The prevalence and antimicrobial resistance phenotypes of *Salmonella*, *Escherichia coli* and *Enterococcus* sp. in surface water

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**Significance and Impact of the Study:** Surface water is prone to bacterial contamination as it receives wastes and animal sources, and contaminated water may expose local populations to health risks. Studies have demonstrated the widespread distribution of pathogenic and antimicrobial resistant bacteria in surface waters of both developing and developed countries, confirming the importance of environmental waters as a reservoir for these bacteria and the need for more attention on the environmental bacteria for emerging antimicrobial resistance. This review discusses the prevalence and diversity of pathogenic and commensal bacteria, namely *Salmonella*, *E. coli*, and *Enterococcus*, present in surface waters and provides a basic understanding of the occurrence and persistence of antimicrobial resistance in these bacteria.

**Keywords**
- antimicrobial resistance
- *Escherichia coli*
- *Enterococcus*
- environmental water
- prevalence
- *Salmonella*
- virulence

**Abstract**

Surface water is prone to bacterial contamination as it receives wastes and pollutants from human and animal sources, and contaminated water may expose local populations to health risks. This review provides a brief overview on the prevalence and antimicrobial resistance (AR) phenotypes of *Salmonella*, *Escherichia coli* and *Enterococcus*, found in natural freshwaters. These bacteria are frequently detected in surface waters, sometimes as etiological agents of waterborne infections, and AR strains are not uncommonly identified in both developed and developing countries. Data relating to *Salmonella*, *E. coli* and *Enterococcus* present in environmental water are lacking, and in order to understand their development and dissemination using the One Health approach, understanding the prevalence, distribution and characteristics of the bacteria present in surface water as well as their potential sources is important. Furthermore, AR bacteria in natural watersheds are not well investigated and their impacts on human health and food safety are not well understood. As surface water is a receptacle for AR bacteria from human and animal sources and a vehicle for their dissemination, this is a crucial data gap in understanding AR and minimizing its spread. For this review, *Salmonella*, *E. coli* and *Enterococcus* were chosen to evaluate the presence of primary pathogens and opportunistic pathogens as well as to monitor AR trends in the environmental water. Studies around the world have demonstrated the widespread distribution of pathogenic and AR bacteria in surface waters of both developing and developed countries, confirming the importance of environmental waters as a reservoir for these bacteria and the need for more attention on the environmental bacteria for emerging AR.
Introduction

Surface water and waterborne outbreaks

Surface waters are constantly influenced by human activities as they are used for recreational activities, such as swimming, kayaking, tubing, surfing and fishing, and serve as a receptacle for wastes and wastewater from surrounding wastewater treatment plants, septic systems and industrial facilities. Contaminations by animal wastes can also occur due to runoff from animal farms, wildlife and parks with domestic pets. Since bacterial pathogens present in the gastrointestinal tract of humans and other warm-blooded animals are shed into environmental water, ingestion or contact with contaminated water can lead to infections with primarily gastrointestinal symptoms by the faecal–oral route, and also with respiratory, eye, ear and skin symptoms (DeFlorio-Barker et al. 2018). Faecal contamination from various human and animal sources can pose health risks to local populations exposed to environmental water through municipal, agricultural and recreational uses; hence, to reduce the public health risk, monitoring the water for any microbial pollutants is essential.

Waterborne diseases still remain the leading cause of morbidity and mortality worldwide, causing about 2.2 million deaths per year (WHO and UNICEF 2000). In developed countries, improved sanitation and water quality have reduced the number of waterborne infections and the severity of their impacts as compared to developing countries, with some of the fatal waterborne infections such as cholera and typhoid fever considerably reduced in number (WHO and UNICEF 2000; Craun et al. 2006; Cabral 2010). However, water-associated outbreaks still occur in developed countries, suggesting the need for attention to water quality (Benedict et al. 2017; Graciaa et al. 2018; Hlavsa et al. 2018). Outbreaks of bacterial diseases associated with contaminated surface water have been frequently reported in North America, responsible for both public health and economic burdens (Olsen et al. 2002; Hrudey et al. 2003; Beach 2004). During 2000–2014, 140 outbreaks and 4 958 illness cases associated with untreated recreational water, such as lakes and oceans, were reported in the United States alone, and a third of the outbreaks with known aetiology were attributed to bacteria such as Shigella, Escherichia coli and Leptospira (Graciaa et al. 2018). While Graciaa et al. reported only the identified outbreaks and illnesses, a study conducted by DeFlorio-Barker et al. estimated approximately 90 million illness cases, including both sporadic and outbreak cases of all levels of severity, that are associated with surface water recreational activities, such as swimming, kayaking, rowing, canoeing, motor boating and fishing, annually in the United States (DeFlorio-Barker et al. 2018; Graciaa et al. 2018). This translated into an economic burden of 2.9 billion US dollars, resulting from healthcare provider visit, medication, hospitalization, sequelae, mortality and lost productivity (DeFlorio-Barker et al. 2018).

Antimicrobial resistance and the one health approach

Antimicrobials have had a significant impact on treating and controlling bacterial infectious diseases, reducing the burden of illnesses since the 20th century (Aminov 2010). Since the introduction of the first antimicrobial agent approximately seven decades ago, antimicrobials have been widely used in both humans and animals for therapeutic use as well as at sub-therapeutic levels in animals for promoting growth, improving feed efficiency and preventing diseases (Aarestrup and Wegener 1999; Aminov 2010). However, resistance to antimicrobials was detected soon after their introduction to the public, and the over- and misuse of the drugs have led to a rapid increase in antimicrobial resistance (AR) (Alanis 2005). Resistance to therapeutic antimicrobials is a public health concern as it renders an antimicrobial ineffective whenever treatment is needed and potentially results in treatment failure (Levy and Marshall 2004). The World Health Organization (WHO) has provided a list of critically important antimicrobials in humans and recommended the prudent use of the drugs to prevent the development and dissemination of resistance in pathogenic bacteria, the consequence of which can be severe for human health (WHO 2000; WHO 2019). The Centers for Disease Control and Prevention (CDC) estimates about 2.9 million infections and 35 900 deaths each year due to AR infections in the United States alone (CDC 2019). This is a more serious problem in developing countries where antimicrobials are available without a prescription and their distributions are not effectively regulated (Alanis 2005). If AR were to continue at the same rate with the same trend as now, infections due to AR bacteria are estimated to cost the world 300 million premature deaths and 100 trillion US dollars by 2050 (Resistance 2014). The emergence of bacteria resistant to medically important antimicrobials due to selective pressure created by sub-therapeutic usage in animals has contributed to the transfer of AR bacteria to humans, and in response, the sub-therapeutic use of antimicrobials for growth promotion and disease prevention has been banned or controlled in multiple countries (FDA 2012; Maron et al. 2013; Holmes et al. 2016).

Antimicrobial resistance has become a threat to the public health worldwide, and the development of effective strategies to combat AR is a global task that needs to be addressed by governments and the public around the
world. The World Health Organization (WHO), along with the Food and Agriculture Organization of the United Nations (FAO) and the World Organization for Animal Health (OIE), has claimed AR as one of the three priority public health threats that needs to be addressed with the help of global collaboration (WHO 2014). To address this challenge and elucidate the emergence, dissemination and fate of AR, the One Health approach has been implemented. One Health approach is defined as collaborative and transdisciplinary efforts working at the local, national and global levels to achieve optimal health for humans, animals and the environment (AVMA 2008). One Health concept, which recognizes that the health of animals, people and the environment is connected, is not a new concept but there has been a rapidly growing interest in One Health approach to better understand and address the worldwide challenge of AR (Robinson et al. 2016).

