ANTIMICROBIAL RESISTANT BACTERIA IN WASTEWATER: GLOBAL OVERVIEW AND CONCEPT: REVIEW

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Abstract: **Antimicrobial resistance (AMR) is a global issue that has raised significant concerns due to its harmful impact on both public health and national economies. The escalating threat of AMR and its sources remain subjects of ongoing research. Wastewater plays a crucial role as a habitat for bacteria and an environment that facilitates gene transfer. This review aims to examine the contribution of wastewater to AMR. Data on AMR in wastewater was gathered from studies published over the last decade, from 2014 to 2024. It was found that wastewater from agricultural practices, pharmaceutical manufacturing, and hospital effluents contribute to the spread of AMR. Additionally, factors such as the presence of antibiotics, heavy metals, pH levels, and temperature can trigger and exacerbate AMR in bacteria residing in wastewater. The AMR observed in these bacteria can be either natural or acquired. Various wastewater treatment methods, including membrane filtration, coagulation, adsorption, and advanced oxidation processes, have been employed to remove resistant bacteria, though their success varies. Wastewater plays a significant role in the propagation of AMR, and understanding this role is crucial to finding sustainable solutions. It is essential to consider the spread of AMR through wastewater as a threat that requires a comprehensive strategy to prevent further harm. The review suggests that pre-treatment of effluents from healthcare and agricultural sectors before their discharge into wastewater treatment plants should be mandatory. Finally, the study calls for collaborative efforts among governments, industries, and communities to curb the spread of AMR and protect both public health and ecosystems.**

Keywords: **Antimicrobial Resistance (AMR), Wastewater Treatment, Hospital Effluents, Public Health Threats, Wastewater Management.**

1. INTRODUCTION

Antibiotics, whether derived from natural sources or synthesised chemically, have been instrumental in safeguarding human, animal, and plant health by effectively treating and preventing bacterial infections. Beyond these critical applications, antibiotics are occasionally utilised as fodder additives and coccidiostatic agents in poultry farming despite being prohibited mainly as growth promoters in animal husbandry across Europe (Singer et al., 2003; Cabello, 2006; Kümmerer, 2009b). This widespread usage has significantly increased global antibiotic consumption (Wright, 2007; Aminov, 2009).

Initially, antibiotics were extracted from natural sources; however, advances in pharmaceutical science have enabled the production of synthetic antimicrobial compounds. These antibiotics are classified into several major groups, including beta-lactams, quinolones, tetracyclines, macrolides, sulfonamides, aminoglycosides, carbapenems, and cephalosporins (Kümmerer, 2009a). Data from the European Surveillance of Antimicrobial Consumption (ESAC) project reveal significant variations in antibiotic consumption among EU countries, with Greece leading at 31.4 defined daily doses (DDD) per 1,000 inhabitants per day between 1997 and 2003, compared to the Netherlands, which reported the lowest usage at 9.8 DDD/1,000 inhabitants per day (Ferech et al., 2006).

The diverse applications of antibiotics have resulted in their residues being introduced into the environment through various pathways, including human and veterinary use. Antibiotics entering ecosystems can disrupt microbial community structures (Aminov & Mackie, 2007) and consequently alter the ecological functions of aquatic environments (Thiele-Bruhn & Beck, 2005; Kotzerke et al., 2008). Concerns have been raised about the environmental impact of antibiotic residues, particularly their potential to contaminate aquatic systems (Sarmah et al., 2006; Wright, 2007; Kemper, 2008).

Beyond chemical contamination, the extensive use of antibiotics has facilitated the emergence of antibiotic-resistant bacteria (ARB) and antibiotic-resistant genes (ARGs), posing serious health risks to humans and animals (Thiele-Bruhn & Beck, 2005). Humans often incompletely metabolise antibiotics, leading to their presence in municipal wastewater and discarded, unused medications (Nagulapally et al., 2009). Consequently, both ARB and ARGs have been consistently detected in wastewater samples (Zhang et al., 2009a; Zhang et al., 2009b; Auerbach et al., 2007; Brooks et al., 2007).

