar factors were mainly critically discussed. Finally, existing research deficiencies and future research directions of ARG horizontal transfer in water environments were discussed.

1. Introduction

Antibiotics are one of the greatest inventions of mankind in the 20th century and the guardian of human life (MacLean and San Millan, 2019). Antibiotics kill sensitive pathogenic bacteria and select antibiotic resistant variants, resulting in antibiotic resistance. Environmental pollution of antibiotic resistance has attracted extensive attention (Martinez, 2008). Antibiotic-resistant bacteria (ARB) have a strong resistance to antibiotics, resulting in the reduction or even complete failure of antibiotic efficacy in ARB infections. In 2008, it was reported that in the European Union, at least 25000 patients died due to infections by multidrug-resistant bacteria every year, resulting in additional medical expenses and productivity loss of at least 1.5 billion euros (Braine, 2011). It is estimated that 10 million people will die of ARB infections worldwide by 2050, with an economic impact of 100 trillion dollars (O'Neill, 2016). Bacterial antibiotic resistance has become one of the most severe problems threatening human health in the 21st century. Human beings need to cooperate to overcome this problem.

There are two ways for bacteria to acquire antibiotic resistance, including producing ARGs by spontaneous gene mutation and obtaining ARGs by horizontal gene transfer (HGT). However, the transmission modes of these two ways are different. The former is mainly transmitted to the offspring through vertical genes transfer (VGT), while the latter has the potential to transmit ARGs faster, which can be transmitted between different bacteria, even different species (Andam et al., 2011; Summers, 2006). HGT mainly consists of three pathways: conjugation, natural transformation, and transduction (Marraffini and Sontheimer, 2008). Multidrug-resistant bacteria are more harmful than single drug-resistant bacteria because they are resistant to various antibiotics (Fiorentino et al., 2015; Kanchanapally et al., 2015; Nikaido, 2009). Single drug-resistant bacteria can obtain exogenous ARGs by HGT to become multidrug-resistant bacteria. Most researchers think that one of the important pathways to multidrug resistance to ARB is the horizontal transfer of ARGs (Qiu et al., 2012). Then the study of the horizontal transfer of ARGs has attracted extensive attention from researchers.

ARB and ARGs entering the environment will spread in urban sewage treatment plants, animal farm wastewater and sediments, medical wastewater, surface water, groundwater, and soil ecosystems through the water cycle, such as surface runoff or soil infiltration, and finally penetrate all corners of the Earth biosphere (Bengtsson-Palme and

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Larsson, 2015; Li et al., 2016; Liu et al., 2012). Studies have shown that most ARGs in wastewater treatment plants are situated on mobile element plasmids (Che et al., 2019). Water environments are hotspots of ARG horizontal transfer. The risk of transmission of ARGs in aquatic environments through horizontal transfer must be a research focus. Since Qiu et al. (2012) first reported that nano Al_2O_3 in water environments promoted the horizontal transfer of ARGs mediated by the RP4 plasmid in 2013, the effects of various environmental materials and factors that may enter the water body or be used for water treatment on the horizontal transfer of ARGs have attracted the attention of researchers (Cui and Smith, 2022). At present, reviews of ARGs in water environments mostly focus on the control technology of ARGs by water supply treatment plants or wastewater treatment plant processes and the distribution of ARGs (Almeida Kumlien et al., 2021; Karkman et al., 2018; Michael-Kordatou et al., 2018; Zhang et al., 2020a); however, reviews on the horizontal transfer of ARGs are lacking. Hasegawa et al. (2018) summarized the natural transformation of plasmids in *E. coli* in the environment but did not analyse conjugation and transduction; Abe et al. (2020) reviewed the HGT on biofilms in water environments but did not analyse suspended bacteria; Wu et al. (2021) reviewed the horizontal transfer of ARGs in soil biofilms but did not consider the analysis of water environments; Liu et al. (2020) summarized the correlation between exogenous compounds and the horizontal transfer of ARGs carried by plasmids from a clinical perspective; however, the analysis of factors in water environments is far from sufficient, and there is a lack of phage-mediated transformation analysis; Jiang et al. (2022) reviews the effects of non-antibiotic factors on ARG horizontal transfer, but the detection technologies are not analysed and the critical discussion of differences caused by the same disinfection methods is lacking, and in fact, antibiotics are also very likely to enter the water environments to affect HGT. In summary, there is still a lack of systematic and comprehensive reviews on the detection technologies and environmental influence factors of ARG horizontal transfer in water environments.

In this review, three modes of HGT, including conjugation, natural transformation, and transduction, were introduced. Second, various analytical methods for detecting the horizontal transfer of ARGs were reviewed, and their limitations were critically analysed. Thereafter, the effects of various factors in water environments on the horizontal transfer of ARGs were reviewed, and the possible reasons for the different effects caused by similar factors were mainly discussed. Next, the mechanisms of various factors affecting the horizontal transfer of ARGs were reviewed, and the relationship between them was summarized. Finally, the limitations of existing studies were analysed, and the future research directions of the horizontal transfer of ARGs in water environments were discussed.

2. HGT modes

Transmission modes of ARGs are vertical transfer and horizontal transfer (Dodd, 2012). VGT refers to the transfer of genes from the parents to the offspring. HGT is the exchange of genetic material between different organisms or between organelles in a solitary cell, including conjugation, natural transformation, transduction and membrane vesicles (MVs).

2.1. Conjugation

Conjugation refers to after the contact between donor and recipient bacteria, a conjugation bridge between the donor and recipient is synthesized through the conjugative pili, and the movable genetic elements (usually plasmids or transposons) in the donor can be transferred to the recipient through the channel (Davison, 1999; Roberts et al., 2001). The conjugative plasmid encodes the conjugation system control genes, which can spontaneously perform conjugation (Qiu et al., 2012), whereas the non-conjugative plasmid can also perform the transfer with the help of the system generated by conjugative plasmid expression (Davison, 1999). Many plasmids are under a wide range of hosts and can spread among bacteria of different species and genera or even between different biological species. For example, the conjugation can occur between bacteria and yeast or between bacteria and plants (Heinemann and Sprague, 1989; Roberts et al., 2001).

2.2. Natural transformation

Natural transformation refers to the direct uptake of free DNA by bacteria from the environment and the acquisition of the corresponding genetic traits (Magasanik, 1999). The occurrence of transformation must change the permeability of the cell membrane; that is, the cell membrane presents a receptive state (Maeda et al., 2004). Transformation must also meet the following conditions: DNA must exist in the extracellular environment; the recipient bacteria must have the ability to transform; the transported DNA must be stable and transferred to the recipient genome through integration or directly into the recipient bacteria (plasmid DNA) (Johnston et al., 2014).

2.3. Transduction

DNA in donor cell is transferred to recipient cell through phage vector infection, resulting in gene changes in the recipient cell, which is called transduction (Chen et al., 2018). When phages proliferate and cleave bacteria in donor bacteria, some DNA phages (called transduction phages) can wrap the bacterial DNA into the head protein capsid by mistake as the DNA of the phage itself in rare cases (once in approximately 10^5–10^7 packaging); after cleaving the donor bacteria, the released phage can carry the DNA of the donor bacteria into the recipient bacteria by infecting the susceptible recipient. If recombination occurs, the recipient bacteria can obtain the DNA fragment of the donor bacteria transferred by the phage vector and obtain the corresponding genetic traits (Dion et al., 2020; Jain et al., 1999).

2.4. Other modes

MVs are mostly lipid bilayer closed particles with a diameter of 20–400 nm released by bacteria (Toyofuku et al., 2019). MVs are released from planktonic cells and biofilm (Abe et al., 2020). Dorward et al. (1989) first reported that MVs could carry the R plasmid for HGT between bacteria in 1989. Several studies confirmed that MVs carried ARGs for HGT between bacteria (Abe et al., 2020). At present, there are no reports on MV-mediated HGT in biofilms, and follow-up studies can continue to examine this aspect. There are also few studies on influencing factors of MV-mediated HGT, so the following discussion mainly involves conjugation, natural transformation, and transduction.