For the improvement of the overall health of humans, animals and the environment, a concerted effort and cooperation among the three sectors is needed. However, previous studies have mainly been focused on AR in pathogenic bacteria in clinical settings or in wastewater, and AR bacteria and their AR genes in the environment are not well described. As aquatic environment is a hot-spot for the development of AR bacteria, it is important to enhance our limited knowledge on the increasing AR of bacteria in the environment (Martinez 2009). Surface water receives pathogenic and non-pathogenic bacteria harbouring AR genes from human and animal wastes as well as antibiotic residues from healthcare, agricultural farms and industries that can serve as selective pressure. Aquatic environments can provide the opportunity for genetic materials, such as AR genes, to be exchanged between bacteria, and the transfer of AR genes to pathogenic bacteria is particularly a concern (Kenzaka et al. 2010; Walsh et al. 2011; Karkman et al. 2018). Such AR bacteria can then be transferred back to human population through exposure to contaminated water through drinking water, irrigation systems or recreational activities. To understand their impact on human health and food safety, AR bacteria in water and their environmental transmission routes to humans need to be assessed.

For this review article, *Salmonella*, *E. coli* and *Enterococcus* were chosen to evaluate the presence of primary pathogens and opportunistic pathogens in natural freshwaters. *Salmonella* has impact on human health as a pathogen, while *E. coli* and *Enterococcus* are not only indicators of faecal contamination of water but are also indicators of potential presence of pathogens. Moreover, *Salmonella*, *E. coli* and *Enterococcus* are commonly used as sentinel organisms for monitoring trends in resistance to antimicrobial agents with activity against Gram-negative as well as Gram-positive bacteria (WHO 2013). Since 1996, the National Antimicrobial Resistance Monitoring System (NARMS) has been monitoring the AR of selected bacteria, including *Salmonella*, *E. coli* and *Enterococcus*, isolated from humans, retail meats and food animals in the United States (U.S.FDA 2013a, 2013b). However, as a part of their One Health goal, NARMS may add an environmental component to their programme to monitor AR in surface water as well (personal communication). As such, a review of the presence of *Salmonella*, *E. coli* and *Enterococcus* in environmental water and their AR is warranted. AR in these three groups of bacteria are public health threats; extended spectrum beta (β) lactamase-producing *Enterobacteriaceae* (ESBL) and vancomycin-resistant *Enterococcus* (VRE) have been categorized as serious threats by the CDC while carbapenemase-resistant Enterobacteriaceae (CRE) has been categorized as an urgent threat (CDC 2019). This work serves to characterize populations of *Salmonella*, *E. coli* and *Enterococcus* present in the environmental water and their AR and fill the key data gap in understanding AR and minimizing its spread.

Salmonella

*Salmonella* as a pathogen

*Salmonella* is an enteric pathogen but is also ubiquitous and can be found in a wide variety of hosts including companion animals, farm animals and wild animals, as well as plants and insects (Brooks et al. 2001; Guo et al. 2002; Natvig et al. 2002; Holt et al. 2007; Dolejska et al. 2009; Mettee Zarecki et al. 2013; Bartholomew et al. 2014; Basler et al. 2016; Bosch et al. 2016). *Salmonella* has been traditionally considered as a foodborne bacteria that is transmitted through contaminated meat and animal products, but *Salmonella* infections through other transmission routes, such as contaminated fresh produce, contact with infected animals and contaminated water and soil, are commonly reported as well (Ailes et al. 2013; Jackson et al. 2013; Marus et al. 2019). *Salmonella* usually causes self-limiting gastroenteritis, and it is one of the leading causes of foodborne illnesses in developed countries; however, it can also be the etiological agent of invasive systemic diseases, for example, typhoid and paratyphoid fever which are rare in the United States but persistent in less developed countries (Lavigne and Blanc-Potard 2008; Levantesi et al. 2012; Chatham-Stephens et al. 2019). Most of *Salmonella* infections result in gastroenteritis that does not require antimicrobial treatments; however, antimicrobial therapy is required for the very young, the very old and immunocompromised patients who may develop extraintestinal infections, such as enteric fever, to prevent further complications (Varma et al. 2005). Infections caused by AR *Salmonella* limit treatment options and can be more severe than infections caused by
susceptible bacteria, increasing the rates of hospitalization, morbidity and mortality as well as the cost of treatment (Lee et al. 1994; Helms et al. 2002; Travers and Barza 2002; Varma et al. 2005; Foley and Lynne 2008). In the United States, 212,500 human infections associated with AR Salmonella are estimated with 70 deaths each year and the numbers are increasing (Lee et al. 1994; CDC 2018a; CDC 2019).

Salmonella in surface water

Life-threatening typhoid and paratyphoid fevers, caused by Salmonella enterica subsp. enterica serovar Typhi (S. Typhi) and Salmonella enterica subsp. enterica serovar Paratyphi (S. Paratyphi), respectively, still remain a major public health concern in developing countries, especially south-central and south-east Asia; and contamination of drinking water has been attributed as the main source of epidemic and endemic outbreaks (Crump et al. 2004; Bhan et al. 2005; Crump et al. 2015; Chatham-Stephens et al. 2019). While typhoidal Salmonella serotypes are involved in waterborne diseases in developing countries, non-typhoidal Salmonella (NTS) serotypes are mostly associated with animal products and increasingly with fresh produce in developed countries (Crump et al. 2015). Salmonella were traditionally considered zoonotic bacteria originating from animals; but recently, environments have been recognized as sources of Salmonella. Contaminated water and fresh produces irrigated with water contaminated with Salmonella from human and animal sources have been suggested to be sources of Salmonella infections (Schuster et al. 2005; Denno et al. 2009; Hanning et al. 2009; Clarkson et al. 2010; Craun et al. 2010; Dewey-Mattia et al. 2018). Salmonella were responsible for several waterborne outbreaks in the United States and Canada; source waters (e.g. groundwater and private wells), distribution systems (e.g. community water systems) and recreational water (e.g. fresh and salt waters) that had been contaminated by animal and human wastes were associated with these Salmonella waterborne outbreaks (Schuster et al. 2005; Denno et al. 2009; Clarkson et al. 2010; Craun et al. 2010). Furthermore, contaminated water has been indicated as the source of Salmonella outbreaks that involved fresh produce, including cucumbers, melons, tomatoes, lettuce, mangoes and sprouts (Hanning et al. 2009; Herman et al. 2015; Dewey-Mattia et al. 2018). In many cases, outbreak strains were isolated from waters used for irrigation and processing of the fresh fruits and vegetables (Hanning et al. 2009; Herman et al. 2015).