Numerous bacterial species now exhibit acquired resistance to one or more antibiotic classes, with antibiotic-resistant bacteria being increasingly identified in aquatic environments (Kümmerer, 2004; Kim et al., 2007; Schlüter et al., 2007; Watkinson et al., 2007; Vanneste et al., 2008; Caplin et al., 2008). This resistance is a significant concern as it renders infections caused by these bacteria more challenging to treat, thus endangering public and animal health. Notable examples include *Enterococci*, *Klebsiella pneumoniae*, and *Pseudomonas* species, which are increasingly prevalent in healthcare settings (Chambers, 2001; Edmond et al., 1999; Jones et al., 2002; Karlowsky et al., 2003; NNIS Report, 2004).

The rise of antibiotic resistance often leads to treatment failures, compounding public health challenges. The economic implications of this phenomenon remain poorly understood, but it is evident that managing antibiotic resistance and curbing the spread of resistant bacterial strains demand significant investments and strategic interventions.

Comprehensive Review of Antimicrobial Resistance in Wastewater

Antimicrobial Resistance (AMR): A Global Challenge

Antimicrobial resistance (AMR) represents a significant global threat with profound implications for public health and economic stability. The rising prevalence of AMR continues to prompt investigations into its sources and mechanisms. Wastewater environments serve as critical reservoirs for bacterial populations and facilitate the horizontal transfer of antibiotic-resistant genes (ARGs). This review focuses on the role of wastewater in exacerbating AMR and highlights strategies for its mitigation.

Given their public health significance, disinfection processes are central to managing ARGs and antibiotic-resistant bacteria (ARB) in wastewater. Surveillance initiatives targeting AMR aim to gather data on its geographical distribution, seasonal patterns, and emerging resistance trends (WHO, 2022; Ferri et al., 2017).

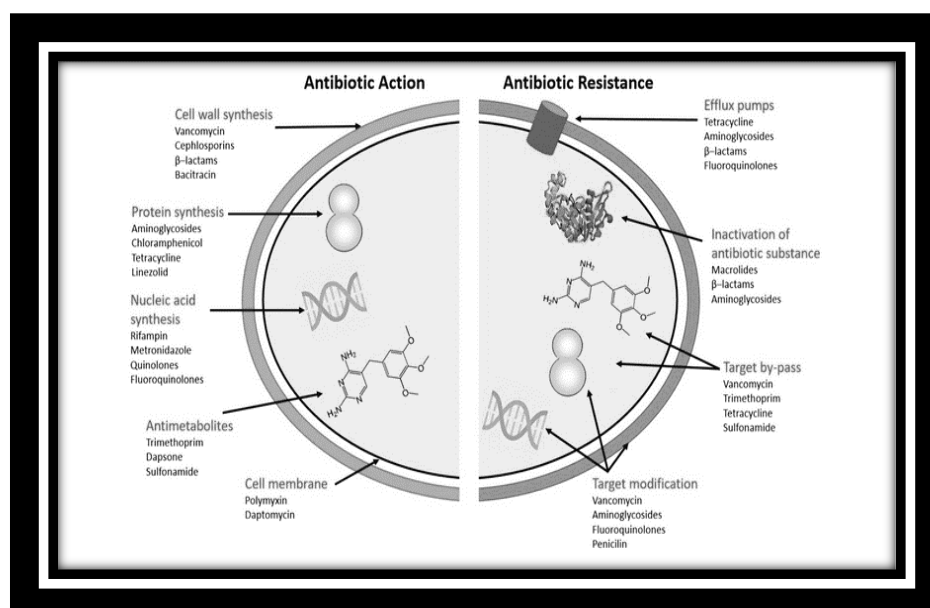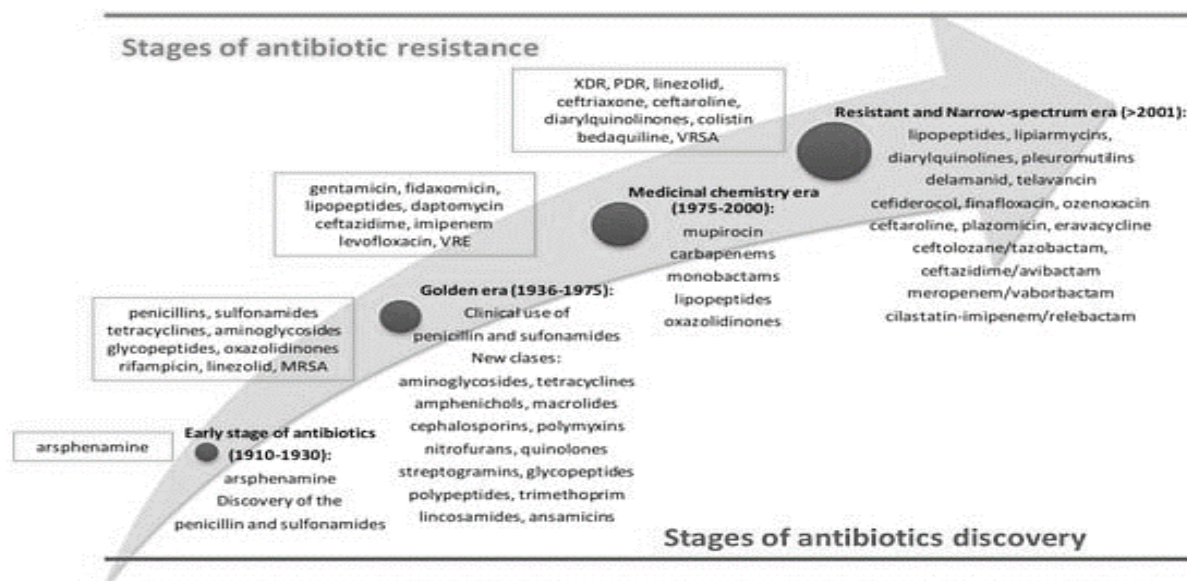**Occurrence and Prevalence of ARB in Wastewater**

