Environmental Research 169 (2019) 483–493

Contents lists available at ScienceDirect

Environmental Research

journal homepage: www.elsevier.com/locate/envres

Human health risk assessment of antibiotic resistance associated with antibiotic residues in the environment: A review

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ARTICLE INFO

Keywords:
Antibiotic residues
Antibiotic resistance
Exposure assessment
Health risk assessment

ABSTRACT

The extensive use of antibiotics leading to the rapid spread of antibiotic resistance poses high health risks to humans, but to date there is still lack of a quantitative model to properly assess the risks. Concerns over the health risk of antibiotic residues in the environment are mainly (1) the potential hazard of ingested antibiotic residues in the environment altering the human microbiome and promoting emergence and selection for bacteria resistance inhabiting the human body, and (2) the potential hazard of creating a selection pressure on environmental microbiome and leading to reservoirs of antibiotic resistance in the environment. We provide a holistic view of health risk assessment of antibiotic resistance associated with antibiotic residues in the environment in contrast with that of the antibiotic resistant bacteria and discuss the main knowledge gaps and the future research that should be prioritized to achieve the quantitative risk assessment. We examined and summarized the available data and information on the four core elements of antibiotic resistance associated with antibiotic residues in the environment: hazard identification, exposure assessment, dose-response assessment, and risk characterization. The data required to characterize the risks of antibiotic residues in the environment is severely limited. The main future research needs have been identified to enable better assessments of antibiotic resistance associated with antibiotic residues in the environment: (1) establishment of a standardized monitoring guide of antibiotic residues and antibiotic resistance in the environment, (2) derivation of the relationship between antibiotic levels and pathogenic antibiotic-resistance development in different settings, and (3) establishment of the dose-response relationship between pathogenic antibiotic resistant bacteria and various infection diseases. After identification of key risk determinant parameters, we propose a conceptual framework of human health risk assessments of antibiotic residues in the environment.

Capsule: A holistic view of human health risk assessment of antibiotic residues in the environment was provided.

1. Introduction

Antibiotics are a class of secondary metabolites produced by microorganisms, as well as chemically synthesized or semi-synthesized analogous compounds, which could inhibit growth and survival of other microorganisms (Demain and Sanchez, 2009). Antibiotics are very useful as therapeutic agents in the treatment of human infectious disease, and nowadays antibiotics are also extensively used in livestock industry and aquaculture (Nisha, 2008). Veterinary antibiotics are originally used in prevention and treatment of animal diseases, but they are gradually added as feed additives as prophylactics and growth promoters, which far outweighs their use as animal therapeutics at present (Gelband et al., 2015). Antibiotics can enter the water and land environment through various pathways (Ashbolt et al., 2013), such as the discharge of municipal sewage, manufacturing industry, animal husbandry, and landfill leachates of antibiotic disposal. Other sources may include runoff from agricultural field containing livestock manure, aquaculture ponds, and urban centers (Fig. 1). Particularly, irrigation

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https://doi.org/10.1016/j.envres.2018.11.040
Received 30 July 2018; Received in revised form 11 November 2018; Accepted 24 November 2018
Available online 26 November 2018

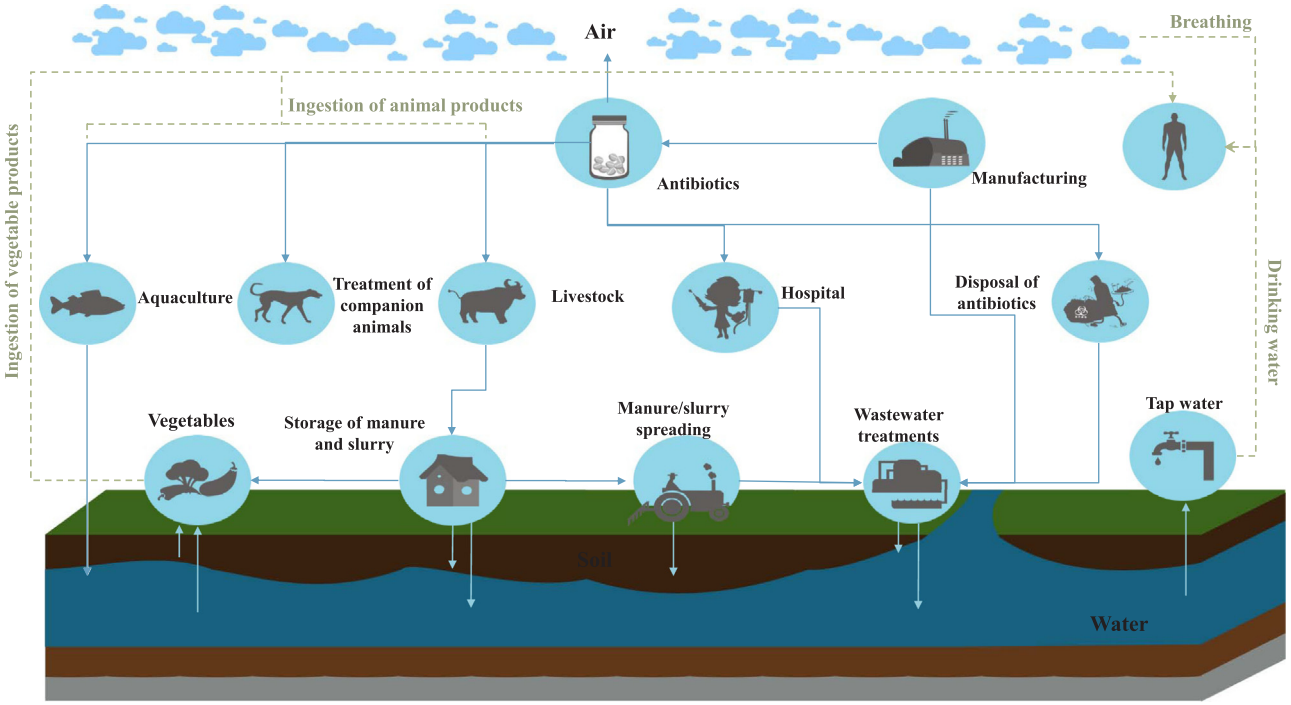

Fig. 1. Human exposure to antibiotic resistance associated with antibiotic residues in the environment.

with treated wastewater and fertilization with livestock manure are high potential pathways for the introduction of antibiotics in the agroecosystem. Although the half-life of most antibiotics is not long (hours to hundred days) (Ji et al., 2010), antibiotic residues that remain in the environment can be considered as a 'persistent' organic contaminant due to frequent and extensive use of antibiotics and uninterrupted emissions (Hamscher et al., 2002).