Recent studies show that Salmonella are frequently detected in surface water from different countries around the world (Lemarchand and Lebaron 2003; Meinersmann et al. 2008; Till et al. 2008; Jyoti et al. 2010; Schriewer et al. 2010; Jokinen et al. 2015). Their widespread occurrence in aquatic environments suggests that water may play a direct or indirect role in the transmission of Salmonella serving either as a vector or a reservoir for the bacteria. The potential contribution of surface water in Salmonella transmission to humans is a public health concern that needs to be addressed to reduce the burden of Salmonella infections in humans. Geographical and seasonal trends in Salmonella infections are apparent, such as increased cases of infections in the Southeastern states of the US and during summer months (CDC 2017, 2018b). Interestingly, studies conducted in the Southeastern US have shown that Salmonella are prevalent in surface water with significantly higher prevalence in summer (Haley et al. 2009; Li et al. 2014). The occurrence and persistence of Salmonella may be associated with seasonal changes in environmental conditions; for example, Salmonella detection levels in these watersheds have been shown to be correlated with both precipitation and temperature (Haley et al. 2009; Li et al. 2014). Seasonal and spatial patterns in the prevalence of Salmonella in the aquatic environment that coincide with the rate of cases of Salmonella infections supports the hypothesis that the environmental water plays a role in the transmission of human infections either directly as a vector or indirectly as a reservoir for Salmonella. However, the relationship between Salmonella occurrence in aquatic water and environmental factors is complex with numerous factors to consider. While studies in the Southeastern US suggested higher Salmonella prevalence in summer months in general, studies carried out in other parts of the world have shown varied seasonal peaks. The prevalence was highest during spring in Canada and during winter in New Zealand, Mexico and Czech Republic while others did not detect any significant seasonality (Simental and Martinez-Urtaza 2008; Till et al. 2008; Dolejska et al. 2009; Jokinen et al. 2010; Schriewer et al. 2010; Thomas et al. 2013). On the other hand, a positive correlation between precipitation and Salmonella detection rate is generally agreed upon (Simental and Martinez-Urtaza 2008; Haley et al. 2009; Jokinen et al. 2010; Luo et al. 2015). Rainfall is believed to transport bacteria from contamination sources into the water, increasing the bacterial load through runoff; however, Vereen et al. did not detect any Salmonella following a very heavy rainfall which suggests a dilution effect of the elevated precipitation on the stream water (Vereen et al. 2013). The same authors also reported no Salmonella detection during droughts reflecting no runoff from contaminated sources (Vereen et al. 2013). As such, predicting a seasonal pattern of Salmonella detection in environmental water is complicated as their occurrence may be influenced by seasonal differences in environmental parameters in different regions, including variable rainfall level and water temperature.
Salmonella were detected in diverse surface water sources of different landscapes with various detection rates ranging from below 10 to 100% (Lemarchand and Lebaron 2003; Till et al. 2008; Patchanee et al. 2010; Schriewer et al. 2010; Vereen et al. 2013; Luo et al. 2015). Several studies have shown that indicator organisms such as faecal coliform, E. coli and enterococci were not a good predictor of Salmonella presence in water as the concentrations of the indicator organisms did not associate with the detection rates of Salmonella, while Vereen et al. demonstrated that enterococci are a better indicator organism as elevated enterococci concentrations were significantly associated with higher Salmonella prevalence (Lemarchand and Lebaron 2003; Meinersmann et al. 2008; Till et al. 2008; Haley et al. 2009; Vereen et al. 2013; Luo et al. 2015). The prevalence of Salmonella increased at highly impacted water near the sources of pollution such as agricultural lands, farms and wastewater treatment plants, suggesting livestock and human sources of the contamination (Lemarchand and Lebaron 2003; Till et al. 2008; Jyoti et al. 2010; Vereen et al. 2013). Wildlife, including reptiles and birds, was also suggested to affect the Salmonella populations in the environment (Dolejska et al. 2009; Maurer et al. 2015). Reptile-associated Salmonella subsps. Arizonae was detected in up to 40-6% of the total Salmonella isolated by Haley et al. (2009), indicating a reptile origin of Salmonella in surface water. The same authors measured the concentrations of Salmonella in surface water which were positively correlated with water temperature and rainfall, with the highest concentrations observed in summer months, especially August (Haley et al. 2009). A high concentration of Salmonella was observed in highly contaminated rivers in India: 10^2–10^4 colony forming unit (CFU) per ml was observed in the rivers that receive untreated domestic wastewater (Jyoti et al. 2010). This measurement was higher by three to eight orders of magnitude compared to other studies carried out in developed countries, including the United States, France and New Zealand (Lemarchand and Lebaron 2003; Till et al. 2008; Haley et al. 2009; Luo et al. 2015). However, regardless of the landscapes, geographical location or sources of water, Salmonella were detected in all of the water environments under study, although there were variations in the detection rate and occurrence level. This suggests that aquatic environments are either a transient or permanent habitat for Salmonella, playing a role as a reservoir or vector for the bacteria to humans.

Diverse Salmonella serotypes were detected in aquatic environments with the total number of serotypes generally ranging from 10 to 43 (Catalao Dionisio et al. 2000; Meinersmann et al. 2008; Simental and Martinez-Urtaza 2008; Thomas et al. 2013; Jokinen et al. 2015; Luo et al. 2015; Maurer et al. 2015). Highest diversity of Salmonella serotypes was observed in the environment near the sources of pollution, such as animal farm that might contribute to the loading of Salmonella in surface water (Simental and Martinez-Urtaza 2008; Haley et al. 2009). Serotypes associated with human and animal infections were commonly recovered from surface water in the United States; however, the most common clinical serotypes such as S. Enteritidis and S. Typhimurium either were absent or constituted only a small fraction of the total Salmonella populations (Meinersmann et al. 2008; Haley et al. 2009; Patchanee et al. 2010; Li et al. 2014; McEgan et al. 2014; Maurer et al. 2015). Some of the most commonly identified serotypes in environmental waters were S. Braenderup, S. Hartford, S. Muenchen, S. Newport and S. Rubislaw, many of which are listed as common clinical serotypes, suggesting a link among Salmonella from humans, animals and the environment (Meinersmann et al. 2008; McEgan et al. 2014; Luo et al. 2015; Maurer et al. 2015). On the other hand, S. Enteritidis and S. Typhimurium formed a considerable portion of the Salmonella populations in countries outside the United States (Simental and Martinez-Urtaza 2008; Dolejska et al. 2009; Thomas et al. 2013; Jokinen et al. 2015). Streams in Mexico had a notably different Salmonella serotype distribution, identifying serotypes not recovered in other water environments, such as S. Vejle, S. Suberu and S. Othmarschen (Simental and Martinez-Urtaza 2008). This suggests that a regional difference in serotypes exists among Salmonella found in aquatic environments. A spatial trend in Salmonella serotype was emphasized by Jokinen et al. as they observed that certain serotypes were widespread throughout the watersheds while others were specific to particular regions, suggesting different sources of contamination (Jokinen et al. 2015).

More than 2 600 Salmonella serotypes have been identified so far; however, only a limited number of serotypes account for most of Salmonella infections in humans with S. Typhimurium and S. Enteritidis as the most common causes globally (Herikstad et al. 2002; Grimont and Weill 2007; Issenhuth-Jeanjean et al. 2014). Serotyping of Salmonella from surface water is important to identify any clinically important serotypes and to compare the composition of Salmonella serotypes in the environment with those in humans and animals, which will help evaluate the role of environmental water in the transmission of Salmonella. Serotypes often associated with humans and animals were frequently recovered from surface water, and this may indicate that a significant population of the bacteria move between human and water environment. Moreover, identification of serotypes that are specific to certain sources can help track the sources of Salmonella contamination. For example, Jokinen et al. frequently...
recovered *S.* Kentucky, a serotype commonly associated with birds, from areas without intense poultry production (Jokinen *et al.* 2015). However, this was during months when birds actively migrate over these regions, allowing this information to suggest the potential source of *Salmonella* contamination as the migratory birds. Identification of *Salmonella* serotypes along with epidemiological data would help us fully understand the connection between water and humans and the directionality of the bacterial transmission.

