Biological HRPs in wastewater

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Chapter outline

few micrometers in length and exist together. Although bacterial cells are much smaller and simpler in structure (Todar, 2013), the sizes of most bacteria range from 0.3 to 3 μm (Gerardi, 2006). There are many kinds of bacteria, which are widely distributed in nature and closely related to human beings. Some beneficial bacteria have been discovered by human beings. However, there are also countless harmful bacteria in the living environment of human beings, which pollute the air, water, soil, and food and pose a great threat to human health. There are a large number of bacteria in wastewater, and various bacteria and even human pathogenic bacteria can spread by water pollution caused by wastewater discharge, which poses risks to human health and ecological security.

Bacteria are an exceedingly diverse group of organisms that differ in shape, size, habitat, metabolism, and other features. A few different criteria are used to classify bacteria, based on the differences in shape, metabolism, cell walls, and genetic makeup. One way of classifying them is based on shape, and there are three basic shapes (Dusenbery, 2009): (1) spherical: the bacterial shape likes a ball and a single bacterium is a coccus; (2) rod-shaped: these bacteria are called bacilli (singular bacillus) and part of the rod-shaped bacteria are curved, which are called as vibrio; (3) spiral: these bacteria are known as spirilla (singular spirillus), and they are known as spirochetes if their coil is very tight. Furthermore, a small number of other unusual shapes have been described, such as icosahedron, cube, or star-shaped (Fritz et al., 2004; Yang et al., 2016). The shapes and structures of bacteria are often reflected in their names (Table 3.1).

The other way of classifying them is according to the metabolism style, and most bacteria are divided into autotrophic and heterotrophic bacteria (Foster and Slonczewski, 2017; Hellingwerf et al., 1994; Nealson, 1999; Zillig, 1991). Autotrophic bacteria, which only need carbon dioxide as their carbon source, make their food by (1) photosynthesis using sunlight, carbon dioxide, and water or (2) chemosynthesis using carbon dioxide, water, and some chemicals (such as ammonia, nitrogen, and sulfur). Heterotrophic bacteria, relying on organic matters as carbon source, get their energy by consuming organic carbon outside the cells. According to their reaction to oxygen, most bacteria can be divided into the following three categories (Joubert and Britz, 1987): (1) aerobic bacteria that can only grow in the presence of oxygen; (2) anaerobic bacteria that can only grow in the absence of oxygen; and (3) facultative anaerobic bacteria that can grow regardless of aerobic and oxygen free.

Table 3.1 Bacteria groups and their corresponding shapes.

Shape	Examples
Spherical	*Streptococcus* group, *Aerococcus*, and *Pediococcus*
Rod-shaped	*Bacillus anthracis*, *Bacillus cereus*, and *Coxiella burnetii*
Spiral	*Leptospira interrogans*, *Treponema pallidum*, and *Borrelia recurrentis*

The structure of bacteria with no membrane structure of the organelles, such as mitochondria and chloroplasts, is very simple. However, the cell wall of most bacteria is divided into two different types based on the reaction of cells to the Gram stain, which classifies bacteria into Gram-positive bacteria and Gram-negative bacteria (Beveridge, 2001). Gram-positive bacteria have a thick cell wall with peptidoglycan and teichoic acids. On the contrary, a relatively thin cell wall consisting of several layers of peptidoglycan that are surrounded by a second lipid membrane with lipopolysaccharides and lipoproteins is possessed by Gram-negative bacteria (Hugenholtz, 2002). Furthermore, some bacteria have cell wall structures that differ from those possessed by Gram-positive and Gram-negative bacteria. For instance, *Mycobacteria*, a clinically important bacterium, have the thick peptidoglycan cell wall and also the second outer layer of lipids (Alderwick et al., 2015).

3.1.2 Typical bacteria in wastewater

Bacteria also can be classified by the roles that they perform in the wastewater based on different condition (Gerardi, 2006). Table 3.2 summarizes the typical groups of bacteria and their corresponding roles in the wastewater according to the study by Gerardi (2006).

3.1.3 Pathogenic bacteria and their typical characteristics and hazards

Pathogenic bacteria are bacteria that can cause disease and are one of the major threats to public health in the world. Pathogenic bacteria are infections and able to contribute to globally important diseases, such as pneumonia, foodborne illnesses, tetanus, typhoid fever, diphtheria, syphilis, and leprosy (Chan et al., 2013; El-Lathy et al., 2009). Wastewater may contain millions of bacteria per milliliter and is also a potential source of various pathogenic bacteria. Recently, a wide range of pathogenic bacteria, such as *Escherichia coli*, *Clostridium perfringens*, *Mycobacterium tuberculosis*, *Legionella pneumophila*, *Pseudomonas aeruginosa*, *Shigella flexneri*, *Salmonella enterica*, *Vibrio cholera*, and *Yersinia enterocolitica*, have been detected in the wastewater (Cai and Zhang, 2013; Dudley et al., 1980; Stevik et al., 2004). Several reports have summarized pathogenic bacteria commonly detected in wastewater (Table 3.3) (Gerardi, 2006; Gerardi and Zimmerman, 2004).

3.1.3.1 Pathogenic Escherichia coli

Escherichia coli, commonly known as nonpathogenic bacteria, are long-lived bacterium found in the intestines of humans and animals. The bacterial antigen type is "O" type, the flagellar antigen type is "H" type, and the surface antigen type is "K" type. According to the different antigen structures, the serotypes of the bacteria can be divided into more than 180 kinds. However, some strains are highly pathogenic and can cause diarrhea and other diseases, which are often called pathogenic *Escherichia coli*. The pathogenic *Escherichia coli* can produce or contain

Table 3.2 Typical bacteria groups in wastewater.

Typical groups	Examples
Acetongenic bacteria	*Acetobacter*, *Syntrobacter*, and *Syntrophomonas*
Coliforms	*Escherichia*, *Citrobacter*, *Enterobacter*, *Hafnia*, *Klebsiella*, *Serratia*, and *Yersinia*
Cyanobacteria	*Anabaena*, *Chlorella*, *Euglena*, and *Oscillatoria*
Denitrifying bacteria	*Alcaligenes*, *Bacillus*, and *Pseudomonas*
Fecal coliforms	*Escherichia*
Fermentative bacteria	*Bacteroides*, *Bifidobacteria*, *Clostridium*, *Escherichia*, *Lactobacillu*s, and *Proteus*
Floc-forming bacteria	*Achromobacter*, *Aerobacter*, *Citromonas*, *Flavobacterium*, *Pseudomonas*, and *Zoogloea*
Gliding bacteria	*Beggiatoa*, *Flexibacter*, and *Thiothrix*
Gram-negative aerobic cocci and rods	*Acetobacter*, *Acinetobacter*, *Alcaligens*, *Nitrobacter*, *Nitrosomonas*, *Pseudomonas*, and *Zoogloea*
Gram-negative facultative anaerobic rods	*Aeromonas*, *Escherichia*, *Flavobacterium*, *Klebsiella*, *Proteus*, and *Salmonella*
Hydrolytic bacteria	*Bacteroides*, *Bifidobacteria*, and *Clostridium*
Methane-forming bacteria	*Methanobacterium*, *Methanococcus*, *Methanomonas*, and *Methanosarcinia*
Nitrifying bacteria	*Nitrosomonas*, *Nitrosospira*, *Nitrobacter*, and *Nitrospira*
Pathogenic bacteria	*Campylobacter jejuni*, and *Leptospira interrogans*
Poly-P bacteria	*Acinetobacter*, *Aerobacter*, *Beggiatoa*, *Enterobacter*, *Klebsiella*, and *Proteobacter*
Saprophytic bacteria	*Achromobacter*, *Alcaligenes*, *Bacillus*, *Flavobacterium*, *Micrococcus*, and *Pseudomonas*
Sulfur-reducing bacteria	*Desulfovibrio* and *Desulfotomaculum*

colonization factors, enterotoxin, K antigen, and related substances, and also has the ability of endotoxin secretion. Pathogenic *Escherichia coli* cause disease outbreaks through the contamination of drinking water, food, and other ways.