3. HGT detection technologies

3.1. Plate culture

The donor (or gene fragment or phage) and recipient bacteria carry different ARGs and are resistant to different antibiotics. Based on the traditional culture method, a culture plate containing different antibiotics is made, and the donor and recipient ARB are screened by culture plates containing specific antibiotics, because bacteria that fail to meet the screening conditions cannot survive on the corresponding plate. HGT bacteria (which refer to the recipient bacteria acquiring the ARGs from donor through HGT) can survive on the plates containing the antibiotics which connected with the donor and recipient bacteria (Rizzo et al., 2013). Therefore, donor, recipient, and HGT bacteria can be distinguished.. Although the horizontal transfer of ARGs from HGT bacteria to recipient bacteria may also occur, the frequency of these cases is exceedingly low and can be ignored because the frequency of HGT (the number of HGT bacteria/the number of recipient bacteria or

donors) is mostly approximately 10^{-11}-10^{-4} in most studies (Lin et al., 2016; Qiu et al., 2012; Zhang et al., 2017). The number of HGT bacteria detected is considered only transferred from donor to recipient bacteria.

The traditional culture method is commonly used and simple to operate; however, it requires a high initial bacterial concentration. There are many microbial species; however, most of them are not culturable, and only approximately 1% can be cultured in the medium. The viable but non-culturable (VBNC) state of bacteria is an inactive form of life which is induced by stressful conditions and undergoes recovery under suitable conditions. It shows low but detectable metabolic activity, expresses genes at low levels and cannot form the colony forming units (CFUs) on culture media (Schottroff et al., 2018). Therefore, many factors such as chlorine will cause bacteria to enter VBNC state, which leads to inaccurate detection results of the culture method. In addition, using the culture method for detection, when there are abundant nutrients in the reaction system, bacterial proliferation occurs. At this time, the first HGT bacteria may vertically transfer the ARGs by natural proliferation. This will result in the fact that not all the bacteria detected by the culture method are HGT bacteria obtained by the horizontal transfer of ARGs.

3.2. Fluorescent labelling technology

Based on the fluorescent marker, this method processes gene fragments, such as plasmids; thus, they emit fluorescence once expressed. At the same time, the bacteria are marked with different colours of fluorescence and the fluorescent signals of different colours are detected by a fluorescence microscope, fluorescence-activated cell sorting (FACS) or confocal laser scanning microscope (CLSM), which can distinguish the receptor bacteria and HGT bacteria (Arias-Andres et al., 2018; Klumper et al., 2015). Among them, when FACS is used, cells can be quickly screened and counted, but single cell imaging cannot be performed; fluorescence microscope and CLSM can be used to observe cell conformation; but CLSM imaging is clearer, and serial layer scanning and three-dimensional imaging can be achieved by CLSM. Although the method requires the addition of fluorescent labelling and the operation is complex, HGT is visualized when ARGs enter the recipients. HGT in complex environments can be studied; however, vertical and horizontal transfers cannot be distinguished when nutrients are sufficient, and it is still difficult to rule out the probable transfer of ARGs in VBNC because the labelled fluorescent proteins cannot be expressed by VBNC.

3.3. Molecular biology

The method based on molecular biology can be utilized to detect HGT (Huang et al., 2019a). There is a replication of the transferred genes in the process of HGT, and the increased number of ARGs is positively related to the horizontal transfer of ARGs. Horizontal transfer genes and donor-specific DNA are quantified, and the occurrence of HGT is reflected by the change in their ratio. This method can monitor HGT between various microorganisms, including non-culturable microorganisms. However, when nutrients are abundant, vertical and horizontal transfers cannot be distinguished. In addition, when studying HGT in complex environments, this method cannot distinguish the modes of HGT, that is, it cannot be able to determine whether the receptor bacteria obtain ARGs through conjugation, transformation, or transduction.

3.4. Microfluidic technology and fluorescent labelling technology

Microfluidic technology is based on a micron-scale fluid engineering system, which can accurately control various parameters and realise the simulation and manipulation of the spatial structure and chemical properties of the microenvironment (Nge et al., 2013). Microfluidic technology combined with fluorescent protein technology and microscopy can also be used to identify key HGT mechanisms in single- or multi-species biofilms. Successful HGT can be designated by fluorescence changes. The transmission of ARGs in the environment usually comes from horizontal and vertical transfer; however, few studies have distinguished the role of these two pathways (Li et al., 2019). Using microfluidic technology, the biofilm formation process on the chip can be dynamically tracked to determine the maximum specific cell growth rate and specific growth rate of HGT bacteria (Nolivos et al., 2019). The ratio of these two values can represent the relative contributions of the horizontal and vertical transfers (Li et al., 2019). Dynamic change of HGT can be identified by fluorescence changes and the process of a single-cell is visualized (Yu et al., 2021).

Differences of HGT detection technologies are summarized in Table 1. Studies on the effects of various environmental factors on the horizontal transfer of ARGs mostly use the plate culture method, and the other three methods are less frequently used. In fact, plate culture is more suitable for pure bacteria experiment, which is helpful to explore the mechanism of HGT. When the research involves real environments, fluorescent labelling technology is recommended, and the test results are more intuitive. Generally, while analysing the composition of the microbial community, molecular biological detection methods are utilized to analyse the horizontal transfer of ARGs and the HGT in VBNC can be identified. Because the horizontal transfer is only a small part of such research, research using this method lacks an explanation for the mechanism of various factors affecting HGT in the relevant environment. Microfluidic technology can be used to study biofilms, and there is still little research on HGT in biofilms affected by various factors. With researchers' attention on HGT in biofilms, this technology will have more applications in the future. In addition, the four technologies have their own characteristics, so comprehensive utilization of them can make best use of the advantages and bypass the disadvantages, and make the research results more transparent and reliable, however, most studies use only one method. The establishment of HGT system in actual water environments and the comprehensive use of the four technologies to track and detect the horizontal transfer of ARGs can be the research direction in the future.

Table 1
The difference of HGT detection technologies.

HGT detection technologies	Principle	Advantages	Limitations
Plate culture	The bacteria are screened by culture plates containing specific antibiotics.	Simple operation.	VBNC bacteria can not be detected; HGT or VGT cannot be distinguished.
Fluorescent labelling technology	The bacteria are distinguished by different colours of fluorescence.	HGT is visualized when ARGs enter the recipients.	Complex operation; VBNC bacteria can not be detected. HGT or VGT cannot be distinguished.
Molecular biology	The ratio of horizontal transfer genes to donor-specific DNA can reflect HGT frequency.	HGT between many microorganisms, including VBNC microorganisms can be detected.	HGT or VGT cannot be distinguished; the modes of HGT cannot be distinguished.
Microfluidic technology and fluorescent labelling technology	Dynamic change of HGT can be identified by fluorescence changes.	Dynamically track the whole process of HGT; HGT or VGT can be distinguished; the process at a single-cell is visualized.	Complex operation; VBNC bacteria cannot be detected.

4. Effects of various factors on ARG horizontal transfer

Studies on the effects of various factors in water environments on HGT have been summarized in Table 2, Table 3, and the reaction medium, transfer genes and mechanisms were listed in Supplementary Information Table S1. Most studies have focused on suspended bacteria in water, and only a few cases of HGT in activated sludge biofilms or other biofilms have been studied. At present, studies on the effects of nanomaterials, oxidants (including disinfectants such as chlorine and chloramine), quorum sensing signal molecules, photocatalysis, organic pollutants, metals, and other factors on the horizontal transfer of ARGs have been reported. Among these studies, the conjugation was completed only with the participation of donor and recipient bacteria and the process model was established in a relatively early time, while the high-frequency transformation commonly required the recipient bacteria to enter the competent state and bacteria were difficult to enter the competent state in general water environments, and the complete system of transduction was still not completely established. Therefore, conjugation has been investigated the most. The role of the disinfection process in water treatment is mainly to inactivate pathogenic microorganisms in water, and it has a significant impact on microbial life activities; so its influence on the horizontal transfer of ARGs should be considered. Therefore, disinfection methods such as chlorine and UV are the most studied. Many researchers also have different views when studying the impact of the same substance on ARG horizontal transfer (Guo et al., 2015; Lin et al., 2016; Zhang et al., 2017), and the reasons for these different views are mainly further analysed in the following. The effects of environmental factors on HGT are not discussed too much because they have been clearly listed in the Table S1.