A few studies were conducted in various parts of the world on the prevalence of AR *Salmonella* in surface water to understand the role of environmental water in the emergence and spread of AR in this genus. The results have shown that AR *Salmonella* are commonly detected in aquatic environments, including irrigation ponds, mixed-use watershed and rural rivers, thus increasing the risk of AR dissemination to humans (Meinersmann *et al.* 2008; Dolejska *et al.* 2009; Patchanee *et al.* 2010; Li *et al.* 2014; McEgan *et al.* 2014; Jokinen *et al.* 2015; Luo *et al.* 2015). In these studies, the detection rate of AR *Salmonella* varied from 10 to 99% with resistance to streptomycin observed in all of the water environments under study (Meinersmann *et al.* 2008; Dolejska *et al.* 2009; Patchanee *et al.* 2010; Li *et al.* 2014; McEgan *et al.* 2014; Jokinen *et al.* 2015; Luo *et al.* 2015). Interestingly, highest prevalence of AR *Salmonella* was observed in Florida while the lowest prevalence was detected in Georgia, both of which are the Southeastern states of the US (Meinersmann *et al.* 2008; McEgan *et al.* 2014; Luo *et al.* 2015). Patchanee *et al.* have shown that nearby pig farms were a potential source of AR *Salmonella* isolates recovered from the watersheds, whereas Dolejsk *et al.* suggested sea gulls as the source of AR *Salmonella* present in the water environment (Dolejska *et al.* 2009; Patchanee *et al.* 2010). Resistance to third-generation cephalosporin β-lactams including ceftriaxone, which is a recommended drug for salmonellosis, especially in children, was often detected in isolates from surface waters (Meinersmann *et al.* 2008; Patchanee *et al.* 2010; Li *et al.* 2014; Luo *et al.* 2015). Also concerning is the widespread presence of multidrug-resistant (MDR)-AmpC *S.* Newport in surface water of the irrigation ponds in Florida (Li *et al.* 2014). This particular strain of MDR *S.* Newport carrying an IncA/C plasmid with resistance to at least nine antimicrobials has contributed to the rapid and widespread emergence of MDR *S.* Newport among cattle and humans in the United States. This strain, identified as MDR-AmpC *S.* Newport, has caused the epidemic spread of the MDR phenotype to other *Salmonella* serotypes through the transfer of its IncA/C plasmid (CDC 2002; Frye and Jackson 2013). Pulse-field gel electrophoresis patterns of the MDR-AmpC *S.* Newport isolates from the irrigation ponds in Florida matched patterns in the CDC PulseNet database, indicating that these environmental isolates had also been recovered from human clinical samples (Li *et al.* 2014). These isolates present a high risk of spreading to humans since the water they were recovered from is used for the irrigation of produce. The presence of AR and MDR *Salmonella* in the environment not only is a public health concern but also suggests an extensive contamination from nearby animal farms or residential areas.

Resistance to β-lactams is particularly a concern in *Salmonella* as fluoroquinolones are not approved for children and pregnant women due to concerns about fluoroquinolones interfering with cartilage formation (Parry and Thriftall 2008). β-lactamases mediate resistance to β-lactams, and the main β-lactamases involved in resistance to extended-spectrum cephalosporins are AmpC β-lactamases, extended spectrum β-lactamases (ESBLs) and carbapenemases (Poole 2004). In *Salmonella*, the most prevalent β-lactamase resistance mechanism is plasmid-encoded AmpC β-lactamases, while ESBLs are comparatively rare in the United States (Winokur *et al.* 2000; Zhao *et al.* 2003; Folster *et al.* 2010; Sjölund-Karlsson *et al.* 2010). Among the AmpC plasmid-mediated β-lactamases, cephamycinas (CMY) encoded by *bla*<sub>CMY</sub> genes are predominant, out of which *bla*<sub>CMY-2</sub> is most prevalent and widely spread (Philippon *et al.* 2002). However, while *bla*<sub>CMY-2</sub> is widespread among humans and animals, the prevalence of this gene in environmental water is not well described. Wei *et al.* and Agga *et al.* attempted to identify *bla*<sub>CMY-2</sub> in their isolates recovered from environmental water in China using PCR and in the United States using microarray, respectively, but none of their environmental isolates carried the gene (Agga *et al.* 2015; Wei *et al.* 2019). Zheng *et al.* and Arai *et al.* sequenced the whole genomes of a few selected *Salmonella* isolates from environmental water, but none of their isolates contained AmpC β-lactamase or ESBL genes (Zheng *et al.* 2017; Arai *et al.* 2018).

While studies on the phenotypic characterization of AR environmental *Salmonella* isolates are occasionally presented, the genotypic characterization of isolates from aquatic environments to investigate their AR genes and associated MGEs involved in horizontal gene transfer are lacking. Comprehensive studies that involve sequence analyses of environmental AR isolates are needed to enhance our understanding of the genetic contents of the isolates, the mechanisms of their AR gene transfer and the potential for AR gene transmission in the environment. Analysis of the resistance mechanisms can also be used to determine the genetic relationship between resistances found in isolates from environmental water and humans. Because of the diversity of genetic elements that leads to AR, it may be possible to determine whether...
resistances seen in bacterial isolates from the environment are closely related to those found in human infections and animal isolates. Similarity between isolates may indicate that environmental water is a direct vector for human infections or a reservoir that harbours bacteria which could develop resistance and then reach humans by some intermediates, such as food animals.

Escherichia coli

Commensal and pathogenic E. coli

*Escherichia coli* is a commensal bacteria residing in the gastrointestinal tract of warm-blooded animals including humans without causing diseases except in immunocompromised hosts or when the gastrointestinal barriers are breached (Nataro and Kaper 1998; Kaper et al. 2004; Croxen et al. 2013). However, certain *E. coli* acquired virulence factors encoded on mobile genetic elements, such as bacteriophages, pathogenicity islands, plasmids and transposons, through horizontal gene transfer becoming pathogenic and causing diarrheal and extraintestinal diseases (Nataro and Kaper 1998; Kaper et al. 2004; Croxen et al. 2013). While extraintestinal pathogenic *E. coli* (ExPEC) causes infections outside of the gastrointestinal tract, such as urinary tract infections, sepsis and neonatal meningitis, diarrheagenic *E. coli* cause diarrhoea and other gastrointestinal diseases (Nataro and Kaper 1998; Kohler and Dobrindt 2011). Most well-described diarrheagenic *E. coli* pathotypes, based on their pathogenesis mechanisms and virulence factors, are enteroinvasive *E. coli* (EIEC), enteropathogenic *E. coli* (EPEC), enterotoxigenic *E. coli* (ETEC), enterohaemorrhagic *E. coli* (EHEC) and diffusely adherent *E. coli* (DAEC) (Kaper et al. 2004). Together, these pathogenic *E. coli* strains are a significant cause of global morbidity and mortality (Nataro and Kaper 1998; Kaper et al. 2004; Croxen et al. 2013). Such pathogenic *E. coli* were responsible for sporadic cases and outbreaks associated with contaminated recreational and drinking water. Recreational water-associated outbreaks occurred in lakes, rivers, ponds, streams and swimming pools (Keene et al. 1994; McCarthy et al. 2001; Craun et al. 2005; Verma et al. 2007; Probert et al. 2017). Drinking water-associated outbreaks were attributed to well water, municipal water and spring water (Rosenberg et al. 1977; Charatan 1999; Olsen et al. 2002; Hrudey et al. 2003; Craun et al. 2010). The outbreak that occurred in Walkerton, Canada in 2000 was the largest outbreak reported associated with drinking water, affecting more than 2,300 individuals with seven deaths (Hrudey et al. 2003). The frequent occurrence of waterborne outbreaks shows that contaminated water is an effective transmission vehicle of *E. coli*. Pathogenic *E. coli* that are resistant to therapeutic antimicrobials have increased during the last decades; *E. coli* resistant to first-line antimicrobial agents, including extended-spectrum cephalosporins and fluoroquinolones, are of particular concern as they complicate effective treatments for *E. coli* infections (WHO 2014). These AR *E. coli* are one of the nine bacteria of international concern according to the WHO report as they are one of the most common causes of infections in the community as well as in hospital settings worldwide (WHO 2014).