Pathogenic *Escherichia coli* are mainly responsible for three types of infections in humans: (1) neonatal meningitis, (2) urinary tract infections, and (3) intestinal diseases. Pathogenic *Escherichia coli* can be divided into several categories according to its serological characteristics and virulence properties, mainly consisting of enterotoxigenic *Escherichia coli*, enterohemorrhagic *Escherichia coli*, enteropathogenic *Escherichia coli*, enteroaggregative *Escherichia coli*, and enteroinvasive *Escherichia coli* (Kaper et al., 2004; Todar, 2008). The most infamous member of enterohemorrhagic *Escherichia coli* is the strain O157:H7 that can cause bloody diarrhea and fever, and it is prominent and important in North America, the United Kingdom, and Japan (Kaper et al., 2004).

Table 3.3 Pathogenic bacteria commonly detected in wastewater.

Bacterium/Bacteria	Disease
Actinomyces israelii	Actinomycosis
Bacillus anthracis	Anthrax
Brucella spp.	Brucellosis (Malta fever)
Campylobacter jejuni	Gastroenteritis
Clostridium perfringens	Gangrene (gas gangrene)
Clostridium tetani	Tetanus
Clostridium spp.	Gas gangrene
Enteroinvasive *Escherichia coli*	Gastroenteritis
Enteropathogenic *Escherichia coli*	Gastroenteritis
Enterotoxigenic *Escherichia coli*	Gastroenteritis
Enterohemorrhagic *Escherichia coli* O157:H7	Gastroenteritis and hemolytic uremic syndrome
Francisella tularensis	Tularemia
Leptospira interrogans	Leptospirosis
Mycobacterium tuberculosis	Tuberculosis
Nocardia spp.	Nocardiosis
Salmonella paratyphi	Paratyphoid fever
Salmonella spp.	Salmonellosis
Salmonella typhi	Typhoid fever
Shigella spp.	Shigellosis
Vibrio cholerae	Cholera (Asiatic cholera)
Vibrio parahaemolyticus	Gastroenteritis
Yersinia enterocolitica	Yersiniosis (bloody diarrhea)

Pathogenic *Escherichia coli* are reported to be usually detected in wastewater. Some pathogenic *Escherichia coli* strains survive during the treatment stages of sewage treatment plants (STPs) and in the surrounding environmental waterbodies of STPs (Anastasi et al., 2012). Strains of *Escherichia coli* O157:H7 have been not only commonly isolated from urban sewage and animal wastewater in Spain but also are present in human and animal wastewaters with other Shiga toxin-producing *Escherichia coli* (Garcia-Aljaro et al., 2005). The level of *Escherichia coli* O157:H7 is about $10-10^2$ CFU/100 mL for municipal sewage and 10^2-10^3 CFU/100 mL for animal wastewater from slaughterhouses (Garcia-Aljaro et al., 2005). Shannon et al. (2007) detected that the level of *Escherichia coli* in raw wastewater was about 1.51×10^7 gene copy number per 100 mL, and had a reduction of 3.52–3.98 orders of magnitude after final treatment while *Escherichia coli* O157:H7 was not present or was below the detection limit in all treatment stages of the investigative STP.

3.1.3.2 Salmonella enterica serovar Typhi

Typhoid is caused by a highly virulent and aggressive intestinal bacterium called *Salmonella enterica* serovar Typhi. This bacterium infects only humans and is usually acquired by ingestion of food or water contaminated by the feces of patients with typhoid or asymptomatic carriers (Dougan and Baker, 2014). There are three strains of *Salmonella enterica* serovar Typhi including *Salmonella typhi*, *Salmonella paratyphi*, and *Salmonella schottmuelleri*. They are Gram-negative, facultative anaerobes, and rod shaped, which do not have spores and capsules. *Salmonella* pathogenicity islands (SPIs), large genomic regions of 10−134 kb, are responsible for most of the virulence factors. Most of the effector molecules associated with complex pathogenesis are encoded by SPIs (Hensel, 2004). *Salmonella typhimurium* is also a potential source of human illness, having the ability to transfer from irrigation water to the edible parts of the plants (Lapidot and Yaron, 2009). They are common in wastewater and can be induced into the viable but nonculturable state after typical wastewater disinfection (Oliver et al., 2005). Because the infectious dose of them is only as low as 20 cells per mL, the residual level of them in wastewater also has potential health risks (Oliver et al., 2005). In addition, strains of *Salmonella* with a greater pathogenic potential have also been isolated from wastewater and activated sludge, and the most frequent serotypes are *Salmonella hadar* (38.1%), followed by *Salmonella enteritidis* (23.8%), *Salmonella london* (14.3%), and *Salmonella anatum* (9.5%) in raw and treated wastewater (Espigares et al., 2006).

3.1.3.3 Shigella dysenteriae

Shigella dysenteriae belongs to the genus of enterobacteriaceae *Shigella*, and it is a Gram-negative bacterium without spore and flagellates. It can grow on common medium and is cold resistant and facultatively anaerobic. Furthermore, *Shigella dysenteriae* is one of the most common pathogenic bacteria leading to dysentery in human and primate, and typical observed symptoms caused by it are diarrhea, abdominal pain, and fever after infection.

The pathogenic mechanism of *Shigella dysenteriae* is summarized as follows (Athman et al., 2005; Jennison and Verma, 2004; Schroeder and Hilbi, 2008): (1) *Shigella dysenteria* upregulates the acidic gene, so that it is possible to survive in the stomach of the host; (2) *Shigella dysenteriae* invades colonic epithelial cells and is tightly linked to the associated proteins to replicate virulence factors; (3) *Shigella dysenteriae* leads to the apoptosis of macrophages and induces the release of interleukin IL-21, resulting in the accumulation of inflammatory cells and polymorphonuclear leukocytes. The accumulated polymorphonuclear leukocytes can pass through the intestinal epithelial cells and destroy the connections between the epithelial cells, allowing more *Shigella dysenteriae* to reach the submucosal layer through the crack; (4) *Shigella dysenteriae* further infects adjacent cells, causing an inflammatory reaction when the number of infected cells reaches a certain level, thereby resulting in typical bacterial dysentery symptoms such as congestion, hemorrhage, and edema of the intestinal mucosa.

Notably, *Shigella dysenteriae*, considered as the most virulent bacterium, is widely distributed in the wastewater, which is considered as a reservoir. For example, the occurrence rates of *Shigella dysenteriae* are up to 40–60% in wastewater effluents and the receiving waterbodies in South Africa (Teklehaimanot et al., 2014, 2015). *Shigella dysenteriae* has also been detected in 35 sewage samples collected from hospital and residential areas (Peng et al., 2002). Furthermore, *Shigella dysenteriae* was isolated from water and riverbed sediment of the Apies River, South Africa (Ekwanzala et al., 2017)

3.1.3.4 Vibrio cholerae

Vibrio cholerae, which is a member of the family Vibrionaceae and is capable of respiratory and fermentative metabolism, is a Gram-negative, comma-shaped, facultative anaerobic, and nonspore-forming bacterium (Morris Jr and Acheson, 2003; WHO, 2004). *Vibrio cholerae* is suitable for survival in salt-containing water and is dependent on aquatic organisms and plankton (Colwell, 1996). It can use chitin on the surface of zooplankton as the carbon source and nitrogen source for growth. Meanwhile, chitin can also induce horizontal gene transfer of *Vibrio cholera* (Kirn et al., 2005).

Some strains of *Vibrio cholerae* can cause the disease of cholera, an acute intestinal infection. *Vibrio cholerae* can be divided into more than 200 serotypes, according to the O antigens located on the lipopolysaccharide of the cell membrane (Longini et al., 2002). After being ingested by humans, *Vibrio cholerae* enters the small intestine through the oral cavity and gastric juice and then colonizes through the mucus layer. Once entering the small intestine, virulence genes of *Vibrio cholerae* are induced and expressed by the host conditions, and the virulence coregulating pili and cholera toxin are important pathogenic factors leading to the virulence of *Vibrio cholera* (Wang, 2015).