The use of antibiotics in large amounts may pose a strong selection pressure on human and natural microbial system (National Academies of Sciences and Medicine, 2018; Qiao et al., 2017). The occurrence of antibiotics in microbial system may lead to genetic or mutational changes in normally sensitive bacteria, allowing the bacteria to survive and further proliferate as antibiotic resistant bacteria (ARB) that carry antibiotic resistant genes (ARGs) (Martinez, 2009). Mobile genetic elements (MGE), including plasmids, integrons, and prophages (Martínez et al., 2015), would further enhance the dissemination and promotion of genetic recombination of ARGs by transformation, conjugation, or transduction collectively referred as horizontal gene transfer (HGT) (Vikesland et al., 2017). ARGs are becoming recognized as an emerging environmental pollutant (Pruden et al., 2006). Concerns over the human health threats of antibiotic resistance associated with antibiotic residues in the environment are mainly (1) the potential hazard of ingested antibiotic residues altering the human microbiome and promoting emergence and selection for resistant bacteria resistance inhabiting the human body namely as human antibiotic resistance (Cho and Blaser, 2012); and (2) the potential hazard of creating a selection pressure on the environmental microbiome and leading to reservoirs of ARGs and ARB referred as environmental antibiotic resistance (Qiao et al., 2017). The clinically relevant antibiotic resistances are associated with the increased hospitalization of patient infection induced by such antibiotic-resistant pathogens, which may result in treatment failure and mortality due to the reduced efficiency in therapeutic antibiotic use (Hidron et al., 2008).

Since antibiotics are extensively used nowadays, risk assessments of human exposure to antibiotic resistance in the environment have attracted increasing attention (Ashbolt et al., 2013). Human health risk assessment is to estimate the probability of illness and deaths caused by the infection associated with ARB (Ashbolt et al., 2013; Manaia, 2017), which included four core elements: hazard identification, exposure assessment, dose-response assessment, and risk characterization. The major purpose of the present study is to provide a holistic view of health risk assessment of antibiotic residues in the environment, based on the effects on ARB, and to address the main knowledge gaps and future research needs in the assessment framework.

2. Hazard identification: what adverse health effects might result from exposure to antibiotic residues in the environment?

2.1. Antibiotic residues, human microbiome and human health

Once entering into human body, antibiotic residues might interact with the human microbiome, which contains a great number of diverse microorganisms inhabiting the human body (National Academies of Sciences and Medicine, 2018). Daily intake of antibiotic residues from the environment may largely enter the human gastrointestinal tract, where approximately 800–1000 different bacterial species and more than 7000 different strains are inhabited (Jernberg et al., 2010). Of these microbes, 95% are beneficial bacteria and others are harmful bacteria and opportunistic pathogens (National Academies of Sciences and Medicine, 2018). There is a stable micro-ecological balance between these bacteria and between bacteria and the human body over time, with predominant taxa of Bacteroidetes and Firmicutes, and minorities of Actinobacteria, Proteobacteria, Verrucomicrobia and other bacteria phyla that have not yet been isolated or identified (Arumugam et al., 2011). Observational, clinical and epidemiological studies have provided increasing evidence that antibiotic exposures are strongly associated with changes in intestinal microbiome composition due to the broad-spectrum influence on the host-associated microbial community rather than one target bacteria (Blaser, 2016). Antibiotic therapy could lead to composition changes of intestinal microbiota with an increase in Firmicutes and a concurrent reduction in Bacteroidetes (Pérez-Cobas et al., 2012), and also induce the emergence of antibiotic-resistant bacteria, which could persist in human intestines for years (Andersson and Hughes, 2011; Jernberg et al., 2010). Once imbalance of intestinal microbiota occurs, it may lead to the proliferation of harmful bacteria and opportunistic pathogens, and further lead to

various diseases such as pseudomembranous colitis, intestinal disorders and colorectal cancer (Damman et al., 2012). Even worse, if intestinal bacteria have developed antibiotic resistance, multiplied in large numbers, and evolved into super-bugs, the diseases caused by these bacteria will lead to death due to incurability. This is not only a risk to human individuals, but to the global human population.

A hypothesis has been put forward, that during the course of antibiotic treatment, the taxa which were low in number and vulnerable to antibiotics may be lost, in contrast with that the antibiotic resistant bacteria would be survived and accumulated (Blaser, 2016). If the taxa serve special metabolic functions, the collateral damage to the host may further lead to human metabolic perturbations and alteration of immunologic development, which may affect adiposity and bone growth (Cho et al., 2012; Cox et al., 2014). This is in particular important in early-years of human life, when the adult microbiome has not been formed and mainly inherits from mother (Blaser, 2016). Compared to the proactive avoidance measure in medicine, the intake of antibiotic residues from the environment is inevitable for children especially after weaning and beginning to consume staple food, which may exert a great influence in the early human life with a critical window in the development of microbiome. Both epidemiological and experimental studies have suggested that the effects of antibiotics may be cumulative in the human individuals, and thus it can expect that the effects of environmental antibiotic exposure on human microbiome may cumulate across generations (Sonnenburg et al., 2016). However, the select and shape influences on human microbial community structure with chronic exposure to antibiotic mixtures and possible interactions among themselves are not fully understood. This critical and important relationship between antibiotic residue exposure and human microbiome and function in terms of human health will require further research.

2.2. Antibiotic residues, environmental microbiome and human health

Antibiotic residues could accelerate the emergency and evolution of ARB and ARGs in the environment (Qiao et al., 2017), and the risks associated with the environmental antibiotic resistome refer to the transmission of environmental ARB and ARGs to humans (Manaia, 2017). Antibiotic residues in the environment create selection pressure on the environmental microbiome and thus generate environmental reservoirs of ARB and ARGs, the phylogenetical bacteria diversity of which belong to phyla such as Actinobacteria, Proteobacteria, or Bacteroidetes, which often possess the capacity to produce or metabolize antibiotics as well as to transfer environmental ARB and ARGs to humans (Forsberg et al., 2012; Manaia, 2017). However, the transmission of environmental ARGs to human could be relied on carrying by pathogenic ARB or human commensal ARB both of which are most likely to be members of the classes Gammaproteobacteria and Betaproteobacteria and the phyla Actinobacteria and Firmicutes (Li et al., 2015; Manaia, 2017; Vaz-Moreira et al., 2014). Pathogenic and the human commensal ARB have the ability to colonize and proliferate in human body, and pathogenic ARB have the ability to penetrate into tissues and cause acute immune reaction and infectious disease. Most commensal ARB are not pathogens, but they could cumulatively reside in the human body and may harbor crucial virulence genes and therefore cause a disease, or they could transfer the genes conferring crucial virulence to other commensal human microbiota.

3. Exposure assessment: what doses of antibiotic resistance associated with antibiotic residues in the environment are occurring in exposed population?

3.1. Antibiotic residues and human microbiome

There are about 200–220 types of antibiotic in the natural environment, out of more than 250 types of semisynthetic or modified natural product-based drugs produced (Bérdy, 2012). Antibiotics can be divided into 10 main categories according to different functional chemical structures: aminoglycoside, β-lactams, lincosamides, macrolides, polypeptides, quinolones, sulfonamides, tetracyclines, chloramphenicols, and others. In these categories, there are about 120 antibiotics available in human health care and livestock husbandry (Wang et al., 2015a). Since antibiotics are extensively used nowadays, biomonitoring of human exposure to antibiotic residues in the environment has become increasingly important.

