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Review

Strategy to Combat Antibiotic Resistance Bacteria and Genes in Wastewater in Developing Countries

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Abstract: Antibiotic resistance is a major public health issue that requires a multifaceted approach. One potential source of antibiotic-resistant bacteria is wastewater in developing countries, which often lacks proper treatment infrastructure and can release high levels of antibiotics and antibiotic-resistant bacteria into the environment. This review article summarizes current knowledge on strategies to combat antibiotic resistance in wastewater in developing countries. Our review highlights the importance of improving wastewater treatment infrastructure to effectively remove antibiotics and antibiotic-resistant bacteria, implementing measures to reduce the release of antibiotics into the environment, and monitoring and surveillance to track the presence and spread of antibiotic-resistant bacteria in wastewater. We also discuss the potential challenges and barriers to implementing these strategies and the need for further research to determine their effectiveness in real-world settings. Overall, this review highlights the need for a comprehensive approach to address antibiotic resistance in wastewater in developing countries and underscores the importance of addressing this issue to protect public health.

Keywords: antibiotic resistance; bacteria; gene; developing countries

1. Introduction

This review elaborates on methods to address issues of bacteria and genes that produce antibiotic resistance in wastewater in underdeveloped nations. The antibiotic resistance gene is dangerous to health and the environment (1). In this case, the antibiotic-resistance gene may be defined as a mutation in a bacterial chromosome that makes it resistant to a particular antibiotic. Increasingly drug-resistant pathogens, which include bacteria and viruses, are spreading in wastewater and causing significant impacts on water quality, public health, and food safety (2). Antibiotic resistance bacteria in wastewater are a severe issue threatening the health of humans and animals worldwide (3). Developing countries have been more vulnerable to this threat than developed countries because of geographical and socioeconomic factors (4). An effective strategy could be to improve water treatment plants in developing countries to remove antibiotic residues, thereby reducing the threat of antibiotic resistance by preventing the entry of these resistant genes and bacteria into essential water sources.

Resistance can lead to treatment failures and the spread of antibiotic-resistant infections, which can be challenging to treat and have serious consequences, including increased morbidity and mortality (3-4). The World Health Organization (WHO) has identified antibiotic resistance as one of the greatest threats to global health. It is estimated that 700,000 deaths worldwide are caused by antibiotic-resistant infections each year. One potential source of antibiotic-resistant bacteria is wastewater, which can harbor high levels of antibiotics and antibiotic-resistant bacteria and genes. In developing countries, where wastewater treatment infrastructure is often inadequate, untreated sewage can be released into the environment, spreading antibiotic-resistant bacteria and genes. Common antibiotic resistance genes found in wastewater include genes conferring resistance to tetracyclines (such as tetA and tetB), beta-lactams (such as blaCTX-M, blaKPC, blaTEM, blaSHV, blaAMPC, and blaTEM), and sulfonamides (such as sul1 and sul2) (38-39). In addition, Common antibiotic-resistant bacteria found in wastewater treatment plants include Escherichia coli, Klebsiella

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pneumonia, Pseudomonas aeruginosa, and Enterococcus species. These bacteria can harbor various antibiotic resistance genes, including those that confer resistance to tetracyclines, beta-lactams, sulfonamides, and multiple classes of antibiotics (such as the multidrug resistance gene, mecA) (39-40). This is a significant issue in nations that are still growing and developing, as these countries often have limited access to effective antibiotics and may be high vulnerable to the negative impacts of antibiotic-resistant infections. This review article summarizes current knowledge on strategies to combat antibiotic resistance in wastewater in developing countries. We focus on three main areas: improving wastewater treatment infrastructure to effectively remove antibiotics and antibiotic-resistant bacteria, implementing measures to reduce the release of antibiotics into the environment, and monitoring and surveillance to track the presence and spread of antibiotic-resistant bacteria in wastewater. We also discuss the potential challenges and barriers to implementing these strategies and the need for further research to determine their effectiveness in real-world settings. Our review highlights the need for a comprehensive approach to address antibiotic resistance in wastewater in developing countries and underscores the importance of addressing this issue to protect public health.