Due to the contribution of wastewater to the spread of cholera, the fate of *Vibrio cholerae* in wastewater and wastewater treatment systems has been investigated. The prevalence of *Vibrio cholerae* has been observed in four wastewater treatment plants (WWTPs) located in Gauteng Province, South Africa (Dungeni et al., 2010). Pathogenic bacteria strains of non-O1 *Vibrio cholerae* have been detected in domestic wastewater with an average abundance between $2.5 \times 10^{1-} - 1.7 \times 10^3$ MPN mL^{-1} (Mezrioui and Oufdou, 1996). The high incidence of *Vibrio cholerae* in wastewater is further validated by other *Vibrio* pathogens to some extent. For example, Nongogo and Okoh (2014) also have found the occurrence of other *Vibrio* pathogens in the final effluents of five WWTPs located in South Africa over 12 months.

3.1.3.5 Legionella

Legionella, a pathogenic group of Gram-negative bacteria, is an intracellular pathogen that is widespread in natural waterbodies and can cause two forms of *Legionella* diseases (including *Legionella* pneumonia and Pontiac fever) (Bartram et al., 2007). *Legionella* survives and proliferates in host cells by the phagocytosis of free-living single-celled protozoa (Kim et al., 2004). *Legionella* can also form a

biofilm with other aquatic microorganisms (Smid et al., 1996). The extracellular polymeric substances in biofilm not only help bacteria to capture and concentrate environmental nutrients, but also protect bacteria against environmental pressures such as antibiotics, disinfectants, dryness, and high temperature (Cooper and Hanlon, 2010; Kim et al., 2002; Wright et al., 1991).

Notably, the occurrence of *Legionella* in wastewater has been reported worldwide in recent years. For example, *Legionella* has been detected in 10 of the 17 investigated WWTPs (58.8%) and also has been observed in hospital, industrial, and domestic wastewater systems in Taiwan (China), respectively (Huang et al., 2009). *Legionella* spp. have been detected at varying concentrations from 4.8 to 5.6 log GU/mL in activated sludge tanks in three WWTPs in Germany (Caicedo et al., 2016). In total, Caicedo et al. (2019) have summarized the occurrence of *Legionella* in several municipal wastewater treatment plants (MWTPs) based on the activated sludge process in France, Norway, USA, China, and other countries (Brissaud et al., 2008; Cai and Zhang, 2013; Kulkarni et al., 2018; Lund et al., 2014).

3.2 Viruses
3.2.1 Definition, morphology, and composition of viruses

Viruses are in the noncellular form, which are composed of the protein and nucleic acid molecule (DNA or RNA), and they are organic species that are parasitic in the living and nonliving body. They are neither biological nor abiotic, and are not attributed to the five kingdoms (including prokaryotes, protists, fungi, plants, and animals). Viruses vary in shape, ranging from simple spirals, regular icosahedral to other complex structures, and their particle size is about 1% of bacteria. Viruses are ubiquitous and are the most abundant biological entities on earth (Bergh et al., 1989; Fuhrman, 2009).

Viruses consist of two or three components: (1) Viruses contain genetic material; (2) All viruses also have capsids made of protein to encapsulate and protect the genetic material; and (3) Some viruses can form a lipid envelope around the cell when they reach the cell surface. Furthermore, viruses can self-replicate using the cellular system of hosts, but cannot grow and replicate independently. Furthermore, viruses can infect almost any living organisms with the cellular structure. However, not all the viruses can cause diseases, because the replication of many viruses cannot cause apparent damage to infected organs. Some viruses, such as human immunodeficiency virus (HIV), can coexist with the human body for a long time and remain infectious without being affected by the immune system of their hosts. In general, viral infections can trigger immune responses that destroy invading viruses. Vaccination can contribute to these immune responses, so that the vaccinated person or animal can immunize the corresponding virus for life. Microorganisms such as bacteria also have effective mechanisms to protect themselves against viral infections (Wilson and Murray, 1991).

3.2.2 Types of viruses

Viruses are mainly classified by phenotypic characteristics, such as nucleic acid type, replication mode, host organism, morphology, and disease types that they cause. Among these classifications, the formal taxonomic classification of viruses is based on the current classification system developed by the international committee on taxonomy of viruses (ICTV). By 2018, ICTV have defined 1 single phylum, 2 subphyla, 6 classes, 14 orders, 5 suborders, 143 families, 64 subfamilies, 846 genera, and 4958 species of viruses (Lefkowitz et al., 2017).

In addition, the Baltimore classification of viruses is based on the mechanism of mRNA production, because viruses must generate mRNAs to produce proteins and replicate themselves. However, this process is different in each virus family. Viruses can be placed in one of the seven following groups (Web book 2008): (1) double-stranded DNA viruses; (2) single-stranded DNA viruses; (3) double-stranded RNA viruses; (4) single-stranded RNA viruses—positive sense; (5) single-stranded RNA viruses—negative sense; (6) positive-sense single-stranded RNA viruses that replicate through a DNA intermediate; and (7) double-stranded DNA viruses that replicate through a single-stranded RNA intermediate.

3.2.3 Common viruses in wastewater

A variety of viruses that are directly harmful to human health are present in wastewater. The implementation of current wastewater treatment processes significantly reduced the levels of virus contamination in wastewater. However, viruses are still widely spread and are disseminated in the environment by discharging untreated or treated wastewater (Bofill-Mas et al., 2006, 2010) to the receiving water environments and food productions (Cantalupo et al., 2011). So the viruses, including enterovirus, hepatitis virus, rotavirus, coronavirus, adenovirus, parvovirus, reovirus, and astrovirus, that are present in wastewater, may deserve special attention.

3.2.3.1 Enterovirus

Enterovirus is a type of single-stranded RNA virus and is associated with several human diseases that mainly infects intestine. Enteroviruses are highly contagious and are transmitted mainly through two routes, including the consumption of contaminated food, water, saliva, etc. and respiratory tract (droplets, cough, etc.).

Enteroviruses are globally distributed, and humans are the only hosts. Some enteroviruses are more resistant to natural conditions and disinfectants than common bacteria (Hu et al., 2011). Moreover, enterovirus can survive in wastewater for several months. For instance, a previous study has also revealed the occurrence and distribution of cultivable enteroviruses in wastewater and surface waterbody of north-eastern Spain (Costan-Longares et al., 2008).

Enterovirus is one of the most common viruses in the water environment, and also one of the most studied viruses in water virology. In the study of the virus safety of water, enterovirus is often used as a representative, because the discharge of the virus is large and detoxification often needs a long time. The incomplete removal of

enterovirus by the wastewater treatment also highlights the resistance and potential health risk for the public after discharging into the natural environment (Battistone et al., 2014).

3.2.3.2 Hepatitis virus

Hepatitis virus is the pathogen causing viral hepatitis, which has been considered as a major public health problem affecting human beings in the world and has considerable morbidity and mortality in the human. Hepatitis viruses are highly resistant to common chemical disinfectants and can survive for months or years in dry or frozen environments.

Human hepatitis viruses include hepatitis A virus (HAV), hepatitis B virus (HBV), hepatitis C virus (HCV), hepatitis D virus (HDV), and hepatitis E virus (HEV). Among them, HAV and HEV are spread through intestinal infection, and other viruses are transmitted by blood and other body fluid. HAV is a small RNA virus that is distinct from other members of picornavirus family in morphology, and it causes infectious or epidemic hepatitis by the route from feces to oral. For HBV, it is a double-stranded DNA virus and belongs to the hepadnavirus group, which is endemic in the human population and hyperendemic in the world. The resistance of HBV to environmental factors is relatively high in vitro that it cannot be inactivated by ultraviolet, heating and chemical disinfectants (such as phenol and thiomersal) in general concentration. Furthermore, they are also widely distributed in wastewater.