3.1.1. Drinking water

Antibiotics have frequently been detected in surface water and wastewater at concentrations generally ranging between 0.01 and 1.0 μg/L (Le Page et al., 2017). That means antibiotic residues have already entered the major sources of drinking water for most people, the occurrence of which may be persistent for several months and may not be removed completely by traditional disinfection technologies in drinking water treatment. However, there is relatively scarce information regarding antibiotic occurrence in drinking water. Quinolones, chloramphenicols, sulfonamides, and macrolides appeared to be the main antibiotics detected in drinking water with the highest concentrations and detection frequencies, with ciprofloxacin exhibiting the highest detected concentration up to 679.7 ng/L (Wang et al., 2010). Based on the analysis from the existing data, the concentration of antibiotic residues of tap water in Guangzhou City (maximum concentrations: 7.9–679.7 ng/L) was higher than that in 42 other cities in China, and Chinese tap water may contain a higher residual antibiotic concentration than other countries (Benotti et al., 2008; Boleda et al., 2014; Simazaki et al., 2015; Zuccato et al., 2000) (Fig. 2**A**).

3.1.2. Air and dust

To our best knowledge, there is currently no report on the concentration of antibiotic residues in the air. However, residual antibiotics have been detected in airborne particles originating from pig-fattening farms, cattle feed yards, and pharmaceutical companies (Farshad et al., 2016; Hamscher et al., 2003; McEachran et al., 2015). Six different antibiotics including tylosin, three tetracyclines, sulfamethazine, and chloramphenicol were detected ranging from not detected (ND) - 12.5 mg/kg in dust samples collected from a pig-fattening farm in Germany during 1981–2000 (Hamscher et al., 2003), while the concentrations of three tetracyclines, tylosin and monensin detected in dust downwind of feed yards in the Southern High Plains, USA ranged from 0.5 to 4.6 mg/kg (McEachran et al., 2015). Penicillin was detected in airborne dust from a pharmaceutical company with a mean concentration of 6.6 mg/m^3 (Farshad et al., 2016). However, there is a lack of information related to antibiotics associated with dust from public areas where general population comes into contact.

3.1.3. Soil

Antibiotics could be introduced into soil through wastewater or reclaimed water irrigation, manure-amended application, or sludge landfill. The concentrations of antibiotics varied greatly across soils from different sources from μg/kg to mg/kg (dry weight) (Fig. 2**E**). Generally, antibiotic residues exhibit higher concentrations in manure amended soils than those of wastewater or reclaimed water irrigated soils. In addition, tetracycline antibiotics in soils showed higher residual levels than quinolones and sulfonamides, with the highest concentration (11.0 mg/kg) of chlorotetracycline detected in livestock manure and amended soils in Northern China (Huang et al., 2013), while sulfonamides showed a higher detection frequency (33.33–100%) in wastewater irrigation soils in Beijing and Tianjin, China (Chen et al., 2014).

3.1.4. Food

3.1.4.1. Meat. Many antibiotics have been used in food-producing animals for therapeutic, prophylactic and growth promotion purposes, and therefore trace quantities of antibiotics may be present

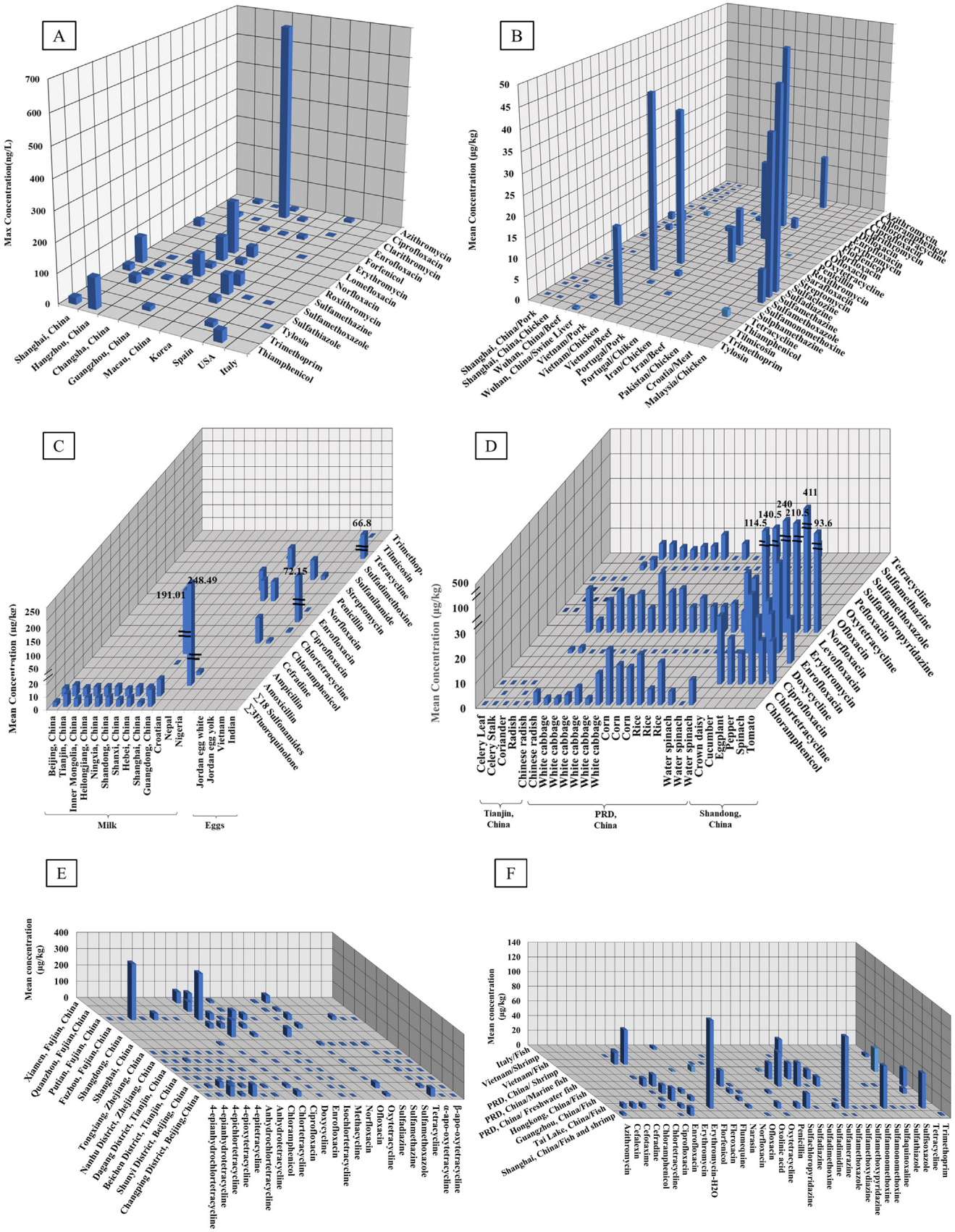

Fig. 2. Maximum concentrations of antibiotic residues in drinking water (A); mean concentrations of antibiotic residues in meat products (B), milk and eggs (C), vegetables (D), soil(E), and fish and shrimp (F); PRD, China: Pearl River Delta, China.

in meat products as residues (Cheong et al., 2010; Tavakoli et al., 2015; Yamaguchi et al., 2015). Antibiotic residues have been found in pork, chicken, beef, and swine liver in different countries (Fig. 2**B**). It seemed that sulfonamides and tetracyclines were presented at higher concentrations and detection frequencies in meat products than quinolones, while aminoglycoside and β-lactams were also commonly detected in meat products. The antibiotic residue concentrations in meat products in Vietnam (Yamaguchi et al., 2015), Iran (Tavakoli et al., 2015), and Malaysia (Cheong et al., 2010) were higher than those in China when comparing residual levels of the same antibiotics.