2. Materials and Methods

The materials and methods section of this review article describes the process we followed to identify and review relevant studies on strategies to combat antibiotic resistance in wastewater in developing countries. Specifically, we conducted a comprehensive literature search using several electronic databases and identified 43 studies that met our inclusion criteria. These studies were published between 2010 and 2021 and were written in English. We used a combination of keywords such as "antibiotic resistance," "wastewater," "developing countries," "treatment," "prevention," and "surveillance" to identify relevant studies. In addition to the electronic database search, we manually searched reference lists of relevant articles to identify additional studies.

Once we had identified the relevant studies, we reviewed and analyzed them to extract information on strategies to combat antibiotic resistance in wastewater in developing countries. This information was organized into the three main areas outlined in the introduction: improving wastewater treatment infrastructure, implementing measures to reduce the release of antibiotics into the environment, and monitoring and surveillance. For each of these areas, we provide a summary of the current state of knowledge, including existing strategies and interventions and potential challenges and barriers to implementation. Our review aims to provide a comprehensive overview of current knowledge on strategies to combat antibiotic resistance in wastewater in developing countries and to identify areas in need of further research. Several methods can be used to detect antibiotic resistance genes and bacteria in wastewater treatment plants, including culture-based methods and molecular biology-based methods.

Culture-based methods involve growing bacteria from wastewater samples on agar plates containing various antibiotics. The bacteria that can grow in certain antibiotics are resistant to those antibiotics. This method can be time-consuming and may only detect some antibiotic resistance genes or bacteria present in a wastewater sample. Molecular biology-based methods, such as polymerase chain reaction (PCR) and quantitative PCR (qPCR), can detect specific antibiotic resistance genes in wastewater samples. This method can be more sensitive and specific than culture-based methods but requires specific reagents, equipment, and trained personnel. Metagenomics approaches such as next-generation sequencing (NGS) can also be used to identify the resistance gene and the bacteria that harbor it. This can give a complete picture of the resistance profile of the wastewater (40). Among the 43 articles we reviewed, our search finds several methods for antibiotic resistance genes in wastewater, including PCR and real-time qPCR. It is noteworthy that the materials most commonly used in PCR are materials used in PCR and real-time qPCR are DNA molecules, thermostable DNA polymerase or DNA replicate, radioactively labeled primers, 5-ATP, MgCl2, and one mM DTT (6). Among these, all PCR, real-time qPCR, and metagenomics methods are best for analyzing scientific samples. These techniques can be used to check the presence or absence of different types of genes in biological samples or even determine their levels in samples. First, prepare the piece and transfer it into a PCR tube. For RNA quantification on real-time qPCR, a specific reaction mix of TaqMan probes directed against the gene of interest must be added. Researchers must understand the various factors that influence their results. PCR, a common technique used to study multiplex strains, relies on single copy and indirect methods and is, therefore, less accurate than traditional microarray techniques (7,8). Real-time qPCR has become an essential tool in modern microbiology.

3. Results

Our review results find there are several antibiotic resistance bacteria and genes that have been detected in wastewater treatment plants. Some common examples include *Escherichia coli*, commonly found in mammals' guts and is often used as a marker for fecal contamination of water sources. Strains of E.coli that are resistant to antibiotics such as ampicillin, tetracycline, and sulfonamides have been discovered in wastewater treatment facilities(39-40). *Klebsiella pneumonia*, this bacterium, can cause respiratory, urinary tract, and bloodstream infections. It is known to be resistant to multiple antibiotics, including carbapenems, which are considered to be a last-line defense against antibiotic resistance. K. pneumoniae resistant to carbapenems has been isolated from wastewater treatment plants. *Pseudomonas aeruginosa*, this bacterium, can cause a wide range of infections and is known to be resistant to multiple antibiotics, including aminoglycosides, cephalosporins, and fluoroquinolones. P. aeruginosa resistant to these antibiotics has been isolated from wastewater treatment plants. Some other antibiotic-resistance genes commonly found in wastewater treatment plants include *blaCTX-M*: A gene that confers resistance to cephalosporins, a group of antibiotics commonly used to treat bacterial infections. *qnr*: a gene that confers resistance to fluoroquinolones, A class of drugs that are utilized to cure various bacterial infections. *sul1*, *sul2*, and *sul3*: Genes that confer resistance to sulfonamides, a group of antibiotics used to treat bacterial infections. *ermB*, *ermC*, and *ermF* are genes that can give resistance to erythromycin (40-41).

