

Review Paper

Potential of waterbodies as a reservoir of *Escherichia coli* pathogens and the spread of antibiotic resistance in the Indonesian aquatic environment

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ABSTRACT

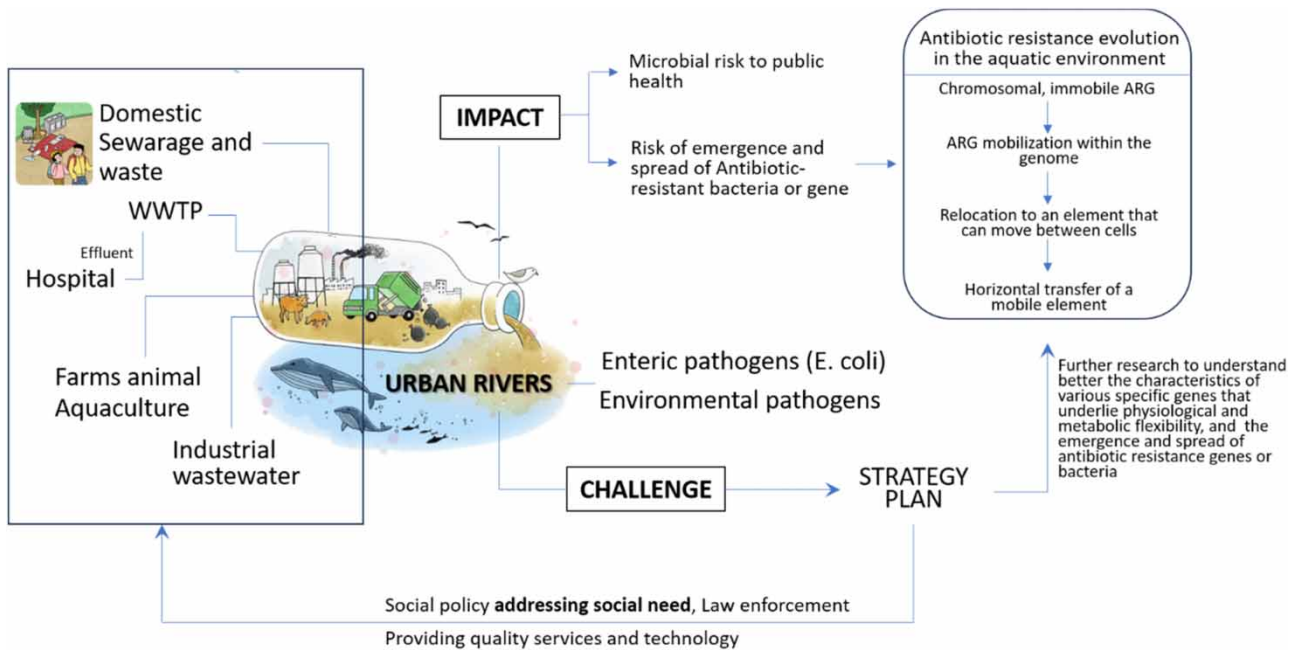
River contamination remains a significant public health problem worldwide, including in Indonesia. In the community, rivers are water resources for many purposes. However, indiscriminate use of antibiotics in the veterinary and medical sectors, the community habit of throwing waste into the river, the inadequate removal of human medicine compounds in WWTP, and poor industrial waste management will induce the spread of pathogenic bacteria and antibiotic resistance between the environment and humans/animals. This review aims to analyse the various factors that influence the emergence of pathogenic *Escherichia coli* and antimicrobial resistance bacteria (ARB) in the aquatic environment, especially in Indonesia. In conclusion, pathogenic *E. coli* can adjust its physiological or metabolic conditions to survive in aquatic environments and was found resistant to antibiotics. Meanwhile, this study also describes the potency of the aquatic environment in Indonesia as a vector for the spread of pathogenic *E. coli* and the growth of ARBs, factors involved in the evolution and spread of antibiotic resistance in aquatic environments, and possible ways to curtail microbial risk to public health and the emergence and spread of antimicrobial resistance in the aquatic environment in Indonesia.

Key words: antibiotic, *Escherichia coli*, resistance, river contamination

HIGHLIGHTS

- Various rivers in Indonesia have the potential to become a reservoir of *E. coli* pathogens.
- Various rivers in Indonesia are efficient vectors for the spread of antimicrobial resistance bacteria.
- The antibiotic resistance can spread in the environment.
- Pathogenic *E. coli* can adjust to survive in aquatic environments.
- *E. coli* pathogens can facilitate the transfer of resistance genes between bacteria.

GRAPHICAL ABSTRACT



1. INTRODUCTION

The quality of river and stream water is susceptible to anthropogenic influences such as intensive livestock and agricultural activities, industrial activities, the disposal of waste, especially untreated wastewater, into rivers, and increased activity of residents living alongside rivers, as well as natural factors, such as precipitation and climate (Kim & An 2015). In addition, various types of pollutants with different properties can contaminate rivers, such as non-biodegradable plastics, synthetic chemicals, heavy metals, and even bacterial pollutants (Rodríguez-Tapia & Morales-Novelo 2017). Bacterial pollutants, especially pathogenic enteric bacteria, can contaminate rivers through untreated domestic wastewater or leakage of sanitary wastewater, making the rivers a reservoir for pathogenic bacteria in the environment.

A study by Na *et al.* showed that the anthropogenic index significantly correlates with the prevalence of antibiotic-resistant bacteria (ARB), including the incidence, distribution, and migration of antibiotic-resistant *Escherichia coli* in a natural aquatic environment (Na *et al.* 2018). This supports the notion that rivers can facilitate the spread of ARB. Several pollutants contribute to the emergence of ARB and the spread of antibiotic-resistant genes (ARGs) among bacteria. For example, microplastic surfaces in aquatic environments harbour antibiotic-resistant microorganisms. Bacterial biofilms found on microplastics in aquatic ecosystems have been shown to contain bacteria with ARGs (Arias-Andres *et al.* 2018). Meanwhile, metal contamination induces antibiotic resistance through co-selection, cross-resistance, and co-regulation (Wales & Davies 2015).

Another critical factor for the emergence of ARB and its spread is the contamination of rivers by antibiotics, which can occur via the waste of humans or animals previously treated with antibiotics (Aminov & Mackie 2007). Bacteria exposed to antibiotics can find a way to survive under the pressure of antibiotics. As a result, they developed mechanisms for resistance and multiply. Some resistant bacteria can confer resistance to other bacteria through horizontal gene transfer (HGT) involving mobile genetic elements (MGEs). HGT has been considered one of the primary mechanisms in spreading ARG from environmental and commensal species to pathogenic species or vice versa in aquatic environments (Lupo *et al.* 2012). MGE can also carry virulence factors, allowing bacteria to replicate and spread these genes to other bacteria. The presence of ARG in MGE leads to the possible emergence and spread of multi-resistant phenotypes (Holt *et al.* 2015).

Various rivers in Indonesia are likely to be efficient vectors for the spread of ARB. A range of conditions supports this potential, as according to the Indonesian Ministry of Environment and Forestry (KLHK), about 59% of rivers in Indonesia are heavily polluted, mainly due to industrial activities, household waste, and livestock. A survey on antibiotics in the

livestock sector in Indonesia using data from iSIKHNAS (Indonesia's new integrated animal health information system) from 2014 to 2016 provided an increase in antimicrobial use (AMU). Most antibiotics were used for non-bacterial diseases such as viral and parasitic diseases (Wasnaeni & Iqbal 2015). In addition, many residents along rivers in Indonesia are accustomed to cleaning themselves, washing clothes and kitchen utensils, and urinating/defecating in rivers. Using detergents related to bathing-washing-latrines can also select resistant bacteria and multidrug-resistant (MDR) strains in rivers where domestic household activities are performed (Coutinho *et al.* 2014).