Plasmid is the most studied MGEs, ARGs carried on plasmids are important parts of the total ARGs in wastewater treatment plants, even the largest proportion (Che et al., 2019, 2022; Dai et al., 2022). Plasmids carrying ARGs transmit antibiotic resistance in water environments through HGT and there are many factors that can promote the horizontal transfer of plasmids, which should be considered. It is worth mentioning that antibiotics can not only naturally select bacteria with the gene mutation to increase ARGs abundance (MacLean and San Millan, 2019), but also increase the abundance of ARB in water environments by promoting ARG conjugation. For example, tetracycline, gentamicin and sulfamethoxazole can promote ARG conjugation (Jutkina et al., 2016, 2018; Zhang et al., 2018c). There are few studies on the effects of antibiotics on natural transformation and transduction, and follow-up studies can continue to consider this aspect. The task of controlling the entry of antibiotics into the water environments should be concerned. Above all, plasmids and antibiotics are essential for the transmission of ARGs.

4.1. Effects of factors on conjugation

Before studying the effects of various environmental factors on conjugation, the basic reaction conditions such as initial bacterial concentration, donor/recipient ratio, metallic activity of mating pairs, and nutrients need to be determined. Generally, in order to facilitate the study of mechanism, the initial bacterial concentration is 10^6–10^9 CFU/mL, the donor/recipient ratio is about 1:1, the logarithmic bacteria are selected as the research object, and the nutritional conditions are configured according to the selected water environment (Guo et al., 2015; Qiu et al., 2012; Zhang et al., 2017). As shown in Table 2, nanomaterials such as nano-alumina can promote the conjugation of ARGs; however, when the concentration is extremely high, the conjugative frequency decreases because of the large number of bacteria killed by nanomaterials (Qiu et al., 2012). Oxidants, such as chlorine and hydrogen peroxide, also showed the same effects as nanomaterials. Most factors can promote conjugation under certain conditions; and some studies have shown that conjugation is inhibited by quorum sensing inhibitors (QSIs) (Zhang et al., 2018c; Zhu et al., 2020), such as

Table 2
Effects of environmental factors on ARG conjugation (More details were listed in Supplementary Information Table S1).

Factors and transfer detection methods	Effects	Reference
Disinfectants and oxidants		
Chlorination (0.5–160 mg Cl min/L); plate culture.	Low chlorine doses (0.5–40 mg Cl min/L) promoted the frequency of conjugation by 2-5-fold, high doses (>80 mg Cl min/L) inhibited the transfer.	Guo et al., (2015)
Low-level chlorination (0.05–0.5 mg/L) mating for 6–48 h; plate culture.	The transfer frequency was not significantly affected by chlorine (0.05–0.2 mg/L); but was decreased to 4.40 × 10^{-5} or below the detection limit with 0.3–0.5 mg/L chlorine.	Lin et al., (2016)
Free chlorine (0.1–10 mg/L), chloramine (0.1–10 mg/L), and H_2O_2 (0.24–60 mg/L) mating for 30 min; plate culture.	Free chlorine (0.1–1 mg/L Cl_2), chloramine (0.1–1 mg/L Cl_2) and H_2O_2 (0.24–3 mg/L) led to increases in conjugation by 3.4–6.4,1.9-7.5, and 1.4–5.4 folds.	Zhang et al., (2017)
Nanomaterials		
Nano-alumina (0.5–50 mmol/L); plate culture	Nano-alumina (5.0 mmol/L) promoted the conjugation by up to 200-fold compared with un-treated cells.	Qiu et al., (2012)
Nano- ZnO (nZnO) at sublethal concentrations (1–10 mg/L) ; plate culture.	Nano- ZnO promoted the conjugation by up to 24.3-fold.	Wang et al., (2018a)
Nano-Fe_2O_3 composited with MoS_2; plate culture.	Nano-Fe_2O_3 promoted conjugation; nano-Fe_2O_3 composited with MoS_2 inhibited the transfer.	Wang et al., (2019a)
Nano-CuO and Cu^{2+} (1–100 μmol/L); plate culture.	Nano-CuO and Cu^{2+} promoted conjugation by up to 3.0-fold.	Zhang et al., (2019)
Nano- CeO_2 (1–50 mg/kg); plate culture.	Nano- CeO_2 (1 and 5 mg/kg) inhibited conjugation by 22–26%, Nano- CeO_2 (25 and 50 mg/kg) promoted transfer by 118–123%.	Yu et al., (2020)
Graphene oxide (GO, 0.1–100 mg/L); plate culture.	GO (50 mg/L) promoted the conjugation by up to 10-fold.	Guo and Zhang (2017)
Natural sphalerite nanoparticles (0.5–500 mg/L); plate culture.	Natural sphalerite nanoparticles (0.5–50 mg/L) promoted conjugation by up to 11-fold.	Li et al., (2020a)
Quorum sensing signal molecules		
Quorum sensing (QS) signal molecules N-(beta-ketocaproyl)-dl-homoserine lactone (3-oxo-C6-HSL, C6) and sulfonamides (SAs); plate culture.	C6 (20 μmol/L) enhanced the inhibitory effect of sulfanilamides (10^{-7}-10^{-3} μmol/L) on the conjugation transfer.	Wang et al., (2018b)
Tetracyclines (TCs); Quorum sensing autoinducers (AIs); quorum sensing inhibitors (QSIs) ; plate culture.	Subinhibitory concentration TCs facilitated the conjugation, which was enhanced by AIs but inhibited by QSIs.	Zhang et al., (2018c)
QSIs (25–800 mg/L); plate culture.	QSIs inhibited conjugation by up to 58-fold.	Zhu et al., (2020)
QSIs; plate culture.	Low concentration QSIs promoted conjugation; while high concentration QSIs inhibited the transfer.	Li et al., (2021)
Photocatalysis or light irradiation		
Ultraviolet (UV, 1–40 mJ/cm^2); plate culture.	Low UV doses (up to 8 mJ/cm^2) had little influence on conjugation; The frequency of ARG transfers was greatly	Guo et al., (2015)

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Table 2 (*continued*)