Table 1. List of antibiotic-resistant bacteria and genes in wastewater treatment plants.

Bacteria	Gene(s)	References:
Escherichia coli	blaCTX-M, qnrS, tetA, sul1	(39-42)
Pseudomonas aeruginosa	blaIMP, sul1, tetA, catB3	(39,41)
Klebsiella pneumoniae	blaCTX-M, blaKPC, mcr-1	(43)
Acinetobacter baumannii	blaOXA-23, blaOXA-24, blaOXA-58	(42-43)
Salmonella enterica	blaTEM, blaSHV, tetA	(38)
Staphylococcus aureus	mecA, ermB, tetK	(38-41)

These are only some of the most frequently observed cases, many other genes responsible for antibiotic resistance have been found in wastewater and new genes are continually being discovered. It is also important to note that the presence of antibiotic-resistance genes in wastewater does not necessarily mean that the corresponding resistance will be found in all bacteria in the wastewater. The presence of these genes serves as an indicator of the potential for the development of antibiotic resistance in the environment. Below are some of the points we can focus on reducing the antibiotic-resistant gene and bacteria in wastewater.

3.1. Improving wastewater treatment infrastructure

Effective wastewater treatment is critical for reducing the levels of antibiotics and antibiotic-resistant bacteria in wastewater and protecting public health. In developing countries, however, wastewater treatment infrastructure needs to be improved, leading to the release of untreated wastewater into the environment. One approach to improving wastewater treatment infrastructure is the implementation of centralized treatment systems. These systems involve collecting, treating, and discharging wastewater at a central location, and they are effective at removing antibiotics and antibiotic-resistant bacteria from wastewater (9). However, the construction and maintenance of centralized treatment systems can be expensive, and there may be challenges in securing funding and overcoming logistical barriers to implementation (10). Another approach is using decentralized

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treatment systems, which are smaller and more locally based. Decentralized systems can be more cost-effective and easier to implement, especially in areas with limited infrastructure (10). One example of a decentralized treatment system is constructed wetlands, which use natural processes to remove contaminants from wastewater (11). However, decentralized systems may not be as effective as centralized systems at removing antibiotics and antibiotic-resistant bacteria (10-11).

Implementing measures to reduce the release of antibiotics into the environment Reducing the release of antibiotics into the environment is another important strategy for combating antibiotic resistance in wastewater in developing countries. Several approaches can be taken to achieve this, including:

3.1.1. Reducing unnecessary antibiotic use

Overuse and misuse of antibiotics are significant contributors to the development of antibiotic resistance (12). Efforts to reduce unnecessary antibiotic use, such as by implementing prescribing guidelines and patient education, can help reduce the levels of antibiotics in wastewater.

3.1.2. Improving the disposal of antibiotics

Proper disposal of antibiotics is also essential to prevent their release into the environment. Improving can be achieved by implementing programs to collect and safely dispose of expired or unused antibiotics (12).

3.1.3. Regulating the discharge of antibiotics from point sources

Point sources, such as hospitals and pharmaceutical manufacturing facilities, can significantly contribute to the levels of antibiotics in wastewater (9). Regulations that limit the discharge of antibiotics from these sources help reduce their release into the environment.

3.2. Monitoring and surveillance

Effective monitoring and surveillance systems are essential for tracking the presence and spread of antibiotic-resistant bacteria in wastewater in developing countries. This information can be used to identify potential outbreaks and inform public health interventions. There are several approaches to monitoring and surveillance of antibiotic-resistant bacteria in wastewater, including:

3.1.1. Routine monitoring

Regular monitoring of wastewater can provide valuable information on the levels of antibiotics and antibiotic-resistant bacteria present. This can be done using monitoring systems that are installed at wastewater treatment plants (10).

3.1.2. Targeted monitoring

Targeted monitoring can focus on specific antibiotics or antibiotic-resistant bacteria of concern (10,11). This can be useful for identifying emerging trends or hotspots of antibiotic resistance.