Against this background, there is a need to obtain an overview of the spread of bacteria, especially pathogenic *E. coli*, in rivers in Indonesia and to determine the mobilisation of various factors that influence the emergence of pathogenic *E. coli* and ARB in the aquatic environment. Understanding how these pathogens survive in their secondary habitats and the antibiotic resistance mechanisms in aquatic environments is also important. It is necessary to determine appropriate river management strategies to ensure safe water resources for communities.

2. METHODS

2.1. Article search and selection strategy

This literature review article is based on scientific information obtained from various literature sources. Literature sources were searched using PubMed-based electronic data until September 2021. Keywords were linked using Boolean operators as follows: (((*Escherichia coli*) OR (coliform) AND (aquatic environment) AND ((water pollution) OR (faecal contamination) OR (antibiotic resistance) OR (antibiotic resistance spread) OR (gene transfer)) AND ((plasmid) OR (mobile genetic element) OR (biofilm) OR (resistance mechanism))). Articles from other sources on relevant topics, such as those obtained by a manual search through the reference lists of the identified papers, were also used in this study. Exclusion criteria were any intervention on the sample that could affect the procedures or results of laboratory tests or the use of pollution indicators other than *E. coli* or coliform bacteria. Results were measured by identifying the presence of *E. coli* isolates in water samples and certain genes related to antibiotic resistance.

2.2. Data extraction

The first and second authors completed data extraction and quality assessment, with the results being re-examined by the third, fourth, and fifth authors, after which disagreements were discussed. Full-text articles were extracted into a table, collecting various data on the author(s), year of publication, water samples, methods, bacterial species/strain, and indicators used to assess the presence of *E. coli* isolates and their sensitivity to antibiotics.

3. RESULT AND DISCUSSION

3.1. Article selection

In the literature search, 46 articles from the PubMed database were obtained using predetermined keywords and 10 articles were obtained from the manual search of the reference lists of articles, as described in the method section. Of these 56 articles, 31 met the exclusion criteria for the following reasons: 10 articles were on studies with a review study design, 2 used indicators other than *E. coli*, and 19 were irrelevant or off-topic. Thus, 25 articles relevant to this study were obtained (Figure 1).

3.2. Spread of *E. coli* in various rivers in Indonesia as an indicator of water pollution

E. coli, a Gram-negative, rod-shaped bacterium belonging to the Enterobacteriaceae family, are commonly used as the primary indicator of faecal pollution and in determining the level of river pollution. In Indonesia, according to government regulation, the water quality criteria are divided into four classes based on the number of coliform bacteria or *E. coli* as follows: class I = mild with a limit of 100 *E. coli* bacteria/100 ml and 1,000 coliform bacteria/100 ml, class II = moderate with a limit of 101–1,000 *E. coli* bacteria/100 ml and 1,001–5,000 coliform bacteria/100 ml, class III = intense, and class IV = very intense with the same limit, namely, 10,000 coliform bacteria/100 ml (Sholeh *et al.* 2018).

A study of the raw water of the Citarum River, one of the largest rivers in West Java Province, showed that the Citarum tributaries, including Cilebak, Cikaro, Cikapundung Hilir, and Cipadulun Hulu, have extremely high numbers of *E. coli* (class IV) (Junando *et al.* 2019). The coliform in the Citarum River is between 23×10^3 to more than 11×10^4 MPN/100 ml, categorised as crossing the water quality standard threshold (Zainun *et al.* 2016). Another study showed that the water of several wells near the Cikapundung River in Bandung, West Java, contained *E. coli*, with the highest index

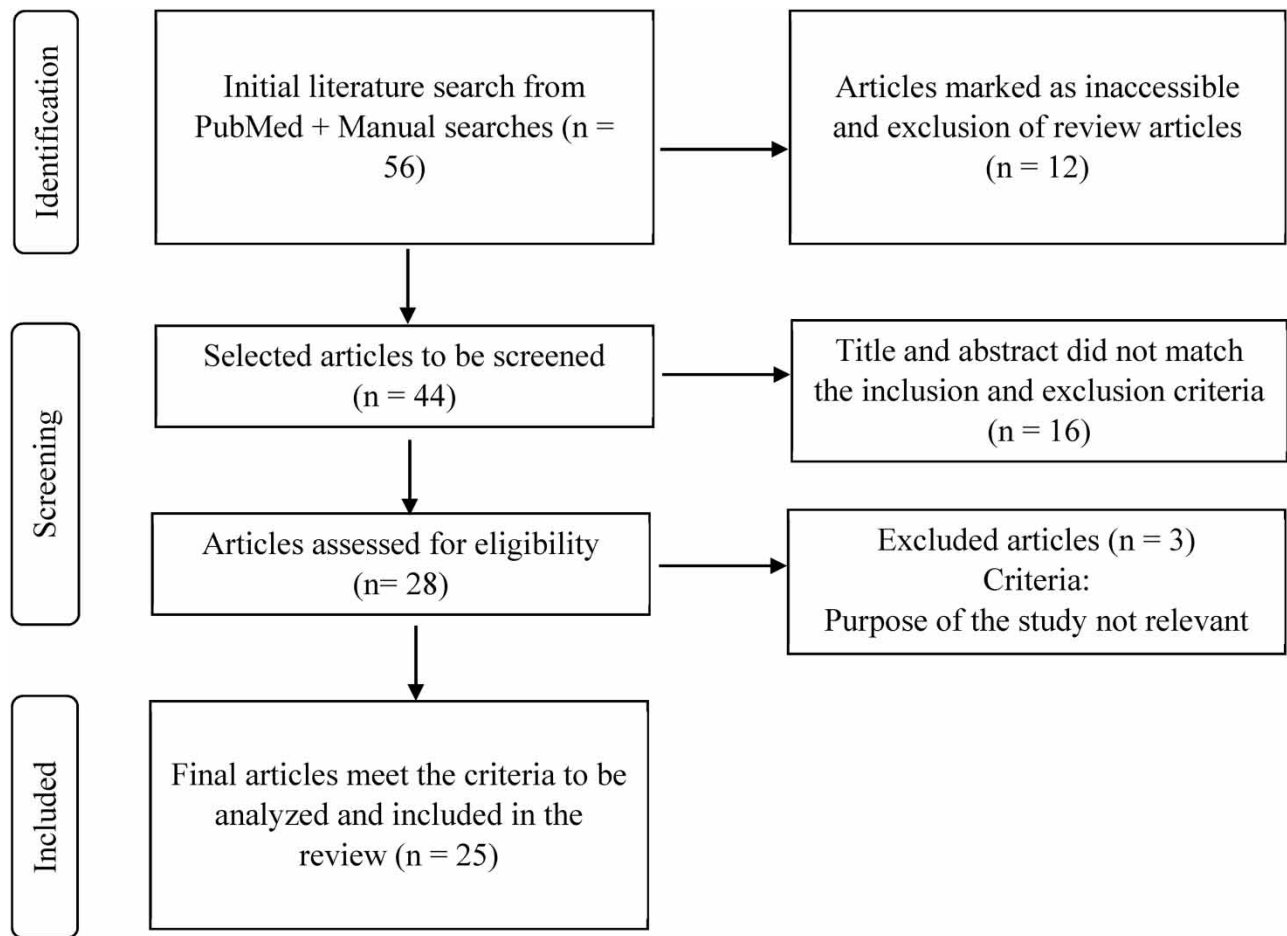


Figure 1 | Steps of literature review selection.

value reaching 210 MPN/100 ml. The distance between the river and the well was also significant: the shorter the distance, the higher the concentration of *E. coli* (Pradiko & Yustiani 2019). Safe levels of coliform bacteria in the water supply in wells are set at 50/100 ml and 10/100 ml, while that for *E. coli* in drinking water is 0/100 ml (Sekarwati *et al.* 2016).