Factors and transfer detection methods	Effects	Reference
	suppressed by high doses of UV (>10 mJ/cm^2).	
UV irradiation (5–100 mJ/cm^2); plate culture.	The transfer frequency decreased from 2.8×10^{-3} to 2.44×10^{-5} after exposure to UV (5–20 mJ/cm^2).	Lin et al., (2016)
Light irradiation including visible light (VL, 60 mW/cm^{-2}), simulated sunlight (SS, 60 mW/cm^{-2}) and ultraviolet light (UV_{254}, 4 μW/cm^{-2}); plate culture.	VL did not affect conjugation; SS slightly promoted the transfer by 2-10-fold; UV promoted the transfer by up to 100-fold.	Chen et al., (2019)
Natural sphalerite (NS) with different light sources (UV_{254}, UV_{365}, simulated sunlight (SS), and visible light (VL)); plate culture.	Natural sphalerite with different light sources promoted conjugation by 1–10 folds.	Yin et al., (2021)
TiO_2/Ag/GO powder (10–100 mg/L) with the light source (500 W Xenon lamp); plate culture.	TiO_2/Ag/GO promoted conjugation by 1–2 folds.	Guo and Tian (2019)
Organic pollutants		
Tetracycline (0–10000 μg/L); plate culture.	Tetracycline (0–1000 μg/L) promoted the conjugation by up to 3.5-fold; while high concentrations (10000 μg/L) inhibited conjugation.	Jutkina et al., (2016)
Antibiotics and common antibacterial biocides; plate culture.	Chlorhexidine (24.4 μg/L), triclosan (0.1 mg/L), gentamicin (0.1 mg/L) and sulfamethoxazole (1 mg/L) increased the transfer.	Jutkina et al., (2018)
Triclosan (TCS, 0.02–2000 μg/L); plate culture.	TCS (0.02–20 μg/L) promoted the conjugation by up to 6.0-fold.	Lu et al., (2018)
Six repre-sentative types of organic compounds in textile dyeing wastewater; plate culture.	Six selected organic compounds in dyeing wastewater (malachite green, 2,4-dichloroaniline, styrene, ethylbenzene, trioxymethylene and o-xylene) promoted conjugation by 4- to 200-fold.	Jiao et al., (2017)
Four petrol and diesel exhaust particles (97 octane petrol, 93 octane petrol, light diesel oil, and marine heavy diesel oil); plate culture.	These four kinds of nano-particles (0–320 mg/L) promoted the transfer by up to 5.1-fold.	Zhang et al., (2018b)
Quaternary ammonium compounds (QACs, 10^{-5}–100 mg/L); plate culture.	QACs promoted conjugation by up to 9.0-fold.	Han et al., (2019)
Carbamazepine (0.05–50 mg/L); plate culture.	Carbamazepine promoted conjugation by up to 8-fold.	Wang et al., (2019b)
Microcystins (MCs, 10^{-5}–5 mg/L); plate culture.	MCs promoted conjugation by up to 25.13-fold.	Xu et al., (2020)
linoleic acid (LA, 0.03–3 mol/L) and α-linolenic acid (ALA, 0.03–3 mol/L); plate culture.	LA and ALA inhibited conjugation by up to 100-fold.	Li et al., (2020b)
Glyphosate (0.1–0.6 mg/L); plate culture.	Glyphosate promoted conjugation by up to 4-fold.	Zhang et al., (2021a)
Non-antibiotic pharmaceuticals (ibuprofen, naproxen, diclofenac, gemfibrozil, propranolol, 0.005–50.0 mg/L); plate culture.	Non-antibiotic pharmaceuticals promoted conjugation by up to 8-fold.	Wang et al., (2021b)
Metals		
Sub-inhibitory concentrations Cu(II), Ag(I), Cr(VI), and Zn (II); plate culture.	Sub-inhibitory concentrations heavy metals	Zhang et al., (2018a)

Table 2 (*continued*)

Factors and transfer detection methods	Effects	Reference
	promoted the conjugation by up to 3.0-fold.	
Zero valent iron (Fe^0, 0.15–3.75 g/g) ; quantitative qPCR.	Fe^0 promoted the conjugation of tetracycline resistance genes in plasmids.	Yang et al., (2018)
Heavy metals (Cu, Cd, Pb, Zn, 0.05–200 μg/L); plate culture.	Cu, Pb and Zn promoted conjugation by up to 16-fold; Cd inhibited the transfer by up to 3-fold.	Wang et al., (2020a)
Cd^{2+} (1–100 mg/L); plate culture.	Cd^{2+} promoted conjugation by up to 10-fold.	Pu et al., (2021)
Cu^{2+} (0.5–100 μmol/L); plate culture.	Cu^{2+} promoted conjugation by up to 2-fold.	Song et al., (2021)
Heavy metal (Cu^{2+} and Zn^{2+}); plate culture.	Heavy metal promoted conjugation by up to 2–10 folds.	Wang et al., (2021a)
Other factors		
1-butyl-3-methylimidazolium hexafluorophosphate ([BMIm][PF6]) (0.001–5.0 g/L); plate culture.	[BMIm][PF6] (1.0 g/L) promoted the conjugation by up to 60-fold compared with un-treated cells.	Wang et al., (2015c)
Different structures of ionic liquid (with 4, 6, and 8 C atoms in the longer alkyl chain); quantitative real-time PCR (qPCR) and plate culture.	Ionic liquid with increasing the alkyl chain length exerts decreased the ability in facilitating conjugation.	Wang et al., (2015a)
Different kinds of ionic liquid (Mainly for [BMIm][PF6], 0–2.5 g/L); plate culture..	[BMIm][PF6] (0.5 g/L) promoted the conjugation by up to 16-fold compared with un-treated cells.	Wang et al., (2015b)
pH (4–10); plate culture.	pHs had no significant impacts in LB medium; treating sludge under acidic conditions benefited the transfer, but reduced the potential of conjugation at alkaline pHs.	Huang et al., (2016)
Aquatic animal guts; plate culture and flow cytometry.	15% faecal bacteria obtained ARGs through conjugation. The hindgut was the most important intestinal region supporting ARG transfer.	Fu et al., (2017)
Low-intensity (≤0.05 W/cm^2) ultrasound ; plate culture.	Low-intensity ultrasound had no effect on bacterial growth and survival rates, but increased the conjugative frequency by 300-fold.	Song et al., (2018)
Microplastics polystyrene (PS); fluorescent labelling.	Compared with suspended bacteria, the frequency of plasmid transfer in bacteria on the surface of microplastics increased.	Arias-Andres et al., (2018)
CO_2 (3000–30000 mg/L); plate culture.	CO_2 promoted the conjugation by up to 1.3–9.0 folds.	Liao et al., (2019b)
Urban fine particulate matter ($PM_{2.5}$, 31.25–500 μg/L); plate culture.	$PM_{2.5}$ promoted conjugation by up to 2.9-fold.	Xie et al., (2019)
Inhalable particulate matter (PM, 31.25–500 μg/L); plate culture.	$PM_{2.5}$ and PM_{10} increased conjugation by 110% and 30%.	Zhou et al., (2021b)
Low-level free nitrous acid (FNA, 0–0.02 mg/L); plate culture.	FNA inhibited conjugation by up to 10-fold.	Huang et al., (2019b)
Soil minerals (kaolinite, montmorillonite, goethite and birnessite, 0.005–5 g/L); plate culture.	Birnessite promoted conjugation by 1.3–3.4 folds; kaolinite and montmorillonite had no effects; the transfer rate increased and then decreased as the goethite concentration increased.	Wu et al., (2020)

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Table 2 (*continued*)

Factors and transfer detection methods	Effects	Reference
Preservatives (sodium nitrite, sodium benzoate, and triclocarbon, 0.01–10 mg/L); plate culture.	Sodium nitrite, sodium benzoate, and triclocarbon promoted conjugation by 1.24–2.63, 6.79–7.05, and 2.17–4.31 folds.	Cen et al., (2020)
Biological powdered activated carbon (BPAC, 20–80 mg/L); plate culture.	BPAC promoted conjugation by 12-fold.	Sun et al., (2020)

N-(beta-ketocaproyl)-dl-homoserine lactone (3-oxo-C6-HSL, C6) (Wang et al., 2018b), free nitrous acid (FNA) (Huang et al., 2019b), linoleic acid (Li et al., 2020b), and α-linolenic acid (Li et al., 2020b). In addition, in most experiments, the frequency of conjugation between bacteria of the same species was usually higher than that of different species (Qiu et al., 2012; Wang et al., 2015d).