3.1.4.2. Egg and milk. Antibiotics are widely used as an antibacterial agent into dairy products and diary livestock feed which may induce deposition of antibiotic residues in milk (Fig. 2**C**). Forty-three antibiotics were analyzed in raw milk samples collected from 10 provinces in China through ELISA (enzyme-linked immunosorbent assay) method, and the results showed that the average concentrations of 3 fluoroquinolones and 18 sulfonamides in raw milk in China were 8.52 and 7.64 μg/kg, respectively, with the highest concentrations detected in samples collected from Guangdong, followed by Tianjin (Zheng et al., 2013). The highest concentration of chloramphenicol (3629.2 μg/L) was noted in milk samples from Shanghai, China (Wang et al., 2017a), while the highest concentration of penicillin G (353 μg/L) was obtained in milk from Nigeria (Khanal et al., 2018). Laying hens fed or treated improperly with antibiotics may produce eggs contaminated with antibiotic residues. In an investigation conducted in Jordan, the concentration of chlortetracycline was up to 8589 μg/kg in egg white and 808 μg/kg in egg yolk (Alaboudi et al., 2013), respectively, while the highest concentration of enrofloxacin (1485 μg/kg) was detected in chicken eggs in Vietnam (Yamaguchi et al., 2017).

3.1.4.3. Vegetables and grains. Since chemical fertilizers are restricted for organic vegetables and grains, manure rich in plant nutrients is considered to be a good alternative in organic farming. However, the use of antibiotics may end up in livestock or poultry manure with a high residual concentration due to the incomplete absorption by animals. Therefore, antibiotic residues may accumulate in crops through uptake from manure-amended cropland. Concentrations of antibiotic residues in vegetables and grains in China are shown in Fig. 2**D**. High concentrations of three quinolones have been detected in vegetables from one of the largest vegetable bases in Shandong Province, China, of which the detected concentration was in the range of 18.2–658.3 μg/kg for norfloxacin, 2.0–32.3 μg/kg for enrofloxacin, and 2.5–27.5 μg/kg for ciprofloxacin, respectively (Li et al., 2014). This was significantly higher than that in crops from the Pearl River Delta in Southern China (Pan et al., 2014). Quinolones and chloramphenicol showed higher concentrations in vegetables than those of tetracycline and sulfonamides. In general, the antibiotic distribution in plant was in the order of sequence of leaf > stem > root, which was consistent with the investigation of antibiotic residues in vegetables irrigated by pharmaceutical wastewater from Lahore, Pakistan with a mean detected concentration range of 0.02–0.85 μg/kg (Hussain et al., 2016). The relatively high concentrations of antibiotic residues in vegetables and grains suggested human exposure to antibiotics through the consumption of edible crops should not be neglected.

3.1.4.4. Fish and shrimp. The use of certain antibiotics as veterinary pharmaceuticals is approved for aquaculture, such as oxytetracycline, thiamphenicol, erythromycin, sarafloxacin, and trimethoprim (Serrano, 2005). Therefore, antibiotics may enter into the aquatic products mainly by oral administration and can also be taken up from the surrounding water or sediments with comparatively lower levels. Antibiotic residues have been detected in aquatic products collected in China (He et al., 2016; Song et al., 2016), Vietnam (Uchida et al., 2016), India (Swapna et al., 2012), Italy (Chiesa et al., 2018), and Korea (Won et al., 2011) (Fig. 2**F**). Quinolone and sulfonamide antibiotics were the most detected antibiotics in fish and shrimp, with a higher concentration and detection frequency. In general, the most developed economic regions in China, Tai Lake and Pearl River Delta, showed a higher antibiotic residue contamination in fish and shrimp (mean concentration: 0.1–120.58 μg/kg fresh weight) than that of Vietnam (0.02–47.23 μg/kg fw) and Italy (055–3.59 μg/kg fw).

According to the above data summary, there is a huge biomonitoring data gap on the residual levels of antibiotics in human exposure sources in the environment, and different detected antibiotic compounds and analytical methods which hindered direct comparison of antibiotic residue concentrations among different studies. For example, bioanalytical method including ELISA (Zheng et al., 2013) and microbiological detection test kits (Aalipour et al., 2013) have been used, but most studies employed chemical analytical methods based on liquid chromatography-based mass spectrometry coupled with different detectors such as mass spectrometry, diode array detector (Salama et al., 2011), spectrofluorometric (Pena et al., 2004), and UV detector (Cheong et al., 2010). Furthermore, most previous studies only focused on < 20 antibiotic compounds, while up to 95 antibiotic compounds have been investigated in the environment or animal-derived products, 72 antibiotic compounds of which exhibited detectable concentrations (Table S1). Possible antibiotic residues in the environment may occur beyond the 72 antibiotics compounds. Therefore, monitoring a wider range of antibiotic residues using unified analytical methods are urgently needed with regard to the establishment of a standardized monitoring guide of antibiotic residues in the environment, which is the prerequisite for a more comprehensive exposure assessment of antibiotic residues in the environment.

3.1.5. Exposure assessment

Previous studies monitored 72 antibiotic residues in drinking water, airborne dust and soil particles, and food (Table S1) with the highest reported concentrations in these matrices being up to 679.7 ng/L (Wang et al., 2010), 11.0 mg/kg (Hamscher et al., 2003) and 15.1 mg/kg (Chen et al., 2015), respectively. These data strongly indicate possible human health risks via drinking water, inhalation, ingestion and dermal contact of particulate matters, and food consumption, due to the unintended intake of antibiotics. A multisite biomonitoring-based study on the measurement of 18 antibiotics in urine samples of more than 1000 children in east China showed that the antibiotics were detected in 58.3% of the urine samples (Wang et al., 2015a). Especially, the detection of antibiotic residues in the children's urine samples which were used exclusively in animal husbandry strongly indicated that food might be the primary exposure source (Wang et al., 2016a), such as chloramphenicol which has been banned in animal-derived food in western developed countries, Japan and China (Nordkvist et al., 2016). However, the current comprehensive data on human exposure to antibiotic residues in the environment is still very limited. Furthermore, human exposure assessments of antibiotic residues through inhalation and ingestion of drinking water and food have not been fully explored and comprehensively evaluated across the globe. Human daily exposure to antibiotics from drinking water has been estimated in several studies which collectively suggested a limited role of drinking water in total human exposure to antibiotics. Therefore, the most likely dominant source would be food intake (Leung et al., 2013; Simazaki et al., 2015; Wang et al., 2016a).