Assessment of the water quality of Ciliwung River, which crosses Bogor Regency and Capital Region Jakarta, showed the growth of coliform bacteria with an index value as high as 1,100 MPN/100 ml (Puspitasari *et al.* 2016). This exceeds the coliform standard for drinking water. In addition, a study in Cimandiri River, West Java, also detected domestic sewage pollution and the presence of *E. coli* and other pathogenic bacteria with varying densities. The highest number of coliforms at the mouth of the Cimandiri River estuary reached 4.56×10^5 CFU/100 ml, while that for *E. coli* was 2.59×10^4 CFU/100 ml. The mean total coliform level was 4.61×10^3 CFU/100 ml, and that of *E. coli* was 7.61×10^2 CFU/100 ml, exceeded the water quality standard from KLHK (Afianti & Sutiknowati 2020).

Of the various factors that affect the quality of river water, throwing household waste into the river is a habit that is hard to get rid of, considering that people living along the river in big cities in Indonesia tend to turn their backs on the river. In addition to affecting the quality of river water, it also shows a lack of personal norms, awareness, and responsibility for the environment (Akmal 2021; Fakhri & Sa'id 2021). The Indonesian Central Bureau of Statistics estimates that an increase in data on the amount of waste in 2020 in 384 cities in Indonesia will reach 80,235.87 tons. Most of the waste dumped into the river is 58.2%, while 37.6% is in landfills (Kusminah 2018).

In the community around the Batang Bakarek-Karek River, Padang Panjang, West Sumatra, the behaviour of disposing of garbage to the river is most related to attitudes, as well as knowledge, infrastructure, and weak policy implementation (Yulida *et al.* 2016). The case of river pollution in the Teluk Dalam River, Banjarmasin, South Kalimantan, reflects the

relationship between river pollution and increased waste production due to population growth, especially household waste, which is not accompanied by concern for waste management (Muzaidi *et al.* 2018). In almost the same context, the Citarum River cleaning program has been carried out for over two decades to achieve open-defecation-free status. However, this has yet to be achieved, mainly due to the low public awareness and knowledge level (Rathomi & Nurhayati 2019).

The intensity of people's activities on the riverside influences the spread of microbial indicators. Research to identify the genetic relationship of *E. coli* isolates along the Riak River, Riau Province, using the random amplified polymorphic DNA polymerase chain reaction (RAPD-PCR) method shows that the level of *E. coli* in the low-risk area of contamination, Taping, is similar to that in the upper Perawang area which is 90 km away. Nevertheless, it differed markedly from those in Perawang 3 and 4 zones, residential areas with extensive bathing, washing, and toilet activities (Haryani *et al.* 2019). Most studies on several other rivers in Indonesia also show that the density of *E. coli* has exceeded the safe level of coliform bacteria in the water supply, as shown in Table 1, which indicates that water in various rivers in Indonesia is contaminated and can cause health problems, especially for people who use these rivers for their daily needs.

Table 1 | Report on identification and density or contamination level of *E. coli* or coliform in several rivers in Indonesia

Authors	Sample	Methods	Bacteria	Results
Pradiko & Yustiani (2019)	Cikapundung River, West Java, Indonesia	Most probable number (MPN)	<i>E. coli</i>	<ul style="list-style-type: none"> All well water from Babakan Ciamis village was positive for <i>E. coli</i>. The well closest to the Cikapundung River has an MPN value of 210/100 ml, and the farthest is 5.6/100 ml.
Puspitasari <i>et al.</i> (2016)	Ciliwung River Water	Most probable number (MPN)	Coliform	<ul style="list-style-type: none"> Coliform growth with as much as 1,100 MPN/100 ml value.
Afianti & Sutiknowati (2020)	Cimandiri River Water, Pelabuhan Ratu, West Java Province	Filter membrane and compact dry EC 'Nissui' for coliform and <i>E. coli</i> .	Coliform, <i>E. coli</i> , <i>Vibrio</i> sp., and <i>Aeromonas</i> sp.	<ul style="list-style-type: none"> Number of coliforms 4.56×10^5 CFU/100 ml and <i>E. coli</i> 2.59×10^4 CFU/100 ml. The mean (geomean) total coliforms were 4.61×10^3 CFU/100 ml and <i>E. coli</i> 7.61×10^2 CFU/100 ml.
Haryani <i>et al.</i> (2019)	Watershed Siak, Riau Province	Random amplified polymorphic DNA (RAPD)-PCR	<i>E. coli</i>	<ul style="list-style-type: none"> At 70% similarity, <i>E. coli</i> isolates from Tapung were in the same cluster with <i>E. coli</i> isolates from Perawang 1 and 2 but differed from Perawang 3 and 4.
Verawaty <i>et al.</i> (2020)	Aquatic ecosystems, Palembang, South Sumatra, Indonesia	Most probable number (MPN)	Coliform <i>E. coli</i>	<ul style="list-style-type: none"> Total coliform (TC) numbers ranged from 0 to >1,600 MPN/100 ml. Sampling sites with high MPN values of $\geq 1,600$ MPN/100 ml were Sekanak watersheds (SW1, 8, 11, 12, 13, and 14), followed by SW2 and SW9 with a value of 1,600 MPN/100 ml.
Adrianto (2018)	Lampung Province River Waters	Most probable number (MPN)	Coliform	<ul style="list-style-type: none"> The highest coliforms were found in the River I location ranging from 25,394 to 24,413 MPN/100 ml; the lowest was found at the location of River VII, ranging from 8,564 to 12,034 MPN/100 ml. River waters are contaminated with coliform bacteria (1,000 Total/100 ml).
Zainun <i>et al.</i> (2016)	Citarum River	Most probable number (MPN)	Coliform <i>Salmonella</i>	<ul style="list-style-type: none"> The coliforms and <i>Salmonella</i> were found in the Citarum River. Coliform growth was 23×10^3 to more than 11×10^4 MPN/100 ml.

3.3. Potential of waterbodies as an *E. coli* pathogen reservoir

Water and soil are less favourable for the survival of *E. coli* than their natural habitat in the digestive tract of humans and animals; however, depending on the environmental conditions, the *E. coli* pathogen (O157:H7) can survive in the environment for days to weeks. Even in oligotrophic environments with deficiencies of food and nutrients, such as surface water or groundwater, pathogenic *E. coli* can survive for 2 months at low densities (Avery *et al.* 2004). Pathogenic *E. coli* O157:H7 can efficiently utilise nutrients, including carbon, phosphate, and nitrogen, or perform physiological adaptations by reducing its cell size to increase the surface/volume ratio, enabling the absorption of nutrients at lower levels. This physiological state is known as the starvation-survival state (Peterson *et al.* 2005).