In conjugation experiments, similar tests reached different conclusions. Guo et al. (2015) found that low chlorine doses (0.5–4.0 mg/L, 10 min, 5–40 mg Cl min/L) promoted the frequency of conjugation by 2–5 times; Zhang et al. (2017) found that free chlorine (0.1–1 mg/L, 30 min, 3–30 mg Cl min/L) led to increases in conjugation by 3.4–6.4 times. After the chlorine dose continued to increase (up to 300 Cl min/L), the conjugation frequency decreased or was even lower than the detection limit. However, Lin et al. (2016) found that the transfer frequency was not significantly affected by chlorine (0.05–0.2 mg/L, 6–48 h, 18–576 mg Cl min/L) but was decreased to 4.40×10^{-5} or below the detection limit with 0.3–0.5 mg/L chlorine concentrations. They all found that a very high dose chlorine (>80 mg Cl min/L) could inhibit conjugation; however, when a low chlorine dose (about 30 mg Cl min/L) was present, some results showed promoting effects, and some results showed no significant effects. After analysis, the effect of chlorine on the conjugation frequency was affected by the chlorine concentration and chlorine action time. A single chlorine dose (the unit is mg Cl min/L.) factor could not determine the effect of chlorine on conjugation. The reason Lin et al. (2016) did not conclude that low concentrations of chlorine promoted conjugation might be that their chlorine action time was too long, and the lowest was still 6 h. When the chlorine concentration was low, its effect on bacteria was limited, and prolonging the reaction time just increased chlorine exposure, but did not achieve the results. Therefore, when studying the effect of chlorine on HGT, it is not appropriate to take mg Cl min/L as the abscissa unit. Different results might be obtained by the same chlorine dose, and it is necessary to comprehensively consider the concentration and reaction time. Designing an appropriate chlorine action time according to the actual situation is essential. In addition, strains and conjugation media selected in the above three tests were also different, which might also be reasons for the different results.

Similarly, Guo et al. (2015) and Lin et al. (2016) found that ultraviolet (UV, 0.17 mW/cm^2, 47–588 s, 8–100 mJ/cm^2) could significantly inhibit conjugation by killing bacteria in large numbers; however, Chen et al. (2019) found that UV (4 μW/cm^2, 300–7100 s, 1.2–28.8 mJ/cm^2) promoted the transfer by up to 100-fold. The above differences might be because the UV light intensity selected in the latter experiment was too low to sterilise, and UV under this light intensity promoted the transfer by producing reactive oxygen species (ROS). Therefore, the effects of chlorine and UV on conjugation are jointly affected by the action concentration or power, and reaction time. Specific problems should be discussed in detail. It is possible to draw opposite conclusions under the same test dose. In the actual disinfection process, UV power, chlorine concentration and reaction time is taken into account comprehensively according to reality. At the same dose, increasing chlorine exposure time and reducing UV irradiation time are helpful to inhibit HGT.

Table 3
Effects of environmental factors on ARG transformation and transduction (More details were listed in Supplementary Information Table S1).

Factors and transfer detection methods	Results	Reference
Natural transformation (Disinfectants)		
NaClO (6 mg/L, 20min); plate culture.	NaClO promoted transformation frequency by up to 550-fold.	Jin et al., (2020)
Chloramine and free chlorine (0.5–10 mg/L, 5–15 min); plate culture.	Chlorine disinfection promoted transformation frequency by up to 10-fold.	Zhang et al., (2021b)
Natural transformation (Nanomaterials)		
Nano-ZnO (1–10 mg/L); plate culture.	Nano-ZnO promoted transformation by up to 3-fold.	Wang et al., (2018a)
Nano-Al_2O_3, nano-ZnO, nano-TiO_2 (0–50 mg/L); plate culture.	Nano-Al_2O_3, nano-ZnO, and nano-TiO_2 decreased transformation by up to 0.79, 0.71, 0.64 folds, respectively.	Hu et al., (2019b)
Metal oxide nanoparticles (AlOOH, CuO, Fe_3O_4, TiO_2, and ZnO, 15.6–1000 μg/L); plate culture.	ZnO nanoparticles promoted transformation by 9-fold, while the other nanoparticles reduced it up to 31-fold.	Otinov et al., (2020)
Nano-Al_2O_3 (10 mmol/L); plate culture.	Nano-Al_2O_3 promoted transformation by up to 10^4-fold.	Ding et al., (2021)
Natural transformation (Photocatalysis or light irradiation)		
UV_{254} (20–25 W/cm^2); plate culture.	The transformation was significantly inhibited by UV.	Chang et al., (2017)
UV_{254} (2.1–10.5 mJ/cm^2); solar irradiation (280–800 nm, 69.6–153.2 mJ/cm^2); plate culture.	UV decreased transformation frequency by 0.8 folds, solar irradiation increased by 2 folds.	Augsburger et al., (2019)
Natural transformation (Organic pollutants)		
Substituted aromatic pollutants; plate culture.	Substituted aromatic pollutants promoted transformation frequency by up to 5-fold.	Shou et al., (2019)
Triclosan (TCS, 0.02–20 μg/L); plate culture.	TCS promoted transformation by up to 1.4-fold.	Lu et al., (2020)
Nonsteroidal anti-inflammatories, ibuprofen, naproxen, diclofenac, the lipid-lowering drug, gemfibrozil, and theβ-blocker propranolol (0.005–50.0 mg/L); plate culture.	Transformation frequency was increased by up to 3.0 folds.	Wang et al., (2020b)
Natural transformation (Other factors)		
Bromoacetic acid (0–600 mg/L); plate culture.	Bromoacetic acid increased transformation by up to 2-fold.	Mantilla-Calderon et al., (2019)
CO_2 (1000–30000 mg/L); plate culture.	CO_2 promoted the transformation by 1.4–5.5 folds.	Liao et al., (2019a)
EPS; plate culture.	Transformation efficiency for *E. coli* without EPS was up to 29-fold of those with EPS.	Hu et al., (2019a)
Montmorillonites (MMTs, 10^{-3}–2 g/L); plate culture.	Transformation frequency first increased and then decreased with the increase of MMTs concentration.	Hu et al., (2020)
Transduction (Nanomaterials)		

(*continued on next page*)

Table 3 (*continued*)

Factors and transfer detection methods	Results	Reference
Nano-TiO_2 (0.05–50 mmol/L); plate culture.	Nano-TiO_2 promoted transduction frequency by up to 4.5-fold.	Han et al., (2020)
TiO_2 photoexcitation (0.5 mmol/L, UV_{365}, 50–300 μW/cm^2, 1 h); plate culture.	TiO_2 photoexcitation promoted transduction frequency by up to 20-fold.	Xiao et al., (2021)

4.2. Effects of factors on natural transformation

There are few studies of the natural transformation of ARGs; however, these have been performed in recent years. Most of the factors studied have promoted the natural transformation of ARGs, such as nanoparticles (Ding et al., 2021), chlorine (Zhang et al., 2021b), and some organic pollutants (Shou et al., 2019), while extremely high concentration showed inhibitory effects because a large number of recipient bacteria died or plasmids lost the transformation ability. In addition, UV (Augsburger et al., 2019) and extracellular polymer (EPS) (Hu et al., 2019a) secreted by cells inhibited the natural transformation of ARGs.

In the study of natural transformation, most studies directly added plasmids containing ARGs in the reaction system, and most of the selected plasmids had a high concentration (>10^8 copies/mL). However, in actual water environments, due to the existence of DNAase, the concentration (about up to 10^2 copies/mL in tap water) of extracellular ARGs (eARGs) is often much lower than that of intracellular ARGs (iARGs), it is only approximately 1% of that of iARGs and bacteria have antibiotic resistance only when ARGs are intracellular (Hao et al., 2019). Therefore, horizontal transfer of iARGs was studied more than that of eARGs. However, eARGs can be absorbed by bacteria through transformation and become iARGs, the risk of horizontal transfer of eARGs through natural transformation should also be concerned. Jin et al. (2020) found that NaClO (6 mg/L, 20 min) could kill a large number of non-chlorine-resistant bacteria; but the RP4 plasmid in dead non-chlorine-resistant bacteria would be released into the water and still maintain a certain activity. Simultaneously, the removal effect of NaClO on chlorine-resistant bacteria was very limited, but it would produce ROS, increase the cell membrane permeability of chlorine-resistant bacteria, and enhance the ability of chlorine-resistant bacteria to receive eARGs from natural transformation, leading to the fact that NaClO promoted transformation frequency by up to 550-fold. Although the concentration of eARGs in water is lower than that of iARGs, changing the iARGs in non-chlorine-resistant bacteria into eARGs after chlorine disinfection will significantly but temporarily increase the concentration of eARGs in water. Thus, risk of ARGs entering chlorine-resistant bacteria through natural transformation should be concerned.