To our best knowledge, only a few studies have so far estimated the daily intake of antibiotic residues through food consumption. The median daily intake among general population in Korea through consumption of drinking water, and consumption of beef, pork, chicken, dairy products and fish was estimated to be 679.3, 88.8, 619.2, and 49.7 pg/kg/day for sulfamethazine, trimethoprim, enrofloxacin, and roxithromycin, respectively (Ji et al., 2010). The daily intake of a mixture of 21 antibiotics was estimated to be 0–0.0125 μg/kg/day for women and 0–0.0111 μg/kg/day for men in Shanghai, China,

respectively, based on exposure sources of only meat products derived from livestock, poultry and aquaculture rather than full consideration of all the exposure sources (Wang et al., 2017a). The estimated daily intake was about 100-fold lower than the result obtained from back-calculation based on the concentration of 21 antibiotics detected in urine, that the daily antibiotic intake of 56.4% in children and 37.4% in pregnant women was in the range of 0–1.0 μg/kg/day, while that of 22% in children and 4.1% in pregnant women was > 1.0 μg/kg/day (Wang et al., 2017b; Wang et al., 2017b, 2016a). Although the reverse dosimetry calculation method may have great uncertainty, the lower external exposure estimation in the study based on the exposure pathway of meat product consumption may greatly underestimate human daily exposure to environmental antibiotic residues due to insufficient consideration of all the exposure sources (Wang et al., 2017a). However, a different result was obtained from a similar survey conducted in Hong Kong, China that the estimated daily intake of single antibiotic using the reverse dosimetry calculation method with the antibiotic concentrations in children urine ranged from 7.7×10^{-4} (ofloxacin) to 0.045 (oxytetracycline) μg/kg/day, which was 10 times lower than that based on food consumption (Li et al., 2017). This may be due to the relatively small investigation scale of 31 urine samples in the study. It is noteworthy that most antibiotic residues in food are heat stable in cooking process, suggesting that cooking process could not completely remove antibiotic residues in food (Li et al., 2017; Manu, 2014).

Current studies on potential hazards of antibiotics on intestinal microbiome are mostly based on the short-term effects by therapeutic clinical or self-medication generally with a single type of antibiotic, which is used at a high-dose exposure level of mg/kg/day. Compared with the exposure of antibiotic targeting specific pathogens during disease treatment, exposure to antibiotic residues in the environment presented a different exposure mode: long-term exposure to a mixture of antibiotics with a high proportion of broad-spectrum veterinary antibiotics used in animal husbandry , at a low-dose exposure level of μg/kg/day. The detected trace amounts of antibiotic residues in the environment may pose a low risk when considered individually. However, chronic exposure to the mixture of hundreds of residual antibiotics may become a more powerful stressor on the selection of antibiotic-resistant bacteria than the antibiotics used in therapeutic purposes, similar to the difference between the toxicity processes of long-term exposure to low dose and short-term exposure to high dose. This may pose a high potential risk to human by the ARB infection associated with human microbiome. Further research is needed to assess the overall human exposure to antibiotic residues in the environment, especially through food consumption, and to clarify the exposure characteristics and the main exposure pathways.

Gastrointestinal absorption of orally administered antibiotics mainly in the range of 30–90% derived from pharmacokinetics experiments with high oral doses used for therapy (MacGregor and Graziani, 1997). Few studies reported absorption of antibiotics intake through inhalation, ingestion of drinking water and food consumption, and the corresponding gut bacteria metabolic response. This is due to the technical challenge to analyze metabolites of the re-ingested antibiotics at the environmental level in the highly heterogeneous gastrointestinal system. Exposure of different single gut bacteria species to xenobiotic mixtures including antibiotics at human relevant levels, demonstrated that the gut bacteria metabolism could be significantly perturbed in a species-specific manner (Zhang et al., 2018). The absorption and metabolism of re-ingested antibiotics should be further investigated due to the importance of their role in selection and maintenance of ARB and ARGs within human body when taking total concentrations of active antibiotics into account.

3.2. Antibiotic resistance in the environment and human exposure assessment

ARB and ARGs have been suggested as emerging environmental contaminants due to the undesirable increased prevalence impacted by human activities and the potential health threats induced by transmission from environment to human (Pruden et al., 2006). The highest ARB and ARGs burden sources are sewage water discharged from home and hospitals, antibiotic manufacturing facilities, and animal feedlots (Vikesland et al., 2017). There is a growing interest in the dissemination of antibiotic resistance in the environment, which has been reviewed (Qiao et al., 2017).

3.2.1. ARB

Based on culture-dependent methods, ARB have been reported in water, soil and atmosphere related to antibiotic contaminated hotspots, with high microbial density, nutrient content and antibiotic concentration. This would enhance the selection and propagation of ARB and ARGs, especially from wastewater treatment plants, livestock farms and hospitals (Qiao et al., 2017). For example, based on the antimicrobial susceptibility test, it was indicated that about more than 50% *Staphylococcus* spp. isolated from the atmosphere of a sewage treatment plant in Poland exhibited resistance to penicillin, with 20% to erythromycin, and 11% to rifampicin, 6% to tetracycline, 3% to gentamicin, 3% to cefoxitin, and 1% to levofloxacin, but totally susceptible to chloramphenicol (Małecka-Adamowicz et al., 2016). 19–75% of the *Enterococcus* spp. and 13–94% of the *Streptococcus* spp. recovered from the indoor air of a large-scale swine-feeding operation contained macrolide, lincosamide, streptogramin, or tetracycline resistant genes (Sapkota et al., 2006). The percentages of *Enterobacteriaceae* (*Aeromonas*, *Enterobacter*, *Escherichia* or *Shigella*, and *Klebsiella*) isolated from the effluents of a municipal wastewater treatment plant in China, resistant to chloramphenicol, penicillin, cephalothin, ampicillin, rifampicin, and tetracycline were 69%, 63%, 55%, 47%, 11% and 2.6%, respectively (Huang et al., 2012). Due to accessible isolation from different settings, *Escherichia coli* (*E. coli*) has also been widely used to indicate bacteria resistance. *E. coli* isolates from chicken, swine and cattle in animal farms in Shandong Province, China presented resistance to 12, 10 and 1 antimicrobial agents with a percentage of 51.61%, 25%, and 30%, respectively (Lu et al., 2010). Almost 50% of *E. coli* isolates had resistance to nine antibiotics from surface water of Wenyu River Basin in Beijing, China (Hu et al., 2008), and the resistant percentages to multiple classes of antibiotics ranged from 64% to 98% of isolates from manure waste discharged from livestock and poultry farms in Beijing and Hebei Province, China (Yang et al., 2004). The high prevalence of antibiotic resistance among bacteria especially pathogenic bacteria indicates a tremendous potential threat on human health.