3.1.3. Surveillance of outbreaks

Surveillance of outbreaks can help to identify the source and extent of an outbreak and inform public health interventions (11,12). This can be done using laboratory-based surveillance systems or using clinical data.

3.3. Why Antibiotic Resistance is a Global Problem

Results suggest that pathogens isolated from various sources such as food processing industries, pharmaceutical industry, livestock farms, sewage systems, and nature have been found to resist the aminoglycoside antibiotics (13,14). For gene transfers to contaminate the environment, several reports indicate that this resistance gene transfer is standard across global regions with a higher presence in developing countries such as Africa and South Asia (15).

Antibiotic resistance is a global problem, hence why this topic is of interest (14,15). Poor sanitation and poverty have led to an environment where pathogens have evolved to rely on antibiotics as their first line of defense against attackers, as research has shown. (15, 16). In addition, Modern illnesses lead to the enormous use of antibiotics. The excessive use of antibiotics is due to the overuse of these medications, which has led to the effects. Many of these strains are pathogenic, and there is the emergence of bacterial strains resistant to antibiotics, a threat of them causing epidemics globally (17). Antibiotic resistance is a significant public health threat related to many aspects of human activity: lifestyle choices, social conditions, economic development, and agricultural production (16-17). Spontaneous growth mechanisms drive antibiotic resistance, not necessarily deliberate actions by humans. However, in many situations, people can unknowingly contribute to the spread of antibiotic-resistant pathogens.

Recently, scientists conducted more research on detecting genes and bacteria in wastewater treatment. The new findings show that antibiotic-resistant bacteria and genes were prevalent in wastewater across the globe (18). Observation shows that the country's waste management systems, rules, and regulations are essential in influencing the prevalence of antibiotic-resistance genes in wastewater(20-22). Further results show that Nepal had a few genes detected, which can be traced back to humans hosted in hospital settings and animal sources as they discuss their environmental impacts (15). Russia had a comparatively lower amount of antibiotic resistance genes detected compared to other countries within Europe (23). In contrast, Hungary mainly had agricultural sources and medical facilities as their main contributors to its total amount of antibiotic resistance bacteria.

3.4. Strategies to Combat Antibiotic Resistance

The research reveals numerous strategies to combat antibiotic-resistant bacteria and genes in wastewater, including sludge screening by toxicology, epidemiology, genetic analysis, and sterility (14). The strategy of implementing waste management systems, rules, and regulations is being implemented in numerous developing countries to combat the spread of antibiotic-resistance genes in wastewater. Antibiotics are a powerful weapon against disease, but their use also creates opportunities to develop antibiotic-resistant bacteria (34). However, there is a need for people to change the current practice of using antibiotics to combat the problem of antibiotic resistance in wastewater (16, 17). Furthermore, antibiotic-resistant bacteria and genes must be monitored closely to determine whether they are increasing, how much they are increasing, and what potential problems this could cause. In this case, people also need to identify and provide information about known sources of resistance (4). The procedure will also help to reduce accidental infections through the inappropriate use of antibiotics.

Table 2. Comparison of developing and developed world in detecting genes and bacteria in wastewater treatment.

Developing Countries	Developed Countries
The study suggested that existing wastewater treatment processes in developing countries need to be improved for the current strategy to combat antibiotic-resistant bacteria and genes in wastewater.	Developed countries have made great strides to improve the efficiency of municipal wastewater discharge treatment and promote a cleaner environment (23).
The study also suggested that testing a new bacterial species may be required to meet the complete defense against multidrug-resistant organisms (7).	In contrast to developing countries, rich nations have built treatment facilities to deal with the problem of spotting genes and bacteria in wastewater treatment (51).
There are challenges in developing countries, such as the high cost of operation and poor awareness among citizens to prevent and manage potential risks (13).	Developed nations destroy bacteria before releasing wastewater into the environment using chlorine, UV light, or both. This makes their cities safer to live in (4,5).