*E. coli* in surface water

Environmental water sources are prone to contamination by *E. coli* from both humans and other warm-blooded animals. Possible human sources include discharge of wastewater, sewage leaks, and failing septic tanks and drain fields. Additionally, partially treated sewage is sometimes directly discharged into rivers, intentionally or unintentionally, often enhanced by storm events that have been shown to increase *E. coli* counts in water by several folds (Sidhu et al. 2012; Sidhu et al. 2013; Cho et al. 2018). Animal sources include runoffs from livestock farms, land application of animal waste, pet wastes from parks, and wildlife such as geese, raccoons and deer (Fairbrother and Nadeau 2006; Somarelli et al. 2007). *E. coli* are ubiquitous in human faeces and the environment; thus, they are used as an indicator of faecal contamination for assessing water quality (U.S.EPA 2012). They are not generally considered pathogens, but their presence indicates potential presence of pathogens as high *E. coli* levels in recreational waters have been shown to be associated with an increased risk of swimming-associated gastrointestinal illness (DuFour 1984; Wade et al. 2003). In addition, simple and unsophisticated methods to culture, detect and enumerate *E. coli* make them a good indicator organism. *E. coli*, along with enterococci, replaced faecal coliforms as an indicator of faecal contamination by EPA in 1986 as they were a better predictor of gastrointestinal illness in recreational water (U.S.EPA 1986). In the United States, EPA standards for *E. coli* are either 126 *E. coli* CFU per 100 ml of water or less based on a geometric mean or a single-sample measurement not greater than 235 CFU per 100 ml in freshwater for the purpose of primary contact recreation such as swimming, surfing, tubing and water skiing, which involves full-body contact with water (U.S.EPA 1986).

On the other hand, the use of *E. coli* as an indicator of faecal contamination has been put into question as ‘naturalized’ *E. coli* have been shown to persist in water environments regardless of the faecal input into the environment (Rivera et al. 1988; Power et al. 2005). Despite the adverse environmental conditions, such as UV radiation, temperature fluctuation, predation and limited nutrients, *E. coli* are present in the environment.

Prevalence and AR of bacteria in surface water

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in a large number outside their primary habitat within the host; half of the entire *E. coli* population are estimated to be outside of the host in this secondary habitat (Savageau 1983). Further studies suggested that a specialized subset of *E. coli* strains can survive and grow in the environment, and a considerable population of these environmentally adapted, or ‘naturalized’ *E. coli*, which is distinct from faecal *E. coli*, can be found in various environments, such as water, soil and sediment (Gordon *et al.* 2002; Byappanahalli *et al.* 2003; Ishii *et al.* 2006; Ishii *et al.* 2007; Walk *et al.* 2007). Walk *et al.* characterized *E. coli* isolates from the aquatic environment using multilocus sequence typing (MLST) and identified environmentally adapted lineages of *E. coli*: ET-1 clade, which is a subset of the B1 phylogenetic group, and cryptic clades CIII, CIV and CV (Walk *et al.* 2007; Walk *et al.* 2009). The authors also suggested that approximately one-fourth of their environmental *E. coli* from freshwater beaches was strains adapted to the environment (Walk *et al.* 2007). Out of the four major phylogenetic groups of *E. coli*, referred to as A, B1, B2, and D, B2 and D groups are associated with extraintestinal infections, and members of A and B1 groups are generally commensal *E. coli* (Picard *et al.* 1999). B1 group was the most commonly identified group in the freshwater beach study by Walk *et al.*, supporting the recent finding that B1 strains are host generalists and environmentally adaptive, in contrast to B2 strains, which are more host-adapted (Walk *et al.* 2007; White *et al.* 2011; Meric *et al.* 2013). In their study, Gordon *et al.* (2002) compared *E. coli* isolated from septic tanks with those isolated from the people using the septic tanks and found out that the strains from the septic tanks were distinct from the human strains and adapted better to the conditions of the external environment. These results underscore the presence of ‘naturalized’ *E. coli* populations that persist better outside of the host in their secondary habitat.

Few studies have determined the presence of virulence genes in *E. coli* isolated from environmental water to examine the frequency of potential pathogenic *E. coli* (Lauber *et al.* 2003; Hamelin *et al.* 2006; Hamelin *et al.* 2007; Ishii *et al.* 2007; Ram *et al.* 2008; Sidhu *et al.* 2013; Titilawo *et al.* 2015b; Cho *et al.* 2018; Haymaker *et al.* 2019). Some of the most commonly detected *E. coli* pathotypes include ETEC, defined by the ability to produce heat-labile (LT) and/or heat-stable (ST) enterotoxins and causes traveller’s diarrhoea; EPEC, defined by the ability to produce intimin adhesin protein and causes infant diarrhoea; and EHEC, defined by the ability to produce Shiga toxin and intimin and are the causative agent of haemorrhagic colitis and haemolytic uremic syndrome (Nataro and Kaper 1998). Virulence genes were detected with varying frequency in different environmental water around the world. Virulence genes were detected in 91, 61.1 and 85% of the isolates from rivers and creeks in Nigeria, India and Australia, respectively (Ram *et al.* 2008; Sidhu *et al.* 2013; Titilawo *et al.* 2015b). On the other hand, virulence genes were detected at a much lower rate, sometimes less than 1%, in the studies by Lauber *et al.* (2003), Ishii *et al.* (2007), Cho *et al.* (2018), Haymaker *et al.* (2019), all of which were conducted in surface waters of the United States. One of the most common virulence genes found in these studies was *eae* A, a gene that encodes intimin protein in EPEC and EHEC; however, while EPEC was frequently isolated from surface water sources, EHEC, which carry additional Shiga toxin genes, *stx1*, *stx2* or both, was not often detected (Lauber *et al.* 2003; Ishii *et al.* 2007; Titilawo *et al.* 2015b; Cho *et al.* 2018; Haymaker *et al.* 2019). Only Ram *et al.* detected more EHEC strains than EPEC strains in the Gomti River, which is one of the most polluted rivers in India, and Sidhu *et al.* detected EHEC as often as EPEC in Australia, while the former study detected more *stx*1 and the latter study detected more *stx*2 genes (Ram *et al.* 2008; Sidhu *et al.* 2013). EHEC with Shiga toxin type 2 (*stx*2) are more commonly associated with severe complications than those producing Shiga toxin type 1 (*stx*1) (Paton and Paton 1998). Shiga-toxin producing *E. coli* (STEC), of which EHEC is a subset, produces Shiga toxin but not intimin protein (Nataro and Kaper 1998). Even though not all STEC strains are considered pathogens, STEC were frequently detected from surface waters as well (Ram *et al.* 2008; Sidhu *et al.* 2013; Titilawo *et al.* 2015b; Cho *et al.* 2018; Haymaker *et al.* 2019). ETEC was detected in surface waters of Australia, India and Nigeria at 5.7, 21.1 and 45% detection rates, respectively, while no ETEC was detected in US waters (Lauber *et al.* 2003; Ram *et al.* 2008; Sidhu *et al.* 2013; Titilawo *et al.* 2015b; Cho *et al.* 2018). As such, a variety of pathotypes seems to be present at higher frequency rates in countries other than the United States. Additionally, it should be noted that many of the chromogenic media used for the isolation of *E. coli* are based on their presence of β-glucuronidase enzyme; however, EHEC, such as O157:H7 strain, lacks β-glucuronidase and as such, the prevalence of EHEC in water is likely underestimated relative to other pathotypes of *E. coli* (Ratnam *et al.* 1988; Maheux *et al.* 2015).