E. coli responds to environmental changes by adjusting physiological or metabolic conditions rather than carrying out structural modifications. *E. coli* grown under nutrient-adequate conditions (such as in faeces) tends to accumulate a reserve carbon source and store it for use in nutrient-poor environments. This high catabolic flexibility enables *E. coli* O157:H7 to survive in secondary habitats such as water and soil environments (Avery *et al.* 2004). *E. coli* O157:H7 cells will enter the general stress response phase in starved or stressed conditions. The regulatory gene *rpoS* (an RNA polymerase subunit involved in survival starvation) regulates the transcription of a series of genes responsible for the general stress response (Hengge-Aronis 2002), thus making *E. coli* adapt and tolerate various stress conditions.

E. coli O157:H7 also responds to environmental changes by facilitating the system for efficiently transporting preferred and easily exploited target nutrients or using other more abundant sources of nutrients. For example, phosphate is a highly preferred nutrient source because it can regulate organisms' growth rates. However, it often becomes limited after use, which is common in freshwater environments. When the availability of inorganic phosphate (Pi) becomes limited (<4 M), *E. coli* activates *Pho*-regulon (Crépin *et al.* 2011). This virulence factor allows efficient use of the limited Pi to maintain survival.

3.4. Potential of watershed for spreading antibiotic resistance

Various conditions contribute to antibiotic-resistant pathogenic strains in the environment, such as the non-selective use of antibiotics in livestock and fisheries, redeemed antibiotics without a prescription, animal husbandry, household waste pollution, industry, and even sewage from hospitals.

3.4.1. Routes by which aquatic ecosystems are exposed to antibiotics

Improper waste management that causes contaminated soil and water can be the main route for developing ARBs and spreading ARGs in the environment. One is hospital wastewater (HWW) containing antibiotic residue and other micropollutants, usually discharged into the global sewage system and then treated with municipal wastewater (UWW) at the nearest WWTP. The problem is that antibiotic substances in aquatic environments cannot be separated from WWTP, and traditional urban WWTP systems are usually not designed for HWW. Therefore, the treated effluent may still contain undegradable pollutants in the WWTP, including antibiotic residues. Furthermore, most HWW treatment technologies in Indonesia do not address the potential for antibiotic contamination.

Kurniawan *et al.* identified several types of antibiotics in the waste treatment unit of a hospital in Indonesia, which showed the potential for antibiotic contamination in HWW (Kurniawan *et al.* 2019). In addition, research conducted in the Midlands, England, UK, indicated that the disposal of WWTP wastewater into rivers increases antibiotic resistance in rivers because they act as reservoirs for clonal lineages mediating ARG (Amos *et al.* 2014). Antibiotic molecules in water can interact with bacteria and induce antibiotic selection pressure or a favoured mutation. However, the ARB and emerging ARG will persist even without pressure. Furthermore, vertical gene transfer or HGT can occur. The subsequent transmission of the resistance gene to human pathogens is of particular concern.

Using antibiotics in livestock also contributes to the pollution of the aquatic environment. The Indonesian government has strictly prohibited the use of antibiotics for livestock growing purposes. However, the implementation still needs to meet expectations. Several reasons include the lack of smooth communication between the central and regional governments due to the tiered decentralisation pattern, so each district tends not to follow central policies. In addition, the number of veterinarians working in the government sector is still limited, and these veterinarians are urgently needed to guide and supervise farmers (Siahaan *et al.* 2022).

Following a two-year study of AMU in livestock in Indonesia, from 2014 to 2016, approximately 61.96–99.56% of the types of antibiotics used were important for animals and primarily for non-bacterial diseases (Yusuf *et al.* 2018). Meanwhile, most chicken farmers in Malang, East Java, have been using the same antibiotics for the past 3 years, with doses based on the

average weight of the chickens (Zalizar *et al.* 2015). Therefore, some bacteria may have been resistant to antibiotics. In chicken farms in Blitar Regency, East Java, a very high prevalence of antibiotic resistance was found around 90–100%, with an MDR *E. coli* prevalence of 95.9% which was correlated with several risk factors such as type of chicken, farmer's knowledge, type of feed, veterinary support, antibiotic programs, and references for antibiotic administration (Wibisono *et al.* 2021). A study among broiler breeders in Subang Regency, West Java, also showed that attitudes, knowledge, and practices on the use of antibiotics were generally low and poor (Purnawarman & Efendi 2020). Breeders' attitudes, perceptions, and preferences are needed to increase knowledge and make better decisions (Lauwere & Bokma 2019). A survey of the perceptions of chicken farmers in West Kalimantan Province revealed a need for more understanding of antibiotic resistance and low mastery of technology due to a lack of human resources for education and supervision (Sumambang *et al.* 2019).

Various antibiotics also have been widely used to treat and prevent infectious diseases in fish and shrimp ponds in Indonesia (Rukyani & Supriyadi 2000). Through the Minister of Marine Affairs and Fisheries Regulation, Indonesia has set values of maximum residue limit (MRL); shrimp cultured in sample ponds must meet this MRL; however, shrimp farmers often apply organic fertilisers such as chicken manure to improve primary productivity in ponds (Supriyadi & Rukyani 2000). It is assumed that the aquatic environment in Indonesia is a vector for the spread of ARG among human and animal populations and is also a pathway for ARG to develop and be introduced into natural ecosystems. Figure 2 presents a schematic diagram of the possible route of antibiotics into the aquatic environment in Indonesia.