3.2.2. ARGs

The different phenotyping of antibiotic-resistant bacteria may arise from different genes conferring resistance to different antibiotics. Genotyping profile of bacteria, namely ARGs, is a more sensitive indicator that facilitates the understanding of the mechanism of antibiotic selection for ARB, and the action mechanism of antibiotic resistance. Based on culture-independent method, thousands of ARGs in the environment could be identified and monitored by quantitative polymerase chain reaction (qPCR) and shotgun sequencing methods (Vikesland et al., 2017). Tetracycline (*tet*) and sulfonamide (*sul*) resistance genes are the most reported in effluent and sludge from wastewater treatments, surface water and sediments, livestock manure and manure-amended soils, which were considered to be commonly widespread in the environment due to the extensive use of the corresponding antibiotics. In addition, more recent attention has been paid to the occurrence of other ARGs, including macrolide resistance genes (*erm*), quinolone resistance genes (*qnr*), and beta-lactams (*bla*) (Qiao et al.,

2017). In the present study, by consulting the relevant literatures, we summarized the detectable ARGs in the environment which add up to 56 genes including 3 MGEs (*intI*1, *intI*2, and Tn916/1545) with an abundance range of ND to 6.7×10^{10} (Zhang et al., 2009) copies/mL in surface water, 7.4–9.7×10^{10} (Luo et al., 2010) copies/g in sediments, ND to 6.6×10^{11} (Cheng et al., 2016) copies/g in manure, 3–7.9×10^{2} (Xie et al., 2018) copies/m^3 in air, ND to 1.4×10^{11} (Rahman et al., 2018) copies/g in soil, and ND to 7.8×10^{10} (Li et al., 2016) copies/mL in sewage and ND to 7.6×10^{11} (Wang et al., 2015b) copies/g in residues from sewage treatment plants. The detail information can be found in Table S2. However, these studies may be limited to the qPCR method of tracking antibiotic resistance, which is generally only able to examine a few ARGs at a time. It has been demonstrated that high-throughput sequencing-based metagenomic analysis could profile the full complement of DNA without a preselection of ARGs and yield a significantly higher diversity of ARGs than that by qPCR alone (Vikesland et al., 2017). However, qPCR arrays have been improved to simultaneously track up to 285 ARGs in the environment to overcome such weakness (Zhu et al., 2013). Similarly, as with antibiotic residue monitoring, different detected ARGs and especially the interpretation of data methods which based on total ARGs abundance, relative abundance of total ARGs, or diversity of ARGs hinders the direct comparison of ARGs levels in the environment among different studies. Furthermore, the ARGs database are still incomplete which can impact both qPCR and high-throughput sequencing approaches as they are intended to look for previously discovered genes. Therefore, further supplements and improvements of the ARGs database are still needed for a more comprehensive exposure assessment of antibiotic residues in the environment.

Although a significant acceleration of selection and expansion of antibiotic resistance caused by antibiotic residues in the environment has been regarded as a common occurrence (Vikesland et al., 2017), the relationship between ARGs and their corresponding antibiotic concentrations or a different antibiotic class was found to be inconsistent. There are different results of strong, weak, or no correlation generated from different studies (Qiao et al., 2017), which may be due to different environmental fates related to ARGs and antibiotics themselves in different environmental settings (Wang et al., 2016b). It is noteworthy that horizontal co-transfer of ARGs and MGEs (Sun et al., 2016), and co-selection for resistance to unexposed antibiotic classes have been observed through metagenomic analysis (Lundström et al., 2016; Murray et al., 2018). The cross-resistance phenomenon is likely to be due to the transferable multi-resistance genes, indicating that HGT may partly contribute to the lack of direct correlation. MGEs are strongly associated with HGT, which could take along ARGs to new hosts to generate new resistant strains. It has been suggested that class 1 integron (*intI*1) consists a good indicator of antibiotic resistance associated with human pollution, due to the positive correlations with ARGs and human pollution (Gillings et al., 2015). With respect to this hypothesis, we integrated 72 sets of ARGs data all of which included 13 groups of genes: 7 *tet* genes (*tetM*, *tetO*, *tetQ*, *tetW*, *tetA*, *tetB*, *tetX*), 3 *sul* genes (*sul*1, *sul*2, *sul*3), 2 *erm* genes (*ermB*, *ermC*), and *intI*1 gene. Correlation analysis among the 13 group of ARGs was conducted based on SPSS (version 25.0), and the results show that *intI*1 is significantly correlated with the other 12 resistant genes (Pearson's correlation coefficients: 0.382–0.926, $p < 0.05$).

3.2.3. Exposure assessment

There is a lack of information on human exposure to ARB or ARGs in the environment. Attempts have been made to estimate the daily intake of *erm*B, *tet*W, *qnrS* and *intI*1 via the inhalation of fine Particulate Matter ($PM_{2.5}$), drinking water and ingestion of soil particle (Xie et al., 2018). Results showed similar daily intakes of *erm*B and *intI*1 by the three exposure pathways, but intake of *tet*W through drinking water or ingestion of soil particle was at least 2 fold higher than that by inhalation of $PM_{2.5}$ (Xie et al., 2018). However, this calculation did not take into account the bioavailability of ARGs, nor other potential exposure routes such as contacts with water, farm animals, etc. Given the human consumption patterns, ARB or ARGs may be killed or thermal degraded via ingestion of drinking water and food, inhalation and dermal contact with particulate matters (soil, dust and airborne particles) may be therefore the main pathway in human intake of ARB or ARGs. Further studies are urgently needed to consider human exposure to ARB and ARGs.

4. Dose-response assessment: what is the relationship between the antibiotic concentration and the probability of the emergence of antibiotic resistance?

Bacterial responses to antibiotics are concentration-dependent with diverse biological responses in bacteria at different concentrations as a longstanding assumption (Gullberg et al., 2011; Bernier and Surette, 2013; Sandegren, 2014) (Fig. S1). The high concentrations of antibiotics would completely inhibit growth of the susceptible bacteria, exhibiting antimicrobial activities. When the concentrations are lower than the inhibitory concentrations, bacteria may trigger different cellular responses or alter gene expression as well as induce gene mutation and HGT, leading to antibiotic resistance. Dose-response assessment of the relationship between the evolution and emergence of antibiotic resistance and the antibiotic concentration requires a metric to indicate the potential of antibiotic concentrations to promote the development of ARB in complex bacterial communities.