While most studies focused on diarrheagenic or intestinal *E. coli*, Hamelin *et al.* included ExPEC in their two studies carried out in Canada and found that a higher percentage of ExPEC was present than the diarrheagenic *E. coli* strains in environmental waters (Hamelin *et al.* 2006; Hamelin *et al.* 2007). Approximately 28% and 26% of the total isolates belonged to ExPEC, including neonatal meningitis-associated *E. coli* (MNEC), uropathogenic...
E. coli (UPEC) and septicemia-associated E. coli (SEPEC), while only 4 and 2% belonged to diarrheagenic pathotypes in their studies covering St. Clair River and Detroit River areas and Lake Ontario, respectively (Hamelín et al. 2006; Hamelin et al. 2007). Nevertheless, it is difficult to make direct comparisons between the results from different studies due to differences in methodology for isolation and detection of pathogenic E. coli (enrichment vs non-enrichment, PCR vs microarray) and the choice of virulence genes screened. Although the detection of virulence genes does not indicate the pathogenic potential of the E. coli isolates, and phenotypic tests need to be performed to establish the ability of these E. coli to cause diseases, the widespread distribution of virulence genes and potential pathogenic E. coli in streams and rivers used for recreations, consumption and irrigation raises human health risks with exposure to these water sources.

Storm events not only increase the number of E. coli and increase the number of E. coli with multiple virulence genes in environmental waters but also evenly distribute the prevalence of E. coli pathotypes in surface water bodies by significantly lowering the number of certain pathotypes and increasing the number of other pathotypes (Sidhu et al. 2013). This uniform distribution of pathotypes after rainfall was suggested to be due to the transport of pathogenic E. coli into the water from both point sources (single identifiable sources) and nonpoint sources (many diffuse sources) (Sidhu et al. 2013). Rain also increases the number of STEC of serotype O157:H7 by disseminating bacteria from point sources, such as animal faeces, especially cattle, which are a known reservoir of STEC, or by release of bacteria from sediments (Hussein and Sakuma 2005; Cooley et al. 2007). Of all the pathotypes of E. coli that are associated with human infections, the O157:H7 strain is the most well-known pathogenic strain, responsible for the majority of E. coli outbreaks reported and mortality worldwide (Nataro and Kaper 1998; CDC 2016). Studies showed that the E. coli O157: H7 and other strains belonging to the serogroup O157 were present in environmental waters, and the presence of these pathogenic E. coli in surface water highlights the potential risk to human health through consumption of untreated water and irrigated raw vegetables, as well as through recreational use of surface water (Cooley et al. 2007; Jokinen et al. 2010; Maal-Bared et al. 2013; Cooley et al. 2014). In fact, the E. coli O157:H7 outbreak in Walkerton, Ontario, Canada in 2000, which caused 2 300 cases of gastrointestinal illness and seven deaths, was attributed to the contamination of well water with cattle manure from a nearby farm following intense rainfall events (Hrudey et al. 2003; Auld et al. 2004). The presence of E. coli O157 in water sources near agricultural lands including cattle farms suggests the animal source of the pathogens (Cooley et al. 2007; Maal-Bared et al. 2013; Cooley et al. 2014). As such, wildlife and livestock that have direct, uncontrolled access to surface waters may be the source of the pathogens, while heavy rainfall are the means whereby pathogens in animal manure may be transported to surface waters.

The prevalence of AR E. coli in environmental water has been shown to vary from <10 to 100%, and these variations may be due to differences in the use and regulations of antimicrobials among countries or differences in the source of faecal contamination impacting the water sources (Servais and Passerat 2009; Agga et al. 2015; Titilawo et al. 2015a; Lyimo et al. 2016; Chen et al. 2017; Cho et al. 2018). However, a direct comparison between these studies is difficult as different antimicrobial drugs were used to assess the antimicrobial susceptibility of environmental E. coli. Nevertheless, resistances to sulphonamides (sulfamethoxazole, trimethoprim/sulfamethoxazole), β-lactams (penicillin, ampicillin and amoxicillin) and tetracycline were generally widespread (Servais and Passerat 2009; Agga et al. 2015; Titilawo et al. 2015a; Lyimo et al. 2016; Chen et al. 2017; Cho et al. 2018). Resistance to third-generation cephalosporins, which are used to treat E. coli infections, was often observed as well from the E. coli isolates recovered from surface waters (Pitout 2012; Blaak et al. 2014; Agga et al. 2015; Bajaj et al. 2015; Jorgensen et al. 2017; Cho et al. 2018). Wastewater released into the waterways were implicated to be the main source of AR bacteria, but the presence of AR E. coli in streams with low or no obvious contamination level suggests that either wildlife and companion animals or runoffs from surrounding lands and sediments enhanced by rainfall contribute to the AR E. coli contamination in waters (Servais and Passerat 2009; Agga et al. 2015; McArthur et al. 2016).

ESBL-producing E. coli were identified not only from recreational water but also from wastewater treatment plant effluents and receiving surface water at a high detection rate (Blaak et al. 2014; Agga et al. 2015; Titilawo et al. 2015a; Kittinger et al. 2016; Lyimo et al. 2016; Jorgensen et al. 2017). In E. coli, resistance to expanded-spectrum cephalosporins is usually due to ESBLs, which destroy β-lactam antimicrobial drugs (Vila et al. 2016). Of all the ESBL genes, blaCTX-M, particularly blaCTX-M-15, and blaTEM, particularly blaTEM-1, were often detected in surface water with blashv and blaOXA occasionally identified (Cho et al. in press; Blaak et al. 2014; Agga et al. 2015; Titilawo et al. 2015a; Kittinger et al. 2016; Lyimo et al. 2016; Jorgensen et al. 2017; Cho et al. 2019a). This observation reflects the current trend of CTX-M (cefotaximase-Munich) enzymes as the dominant type of enzymes among ESBL-producing E. coli, of which CTX-M-15 is most widely distributed in community and hospitals.
(Canton and Coque 2006; Coque et al. 2008). Escherichia coli ST131, which is a pandemic E. coli strain that can be MDR, and is globally disseminated causing hospital and community-acquired infections around the world, were also prevalent in surface water (Nicolas-Chanoine et al. 2008; Johnson et al. 2010; Hu et al. 2013; Nicolas-Chanoine et al. 2014; Petty et al. 2014; Assawatheptawee et al. 2017; Jorgensen et al. 2017; Runcharoen et al. 2017; Cho et al. 2018).

Carbapenem-resistant Enterobacteriaceae (CRE) have increased recently, and the CDC has recognized them as an urgent public health threat as these bacteria are resistant to nearly all of the available antimicrobial drugs and the mortality rate of the CRE infections is high (CDC 2013; Thaden et al. 2014). CRE have been frequently detected beyond human healthcare in water environments around the world. Researchers have detected carbapenemase-producing Enterobacteriaceae, such as Enterobacter sp., Klebsiella sp. and Klebsiella sp., and various concentrations of carbapenemase genes, such as blaKPC, blaNDM and blaoXA-48, in surface waters (Ahammad et al. 2014; Montezzi et al. 2015; Proia et al. 2018; Mathys et al. 2019). Carbapenemase-producing E. coli have been identified in surface waters from different countries, including China, Ireland, Lebanon, Portugal and the United States, especially downstream of wastewater treatment plants (Poirel et al. 2012; Xu et al. 2015; Kieffer et al. 2016; Mahon et al. 2017; Yang et al. 2017; Diab et al. 2018; Mathys et al. 2019). The detection of CRE in both effluents and receiving waters suggests that CRE dissemination into the environment occurs through wastewater. Furthermore, the presence of mcr-1, which encodes resistance to colistin, a drug of last resort, in environmental waters, though at a very low rate, is daunting (Nation and Li 2009; Schwarz and Johnson 2016; Yu et al. 2016; Zurfuhr et al. 2016; Jorgensen et al. 2017; Runcharoen et al. 2017). Colistin has been in use since 1950s but, due to its toxicity, the drug has not been in use until recently when MDR became a worldwide problem, which has resulted in colistin being considered the drug of last resort (Lim et al. 2010). Colistin resistance was chromosomally encoded and was not of a concern; therefore, when plasmid-mediated mcr-1 gene was described for the first time in E. coli by Liu et al. (2016), many scientists underwent a retrospective screening of their old E. coli isolates for mcr-1 gene detection and mcr-1-carrying isolates were reported as early as in the 1980s (Liu et al. 2016; Shen et al. 2016). The identification of mcr-1 gene from a Malaysian pond water in a retrospective study by Yu et al. and from recent studies by Jorgensen et al. and Runcharoen et al. in Norway and Thailand, respectively, suggests the potential widespread distribution of colistin-resistant E. coli in the environment, which may not be a recent emergence or on a rising trend (Yu et al. 2016; Jorgensen et al. 2017; Runcharoen et al. 2017).