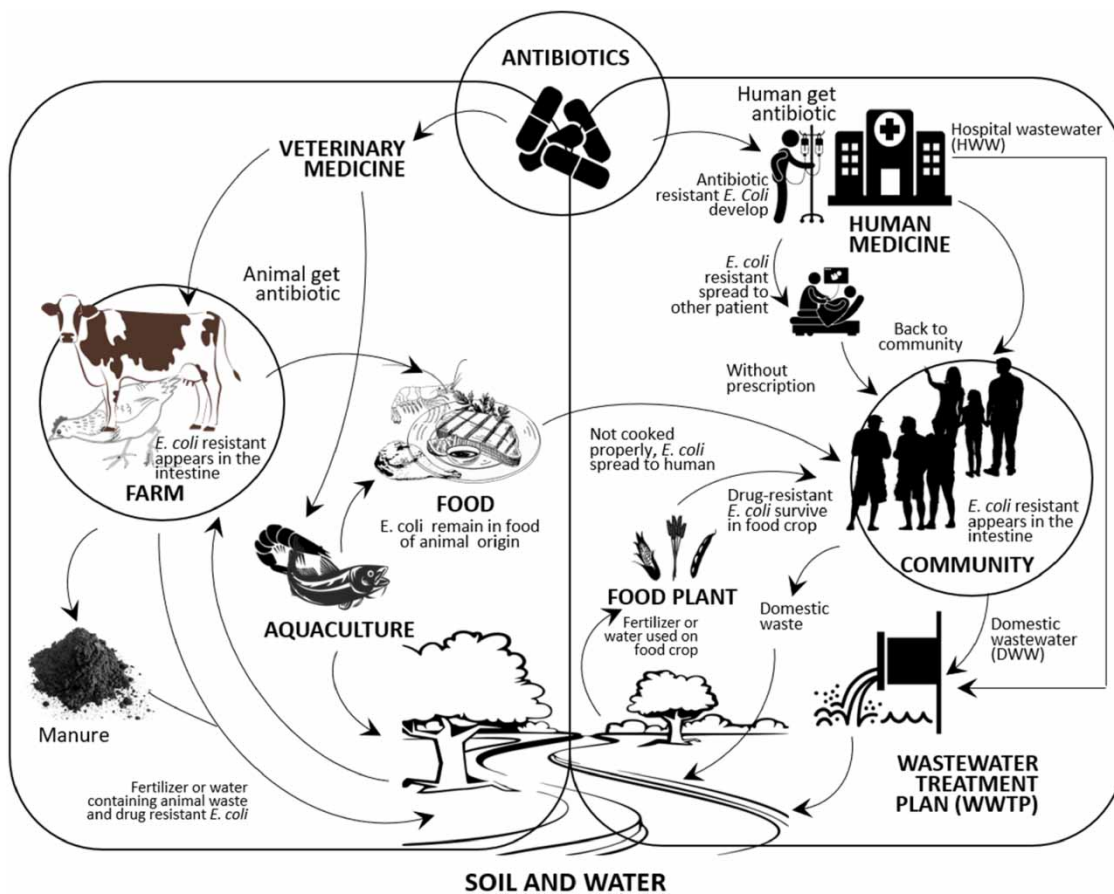


Figure 2 | Possible routes of antibiotic exposure to aquatic ecosystems in Indonesia. This image shows the environmental compartments where animals and humans use antibiotics are mobilised. The arrows indicate the relationship between the compartment and the aquatic ecosystem. The presence of veterinary antibiotics in soil and aquatic systems is mainly due to their use in aquaculture or animal husbandry. The antibiotics administered are removed from the animals, and the application of animal manure to fertilise the soil is a route to spread the antibiotics in the environment. Residents' habit of throwing waste into the river also affects river water quality. On the other hand, antibiotics used in human medicine can enter natural ecosystems mainly due to the inadequate removal of these compounds in WWTP.

3.4.2. Mechanisms of antibiotic resistance and spread of resistance genes in aquatic environments

3.4.2.1. Biofilm facilitates tolerance and resistance of *E. coli* to antibiotics. One of the essential strategies by which bacteria deal with antibiotics is forming a biofilm. There are several reasons why forming a biofilm is beneficial to bacteria. First, the matrix polymeric substance prevents drug diffusion at bactericidal concentrations (Pinto *et al.* 2020). Second, biofilm-forming bacteria tend to produce β -lactamase enzymes in the biofilm matrix or enzymes that can promote the release of antibiotics through efflux pumps (Ciofu & Tolker-Nielsen 2019). Third, some biofilm-forming bacteria have extracellular DNA (eDNA). Although not widely studied, antibiotic-resistant *E. coli* has a *tnaA* gene that encodes the enzyme tryptophanase, associated with pH regulation. Transcriptomic analysis revealed that *tnaA* strains downregulated pH homeostatic pathways to perform specialised functions, including enabling survival after antibiotic treatment (Goode *et al.* 2021). Fourth, the biofilm matrix comprises a community of microorganism cells and an increase of genetic competence and accumulation of MGE that enable the biofilm to promote the acquisition and spread of ARGs (Balcázar *et al.* 2015). Bacteria in biofilms have also shown the ability to generate various molecular strategies to protect themselves from hostile conditions, including slow growth rates, changing their metabolic status to inert or 'persister' (at which stage antibiotics would be ineffective), or other genetically based resistance mechanisms (Ito *et al.* 2009; Pinto *et al.* 2020).

3.4.2.2. Co-selection in the mechanism of antibiotic resistance of *E. coli*. Bacteria respond to the antibiotic exposure by developing strategies that can be genetically mediated through acquiring resistance genes from other bacteria or intrinsic resistance. However, the relationship between antibiotic use and antibiotic resistance is complex. Bacteria may not only choose resistance to the same antibiotic or 'selection' but also for resistance to other antibiotics and also various non-antibiotic compounds such as biocides and heavy metals, otherwise known as 'co-selection'. Metals are essential micronutrients for cellular functions and biological macromolecular components. However, they can trigger the co-selection of antibiotic resistance if they accumulate to critical concentrations (Seiler & Berendonk 2012).

In aquatic ecosystems, exposure of bacteria to various chemical pollutants, including metals, allows them to develop resistance to different compounds, even to compounds to which they have not been exposed, thereby increasing the risk of selection of organisms that can adapt to antibiotic agents. Elfidasari *et al.* identified that the concentrations of lead (Pb), Cd, and Hg in Ciliwung River, Jakarta, Indonesia, had exceeded the pollution threshold standard based on Indonesian government regulation (Elfidasari *et al.* 2019). Meanwhile, the estimated total metal pollutant loads of Pb, Cu, and Zn showed that the waters of the Siak River in the administrative area of Pekanbaru City, Riau Province, have suffered very high metal pollution (Agustina & Amin 2012). The Pb concentration in Cirata Reservoir, West Java, Indonesia, has also been reported to have exceeded the quality standard threshold. The heavy metal Pb in Cirata Reservoir is mainly produced from ship/boat traffic, industrial waste, and household waste flowing into river water that enters the reservoir (Nurfadhilla *et al.* 2020).

3.4.2.3. Spread of antibiotic resistance bacteria and gene in aquatic environments. Several studies have shown a link between *E. coli* isolates and the prevalence and pattern of antibiotic resistance in Indonesian aquatic environments. A study in river water and household water along Code River in Sleman, Jogjakarta, showed that *E. coli* strains were particularly resistant to amoxicillin and streptomycin (Sasongko 2014). In lake water at the National Institute of Science and Technology (ISTN) in Jagakarsa, Jakarta, 100% of *E. coli* isolates were found to be resistant to amoxicillin, but 75% were still sensitive to tetracycline and chloramphenicol, and 100% were still sensitive to ciprofloxacin (Syafriana *et al.* 2020). Antibiotic-resistant *E. coli* are also isolated from several aquatic ecosystems in Palembang (Verawaty *et al.* 2020). Another study in Siak Watershed, Riau Province, showed that all *E. coli* isolates resist erythromycin antibiotics (Haryani *et al.* 2019). Data from various reports of antibiotic resistance patterns of *E. coli* isolates from samples of the aquatic environment in Indonesia are shown in Table 2.

Globally, there has been a tendency for MDR *E. coli* to increase and spread in aquatic ecosystems. Fifteen percent of bacterial isolates from Basaseachic Falls, Chihuahua, Mexico, were MDR phenotype (Delgado-Gardea *et al.* 2016). Meanwhile, 19% of *E. coli* isolates from the Tagus estuary in southern Portugal were multi-resistant (Pereira *et al.* 2013). Moreover, 9.48% of *E. coli* isolates from several water sources in Ghana were resistant to two or more antibiotics (Odonkor & Addo 2018). Research in Uthi River, Kenya, showed that about 33% of *E. coli* isolates had the *bla*CTX-M gene (Wambugu *et al.* 2018). This gene encodes plasmid-mediated extended-spectrum β -lactamase (ESBL), mainly conferring third-generation cephalosporin resistance. As well as polluted urban wetlands in Ibadan, southwest Nigeria, contained MDR *E. coli* with the

Table 2 | Report on resistance patterns and antibiotic-resistance genes in *E. coli* isolates from several aquatic environments in Indonesia

Authors	Sample	Methods	Bacteria	Results
Haryani <i>et al.</i> (2019)	Siak Watershed, Riau Province	Random amplified polymorphic DNA (RAPD)-PCR	<i>E. coli</i>	– All isolates of <i>E. coli</i> are resistant to erythromycin.
Sasongko (2014)	Boyong River Water, Sleman Regency	Kirby-Bauer method modified Mueller Hinton Agar (MHA)	<i>E. coli</i>	– Resistance to amoxicillin 80 and 66.7%, chloramphenicol: 20 and 6.7%; sulfamethoxazole: 33.3 and 46.7%, and streptomycin 73.3 and 86.7%
Verawaty <i>et al.</i> (2020)	Aquatic ecosystems, Palembang, South Sumat	Kirby-Bauer method	Coliform <i>E. coli</i>	– 82% of <i>E. coli</i> were resistant to ampicillin, 57% to tobramycin, and 71% to tetracycline. – The isolates showed an intermediate profile to kanamycin (50%), 57% cotrimoxazole, 50% cefixime, and 54% gentamycin.
Syafriana <i>et al.</i> (2020)	Lake Water ISTN Jakarta	Disc diffusion method – MHA	<i>E. coli</i>	– <i>E. coli</i> isolates showed 100% resistance to amoxicillin. – 75% sensitivity to tetracycline and chloramphenicol, and 100% to ciprofloxacin.