In transformation experiments, similar tests reached different conclusions. Hu et al. (2019b) found nano-Al_2O_3 (50 mg/L) decreased transformation frequency by 0.79 folds, Ding et al. (2021) found nano-Al_2O_3 (10 mol/L) increased transformation frequency by up to 10^4 fold. The difference may be caused by the difference of material concentration, transformation medium, reaction conditions, plasmid and strain. The most probable reason is that the reaction conditions. The transformation condition of the former is that the mixed liquid was placed in an ice-water bath for 30 min, followed by heat-shock at 42 °C for 90 s and placing in the ice-water bath for 3 min; while the latter is only that the mixed liquid was cultured at 37 °C for 9 h. Obviously, the former conditions are more conducive to plasmid transformation, but ice bath and heat-shock are difficult to achieve in real water environments, and the latter is more practical. This difference may be expected to result in contrary experimental results. It is necessary to select appropriate HGT conditions according to the actual situation.

4.3. Effects of factors on transduction

Transduction requires phages to infect donor bacteria to obtain ARGs and then to infect recipient bacteria. After ARGs in phages are transfected into recipient bacteria, recipient bacteria express ARGs to obtain antibiotic resistance; thereafter, the transduction process is completed. Because the process is highly complex and it is difficult to design experiments, transduction is the least studied among the three horizontal transfer modes. Han et al. (2020) found that Nano-TiO_2 promoted transduction frequency by up to 4.5-fold, and Xiao et al. (2021) found that TiO_2 photoexcitation promoted transduction frequency by up to 20-fold. However, in their experiments, only half of the transduction processes were completed. They directly used phages containing ARGs to infect recipient bacteria, lacking the key process of phages infecting donor bacteria to obtain ARGs. Therefore, in future studies, it will be necessary to construct a complete transduction process and use complete experiments to confirm whether various factors can significantly affect the transduction frequency of ARGs.

4.4. HGT not classified

Some studies directly used molecular biology to explore HGT in complex environments; however, they did not distinguish the types of HGT. Huang et al. (2019a) found that CuO and ZnO nanoparticles promoted the horizontal transfer of ARGs during sludge anaerobic digestion through metagenome sequencing technology. Shi et al. (2019) found that zinc oxide and zero-valent iron nanoparticles could promote and inhibit the horizontal transfer of ARGs in landfill leachate by changing the number of mobile genetic elements (MGEs). Shi et al. (2020) found that 50–100 and 200–500 nm microplastics promoted the horizontal transfer of ARGs by producing ROS and increasing the permeability of receptor cell membranes. In addition, free ammonia (FA) (Zhang et al., 2020b) and selenate (Shi et al., 2021) promoted HGT, whereas pyroligneous acid (Zheng et al., 2020), zeolite (ZL), and biochar (BC) (Zhou et al., 2021a) showed inhibitory effects. The above studies did not distinguish whether the specific horizontal transfer mode was conjugation, transformation, or transduction. They only detected HGT frequency through changes in the abundance of MGEs and ARGs. If it is necessary to distinguish the specific HGT mode, further experiments are needed. In addition , during waste activated sludge fermentation, NaClO treatments with acute effects might promote the HGT of certain ARGs, such as *aadA1* and *tetQ* (Luo et al., 2021a), and allicins inhibited the HGT of ARGs by altered energy level and membrane integrity (Luo et al., 2021b). Therefore, as for the impact of environmental variables on the fate of ARGs, more aspects might be concerned, such as the different pretreatments and some new-emerging pollutants interaction.

4.5. HGT in biofilms

Biofilms exist in many water environments, such as rock surfaces, water treatment systems (especially activated sludge), pebbles, and microplastics (Abe et al., 2020; Hu et al., 2021). Bacterial cells in biofilms are embedded in an extracellular polymeric substance (EPS) matrix with high density, which is a biopolymer produced by cells in biofilms and is mostly composed of extracellular polysaccharides, amyloid proteins, lipids, and extracellular DNA (eDNA) (Fulaz et al., 2019). Several studies have confirmed that biofilms in water environments are also an important repository of ARGs and a hotspot of HGT (Balcazar et al., 2015). Angles et al. (1993) found that the frequency of intercellular transfer in biofilms attached to glass beads was higher than that in the aqueous phase. Similarly, Arias-Andres et al. (2018) found that compared with suspended bacteria, the frequency of plasmid transfer in bacteria of the microplastics biofilm increased. Therefore, HGT in biofilms should be concerned more. There are many reports on the effects of various factors on HGT among suspended bacteria; however, there are few reports among biofilms. Only Zhu et al. (2020) reported that

quorum sensing inhibitors (QSIs) inhibited conjugation in biofilms by up to 58-fold. It is worth mentioning that, since chlorine has a significant impact on HGT among suspended bacteria, the residual chlorine in urban water supply distribution system may also have an impact on HGT in pipe wall biofilm. There is still a lack of research in this regard. Subsequent studies should pay more attention to the effects of various factors on HGT in biofilms, particularly activated sludge, new pollutant microplastic biofilms and pipe wall biofilms.

5. Mechanisms of various factors affecting ARG horizontal transfer

The mechanisms of conjugation, natural transformation and transduction are mostly similar, and it is basically aimed at bacteria. The specific details are listed in the Supplementary Information Table S1. Natural transformation only involves the mechanism of receptor bacteria, while the other two involve donors and receptors. Three HGT modes are all related to nutrients and cell activity, ROS, membrane permeability, genes expression and protein synthesis related to HGT, intracellular ion concentration and ATP. Thus, mechanisms of the three HGT modes are comprehensively discussed.

5.1. Nutrients and cell activity

Horizontal transfer of ARGs between bacteria is affected by nutrients and cell activity. For example, appropriate concentrations of carbon (C), nitrogen (N), and phosphorus (P) in water can promote conjugation (Guo et al., 2015) because the required transfer energy and sufficient applied substances can provide adequate energy. Appropriate concentrations of K^+ and Ca^{2+} can promote natural transformation (Jin et al., 2020) because they make it easier for the recipient bacteria to enter the state of competent cells. In addition, because the horizontal transfer of ARGs among bacteria requires a specific bacterial concentration, the frequency of HGT will decrease significantly when the recipient bacteria die quantitatively under the influence of some factors (Guo et al., 2015).

5.2. ROS

ROS is a collective term that describes the chemical substances formed during the incomplete reduction of oxygen, including one electron reduction product of oxygen superoxide anion (O_2·-), two electron reduction products hydrogen peroxide (H_2O_2), three electron reduction products hydroxyl radical (·OH), and singlet oxygen ($^1O_2$). ROS play a key role in various signal transduction and pathological processes, but excessive ROS produced by exogenous stimulation can lead to cell death by causing DNA, protein or lipid oxidation (Winterbourn, 2008). Some factors affect the oxidative antioxidant system of bacteria, causing bacteria to produce an oxidative stress response and increasing the concentration of ROS in cells. ROS can directly attack bacterial DNA, proteins, or cell membranes, cause direct damage to cells, and change the expression of some DNA and protein synthesis, thus changing the bacterial state and affecting the horizontal transfer of ARGs between bacteria. When detecting ROS, the total ROS can be measured directly, and the concentration changes in ·OH and H_2O_2 can be evaluated. At the same time, different ROS quenchers can be utilized to verify which ROS have the greatest impact on HGT. In addition, the concentrations of enzymes related to oxidative stress, such as catalase (CAT), superoxide dismutase (SOD), and glutathione peroxidase (GSH-PX), were also measured. These enzyme concentrations are generally positively correlated with the degree of intracellular ROS. Under the condition that bacterial cells can still metabolise normally, after bacteria are affected by external factors, the content of ROS in the body increases and the phenomenon of oxidative stress increases, which will enhance the permeability of the cell membrane, thus promoting the horizontal transfer of ARGs among bacteria.