Enterococcus
Commensal and pathogenic Enterococcus
Enterococci are Gram-positive bacteria that naturally reside in intestinal tracts of humans and animals including mammals, insects, reptiles and birds (Mundt 1963b; Martin and Mundt 1972). They are also found in the environments including water, soil and plants due to their high tolerance to different conditions (Mundt 1963a; Fujioka et al. 1999; Badgley et al. 2010). They can survive in a wide range of temperatures (5–60°C) and pH (4–9), in the presence of high salt concentration (6–9% NaCl) and bile salts (40%), and in prolonged desiccation (Fisher and Phillips 2009). Enterococci have been a major nosocomial pathogen since the 1970s and has been considered the fifth most common healthcare-associated pathogen in the United States in 2015 (Jett et al. 1994; Magill et al. 2018). Of the 58 enterococcal species identified, E. faecalis and E. faecium, which are also most common enterococcal species in the intestinal microbiota of humans, account for most of the enterococcal infections in humans (Tannock and Cook 2002; Parte 2018). Other species, including E. avium, E. casseliflavus, E. durans, E. gallinarum, E. hirae, E. munditii and E. raffinosus, are also responsible for human infections (Tannock and Cook 2002). Enterococci have become a major nosocomial pathogen due to their resistance to several antimicrobials and ability to acquire and disseminate AR determinants. They are intrinsically resistant to a broad range of antimicrobials such as β-lactams and aminoglycosides (Arias and Murray 2012). Furthermore, they have acquired resistance to almost all currently used and new clinical antimicrobial drugs, further complicating antimicrobial treatments (Arias and Murray 2012). However, it was not until the acquisition of vancomycin resistance that enterococci have drawn attention as an MDR pathogen. Vancomycin-resistant Enterococcus (VRE) is considered a serious threat level by the CDC as only a few antimicrobials are available for the treatment of VRE infections (CDC 2019). Linezolid, quinupristin/dalfopristin, dapto mycin and tigecycline are used for the treatment of VRE infections; but unfortunately, resistance to these drugs is increasingly reported making the VRE infections difficult to treat (Arias and Murray 2012; Rossolini et al. 2014; Guzman Prieto et al. 2016; Cho et al. 2019b).

Enterococcus in surface water
Along with E. coli, enterococci are used as an indicator of faecal contamination in freshwater, and in marine water, enterococci are considered a better indicator of faecal
pollution than *E. coli* as their concentrations in the marine water are positively associated with swimming-associated gastrointestinal illness rates (Cabella 1983; Dufour 1984). U.S. EPA recommends no more than either a geometric mean of 33 *Enterococcus* CFU per 100 ml of water or a single-sample measurement of 61 CFU per 100 ml for freshwater, and either a geometric mean of 35 *Enterococcus* CFU per 100 ml or a single-sample measurement of 104 CFU per 100 ml for marine water as the water quality criteria for primary contact recreational activities with a high probability of water ingestion, such as swimming, diving, surfing and bathing (U.S. EPA 1986).

Certain species of *Enterococcus*, such as *E. faecalis*, *E. faecium*, *E. casseliflavus*, *E. gallinarum*, *E. mundtii*, *E. hirae*, *E. durans* and *E. avium*, are consistently recovered from surface water around the world, although in different proportions. Research has shown that *E. faecalis* is usually the most common species in this niche (Lata et al. 2009; Alipour et al. 2014; Sidhu et al. 2014; Molale and Bezuidenhout 2016). In fact, *E. faecalis* has a broad host range and is the most widely distributed enterococcal species as it has been isolated from humans (hospital and community settings), animals (livestock, companion animals and wild animals) and the environment (Guzman Prieto et al. 2016). While *E. faecalis* appears to be the most abundant species in natural water in a majority of studies, other species such as *E. faecium*, *E. casseliflavus*, *E. hirae* and *E. mundtii* were found to be dominant or as frequent as *E. faecalis* in some water environments (Svec and Sedláček 1999; Meinersmann et al. 2008; Ran et al. 2013; Molale and Bezuidenhout 2016; Cho et al. 2019b).

This difference in species compositions at different locations could be due to spatial or temporal variability. Environmental factors were suggested to influence the species compositions, with warm temperature favouring the bacterial growth and rainfall transporting bacteria into water along with runoff from surrounding vegetation and soil, animal faecal matter, sewage leakage and anthropogenic activities (Ran et al. 2013; Sidhu et al. 2014). Ran et al. reported an increase in the frequency of *E. faecalis* during summer months and after rainfall events potentially due to runoff transporting more of the faecal origin species, while Sidhu et al. reported a decrease in the frequency of *E. faecalis* after rainfall events possibly due to other species of *Enterococcus* being added into the waterbodies from various contamination sources, thus decreasing the proportion of *E. faecalis* of all *Enterococcus* species isolated (Ran et al. 2013; Sidhu et al. 2014). On the other hand, Kühn (2003) reported a spatial variability in the species composition as surface water receiving rural runoff contained almost no *E. faecalis* and exhibited a similar species distribution to the surrounding farmland.

There has been a debate on the identification of potential contamination source based on the presence of specific *Enterococcus* species associated with the source. Presence of soil and plant origin *E. casseliflavus* and *E. mundtii* suggests plants and soil as a source of enterococci, while the presence of faecal origin *E. faecalis* and *E. faecium* suggests human and animal as a source of enterococci in water, increasing the potential health risk due to pathogens originating from humans and animals. Sidhu et al. (2014) found that *E. faecalis* was more prevalent in creeks within high urbanization landscape with more human and animal faecal input, and *E. casseliflavus* and *E. mundtii* being more prevalent in creeks with low urbanization and high vegetation. Ferguson et al. (2013) also reported a similar finding with a higher proportion of *E. casseliflavus* and *E. mundtii* in beach and surface water samples compared to wastewater samples that were dominated by *E. faecalis* and *E. faecium*. Alternatively, some studies were sceptical of the use of a host-specific species as an indicator of either human faecal contamination or environmental residues as similar patterns of *Enterococcus* species composition were seen from various hosts (Layton et al. 2010; Lebreton et al. 2014). A number of *Enterococcus* species have been isolated from human faeces, animal faeces, environment and food, so it is a challenging task to identify a single host for each species found in the environmental water.

Different cultivation methods used for the recovery of *Enterococcus* isolates could contribute towards the variability in species composition found in different water studies. The nature of culture media, either solid or liquid, and the composition of media with different substrates, selective agents and indicator molecules were suggested to affect species populations recovered (Jackson et al. 2005; Ferguson et al. 2013). In addition, culture conditions may account for species variability. As different enterococcal species have different physical properties and growth characteristics, a change in incubation temperature had an effect on the recovery and selection of certain species (Domig et al. 2003; Jackson et al. 2005). According to the study conducted by Jackson et al. (2005), *E. faecalis*, *E. casseliflavus* and *E. durans* were more temperature sensitive than *E. faecium* and *E. hirae*, as more isolates were recovered at 37°C than at higher temperatures of 42 or 45°C.