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In addition, there is a significant difference in the prevalence and diversity of antibiotic resistance genes and bacteria in developed and developing countries. Some of the main differences are:

3.4.1. Prevalence

Studies have shown that antibiotic resistance genes and bacteria prevalence is generally higher in developing countries than in developed countries. It is likely due to various factors, such as higher levels of antibiotic use and inadequate wastewater treatment infrastructure in developing countries (43).

3.4.2. Diversity

The diversity of antibiotic resistance genes and bacteria is generally higher in developing countries than in developed countries. It is likely due to a combination of factors, such as greater exposure to different antibiotic sources, including antibiotics in agriculture and aquaculture, and greater genetic diversity among bacterial populations in developing countries (44).

3.4.3. Antibiotic resistance mechanisms

In developed countries. These strains are often multidrug-resistant and may carry many resistance genes, while in developing countries, resistance is often associated with the spread of many resistant strains, each carrying a small number of resistance genes (43-44).

3.4.4. Type of resistance

Developed countries often show resistance to a broad-spectrum antibiotics such as beta-lactams, fluoroquinolones, and erythromycin. Developing countries often resist drugs such as tetracyclines and sulfonamides, commonly used in agriculture and aquaculture, and have less access to newer, more expensive antibiotics (45-46).

It is vital to note that these are general trends, and there can be significant variation within and between countries.

A combination of approaches is likely most effective in combating antibiotic resistance in wastewater in developing countries. Additional studies are necessary to establish the best combination of the above-mentioned methods and identify any potential obstacles or hurdles to their execution.

4. Discussion

The results of our review suggest that a multifaceted approach is needed to combat antibiotic resistance in wastewater in developing countries effectively. Improving wastewater treatment infrastructure, implementing measures to reduce the release of antibiotics into the environment, and monitoring and surveillance are all essential strategies that can help to address this issue. Improving wastewater treatment infrastructure is critical for removing antibiotics and antibiotic-resistant bacteria from wastewater and protecting public health. While centralized treatment systems effectively remove these contaminants, they can be expensive and challenging to implement in specific settings. Decentralized treatment systems, such as constructed wetlands, can be more cost-effective and easier to implement, but they may need to be more effective at removing antibiotics and antibiotic-resistant bacteria. Reducing the release of antibiotics into the environment is another important strategy for combating antibiotic resistance in wastewater. Efforts to reduce unnecessary antibiotic use and improve the disposal of antibiotics can reduce the levels of these substances in the environment. In addition, regulating the discharge of antibiotics from point sources, such as hospitals and pharmaceutical manufacturing facilities, can also help to reduce their release into the environment. Effective monitoring and surveillance systems are essential for tracking the presence and spread of antibiotic-resistant bacteria in wastewater. This information can identify potential outbreaks and inform public health interventions. Routine and targeted monitoring can provide

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valuable information on the levels of antibiotics, antibiotic-resistant bacteria, and genes in wastewater. Surveillance of outbreaks can help identify the source and extent of an outbreak and inform public health interventions. Overall, our review highlights the need for a comprehensive approach to address antibiotic resistance in wastewater in developing countries. Improving wastewater treatment infrastructure, reducing the release of antibiotics into the environment, and implementing effective monitoring and surveillance systems are all essential strategies that can help to protect public health and address this growing global health concern.

5. Conclusion

In conclusion, antibiotic resistance is a significant public health issue that requires a multifaceted approach. One potential source of antibiotic-resistant bacteria is wastewater in developing countries, which often lacks proper treatment infrastructure and can release high levels of antibiotics and antibiotic-resistant bacteria into the environment. Our review highlights the importance of improving wastewater treatment infrastructure, implementing measures to reduce the release of antibiotics into the environment, and monitoring and surveillance to track the presence and spread of antibiotic-resistant bacteria in wastewater. We also discussed the potential challenges and barriers to implementing these strategies and the need for further research to determine their effectiveness in real-world settings.

A comprehensive approach is needed to address antibiotic resistance in wastewater in developing countries and protect public health. This approach should involve a combination of strategies, including improving wastewater treatment infrastructure, reducing the release of antibiotics into the environment, and implementing effective monitoring and surveillance systems. By working together, we can help reduce the threat of antibiotic resistance and protect the health of communities worldwide.

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