3.2.3.3 Rotavirus

Rotavirus is a nonencapsulated and icosahedral symmetrical virus, affiliated to the family of *Reoviridae* and the genus of *Rotavirus*. Rotavirus, with highly contagious, can survive for several weeks on the surface of water and has strong adaptability to change in physical and chemical factors. It is resistant to some organic reagents (such as chloroform ether), and still has an activity after repeated freezing and thawing as well as ultrasonic treatment. Furthermore, this virus remains infectious under the pH ranging from 3.5 to 10.0. However, ozone, iodine, chlorine, phenol, and other reagents can make it inactive.

Moreover, 285 sewage samples from four Italian cities were tested for rotavirus VP7 and VP4 genes, which revealed that rotavirus was detected in 172 (60.4%) samples and 26 samples contained multiple rotavirus G genotypes (Ruggeri et al., 2015). In China, 46 sewage samples were monthly collected for the detection of rotavirus, and 93.5% of samples were found to be positive (Zhou et al., 2016).

Rotavirus is the primary cause of acute diarrhea in children worldwide, and the peak of disease outbreak is mainly in autumn and winter. It is estimated that more than 140 million children worldwide suffer from rotavirus gastroenteritis every year. Rotavirus is mainly transmitted through the oral−fecal route and can be transmitted by consuming contaminated drinking water, eating contaminated food, or contacting contaminated objects. At present, effective treatment of humans or animals infected with rotavirus is still not available, and its viral harm cannot be

fundamentally eliminated. On this account, the social impact and the economic burden of the family caused by rotavirus are enormous.

3.2.3.4 Coronavirus

Coronavirus (CoV), belonging to the family of Coronaviridae and the order of *Nidovirales*, consists of four genera (including α-CoV, β-CoV, γ-CoV, and δ-CoV) based on phylogeny. Among them, α-CoV and β-CoV mainly infect mammals; γ-CoV mainly infects birds; and δ-CoV can infect both birds and mammals. CoV is an enveloped virus with a diameter of about 60−200 nm, and its average diameter is approximately 100 nm. The length of the single-stranded linear genome is about 12−30 kb, so CoV is the longest positive single strand RNA virus in the genome. The composition and expression of genomes of all the CoVs are similar. Nucleocapsid protein is the main structural protein of CoV and also the most abundant protein in CoV coding protein, exhibiting multiple functions in replication and immune regulation. Since the emergence of severe acute respiratory syndrome coronavirus (SARS-CoV) in 2003, the research on the CoV is becoming a global concern (Zhong et al., 2003). Recently, with the change of environment conditions, the influence of immune pressure or other factors, new mutant strains of CoV have emerged and been prevalent. Novel CoVs have been successive identified, such as Middle East respiratory syndrome coronavirus and Porcine deltacoronavirus, and the emergence and reemergence of these novel CoVs cause high morbidity and mortality in human and animal and pose heavily public health threat and heavily economic losses (Dong et al., 2016; Li et al., 2012b; Zaki et al., 2012; Zhao et al., 2013).

Among the human respiratory viruses, human CoVs are the most difficult to detect in the laboratory and are not detected in most routine diagnostic virology laboratories (Mackie, 2003). In addition, CoVs were reported to die off rapidly in wastewater (Gundy et al., 2009). A previous study had demonstrated that the RNA of SARS-CoV are detectable in the hospital sewage concentrates before disinfection and occasionally after disinfection though there was no live SARS-CoV (Wang et al., 2005).

3.2.3.5 Adenovirus

In 1954, adenovirus was first isolated from tonsil tissue and had been discovered for more than 60 years (Hllleman and Werner, 1954). Adenovirus is a virus without an envelope and is one of the largest and most complex viruses among the viruses that have been discovered so far. Its genome consists of linear double-stranded DNA molecules with the length ranging from 25 to 45 kb (Smith et al., 2008). Compared with CoVs, many members of adenovirus are readily propagated in routine laboratory cell culture systems (Mackie, 2003).

Adenovirus can cause respiratory disease in a small group of normal people, but it can cause 5%−10% of respiratory diseases in children and people with low immunity. Furthermore, human adenoviruses can lead to respiratory infections, conjunctivitis, gastroenteritis, hepatitis, nervous system disorders, and other diseases. For human adenoviruses, the most common infections are in the upper respiratory tract

and the epithelial cells of eyes. Adenovirus can be transmitted through a variety of routes, including humans, water, and equipment. Generally, adenovirus is more susceptible to people with low immunity, such as children, HIV-infected patients, patients with immune genetic defects, etc. For human adenovirus, belonging to the family of Adenoviridae, there are 7 subgroups and 84 kinds of adenoviruses have been reported so far (Kaján et al., 2017). Adenovirus was also frequently detected in wastewater samples. For instance, adenoviruses were detectable in wastewater, and combined sewer overflows discharged samples from a WWTP in Michigan between August 2005 and August 2006 (Fong et al., 2010).

3.2.3.6 Norovirus

Norovirus is a positive-stranded RNA virus of the Caliciviridae family, which has 5 genera (including *Vesivirus*, *Lagovirus*, *Nebovirus*, *Sapovirus*, and *Norovirus*). The norovirus genus contains at least 5 genotypes (GI-V). Among the 5 genotypes, GI, GII, and GIV can infect human beings and cause acute gastroenteritis, and GII, GIII, and GV have also been isolated from pigs, cattle and sheep, and mice, respectively (Thorne and Goodfellow, 2014).

At present, norovirus is considered to be the main cause of gastroenteritis in developed and developing countries (Glass et al., 2009; Hall et al., 2013). This virus is transmitted through the fecal—oral route and usually causes acute self-limiting gastrointestinal infections. Norovirus infection is also associated with many clinical symptoms, such as necrotizing enterocolitis, infantile epileptic encephalopathy, emphysema enteropathy, and sporadic intravascular coagulation (Chan et al., 2010; Centers for disease control and prevention 2002; Medici et al., 2010; Turcios-Ruiz et al., 2008). In developing countries, norovirus infection causes approximately 200,000 deaths of children fewer than 5 years old each year (Hall et al., 2013). Recent reports indicate that norovirus is the second leading cause of gastroenteritis-related deaths in the United States, resulting in approximately 797 deaths each year (Hall et al., 2013; Papafragkou et al., 2013). A previous study quantified norovirus genogroups I (GI) and II (GII) in wastewater in France and explored their removals by treatment systems of waste stabilization pond, activated sludge, and submerged membrane bioreactor treatments, which found that all the treatment systems efficiently decreased the level of norovirus contamination in receiving waterbodies (da Silva et al., 2007).

3.3 Protozoa

3.3.1 Biological characteristics of protozoa

Protozoa are the same as multicellular animals, with physiological functions including metabolism, exercise, reproduction, reaction to an external stimulus, and adaptability to the environment. Some common protozoa are distinguished from sizes, and protozoa individuals range in size from as little as 1 μm to several

millimeters, or more (Singleton and Sainsbury, 2003). Moreover, all the protozoa have cell membranes, and the cell membranes of most protozoa are strong and elastic, so that protozoa could remain a certain shape. Generally, protozoa have one or more nuclei, which are various in shapes. However, there are also some protozoa, such as ameba, which have only one layer of the very thin plasma membrane and cannot maintain a fixed shape. Protozoa produce morphological differentiation in their cells and form organelles capable of performing various life activities and physiological functions. In terms of movement organelles, there are flagella, pseudopods, and cilia. Furthermore, some types of protozoa have myofilaments distributed in the cell membrane, which has the function of contraction and deformation.

There are three types of protozoa that are common in water treatment systems: (1) sarcopods, whose cytoplasm is flexible enough to form a pseudopod acting as an organelle for exercise and feeding; (2) flagellates, which have one or more flagella; (3) infusorians, which have cilia on the body or part of its surface acting as a tool for action or feeding. Some of the protozoa have two stages of the life cycle, alternating between proliferative stage (trophozoite) and dormant stage (cyst). When protozoa are in the stage of trophozoite, they can actively feed. As cysts, protozoa can survive in the harsh condition including extreme temperatures, harmful chemicals, and fewer nutrients, water or oxygen for a long time. The conversion from trophozoite to cyst is known as encystation, while the process of transforming from cyst to trophozoite is known as excystation.