4.1. MICs

The Minimum Inhibitory concentration (MIC), based on bacteria lethality is used as the common metric of bacteria population resistance. *Acinetobacter*, *Burkholderia*, *Stenotrophomonas*, *Neisseria*, *Enterococcus*, *Staphylococcus*, *Streptococcus*, and *Haemophilus* have been suggested as bacterial indicators to assess MICs of antibiotics in clinical settings (Patel, 2017; Reller et al., 2009). MICs are generally based on the inhibition of bacterial growth rate at different gradient antibiotic concentrations and are measured by broth dilution test or disk diffusion test (Reller et al., 2009) (Fig. S1). The broth dilution test is to inoculate standard bacteria suspensions in different liquid media containing a gradient of antibiotic concentrations at 35 °C overnight, and MIC is the minimum concentration that can inhibit bacteria growth rate which is determined by visual observation of the turbidity of each medium. Disk diffusion test is to place disks containing different antibiotic concentrations on the surface of solid agar medium inoculated with a standard bacteria suspension at 35 °C overnight. The zone diameters of growth inhibition around each of the antibiotic disks are measured and interpreted according to the criteria published by the Clinical and Laboratory Standards Institute (CLSI) (Patel, 2017) or the US Food and Drug Administration (FDA) (Reller et al., 2009). It is a qualitative method to derive susceptibility classification as susceptible, intermediate, or resistant relative to an MIC value (Reller et al., 2009). The EUCAST database (European Committee on Antimicrobial Susceptibility Testing) has published the data of MICs for more than 100 antibiotics/antibiotics combinations and for clinically relevant bacteria species (Bengtsson-Palme and Larsson, 2016; EUCAST, 2018). In addition, based on the MICs for 111 antibiotics from the public EUCAST database, predict no effect concentrations (PNECs) for resistant selection were assessed with an assessment factor of 10 (Bengtsson-Palme and Larsson, 2016), corresponding to the median MIC/MSC (Minimal Selective Concentration) ratio estimated by Gullberg et al. (2011).

4.2. MSCs

The use of MICs is more relevant for clinical breakpoints of therapeutic process, but it is inadequate to assess bacterial antibiotic susceptibility and resistance evolution with the low levels of antibiotics.

MSC of bacteria is defined as the concentration below MIC that favor the sensitive variant and would not compensate for growth impairment of the resistance mutation (Kraupner et al., 2018). MSC is a more sensitive metric to reflect the selective pressures at lower antibiotic concentrations than MICs (Sandegren, 2014). It has been proposed to obtain MSC in a two-strain competition system including susceptible and resistant genotypes as the lowest antibiotic concentration that could give a more competitive advantage of growth rate to the resistant strains than the susceptible strains (Fig. S1). Although MSC could better reflect enrichment possibility of resistant bacteria where low concentrations of antibiotics are present, the MSCs obtained from competition experiments are highly depended on the selected susceptible and resistant strains, the latter of which is generally the susceptible mutant carrying specific resistant-genes based on genetic modification technology (Sandegren, 2014).

What needs to be firstly emphasized is that the composition and structure of bacterial community differ greatly in environmental and clinical settings. In addition, antibiotic residue concentrations are highly variable in different environmental media and human body in which bacteria could evolve different mechanisms to respond accordingly. This strongly implies that the relationship between the antibiotic concentration and the probability of the emergence of antibiotic resistance may be different in different settings. Therefore, the reliability of the MSCs measured according to two closely related strains is not likely to be directly applicable when extended to more complex microbial communities.

Aware of the limitation of MSCs derived from single-species competition experiments, selective concentrations of antibiotics in complex communities have been assessed in recent studies, including those of tetracycline (≤ 1 μg/L) in complex aquatic bacteria biofilm (Lundström et al., 2016), ciprofloxacin (1 μg/L) in *E.coli* in complex aquatic bacteria biofilms (Kraupner et al., 2018), and cefotaxime (0.4 μg/L) in sewage-derived bacteria communities (Murray et al., 2018). It is worth noting that Murray et al. (2018) observed a selection plateau as antibiotic concentration increased over a 2-order-magnitude range. This indicated that bacterial selection responses to antibiotics may not occur in a concentration-dependent manner in complex bacteria communities, presumably due to a cross protective effect that the resistant bacteria in the community may break down the extracellular antibiotics to the benefit of the nearby susceptible bacteria.

4.3. Determination method of MSCs

However, MICs are generally based on the function of growth rate of a single clinical isolate, which is actually not sensitive and representative enough to reflect the evolution of bacteria antibiotic resistance. In contrast with MICs, MSCs have been assessed using both phenotypic endpoints (culture-based method), taxonomic endpoints (culture-based method), and genotypic endpoints (metagenomic analysis and qPCR method) (Lundström et al., 2016; Kraupner et al., 2018; Murray et al., 2018). Genetic resistance markers have been demonstrated to be more sensitive than phenotypic endpoints (Lundström et al., 2016). Several approaches have been proposed to define the genetic resistance of antibiotic, including total ARG abundance, relative abundance of total ARGs, diversity of ARGs, and specific ARGs of interest. It is essential to emphasize that in a health risk assessment framework, link-bridges should be established to enable direct comparison and conversion of antibiotic resistance in different environmental compartments, which is also further relevant to human exposure and subsequent infections. With respect to health risk assessment, relative abundance normalized to bacterial 16S rRNA genes (type of pathogens) or pathogenic bacteria number (infection dose of pathogens) is recommended due to the correlation with human infection by antibiotic resistant pathogens. Further specific ARG encoding to last resort antibiotics (e.g, carbapenems, vancomycin, colistin, etc) normalized to bacterial 16S rRNA genes or pathogenic bacteria number is also of great concern due to the correlation with treatment failure of antibiotic resistant pathogen infection. Furthermore, HGT is the core challenge of assessing the spread of antibiotic resistance in the environment. MGEs are of the greatest concern due to the association with HGT, which are recommended to be included in the target genes in the response assessment. High levels of co-selection were found in selection for antibiotic resistance in complex microbial communities exposed to a single antibiotic (Lundström et al., 2016; Kraupner et al., 2018; Murray et al., 2018), indicating the presence of multi-resistance plasmids which may colonize multiple genes and could confer resistance to a wide range of different antibiotics (Gullberg et al., 2014).

4.4. Dose-response assessment in different compartments

Selection of antibiotic resistance at very low concentrations indicates a high potential selection of antibiotic resistant bacteria in or on human bodies and the external environment. To obtain a representative set of species or a prevailing species in different settings relevant for risk assessment is challenging due to the difficulty in cultivating the vast majority of environmental and human bacteria. Therefore, holistic parameters of antibiotic resistance evolution are needed, to show the emergence and dynamics of ARB and ARGs in different environmental and human compartments as condition changes such as pH value, temperature, oxygen level, and oxidation reduction potential which may be related to the spread of pathogenic resistance. In our opinion, holistic parameters should include three factors: antibiotic resistance definition, representative bacteria genotypes, and living conditions.

The use of either different representative bacteria structure in different settings or the same bacteria indicator is a feasible choice. It has been suggested that *E. coli* may be a good indicator of resistance since it is ubiquitous in the environment and serves as a representative of many pathogens. The reliability of *E. coli* representing the emergence and dynamic of antibiotic resistance evolution should be critically validated in different settings as different living conditions may impact the antimicrobial activities of antibiotics and the evolution of bacterial antibiotic resistance in different environmental or human compartments. This could be achieved by setting up different simulation systems as close as possible to imitate the living conditions, including pH value, temperature, oxygen level, oxidation reduction potential, etc. If the relationships between antibiotic concentrations and ARG abundance in different settings have been observed, further relationships between ARGs and ARB should be established in order to identify pathogens since that the risk of antibiotic resistance to humans must be assessed based on ARB rather than ARGs. Thus, in the conceptual model proposed to assess the health risk of antibiotic resistance associated with environmental antibiotic residues, pathogenic ARB would be the end-of-the pathway for environmental antibiotic resistance transmission to humans (Manaia, 2017).