*bla*CTX-M-15 gene on the *IncF* plasmid (Adelowo *et al.* 2020). Meanwhile, the *bla*CTX-M-15 gene in *E. coli* isolates was also detected from river sediments that serve as WWTP disposal sites in the Midlands, England, UK, with *bla*CTX-M-15 increasing dramatically downstream of the WWTP (Amos *et al.* 2014). Moreover, the emergence of extensively drug-resistant (XDR) *E. coli* strain N7, resistant to more than 15 antibiotics and carbapenems, was recently reported in the influent of Jungnam WWTP in Han River, Seoul, South Korea (Shin *et al.* 2021). Reports of antibiotic resistance of *E. coli* isolates from the aquatic environment in several regions worldwide are shown in Table 3.

3.4.3. Mechanisms of antibiotic resistance in aquatic ecosystems

Conjugation is one of the main strategies behind the spread of antibiotic resistance in aquatic ecosystems. Conjugation involves the transfer of genetic material by cell-to-cell contact and MGE. The key players in this mechanism are plasmids, transposons, and integrons, which ensure genetic exchange in bacteria. The conjugative plasmids and antibiotic resistance in *E. coli* isolates are detected from water samples in several regions of Tanzania, the most common being the plasmid *IncF*, followed by *IncI1* and *IncA/C* (Lyimo *et al.* 2016). In addition, clinically relevant carbapenemase genes were present in plasmids in *Enterobacteriaceae* isolates from rivers and canals throughout Switzerland (Bleichenbacher *et al.* 2020). Moreover, in the coastal waters of the Berlenga Archipelago, Portugal, *E. coli* isolates were shown to possess plasmid-mediated resistance to quinolones, *qnrS1* and *qnrB19*, as well as the *bla*CTX-M-15 gene (Alves *et al.* 2014). Furthermore, 50% of the total strains isolated from the Nile in Egypt had plasmids conferring resistance to half of the antibiotics tested (Abdel Rahim *et al.* 2015). Meanwhile, *E. coli* isolates from river water from Guanabara Bay showed an MDR profile with at least one plasmid band (Dias Gonçalves *et al.* 2019).

An integron is a genetic platform that catalyses co-selection because it allows bacteria to cut and rearrange gene cassettes, especially when faced with environmental stresses. Integrons can be classified into two types: (1) mobile integrons, which are closely related to plasmids and transposons and are the primary vehicle for multi-resistance in bacteria and (2) chromosomal integrons, which are the most prominent and static element (Escudero *et al.* 2015). Integrating integrons into plasmids enables them to function as highly efficient molecular instruments by which bacteria can acquire or express ARGs.

Class 1 integrons are the most common type found in enteric bacteria, primarily in conjugative plasmids. Approximately 10% of *E. coli* isolates from WWTP waste contain class 1 integrons, mainly the *aadA1/dhfrI* gene cassette. All of these integron-containing isolates were shown to be resistant to ciprofloxacin and cephalothin (Ferreira Da Silva *et al.* 2007). In *E. coli* isolates from farmed animals, the rate of class 1 integrons ranged from 23 to 52%, almost comparable to the incidence of human clinical *E. coli* isolates. Furthermore, *E. coli* isolates in livestock and humans were shown to most often contain the gene cassettes *dfrA17-aadA5*, *dfrA12-orfF-aadA2*, and *aadA1* (Kang *et al.* 2005), indicating that class 1 integrons disseminate in both human and animal *E. coli* strains. Perez-Etayo *et al.* also identified widely distributed class 1 integrons, especially the *intI1* gene, in several food products, livestock and feed, aquatic environments, and humans (Pérez-Etayo

Table 3 | Global report on resistance patterns and antibiotic-resistance genes in *E. coli* isolates from aquatic environments

Authors	Sample	Methods	Bacteria	Results
Delgado-Gardea <i>et al.</i> (2016)	Basaseachic Falls, Chihuahua National Park, Mexico	Biochemical identification with AutoSCAN-4 system, broth dilution susceptibility test	<i>Klebsiella oxytoca</i> <i>E. coli</i> <i>Enterobacter cloacae</i>	<ul style="list-style-type: none"> – 33% bacteria were isolated; 53% were resistant to one antibiotic, and 15% had MDR phenotype. – MDR were identified as <i>K. oxytoca</i>, <i>E. coli</i>, and <i>E. cloacae</i>. – Some resistant to cefazolin, cefuroxime, ampicillin, and ampicillin-sulbactam.
Amos <i>et al.</i> (2014)	Sediment from rivers 300, 600, and 900 m upstream and downstream of a large WWTP in the Midlands, England, UK	qPCR 16S rRNA gene	<i>E. coli</i>	<ul style="list-style-type: none"> – Detection of the <i>blaCTX-M-15</i> gene – The prevalence of <i>blaCTX-M-15</i> increased dramatically downstream of the WWTP.
Odonkor & Addo (2018)	Multiple water sources, including dams, rivers, and canals in Ghana	API and conventional methods and antibiotic susceptibility testing: Kirby-Bauer method	<i>E. coli</i>	<ul style="list-style-type: none"> – <i>E. coli</i> represented 18.7% of the total bacterial isolates. – 9.48% of <i>E. coli</i> isolates were resistant to two or more antibiotics.
Wambugu <i>et al.</i> (2018)	Athi River, Kenya	PCR	<i>E. coli</i>	<ul style="list-style-type: none"> – 22% of isolates had <i>blaTEM</i>, 33% had <i>blaCTX-M</i>, and 28% had <i>blaCMY</i>. – 36% of class 1 integron-positive isolates are mostly isolated near wastewater from WWTP.
Pereira <i>et al.</i> (2013)	Tagus Estuary in southern Portugal	REP-PCR	<i>E. coli</i>	<ul style="list-style-type: none"> – The highest resistance to streptomycin and tetracycline, followed by β-lactams and sulfonamides. – 65.16% of <i>E. coli</i> isolates resisted one antibiotic and 19% were multi-resistant. – Phylogenetically, <i>E. coli</i> groups A and D were more dominant and were characterised by a higher prevalence of antibiotic resistance. – In 12% of isolates carrying class 1 integrons, <i>intI1</i> and <i>intI2</i> genes were found, and there was a strong correlation between the presence of integrons and MDR.
Pérez-Etayo <i>et al.</i> (2018)	The aquatic environment and from other sources such as humans, food products, livestock, and feed in Southern Spain	PCR	<i>E. coli</i>	<ul style="list-style-type: none"> – Class 1 integron (<i>intI1</i>) was widely distributed in most <i>E. coli</i> and all sources, while the prevalence of <i>intI2</i> was lower.
Adelowo <i>et al.</i> (2020)	Urban wetlands in southwest Nigeria	Whole genome sequencing and qPCR	<i>E. coli</i>	<ul style="list-style-type: none"> – The <i>blaCTX-M-15</i> was identified on the <i>IncF</i> plasmid. – All isolates carrying <i>blaCTX-M-15</i> showed resistance to aztreonam, ceftazidime, ciprofloxacin, and sulfamethoxazole.