3.3.2 Hazards and risks of protozoa

The outbreak of diseases associated with exposure to low levels of waterborne protozoa has received more concern in the world, so the occurrence, hazards, and risks of protozoa are frequently investigated. Some protozoa have a hazardous effect on human health, which can cause clinical diseases and also are responsible for outbreaks of waterborne diseases. The top 12 diseases caused by some protozoa include malaria, amebiasis, trypanosomiasis, chagas disease, lambliasis, babesiosis, cryptosporidiosis, sappinia amebic encephalitis, blastocystosis, trichomoniasis, toxoplasmosis, and schistosomiasis (Kirsten, 2018). These diseases are found in very different parts of human beings and have been documented around the world. There were 214 million cases of symptomatic malaria reported in 2015 (Saheb, 2018). For toxoplasmosis caused by the infection of *Toxoplasma gondii*, approximately 14% of the individuals in the United States are seropositive to it by the age of 40 years (Saheb, 2018). At the same time, the prevalence of *Toxoplasma gondii* infection also has been found in some American countries (Cong et al., 2015).

Moreover, risk assessment methods for these pathogenic protozoa have been explored over past several years, and a semiquantitative risk assessment is possible for most protozoa disseminated in the environment including *Cryptosporidium*, *Giardia*, *Toxoplasma*, and *Entamoeba* (Filipkowska, 2003). Furthermore, the risk assessment for *Cryptosporidium* and *Giardia* has been established to highlight the full control and adequate prevention of protozoa infections to protect human health

(Gibson et al., 1999). For *Cryptosporidium*, source water used by drinking treatment plants with an average concentration of oocysts of less than 2 oocysts per 100 L would meet the acceptable low-risk level (Haas et al., 1996). For *Giardia*, source water used by drinking treatment plants with geometric mean concentration of less than 0 ± 7 cysts per 100 L would result in acceptably low-risk level (Regli et al., 1991).

3.3.3 Common pathogenic protozoa in wastewater

Compared with waterborne epidemics caused by inorganic poisons, organic pesticides, and bacteria, diseases caused by pathogenic protozoa such as *Giardia* and *Cryptosporidium* have characteristics including high outbreak rate, high patient populations, and poor effect of treatment. It can be seen that pathogenic protozoa are the main causes of waterborne diseases among various pathogenic microorganisms. Table 3.4 summaries the common pathogenic protozoa lived in different types of wastewater (Cacciò et al., 2003; Ferrari et al., 2006; Hench et al., 2003; King et al., 2017; Sukprasert et al., 2008).

3.3.3.1 Cryptosporidium

Cryptosporidiosis is one of the most common zoonotic infectious diseases. The main symptom is diarrhea, and the severity of diarrhea depends on the characteristics of the host and the parasite (Cama et al., 2008). Most people can recover from the disease in a short period of time, but immunocompromised people may face prolonged infection and fatal danger (Chen et al., 2002). The outbreak of cryptosporidiosis is often associated with drinking water contaminated with oocysts as well as fresh food contacted by infected hosts. *Cryptosporidium* is the causative pathogen of cryptosporidiosis, which is a kind of intracellular parasitic eukaryotic single-celled organism. Many studies have shown that *Cryptosporidium* can infect humans and more than 170 kinds of animals, and mammalian *Cryptosporidium* infection plays an important role in the transmission of human cryptosporidiosis (Zhang and Jiang, 2001; Zhang et al., 2013b). *Cryptosporidium hominis* and *Cryptosporidium parvum* are two of the most common species infecting humans (Molloy et al., 2010). After entering the human body and animal body via fecal−oral route, they mainly parasitize on the intestinal epithelial cells as well as organs (including stomach, respiratory tract, lung, liver, tonsil, pancreas, gallbladder, and others) (Ortega and Sanchez, 2010; Ryan et al., 2014; Xiao and Feng, 2008).

Table 3.4 Common pathogenic protozoa in different types of wastewater.

Protozoa	Different types of wastewater
Cryptosporidium	Municipal wastewater, industrial wastewater
Giardia	Municipal wastewater, industrial wastewater
Entamoeba histolytica	Municipal wastewater

Moreover, *Cryptosporidium* is seriously harmful to the safety of drinking water. Compared to *Giardia*, *Ameba*, *Toxoplasma*, *Neisseria*, and *Cyclospora*, *Cryptosporidium* survives longer in the environment and has the strongest resistance to chemical disinfection. Furthermore, it is difficult to be removed by filtration and chemical disinfection. Therefore, *Cryptosporidium* is considered as an indicator of pathogenic protozoan parasites in the public water supply system (WHO, 2009).

3.3.3.2 Giardia

Giardia is an anaerobic flagellated protozoan parasite and colonizes and reproduces in the small intestines of several vertebrates, which can cause abdominal pain, diarrhea, and indigestion as well as giardiasis. Moreover, *Giardia* has two different developmental stages in the life cycle, including swimming trophozoite and infective cyst. *Giardia*, which can be discharged into the water via human or animal feces, is more resistant to environmental factors such as water temperature and other chemicals and can persist in a water environment (King and Monis, 2007). Waterborne transmission is the main route of human infection with *Giardia*. People can infect *Giardia* by directly contacting with contaminated water (diving, swimming, bathing, etc.) or eating contaminated food (Baldursson and Karanis, 2011; Feng and Xiao, 2011; Santín and Fayer, 2011).

3.3.3.3 Entamoeba histolytica

Entamoeba histolytica is an anaerobic parasitic amoebozoa (Ryan and Ray, 2004). The life cycle of *Entamoeba histolytica* includes trophozoite and cyst. The trophozoite is an active, feeding, and proliferating stage, and it is divided into small trophozoite and large trophozoite. Small trophozoite can develop to the stage of the cyst, which is nonactive and nonfeeding. *Entamoeba histolytica* can cause tissue destruction that leads to clinical disease, and the tissue damage is induced by direct host cell death, inflammation, and parasite invasion (Ghosh et al., 2019). It is estimated that *Entamoeba histolytica* infection kill more than 55,000 people each year (Shirley et al., 2018). The source of infection is food, drinking water, or utensils contaminated with feces containing mature cysts.

3.3.4 Roles of protozoa in wastewater treatment

Protozoa play an important ecological role not only in the matter cycling and self-purification in a natural ecosystem but also in the artificial system of WWTPs (Madoni, 2011; Pauli et al., 2001). Activated sludge, a widely used wastewater treatment all over the world, has a flocculent structure with protozoa living or crawling around it. There are about 230 species of protozoa observable in the activated sludge system, including Mastigophora (such as *Peranema deflexum* and *Anisonema acinus*), Sarcodina (such as *Mayorella penardi* and *Arcella hemisphaerica*), ciliated protozoa, and others. Among them, ciliates account for about 70% of the total protozoa in the sludge. During the wastewater purification process, protozoa are responsible for improving the quality and safety of the effluent by keeping the density of

dispersed bacteria (Madoni, 2011). The roles of protozoa in wastewater treatment process are (1) direct use of organic matter in wastewater; (2) promotion of flocculation process; and (3) capable of swallowing bacteria and other microorganisms. Moreover, protozoa have a faster rate of bacterial predation and shorter generation time. However, their feeding range is narrow, and they mainly feed on free bacteria, so they are not suitable to be the main predator in the sewage treatment system. Furthermore, other physiological characteristics of bacteria in the sludge, such as athletic ability, morphology, and surface characteristics, can affect the predation of protozoa.

3.4 Helminths
3.4.1 Biological characteristics of helminths

Helminths are oligochaete annelids and worm-like parasite that can generally be seen with the naked eye in their adult stages, including both free-living and parasitic worm in nature. Helminths are the most common parasites in the world, which have three main life-cycle stages, including eggs, larvae, and adults. Different from other pathogens (such as viruses, bacteria, protozoa, and fungi), helminths cannot multiply within their hosts when they are in adult form (Castro, 1996). Compared to other infectious pathogens, helminths develop slowly, so any resultant diseases are slow in onset.