Not only does isolation method affect the species composition but so does the species identification method. Enterococcal species identification has been based on either phenotypic methods, such as conventional morphological and biochemical tests and VITEK, or genotypic methods, such as 16S rRNA gene sequencing and multiplex PCR (Patel et al. 1998; Jackson et al. 2004). Discrepancy in species identification occurs between different
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methods, either overestimating or underestimating certain species (Patel et al. 1998; Fang et al. 2012; Ferguson et al. 2013). A simple standardized method to identify enterococci to the species level is required to enable direct comparisons of species distribution; however, methodology is beyond the scope of this review. More recently, novel Enterococcus species have been identified from various water samples including E. rivotrumbus, E. haemoperoxidus, E. moraviensis, E. aquimarinus, E. silesicus, E. ureasiticus, E. quebecensis, E. urelyticus and E. rotai (Svec et al. 2001; Svec et al. 2005; Svec et al. 2006; Niemi et al. 2012; Sistek et al. 2012; Sedlacek et al. 2013). Since the list of Enterococcus species is expanding as their habitats are explored, and as enterococci are isolated from diverse sample types owing to their ubiquity, a consistent and reliable standardized method to isolate enterococci and identify their species is needed, especially if the species identification could point us to the contamination source.

Several studies have demonstrated that enterococci in environmental water are resistant to a wide range of antimicrobials, the results of which indicate that AR enterococci are not limited to the clinical settings and are prevalent and persistent in the environment (Rice et al. 1995; Meinersmann et al. 2008; Moore et al. 2008; Lata et al. 2009; Cho et al. 2019b). Surface waters receive AR enterococci from hospitals and other sources through discharged sewage or other means, increasing the prevalence of these bacteria in the environment and thus increasing the risk to human health through drinking and recreational activities. Even though VRE are not frequently isolated from uncontaminated aquatic environments, discharged sewage can release VRE from hospitals into aquatic environments (Harwood et al. 2001; Moore et al. 2008; Young et al. 2016). Although not frequently, VRE have been detected in aquatic environments, outgoing treated sewage and receiving surface water around the world (Iversen et al. 2002; Novais et al. 2005; Lata et al. 2009; Rosenberg Goldstein et al. 2014; Young et al. 2016). Their origin has been suggested to be surrounding hospitals and community as healthy individuals have also been shown to carry VRE, and drugs released into surface water may sustain VRE in the environment (Wendt et al. 1999; Padiglione et al. 2000; Novais et al. 2005). Iversen et al. (2002) demonstrated in their study in Sweden that VRE were only detected in sewage from the hospital that had used 10 times as much vancomycin as the other hospital from where no VRE was identified, suggesting that the amount of the drug used in the hospitals may influence the prevalence of VRE.

The prevalence of VRE in the United States outside the clinical setting is lower than in other countries, and no VRE have been detected in US surface water without the obvious contamination after a spill of sewage (Harwood et al. 2001; Young et al. 2016). On the other hand, VRE have been detected in surface water in several European countries (Iversen et al. 2002; Blanch et al. 2003; Novais et al. 2005). This could be because avoparcin, which belongs to the same glycopeptide antimicrobial class as vancomycin, was never allowed in animals as growth promoter in the United States, while in some European countries, it was used at sub-therapeutic doses as a growth promoter in animal farms, providing selective pressure for vancomycin resistance in enterococci among livestock (Bager et al. 1997; McDonald et al. 1997; Kruse et al. 1999). Concerns regarding the cross-resistance between avoparcin and vancomycin and the farm animals as a reservoir of VRE have led to the ban of avoparcin in the entire European Union from 1997, and decreased prevalence of VRE was reported among animals and healthy humans in the community following the discontinued use of the drug (Bager et al. 1999; Klare et al. 1999).

E. casseliflavus and E. gallinarum with reduced susceptibility to vancomycin carrying vanC were often detected in environmental waters (Nam et al. 2013; Nishiyama et al. 2015). E. casseliflavus and E. gallinarum are intrinsically resistant to vancomycin but have attracted less attention compared to vancomycin-resistant E. faecalis and E. faecium for several reasons (Clark et al. 1998). First of all, E. casseliflavus and E. gallinarum are considered to be ‘environmental’ species that are primarily associated with plants and birds (Mundt and Graham 1968; Collins et al. 1984). They are not considered clinically significant pathogens as these two enterococcal species cause human infections less frequently compared to E. faecalis and E. faecium (Toye et al. 1997). Also, MIC of E. casseliflavus and E. gallinarum for vancomycin is not usually higher than the CLSI breakpoint of 32 µg ml⁻¹, making the treatments of infections due to these bacteria relatively less challenging (Gold 2001). In addition, vanC gene, which encodes intrinsic resistance to vancomycin in E. casseliflavus and E. gallinarum, is chromosomally encoded and not transferable (Gold 2001). However, the clinical significance of vanC-carrying species should not be overlooked. Although not frequently, global incidence of infections due to VanC-type E. casseliflavus and E. gallinarum has been identified, for which vancomycin therapy failed in some cases (Green et al. 1991; Reid et al. 2001; Choi et al. 2004; de Perio et al. 2006; Contreras et al. 2008; Cooper et al. 2008). Furthermore, E. casseliflavus and E. gallinarum exhibiting high-level vancomycin resistance phenotype have been detected in clinical isolates with the acquisition of vanA and vanB genes which are transferable to other enterococci (Dutka-Malen et al. 1994; Liassine et al. 1998; Corso et al. 2005; Merquior et al. 2008). The increasing cases of infections
and outbreaks of these less common enterococci suggests a need for increased clinical attention.

Due to their ubiquity in the animal gut, resilience to environmental stress, resistance to clinically important antimicrobials and ability to acquire and transfer resistance, enterococci are used as sentinel organisms for AR with activity against Gram-positive bacteria, for example, by the NARMS for their monitoring of retail meat, food animals and humans. However, enterococci are generally not considered important pathogens outside the hospitals and healthcare settings, due to which studies reporting enterococci and their AR from the environment are scarce. Studies on water sources are focused either on human-specific species, E. faecalis and E. faecium, on certain antimicrobial agents, such as vancomycin, or on polluted water environments such as hospital-impacted wastewater and agricultural runoff (Sadowsy and Luczkiewicz 2014; Nishiyama et al. 2015; Young et al 2016). Prevalence and persistence of AR enterococci released into surface water are of particular interest not only because they can spread to different environments and increase the chance of exposure to these bacteria through water-related activities but also because they can transfer AR genes to other bacterial species of higher human health concern. Studies that address the specific origin of AR enterococci in aquatic environments, their survival mechanisms in such environments and the transfer of AR genes are needed in addition to identifying potential environmental reservoirs of AR enterococci.

Conclusion

The environment is one of the three pillars of the One Health but also the least studied sector among the three. This review discussed the prevalence and diversity of pathogenic and commensal bacteria, namely Salmonella, E. coli and Enterococcus, present in surface waters and provided a basic understanding of the occurrence and persistence of AR in these bacteria. The widespread distribution of pathogenic and AR bacteria in surface waters of both developing and developed countries demonstrated the importance of environmental waters as a reservoir for these bacteria. Since AR pathogens present an increasing public health challenge worldwide, much more attention needs to be given on persisting as well as emerging AR in the environmental water, which is essential for better understanding the AR in humans and animals and thus the overall trend in AR.

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Conflict of Interest

No conflict of interest declared.

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samples associated in time and location. PLoS ONE 12, e0186576.


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