(Continued.)

Table 3 | Continued

Authors	Sample	Methods	Bacteria	Results
Singh <i>et al.</i> (2018)	Yamuna River, Delhi, India	PCR	<i>E. coli</i>	<ul style="list-style-type: none"> – The <i>blaTEM-1</i>, <i>blaCTX-M-15</i>, and <i>blaCMY-42</i> genes were identified in <i>E. coli</i> strains in Indian urban waters. – The <i>E. coli</i> isolate containing the <i>blaCTX-M-15</i> gene had an upstream <i>ISEcp1</i> insertion sequence <i>blaCTX-M-15</i>.
Shin <i>et al.</i> (2021)	WWTP stream in Jungnang, Han River, Seoul, South Korea	PCR and whole genome sequencing	<i>E. coli</i>	<ul style="list-style-type: none"> – Identification of <i>E. coli</i> strain N7, which produces the New Delhi metallo-β-lactamase (NDM-5) variant. – Extensive drug resistance (XDR) to 15 antibiotics and carbapenems at concentrations of doripenem (512 mg/L) and meropenem (256 mg/L).
Lyimo <i>et al.</i> (2016)	Water from several areas in Tanzania (Kilimanjaro, Arusha, and Manyara)	Membrane filtration and PCR	<i>E. coli</i>	<ul style="list-style-type: none"> – Detection of conjugative plasmids and antibiotic resistance in <i>E. coli</i>. – <i>IncF</i> plasmid was detected most frequently (49% of isolates), followed by <i>IncI1</i> and <i>IncA/C</i> types.
Bleichenbacher <i>et al.</i> (2020)	Rivers and canals throughout Switzerland	Phenotypic and NGS-based genotypic methods	<i>Enterobacteriaceae</i> <i>E. coli</i>	<ul style="list-style-type: none"> – Carbapenemase-producing <i>Enterobacteriaceae</i> (CPE) were detected. – Carbapenemase genes replicate and develop river ecosystem pollutants
Alves <i>et al.</i> (2014)	The coastal waters of the Berlenga Islands, Portugal	Disc diffusion method – MHA, PCR	<i>E. coli</i>	<ul style="list-style-type: none"> – Identified the resistance genes <i>blaTEM</i>, <i>sul1</i>, <i>sul2</i>, <i>tet(A)</i>, and <i>tet(B)</i>. – Detected genes that confer resistance to third-generation cephalosporins (<i>blaCTX-M-1</i> and <i>blaSHV-12</i>). – Genes resistance to quinolones (<i>qnrS1</i> and <i>qnrB19</i>) mediated by plasmids.
Abdel Rahim <i>et al.</i> (2015)	Nile water and groundwater in Sohag province, Egypt	PCR	<i>Shigella</i> <i>Salmonella</i> <i>E. coli</i> <i>Pseudomonas</i>	<ul style="list-style-type: none"> – Isolated 39 strains of bacteria, including <i>E. coli</i>. – 81% of strains were cefepime resistant, while 93.75% were sensitive to ciprofloxacin. – 50% of the total strains had plasmids resistant to 50% of antibiotics. – The plasmid-free strain (50%) was resistant to 48.7% of antibiotics.
Gonçalves <i>et al.</i> (2019)	Guanabara Bay, river water, Brazil and hospital clinical samples	Antibiotic susceptibility test, DNA plasmid extraction and PCR	<i>K. pneumoniae</i> subsp. <i>pneumoniae</i>	<ul style="list-style-type: none"> – AST on isolates from water samples similar to MDR profile to isolates from clinical materials.

(Continued.)

Table 3 | Continued

Authors	Sample	Methods	Bacteria	Results
			<i>K. pneumoniae</i> subsp. <i>ozaenae</i> <i>E. coli</i>	<ul style="list-style-type: none"> – All isolates from water samples showed at least one plasmid band. – 7.4% of isolates from water samples showed amplification products for three antimicrobial classes.
Koczura <i>et al.</i> (2015)	Water from four recreational lakes in Poznan, Poland	PCR	<i>E. coli</i>	<ul style="list-style-type: none"> – MDR coliform bacteria containing integrons carrying virulence genes. – The integron-carrying <i>E. coli</i> carried up to nine virulence-associated genes, with the highest frequencies being <i>kpsMT</i> (84.6%) and <i>traT</i> (78.3%).
Chakraborty <i>et al.</i> (2018)	WWTP wastewater and ponds in Dhaka, Bangladesh	PCR with gene-specific primers, antibody-blocking and phage-neutralization assays	<i>E. coli</i>	<ul style="list-style-type: none"> – The ETEC-CFs specific phages were found.

et al. 2018). Horizontal transfer of class 1 integrons via conjugative plasmids appears to be responsible for the widespread distribution of certain types of such integrons.

Several studies identified class 1 integrons in *E. coli* strains in aquatic ecosystems. Approximately 12% of *E. coli* isolates from the Tagus estuary, Portugal, were shown to contain *intI1* and *intI2* genes. In addition, a strong correlation was identified between integrons and multi-resistance isolates of *E. coli* carrying a class 1 integron (*intI1* gene) in rivers and WWTPs in Navarra, Spain (Pereira *et al.* 2013; Pérez-Etayo *et al.* 2018). Fluoroquinolone-resistant *E. coli* strains in San Pedro River, Aguascalientes, Mexico, were also shown to contain integrons (Ramírez *et al.* 2013). Meanwhile, Wambugu *et al.* (2018) identified that 36% of *E. coli* isolates in Athi River, Kenya, were positive for class 1-bearing integral plasmids, most of which were near WWTP wastewater areas. This finding is not markedly different from what Koczura *et al.* found in Poland, which identified more integron-1 genes downstream of a WWTP (Koczura *et al.* 2012; Wambugu *et al.* 2018). Integron-carrying *E. coli* isolated from four recreational lakes in Pozna, Poland, were shown to carry nine virulence-related genes, with the most common being *kpsMT* (84.6%) and *traT* (78.3%) genes (Koczura *et al.* 2015). The ability of *E. coli* to carry plasmid-borne class 1 integron suggests that the ARG encoded can easily be transmitted between bacteria and even between commensal and pathogenic *E. coli* strains. Singh *et al.* (2018) stated that *E. coli* isolates with class 1 integron from Yamuna River, Delhi City, India, were more common than reported globally and presented together with the *blaTEM-1* gene, *blaCTX-M-15*, and *blaCMY-42* encoding the β -lactamase enzyme (Singh *et al.* 2018), indicated the simultaneous transfer of the resistance gene cassette and the β -lactamase gene. Figure 3 shows the proposed mechanism of antibiotic resistance of *E. coli*.

In addition to MGE, bacteriophages, viruses that infect bacteria, can also act as efficient vectors for acquiring and disseminating ARGs. Therefore, they can be an essential source of antibiotic resistance to promote high survival capacity and viability and maintain abundance or density in aquatic environments. Phage-specific ETEC-colonization factors (CFs) were present in samples of WWTP and five ponds in Dhaka, Bangladesh (Chakraborty *et al.* 2018). Aquatic ecosystems such as rivers are secondary habitats for *E. coli* pathogens. If river sanitation is poor and there is contamination with faecal matter, *E. coli* pathogens can spread and maintain resistant clones. Therefore, controlling the use of antibiotics and improving the sanitation of aquatic or river ecosystems should help to reduce antibiotic resistance, especially in Indonesia.