Helminths are invertebrates characterized by elongated, flat, or round bodies. Helminths can be transmitted to humans via water, food, and soil as well as vectors of arthropod and molluscan. It is noteworthy that helminths can infect many organs and are prevalent in intestines. So the most common parasitic infections in humans is helminth infection in the world. The highest prevalence of helminth infections occurs in tropical countries where food supplies are inadequate, and parasite eggs, insects, and other invertebrate vectors are abundant. Moreover, most helminth infections can be prevented by avoiding contacting with vectors, developing sanitation, and avoiding consuming foods and water that might be contaminated with helminths.

3.4.2 Classification of helminths

The definitive classification of helminths is based on the external and internal morphology of the adult stage, larval, and egg (Castro, 1996). Recently, most parasitic helminths are in three major assemblages: flukes (trematodes), tapeworms (cestodes), and roundworms (nematodes) (Castro, 1996). The further subdivision is based on the host organs. Table 3.5 is adapted from previous studies (Garcia, 1997; Cohen et al., 2017) and summarizes the major helminths in the environment.

Flukes (trematodes) are leaf-shaped flatworms, which vary in length from a few millimeters to 8 cm. Flukes are hermaphroditic except for blood flukes, which are bisexual. Flukes go through several larval stages before reaching adulthood. Tapeworms (cestodes) are hermaphroditic and vary in length from 2 mm to 10m,

Table 3.5 Major helminths in the environment (Garcia, 1997; Cohen et al., 2017).

Classification of helminths	Host organs	Major helminths
Flukes (trematodes)	Intestinal	*Fasciolopsis buski*
		Echinostoma ilocanum
		Heterophyes heterophyes
		Metagonimus yokogawai
	Liver/lung	*Clonorchis* (*opisthorchis*) *sinensis*
		Opisthorchis viverrini
		Fasciola hepatica
		Paragonimus westermani
		Paragonimus mexicanus
		Paragonimus heterotremus
		Paragonimus skrjabini
		Paragonimus spp.
	Blood	*Schistosoma mansoni*
		Schistosoma haematobium
		Schistosoma japonicum
		Schistosoma intercalatum
		Schistosoma mekongi
Tapeworms (cestodes)	Intestinal	*Diphyllobothrium latum*
		Dipylidium caninum
		Hymenolepis nana
		Hymenolepis diminuta
		Taenia solium
		Taenia saginata
	Tissue	*Taenia solium*
		Echinococcus granulosus
		Echinococcus multilocularis
		Multiceps multiceps
		Taenia multiceps
		Spirometra mansonoides
		Diphyllobothrium spp.
Roundworms (nematodes)	Intestinal	*Ascaris lumbricoides*
		Enterobius vermicularis
		Ancylostoma duodenale
		Necator americanus
		Strongyloides stercoralis
		Trichostrongylus spp.
		Trichuris trichiura
		Capillaria philippinensis
	Tissue	*Trichinella spiralis*

Table 3.5 Major helminths in the environment (Garcia, 1997; Cohen et al., 2017).—*cont'd*

Classification of helminths	Host organs	Major helminths
	Blood and tissues	Visceral larva migrans (*Toxocara canis* or *Toxocara cati*)
		Ocular larva migrans (*Toxocara canis* or *Toxocara cati*)
		Dracunculus medinensis
		Neural larva migrans (*Baylisascaris procyonis*)
		Angiostrongylus cantonensis
		Angiostrongylus costaricensis
		Gnathostoma spinigerum
		Anisakis spp. (larvae from saltwater fish)
		Phocanema spp. (larvae from saltwater fish)
		Contracaecum spp. (larvae from saltwater fish)
		Capillaria hepatica
		Thelazia spp.
		Wuchereria bancrofti
		Brugia malayi
		Brugia timori
		Onchocerca volvulus
		Mansonella ozzardi
		Mansonella streptocerca
		Mansonella perstans
		Dirofilaria spp. (may be found in subcutaneous nodules)

colonizing the human intestinal lumen. Adult tapeworms are elongated, flattened, and segmented. Adult roundworms (nematodes), which are cylindrical in structure, are usually bisexual (Castro, 1996). Most nematodes inhabit in the intestine and extraintestinal sites. The main developmental stages in nematodes include egg, larval, and adult stage. The eggs lay by most nematodes that are parasitic in humans may include either the zygote, blastomere, or formed larva (Castro, 1996).

The concentration of helminth eggs was high in wastewater and sludge, especially in developing countries (Amoah et al., 2018). The soil-transmitted helminth eggs relate to infection risk through different exposure routes. For instance, wastewater irrigation to the soil, which transmitted helminth infections were found among vegetable farmers in Ghana (Amoah et al., 2016). The raw and treated wastewater samples collected from 8 WWTPs in Tehran and 2 WWTPs in Isfahan were explored for the presence of helminth eggs during 2002−03, revealing that level of eggs was

high in influent and it significantly reduced after treatment (Mahvi and Kia, 2006). Moreover, helminth ova are considered as one of the main target pollutants and should be removed from reused wastewater for agriculture and aquaculture, and their fates should be further investigated during different wastewater treatment processes (Jimenez, 2007).

3.5 Biotoxins
3.5.1 Characteristics and classification of biotoxins

Biotoxins mainly refer to toxic substances that can be produced by animals, plants, or microorganisms in certain conditions. Biotoxins, which are difficult to achieve by chemical synthesis, are with high toxicity and have numerous kinds. Biotoxins can be extremely hazardous even in minute quantities and are threats to human health, which are classified as biological hazards. They are fairly stable in undiluted forms, but, usually, do not persist for long periods in certain environments. Table 3.6 summarizes some biotoxins and their corresponding LD50 (μg/kg) (Institutional biosafety committee et al., 2014; OHSU research integrity Office, 2014).

In addition, many biotoxins may be further classified according to the effects on the human body, such as cytotoxins and neurotoxins. According to the source of biotoxins, it also can be divided into plant toxins, animal toxins, bacterial toxins, mycotoxins, and marine biotoxins.

3.5.2 Bacterial toxins

Among different biotoxins, bacterial toxins are mostly secondary metabolites during their growth and reproduction, which can disable the immune system and directly damage the tissue to contribute to infection and disease. Bacterial toxins include exotoxins and endotoxins. Exotoxins are highly toxic and relatively unstable, and they are cellular products excreted from certain viable Gram-positive and -negative bacteria (Chatterjee and Raval, 2019), such as *Corynebacterium diphtheriae*, *Clostridium tetani*, *Clostridium botulinum*, and *Staphylococcus aureus*. Endotoxins are moderately toxic and relatively stable, and they are lipopolysaccharide complexes derived from the cell membrane of Gram-negative bacteria (Galanos, 1998; Todar, 2018). The properties of endotoxins and classic exotoxins have been summarized by the microbiologist Kenneth Todar of the University of Wisconsin, who found that exotoxins have relatively higher potency (1 μg) than that of endotoxins (>100 μg) as well as higher specificity than that of endotoxins (Todar, 2018). Furthermore, exotoxins have enzymatic activity while endotoxins have no enzymatic activity (Todar, 2018).

Endotoxin is a kind of proinflammatory factors and pyrogen materials. Endotoxin exposure can cause a variety of symptoms, including fever, diarrhea, vomiting, wheezing, dyspnea, shock, and intravascular coagulation (Anderson et al., 2002; Basinas et al., 2015; Liebers et al., 2008). Furthermore, endotoxin may enhance

Table 3.6 Some biotoxins and their corresponding LD50.