5.3. Membrane permeability

Horizontal transfer of ARGs must pass through the bacterial cell membrane, which is a process of bacterial transmembrane uptake of ARGs. In this process, the state of the bacterial cell membrane and permeability of the membrane are important factors (Zhang et al., 2017). When some factors in the environment directly attack the cell membrane or cause an increase in ROS in bacteria, these ROS can also attack the cell membrane, resulting in the enhancement of the permeability of the bacterial cell membrane, which is conducive to the passage of ARGs through the cell membrane by HGT, thus promoting the horizontal transfer of ARGs between bacteria. The main detection methods include direct observations of cell membrane states by scanning electron microscopy (SEM) and transmission electron microscopy (TEM), analysis by flow cytometry, and detection of bacterial lactate dehydrogenase (LDH) release. Flow cytometry is based on the principle that dyes can easily enter the cell membrane, which enhances the permeability, and the strength of dyes in cells is analysed to detect the permeability of the cell membrane. The principle of LDH detection is that, after the bacterial cell membrane permeability is enhanced, the release of LDH from bacteria to the outside is enhanced, so the release of LDH is positively correlated with cell membrane permeability.

5.4. mRNA and protein analysis

At the molecular level, HGT is a complex regulatory process involving a series of genes. Therefore, the mechanism of various factors affecting HGT can be explained by quantifying HGT-related mRNA. Methods for detecting mRNA during HGT include reverse transcription-quantitative polymerase chain reaction (RT-qPCR) and transcriptome analysis. In addition, the products of mRNA expression (various proteins) are really involved in the regulation of HGT; therefore, the change in HGT frequency can also be explained by detecting the concentration change of related proteins through the proteome.

The types of mRNA analysis mainly include measuring the expression levels of global regulatory factors (e.g. *korA*, *korB*, *trbA*, inhibitory regulation), conjugative mating pair formation system (Mpf, e.g. *trbBp*, promote regulation); DNA transfer and replication system (Dtr, e.g. *trfAp*, promote regulation), conjugative pili coupler (CP, e.g. *traA*, *traG*, promote regulation), membrane permeability (e.g. *ompA, ompC*, promote regulation), SOS response (e.g. *recA*, promote regulation), and ROS production or oxidative stress (e.g. *sodA, ahpC*, promote regulation) (Huang et al., 2019b; Qiu et al., 2012; Zhang et al., 2017). Among these, the enhancement of the SOS response can promote intrachromosomal recombination, enhance cell membrane permeability, and change the expression of some DNA (Zhang et al., 2017). Similarly, protein analysis can also use proteome technology to detect the synthesis of corresponding proteins regulated by the above-mentioned genes. In general, the detection results for mRNA and protein are consistent, but sometimes there are differences, because mRNA analysis is instantaneous, while protein analysis is the total amount of protein synthesized over a period of time, and a single protein synthesis may be regulated by multiple genes.

5.5. Intracellular ion level and ATP concentrations

Horizontal transfer of ARGs between bacteria requires energy; therefore, it is affected by intracellular ATP concentrations. In addition, Mg^{2+} can affect ATP synthesis; therefore, HGT is also affected by intracellular Mg^{2+} concentration. For example, FNA treatment led to the release of Fe^{2+} in cells, which triggered a decrease in the Mg^{2+} concentration in cells. The decrease in Mg^{2+} affected the enzymatic metabolism, then reduced the concentration of ATP that could be used in conjugation. This decreased the expression of genes involved in the conjugation and the production of corresponding proteins, which inhibited conjugation between bacteria (Huang et al., 2019b).

5.6. Type IV and VI secretory system

Type IV secretory system (T4SS) is encoded by a gene cluster composed of a series of genes, and it regulates the process of conjugation or transformation (Ding et al., 2003). The detailed molecular process of conjugation is as follows: When the conjugative plasmids in the donor bacteria express conjugative pili by T4SS, they can rapidly touch the nearby cells to connect the two cells (Lawley et al., 2003); the cell membranes of the two cells partially fuse and form a conjugation bridge; then the plasmid DNA in the donor is cut from the oriT region to form a single strand, which passes through the conjugation bridge and enters the recipient bacteria (Achtman et al., 1978); thereafter, the single-stranded DNA in both donor and recipient bacteria replicates and forms the completed double-stranded plasmid. T4SS also regulates DNA release and uptake system, and it transports DNA to the extracellular matrix or gets DNA from the extracellular matrix, to promote natural transformation. Type VI secretory system (T6SS) is a multi-component protein export device, which performs its function by secreting effector proteins into the external environment or directly injecting them into adjacent bacteria or eukaryotic cells (Hood et al., 2017). Many Gram-negative bacteria release DNA from target cells through T6SS mediated non-themself cytolysis, thus promoting transformation (Borgeaud et al., 2015). In summary, environmental factors can affect ARG horizontal transfer by changing T4SS or T6SS.

5.7. Other mechanisms

In addition to the above mechanisms analysed in most studies, a few studies explain the mechanisms of various factors affecting HGT from other aspects. For example, the formation of microplastic biofilm increased the density of bacteria in the biofilm and improved the contact frequency between cells to promote conjugation (Arias-Andres et al., 2018); CO_2 decreased cell surface charge and increased cell surface hydrophobicity to increase cell contact frequency, then induced the release of intracellular Ca^{2+} and increased proton motive force to enhance DNA uptake ability, finally promoting conjugation (Liao et al., 2019b); nano- CeO_2 (1–50 mg/kg) inhibited the synthesis of exopolysaccharides to reduce cell contact, and inhibited the conjugation (Yu et al., 2020); reactive nitrogen species (RNS), similar to ROS, could cause oxidative stress in cells, affecting HGT (Cen et al., 2020); EPS could bind to plasmid DNA or hydrolytic plasmid DNA to prevent plasmid from entering cells to inhibit natural transformation (Hu et al., 2019a; Shou et al., 2019); nano-Al_2O_3 combines with plasmid to form high-density packages performing transmembrane transport to promote the natural transformation of plasmids (Ding et al., 2021).

The above elements work alone or together, and collectively constitute the mechanism of various factors in water environments that affect the horizontal transfer of ARGs. As shown in Fig. 1, under the condition that bacteria have HGT activity, ROS and RNS can affect cell membrane permeability, produce the SOS response, and cause changes in DNA expression and protein synthesis. The SOS response can also change the cell membrane permeability and DNA expression. The expression of *OmpA* and other DNA molecules can also affect cell membrane permeability. Changes in intracellular ion levels can affect the synthesis of intracellular ATP, thus affecting the energy supply required for DNA expression and protein synthesis (Huang et al., 2019b). These mechanisms work together to regulate the horizontal transfer of ARGs (Wang et al., 2015b; Xiao et al., 2021; Zhang et al., 2021b).

6. Conclusions and prospects

As shown in Fig. 2, the horizontal transfer of ARGs between suspended bacteria by various factors in water environments has been studied; however, there is still little research on biofilms. Biofilms in water environments are also important repositories of ARGs and hotspots for HGT. Further studies should explore the impact of various factors on HGT in biofilms. HGT in activated sludge biofilms, pipe wall biofilms and microplastic biofilms should be particularly considered. Microfluidic technology can be used to dynamically track the whole process of HGT on biofilm.