4. CONCLUSION

Household activities in rivers, intensive livestock and agriculture, and domestic wastewater are critical factors in the spread of *E. coli* in aquatic ecosystems in Indonesia. The ability of pathogenic *E. coli* to adapt efficiently in secondary habitats is also a

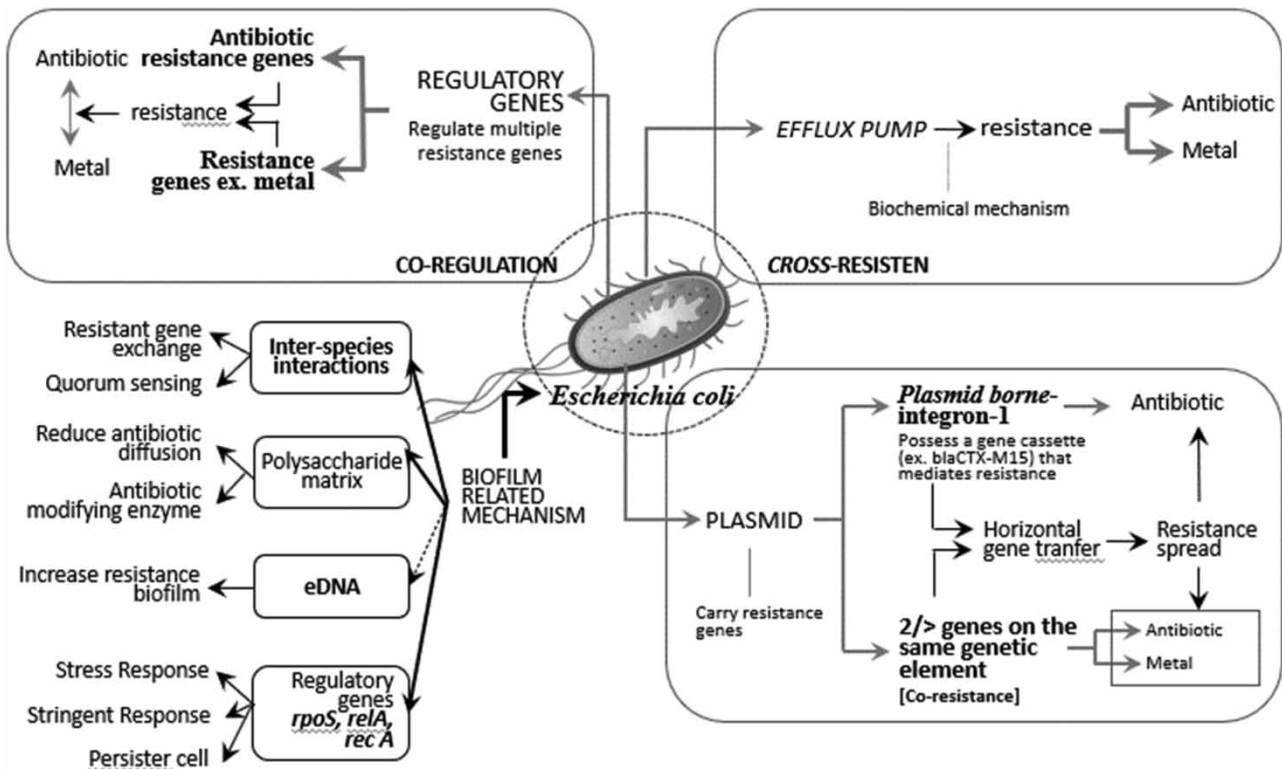


Figure 3 | Proposed mechanism of antibiotic resistance of *E. coli*. Antibiotic resistance *E. coli* can be selected through the following mechanisms: (1) Co-resistance occurs when two or more genes are located on the same genetic element (such as a plasmid), (2) Cross-resistance occurs when one resistance mechanism provides resistance to different substances (e.g. antibiotics and metals), and (3) Co-regulation which occurs when resistance genes for different substances (e.g. antibiotics and metals) are regulated by one regulatory gene. Biofilms facilitate survival and resistance to environmental stress and antibiotics, including through the role of the extracellular polysaccharide matrix.

crucial factor. Meanwhile, the presence of resistance genes and plasmid-borne class 1 integron in the genetic makeup of *E. coli* facilitates the transfer of resistance genes between bacteria, including between pathogenic and commensal *E. coli* in aquatic environments, which indicates that water in various rivers in Indonesia has the potential to become a reservoir of *E. coli* pathogens, as well as the spread of antimicrobial resistant strains.

Indonesia has implemented a policy to suppress the unwise use of antibiotics in the livestock sector. However, it has not been optimal due to several obstacles, such as the lack of strict law enforcement officers, limited budget, facilities and infrastructure, weak coordination, and low knowledge and compliance of farmers with applicable regulations. Staff resources in charge of animal husbandry and livestock health play an essential role in the supervisory function. Therefore, it is necessary to provide resources and professionalism in the process of supervision to prevent irregularities and violations of the use of antibiotics in livestock. In addition, there is a need for ongoing guidance on the use of antibiotics and adequate budget availability.

Pollution of rivers by garbage is still part of the waste problem in Indonesia, primarily related to the entry of waste on land into the aquatic environment. This condition is not only triggered by inadequate waste disposal and management facilities; but also related to the behaviour of throwing garbage into the river due to a lack of awareness of personal norms, awareness of consequences, and a sense of responsibility for the environment. Law enforcement and quality services, infrastructure, and waste management are needed. In its implementation, counselling and outreach to the community should be preceded, preferably by involving officers from related agencies, non-governmental organisations, and local community leaders so that messages can be received and conveyed to the community. The empowerment that involves local wisdom is likely to be effective in building habits and changing character and behaviour to respect the surrounding environment.

Meanwhile, to avoid pollution of water sources due to industrial waste, the Government of Indonesia has regulated procedures and requirements for managing hazardous and toxic waste materials. Policy implementation must involve every

related element, such as the bureaucracy, society, the private sector, and non-governmental organisations, in every aspect of activity or business. Staff resources to implement industrial waste control policies are essential in quantity, quality, financing, and experience. In addition, it is necessary to pay attention to the bureaucratic structure related to the division of tasks according to the field, procedures, and coordination between work units. The need for a more in-depth understanding of the private sector that produces waste to carry out its obligations also requires more serious attention.

The study limitation encountered while compiling this literature review was the difficulty in finding literacy, primarily due to limited studies or articles in Indonesia that matched the title of the literature review. In addition, heterogeneity analysis was not carried out in each study used. This limitation also opens up opportunities for preparing a meta-analysis to assess data heterogeneity and form more quantitative conclusions in the future. Finally, given the limited data in Indonesia regarding the pattern of distribution and mechanism of antibiotic resistance in pathogenic *E. coli* in aquatic environments, further research is needed through the next-generation sequencing approach and the reference pathogen database in explaining the various specific genes that underlie physiological and metabolic flexibility, co-selection or emergence, and transfer of ARGs, as well as knowing the diversity of enteric and environmental pathogens to gain a better understanding of the characteristics of *E. coli* in Indonesian aquatic ecosystems.

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DATA AVAILABILITY STATEMENT

All relevant data are included in the paper or its Supplementary Information.

CONFLICT OF INTEREST

The authors declare there is no conflict.

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