These recommendations attempt to sharpen the focus of antibiotic resistance in the environment to derive the relationship between antibiotic levels and antibiotic-resistance development and suggest a standardized method of resistance testing, taking into account of antibiotic resistance definition, representative bacteria genotypes, and living conditions, which could be directly applicable in different environmental and human settings. This is a pre-requisite for obtaining a global view of antibiotic resistance dynamic evolution impacted by antibiotic residues in environmental and human compartments, and further evaluating the health risk of antibiotic resistance associated with antibiotic residues in the environment.

5. Risk characteristic: how dangerous are the adverse effects of human exposure to antibiotic resistance associated with environmental antibiotic residues?

The overall goal of health risk assessment of exposure to antibiotic resistance is to estimate the number of infection diseases caused by

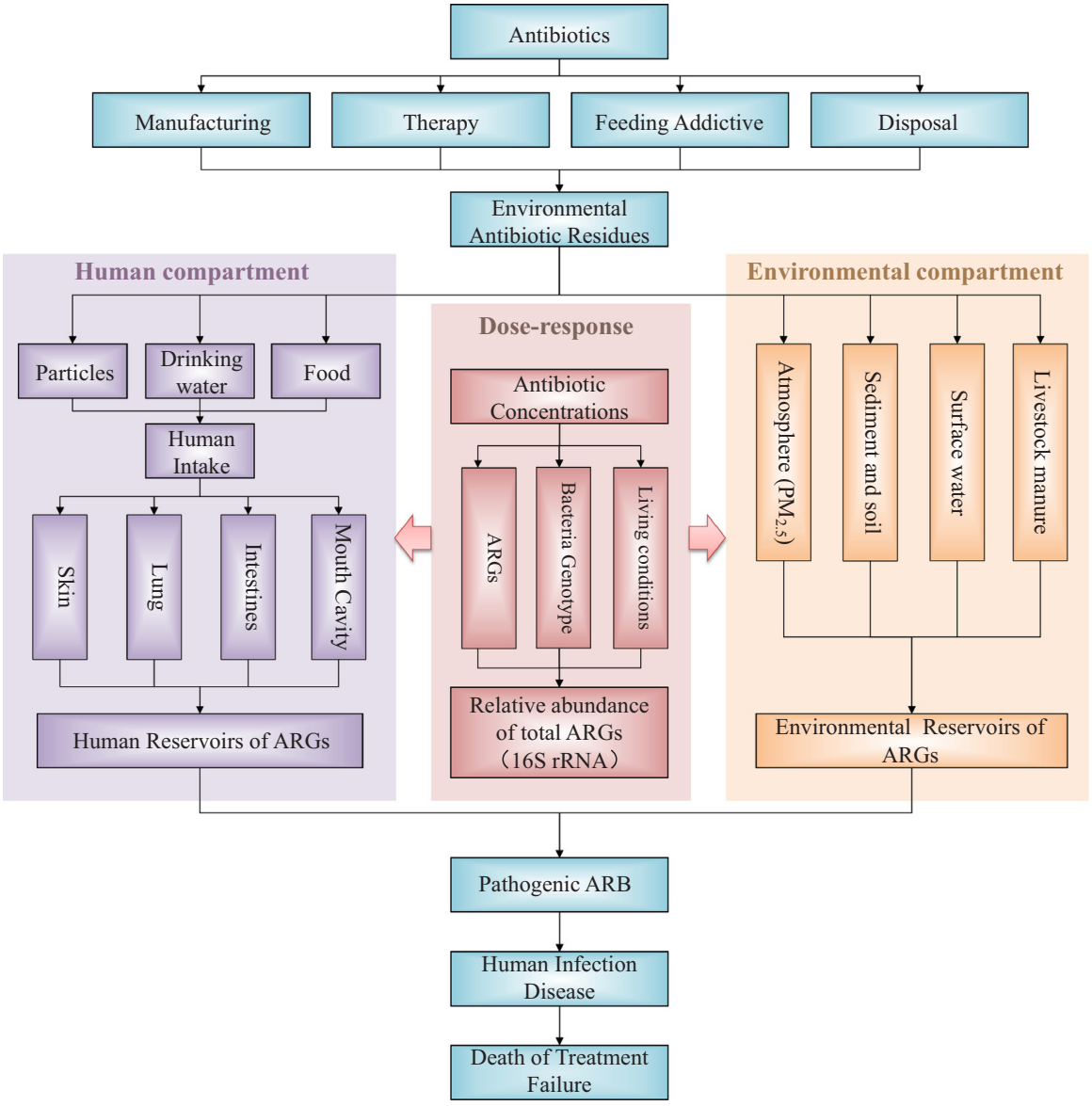

Fig. 3. Conceptual framework of human health risk assessments of antibiotic resistance associated with antibiotic residues in the environment.

antibiotic-resistant bacteria driven by environmental antibiotic residues and the number of deaths resulting from those infections. Pathogenic ARB are the most crucial component for assessing risks, the emergence probability of which could be predicted on account of antibiotic concentrations in human and environmental compartments, based on the presumptive quantitative relationship between antibiotic concentration and ARB development in different settings. Further estimation of pathogen risks also requires dose-response assessments that quantify the relationship between pathogen dose and the likelihood of infection. This would require the capacity of pathogenic ARB to infect human body at species or even strains level to characterize the exposure risk, which could be designated as the number of bacterial cells that are required to infect a host (Leggett et al., 2012). The pathogen-specific estimates of morbidity and mortality from antibiotic resistant infections have been suggested for each pathogenic ARB, according to the surveys of healthcare-associated infections and deaths in hospitals (Roberts et al., 2009). Therefore, on the combination with the probability of emergence of pathogenic ARB and the antibiotic resistant pathogen-specific likelihood of infection and death, the probability of infection and death driven by antibiotic residues in the environment would be derived.

In the present study, a coherent conceptualized model is suggested to undertake human health risk assessment of antibiotic resistance in the environment (Fig. 3). The model established is to assess the risks of infection and deaths arising from human exposure to antibiotic resistance driven by environmental antibiotic residues. It takes into account of compartment-dependent aspects of antibiotic concentration-dynamic resistance response on the basis of experimental and modeling approaches. However, given much of the data required to work on the model is severely limited, it would be very important to conduct extensive research to fill these data gaps. As progress is made, the assessment model would be very helpful to form targeted policies in monitoring of antibiotic residues in the environment and mitigating the dissemination of environmental sources of antibiotic resistance. This would also provide a theoretical basis for reducing health risks of human exposure to antibiotic resistance in the environment.

Acknowledgements

Financial support from the National Natural Science Foundation of China (grant 41330632) and the leading talents of Guangdong Province program is gratefully acknowledged.

Competing financial interests

The authors declare they have no actual or potential competing financial interests.

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.envres.2018.11.040.

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