Toxin	LD50 (μg/kg)
Abrin	0.7
Aerolysin	7
Botulinum toxin A	0.0012
Botulinum toxin B	0.0012
Botulinum toxin C1	0.0011
Botulinum toxin C2	0.0012
Botulinum toxin D	0.0004
Botulinum toxin E	0.0011
Botulinum toxin F	0.0025
β-bungarotoxin	14
Caeruleotoxin	53
Cereolysin	40—80
Cholera toxin	250
Clostridium difficile enterotoxin A	0.5
Clostridium perfringens delta toxin	5
Clostridium perfringens epsilon toxin	0.1
Clostridium perfringens lecithinase	3
Clostridium perfringens perfringolysin O	13—16
Conotoxins	12—30
Crotoxin	12—30
Diacetoxyscirpenol	1000—10,000
Diphtheria toxin	0.1
HT-2 toxin	5—10
Leucocidin	50
Listeria listeriolysin or hemolysin	3—10
Listeriolysin	3—12
Modeccin	1—10
Nematocyst toxins	33—70
Notexin	25
Pertussis toxin	15
Pneumolysin	1.5
Pseudomonas aeruginosa exotoxin A	3
Ricin	2.7
Saxitoxin	8
Shigella dysenteriae neurotoxin	1.3
Staphylococcus enterotoxin B	25
Staphylococcus enterotoxin F	2—10
Staphylococcus enterotoxins A, C, D, and E	20 (A); <50 (C)
Streptolysin O	8
Streptolysin S	25

Continued

Table 3.6 Some biotoxins and their corresponding LD50.—*cont'd*

Toxin	LD50 (μg/kg)
T-2 toxin	5—10
Taipoxin	2
Tetanus toxin	0.001
Tetrodotoxin	8
Viscum Album lectin 1	2.4—80
Volkensin	1.4
Yersinia pestis murine toxin	10

the toxic effects of other toxic substances, such as algal toxins (Best et al., 2002; Roth et al., 1997). Therefore, some control standards of endotoxin have proposed that the endotoxin content of water for injection should be less than 0.25 EU/mL (Anderson et al., 2002). For the workers in the wastewater treatment plant, endotoxin exposure ranged from 0.6 to 2093 EU/m^3, and the geometric mean exposure was low (27 EU/m^3) (Smit et al., 2005). Some symptoms appeared to be more prevalent in workers when they were exposed to endotoxin with a level higher than 50 EU/m^3 (Smit et al., 2005). Endotoxin activity has been assessed in several WWTPs using the samples collected from influent, effluent, return sludge, and advanced treatment effluent in Sapporo and Japan, which have revealed that active endotoxin materials occurred in wastewater and endotoxin activity was high in wastewater (Guizani et al., 2009). Moreover, the discharge of effluent of treatment plants has increased the endotoxicity in the receiving river water (Ohkouchi et al., 2007). Guizani (2010) have reported that biological treatment cannot control endotoxicity and can produce organic matters with endotoxicity during wastewater reclamation.

3.6 Antibiotic resistance
3.6.1 ARGs and ARB

Recently, extensive use and abuse of antibiotics may induce the development of ARGs and ARB in the environment, which has conferred enormous and complicated impacts on human health and environmental safety (Blaser, 2011; He et al., 2016; Rizzo et al., 2013). As early as in 2004, American scholars Rysz and Alvarez (2004) considered ARGs as a new type of environmental pollutants. In 2006, Pruden et al. (2006) put forward ARGs as a new type of environmental pollutants, which has drawn more and more attention in the research field of environmental sciences. ARGs can spread and transfer between different bacteria and have more adverse effects on the environment than the ARGs themselves, which is one of the reasons for the growing pollution in the environment (Dodd, 2012; Jiao et al., 2017). In the meantime, increasing ARB has been detected in the environment. It has been

reported that multiresistant New Delhi Metallo-β-lactamase-1 (NDM-1) emerges in wastewater of two STPs in Haihe River basin of China, and the ARGs of NDM-1 can transfer to the indigenous bacteria in the receiving river providing source water for millions of people nearby, which poses huge health risk (Luo et al., 2013). The purpose of this section is to thoroughly summarize the pollution status of ARGs and ARB in the wastewater and propose future research directions.

3.6.2 Mechanisms of antibiotic resistance in bacteria

Bacterial antibiotic resistance, especially for the multiple-antibiotic resistance, has emerged as both medical and social problems in the world, which poses a significant threat against antiinfective therapy in the environment. The antibiotic resistance in bacteria can be mediated via several mechanisms, which fall into four main groups (Blair et al., 2015; Munita and Arias, 2016; Ramirez and Tolmasky, 2010; Zhang et al., 2009):

3.6.2.1 Enzyme-catalyzed inactivation of antibiotics

The inactivation or passivation enzyme can destruct antibiotic molecules by hydrolysis or modification, which induces antibiotic resistance in bacteria. For example, β-lactamase can destroy the amide bond of the β-lactam ring of penicillin and cephalosporin antibiotics, rendering the antimicrobial ineffective. Aminoglycoside modifying enzymes inactivate some active groups in the molecules of aminoglycoside and quinolones so that their binding ability to the target is reduced.

3.6.2.2 Changes in antibiotic targets

Some antibiotics are specifically combined with bacterial target sites and affect their normal physiological functions, leading to bacterial death. Changes of the genes coding the corresponding targets will lead to the change of targets structure that prevents efficient antibiotic binding and target recognition. For example, methylase synthesized by the bacteria resistant to macrolides, leading to methylation of 23S rRNA adenine in the 50S subunit of the ribosome, which prevents the binding of antibiotics to binding sites and exhibits resistance to macrolides.

3.6.2.3 Bacterial efflux pumps

Bacterial efflux pumps are capable to actively extrude many antibiotics out of the cell, and can also result in antimicrobial resistance. The efflux pump systems are common in all kinds of bacteria and are major contributors to the intrinsic resistance to many drugs. For example, tetracycline efflux pump genes can encode related membrane proteins to extrude the tetracycline out of the cell, which reduces the concentration of intracellular tetracycline.

3.6.2.4 Changes in the permeability of bacterial cell walls or cell membranes

Changes in the outer membrane proteins of bacteria can reduce the permeability of the outer membrane, and limit antibiotic entry into the target sites of the bacterial

cell, leading to cross-resistance to different types of antibiotics, especially for β-lactam and quinolone antibiotics.

For intrinsic resistance, bacteria also acquire or develop resistance to antibiotics via mutations and by horizontal gene transfer (Munita and Arias, 2016). Acquisition of external DNA through horizontal gene transfer by three main strategies (transformation, transduction, and conjugation) is one of the most important drivers of bacterial evolution, and it is frequently responsible for the development and dissemination of resistance to many frequently used antibiotics.

3.6.3 Fates of ARGs and ARB in wastewater

As a reservoir for ARGs and ARB, wastewater is one of the important contamination sources for the antibiotic resistance dissemination in the environment. Recently, a variety of ARG types (tetracycline, sulfonamide, multidrug, aminoglycoside, bacitracin, chloramphenicol, β-lactam, quinolone, trimethoprim, polymyxin, and vancomycin as well as other types) have been found in medical wastewater, pharmaceutical wastewater, domestic wastewater and wastewater from aquaculture systems, and livestock breeding (Guo et al., 2018; Jia et al., 2017; Tong et al., 2019; Zhao et al., 2018). Furthermore, ARB and multiple ARB, such as *Pseudomonas aeruginosa*, *Escherichia coli*, *Acinetobacter* spp., and Enterobacteriaceae are observed in wastewater (Huang et al., 2018; Kümmerer, 2009). The main sources and distribution of ARGs in wastewater were reviewed:

3.6.3.1 Medical and pharmaceutical wastewater

Medical and pharmaceutical wastewater contains a large amount of ARGs and ARB, which is the main source of ARGs and ARB in the water environment. Rodriguez-Mozaz et al. (2015) have detected ARGs in hospital sewage, urban sewage treatment system, and the receiving river in Spain, and found that the abundances of bla_{TEM}, qnrS, sulI, and tet(W) in hospital sewage were higher than those in the urban sewage treatment system and the receiving river. ARGs within β-lactam (bla_{VIM} and bla_{SHV}), aminoglycosides (aacC2), chloramphenicol (catA1 and floR), macrolide—lincosamide—streptogramins (emrA and mefA), sulfonamide (sulI and sulII), and tetracycline (tet(A), tet(B), tet(C), tet(O), and tet(W)) associated with transposons have been found in Romanian hospital sewage (Szekeres et al., 2017). In addition, sulfonamide (sulI and sulII)), tetracycline (tet(O), tet(T), tet(M), tet(Q), and tet(W), β-lactam (bla_{OXA-1}, bla_{OXA-2}, and bla_{OXA-10}), and macrolide (ermB) resistance genes were detected based on quantitative PCR analysis in typical pharmaceutical wastewater treatment systems (Zhai et al., 2016). The maximum concentrations of ARGs detected in the final effluents of pharmaceutical WWTPs were up to 3.68×10^6 copies/mL by Wang et al. (2015) and 2.36×10^7 copies/mL by Zhai et al. (2016), respectively, which were much higher than the concentration in MWTPs as revealed by Mao et al. (2015).