Most studies focus on pure bacteria test, and an appropriate ARG

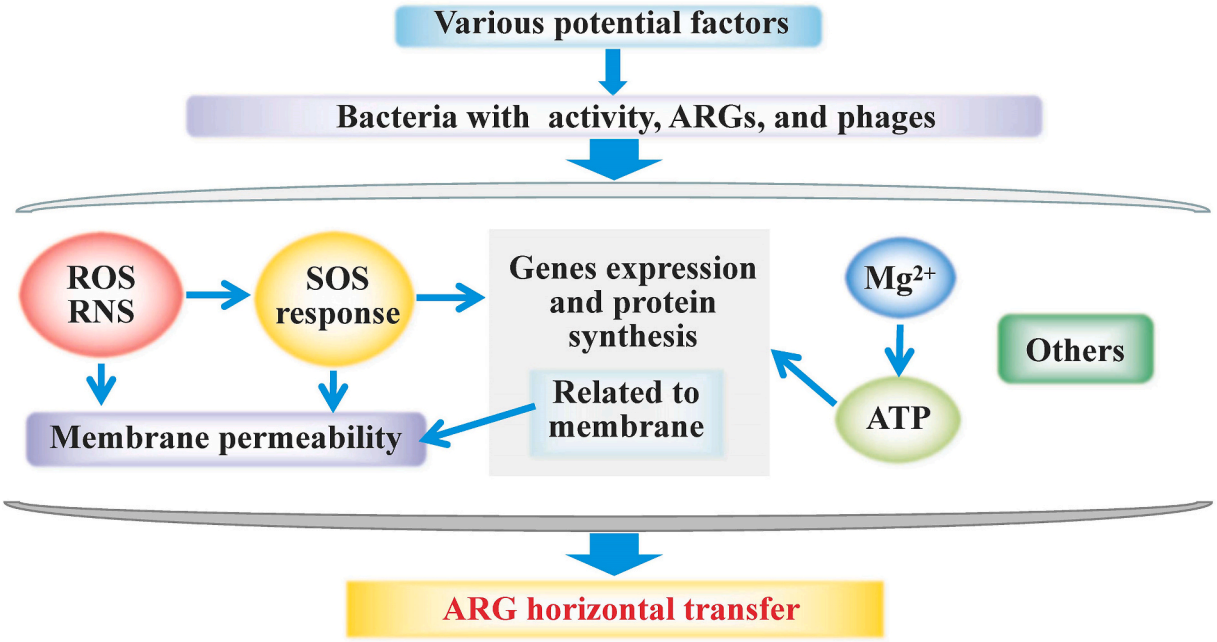

Fig. 1. The mechanisms of various factors affecting ARGs horizontal transfer. (1). When ARGs and bacteria which have HGT activity are affected by various environmental factors, the ROS, RNS, genes expression, ATP and others are affected. (2). ROS and RNS can affect membrane permeability, cause SOS response, and affect genes expression and protein synthesis. (3). The SOS response can affect membrane permeability and genes expression. (4). The expression of *OmpA* and other genes can affect membrane permeability. (5). Changes in intracellular ion levels can affect the synthesis of intracellular ATP, then affect the energy supply required for genes expression and protein synthesis. (6). These mechanisms above work together to regulate the horizontal transfer of ARGs.

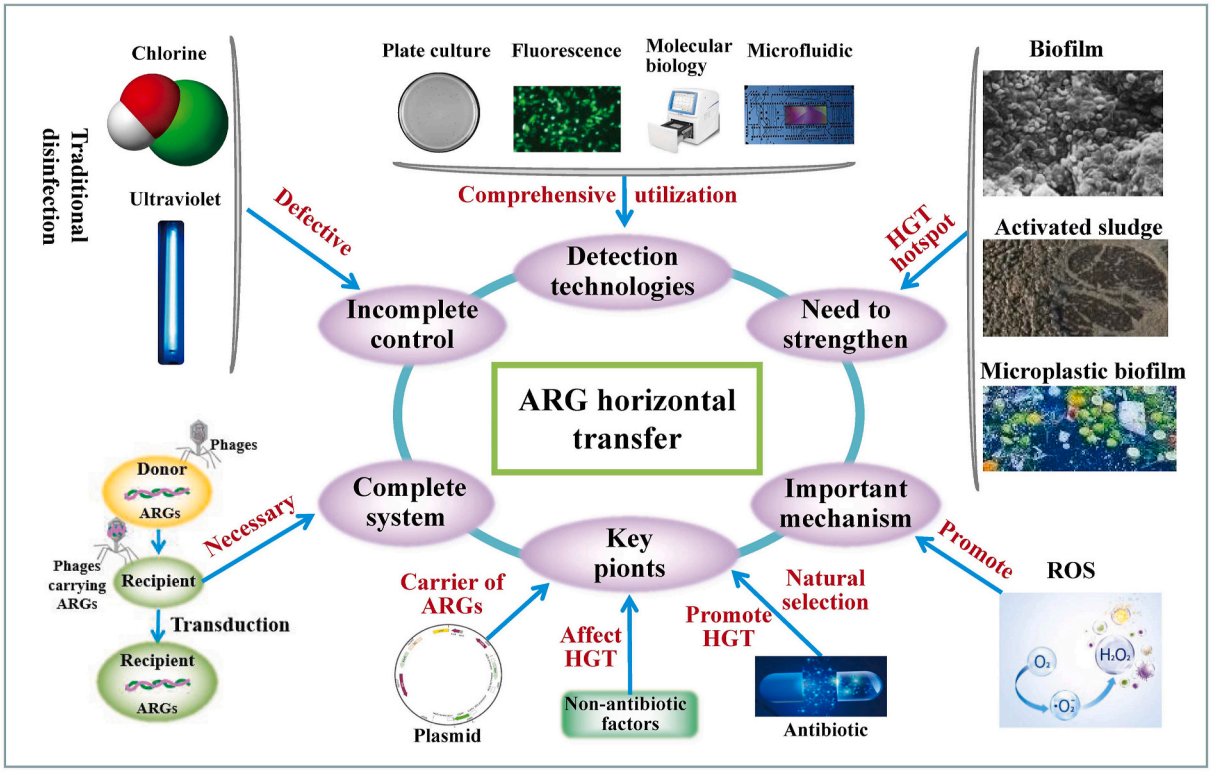

Fig. 2. Conclusions and prospects of the detection and factors of HGT. (1). Detection technologies: comprehensive utilization of various detection technologies is essential. (2). Need to strengthen: the research on HGT in biofilm should be strengthened. (3). Important mechanism: ROS is an important mechanism of ARG horizontal transfer. (4). Key points: plasmids and antibiotics are important to the transmission of antibiotic resistance. In addition, non-antibiotic factors should also be concerned. (5). Complete system: a complete ARG transduction system should be established. (6). Incomplete control: the traditional disinfection methods, such as UV and chlorine, cannot completely control the horizontal transfer of ARGs.

horizontal transfer test system which was tracked and detected by comprehensive utilization of multiple technologies in real water environments still needs to be established.

The traditional disinfection methods, such as UV and chlorine, cannot completely control the horizontal transfer of ARGs. Most disinfection methods may produce ROS to promote HGT to a certain extent. More seriously, most HGT promoting factors, such as CO_2, cannot be completely prohibited from entering water environments. It is extremely difficult to completely remove ARGs in the current water treatment process (Wang and Chen, 2022; Zhang et al., 2020a). Developing more efficient new disinfection technologies to remove ARGs is necessary. Utilization of HGT inhibitory factors, such as FNA, QSIs, should be considered to prevent the spread of ARGs by HGT, but they cannot directly eliminate ARGs completely from the water environments.

Plasmids carrying ARGs transfer horizontally among bacteria through conjugation, natural transformation, and transduction. In addition, it is also the extensive carrier of ARGs in sewage treatment plants (Che et al., 2019). ARGs are more difficult to remove by disinfection than ARB (Zhang et al., 2020a). The role of plasmids in the transmission of antibiotic resistance should be considered particularly. Antibiotics can not only naturally select ARB with gene mutation, but also promote the horizontal transfer of ARGs, which will be extremely conducive to produce multidrug-resistant bacteria. The challenge of controlling the entry of antibiotics into water environments should be concerned. The effects of antibiotics on natural transformation and transduction still need to be studied. In addition, non-antibiotic factors should also be concerned.

ROS production is the main mechanism by which most factors in the water environment affecting HGT. ROS control technology can help to inhibit the horizontal transfer of ARGs.

Finally, the research on the effects of factors on ARG transduction remains imperfect. Follow-up research needs to establish a complete transduction system in which the phage infects donors to obtain ARGs, and then infects receptors to import ARGs.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

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