In hospital sewage in New Delhi, Lamba et al. (2017) have detected 748 extended-spectrum β-lactam resistant bacterial strains (including *Escherichia coli*,

Klebsiella, and *Pseudomonas putida*) and 953 carbapenem-resistant Enterobacteri-aceae strains (including *Klebsiella pneumoniae*, *Pseudomonas putida*, and *Klebsiella pneumonia* subsp. Pneumonia). ARB resistant to carbapenem also existed in hospital sewage in China, Croatia, and other countries in the world (Zhang et al., 2013a; Hrenovic et al., 2015).

3.6.3.2 The domestic wastewater treatment system

Domestic wastewater treatment system is the node where different types of waste-water converge, containing a large number of exogenous ARGs and ARB. At the same time, the activated sludge in the domestic wastewater treatment plant is an ideal habitat for bacteria due to its rich nutrition and large amount of aeration, which also makes the domestic wastewater treatment system to become an important pollu-tion source of ARGs and ARB in the water environment (Donlan, 2002; Li et al., 2019; Yang et al., 2013). Hrenovic et al. (2017) have detected carbapenem-resistant bacteria in influent, activated sludge, effluent, digested sludge, and stabi-lized sludge of the largest Croatian secondary wastewater treatment plant in Zagreb, and found that the number of carbapenem-resistant bacteria in effluent was reduced by more than 99% compared with influent while the relative abundance increased. Hembach et al. (2017) have detected *mcr-1* gene and some clinical-related genes (including $bla_{CTX-M-32}$, bla_{TEM}, bla_{CTX-M}, tet(M), bla_{CMY-2}, and ermB) in the influent and effluent of seven German WWTPs. Luo et al. (2013) have found the NDM-1 gene in disinfected effluent from two STPs in north China with the absolute abundance of 1316 ± 232 and 1431 ± 247 copies/mL, respectively. It is worth noting that ARGs cannot be completely removed by STPs, and ARGs can be trans-mitted in the receiving river (Rodriguez-Mozaz et al., 2015).

The research group of Professor Zhang from the University of Hong Kong con-ducted a detailed and deep research on the fate of ARGs in STPs. *Tet*(A), *tet*(C), *tet*(G), *tet*(M), *tet*(S), and *tet*(X) have been detected in all the 15 STPs across China, Canada, the United States, and Singapore (Zhang and Zhang, 2011). Yang et al. (2013) have investigated the variation of ARGs in activated sludge over a 4-year period, which revealed that more than 200 ARG subtypes have been detected in the activated sludge, and aminoglycoside and tetracycline resistance genes have highest abundances followed by sulfonamide, multidrug, and chloramphenicol resis-tance genes. On this basis, a total of 271 ARG subtypes within 18 ARG types were detectable in the influent, activated sludge, effluent, and anaerobic digestion sludge in a sewage treatment plant in Hong Kong from 2011 to 2012, and the abundance of ARGs was highest in influent followed by effluent, activated sludge, and anaerobic digestion sludge (Yang et al., 2014). Meanwhile, the research group also found 78 persistent ARGs in the process of biological wastewater and sludge treatment, revealing that anaerobic digestion of sludge cannot completely remove ARGs (Zhang et al., 2015). Furthermore, the abundance of ARGs is correlated with anti-biotic resistance phenotypes in municipal sewage, and the abundance of ARGs in resistant coliforms is also relatively high and up to 33.8 ± 4.2 copies per cell (Li et al., 2017).

3.6.3.3 Wastewater from aquaculture system and livestock breeding

The use of antibiotics in aquaculture system and livestock breeding could increase the potential risk of antibiotic resistance in products and ecosystems (He et al., 2016; Huang et al., 2015). Increasing studies have shown that aquaculture and animal farms are important sources of ARB and ARGs in the water environment, where ARB and ARGs are abundant and may infect humans through the food chain (Huang et al., 2015; Jia et al., 2017). Currently, antibiotic resistant pathogens such as *Aeromonas* (Penders and Stobberingh, 2008), *Vibrio* (Oh et al., 2011), and *Salmonella* (Budiati et al., 2013) have been found in aquaculture systems. A total of 4767 strains nonsusceptible to sulfamethoxazole/trimethoprim, tetracycline, erythromycin or cefotaxime were isolated from fish intestine, fish surface rinsing water, fish feed, pond mud, and pond water from an American aquaculture farm, and 80% of them exhibited multiple antibiotic resistance (Huang et al., 2015). Furthermore, strains of *Escherichia coli* were isolated in pigs and poultry in China which had high rates of resistance to ampicillin, tetracycline, doxycycline, trimethoprim—sulfamethoxazole, amoxicillin, streptomycin, and chloramphenicol (Jiang et al., 2011). Notably, antibiotic resistance was observed in aquaculture and livestock breeding system as well as clinically isolated strains, indicating that potential antibiotic resistance could be transmitted between food and humans (Brooks et al., 2014; Huang et al., 2015). Xiong et al. (2015) found a total of 15 ARG subtypes (*sul*I, *sul*II, *sul*III, *tet*(M), *tet*(O), *tet*(W), *tet*(S), *tet*(Q), *tet*(X), *tet*(B/P), *qep*A, *oqx*A, *oqx*B, *aac(6′)-Ib*, and *qnr*S) within four ARG types (sulfonamide, tetracycline, aminoglycoside, and quinolone) in fresh water aquaculture environment and the relative abundance was up to 2.8×10^{-2} (ARG copy/16S rRNA copy). Tajbakhsh et al. (2015) collected 150 water samples from aquaculture water of fish fields in different geographical regions in Iran and found 18% of the water samples contained *Escherichia coli* resistant to ampicillin, ciprofloxacin, gentamycin, chloramphenicol, tetracycline, and imipenem. Furthermore, Zhu et al. (2013) comprehensively evaluated the diversity and abundance of ARGs in a pig farm and detected a total of 149 ARG subtypes resistance to aminoglycoside, β-lactam, tetracycline, vancomycin, and other antibiotics. At the same time, their abundances significantly correlated with levels of antibiotics (Zhu et al., 2013). Quinolone-resistant genes *qnr*S and *oqx*A have been detected in pig farm wastewater using real-time quantitative PCR technology, and they have spread to the surrounding environment based on the comparison with the levels of them in the surrounding soil (Li et al., 2012a).

3.7 Summary

Characteristics, classification, fates, functions, and health implications of different biological HRPs in wastewater are summarized in this chapter. A variety of biological HRPs are prevalent in wastewater, including bacteria, viruses, protozoa, helminth, biotoxins, ARGs, and ARB. The biological HRPs in wastewater possibly contact with

human bodies along with wastewater discharge into the environment or reuse for different purposes, which can induce serious infection to affect public health. Notably, some wastewater treatment processes have the potential to reduce the level of various biological HRPs and the induced risk on environment and health. However, due to the lack of reliable risk assessment methodology for the biological HRPs in the wastewater, whether the treated wastewater is safe enough for public health currently remains known. Thus, the health risk associated with these biological HRPs in the wastewater deserves greater concerns, and more efforts have to be devoted to extending our knowledge regarding their health hazards and risks and developing effective technologies to prevent, remove, or kill biological HRPs in the wastewater.

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