Fig. 1 **Antibiotics action and its Resistance**

The evolution of antibiotics has paralleled the emergence of resistant strains, including methicillin-resistant *Staphylococcus aureus* (MRSA), vancomycin-resistant *Staphylococcus aureus* (VRSA), and extensively drug-resistant (XDR) pathogens (Fair et al., 2014; Hutchings et al., 2019; Peraman et al., 2021; Annunziato et al., 2019).

Factors contributing to the proliferation of AMR include:

• Bacterial evolution through genetic mutations.

• Overuse and misuse of antibiotics in human health, agriculture, and aquaculture.

• Increased global travel and trade, facilitating the spread of resistant microorganisms.

• Inadequate public awareness of proper antibiotic use and AMR risks.

• Gaps in regulatory measures, including infection management and healthcare infrastructure (Dagoster et al., 2019; WHO, 2022).

Types and Mechanisms of Resistance

AMR poses one of the most critical challenges of the 21st century (Li et al., 2022). Resistant pathogens include *Staphylococcus aureus*, *Streptococcus pneumoniae*, *Clostridium difficile*, and carbapenem-resistant *Enterobacteriaceae* (Sandiumenge et al., 2006; Pakyz et al., 2008). These pathogens exhibit diverse resistance mechanisms, complicating treatment options.

Strategies to combat AMR include employing diverse antimicrobial agents and combination therapies, which use drugs with distinct mechanisms of action to reduce the selection pressure for resistance (Sandiumenge et al., 2006; Pakyz et al., 2008).

AMR: A Multisectoral Threat

AMR impacts human and animal health, ecosystems, food security, and economic development (Zhang et al., 2021). The *One Health* perspective underscores the interconnectedness of these domains, where contaminants quickly transfer across sectors, exacerbating the crisis. Between 2000 and 2015, global antibiotic use rose by 65%, driven largely by increased consumption in low- and middle-income countries (Klein et al., 2018).

Bacterial populations exhibit high genetic plasticity, allowing rapid acquisition and dissemination of resistance genes. Contrary to previous assumptions, antibiotics are not the sole drivers of horizontal gene transfer but play a significant role in selecting for resistance mechanisms (WHO, 2021). The WHO has declared AMR one of the top global health threats, with AMR-related deaths reaching 1.27 million globally in 2019 (Murray et al., 2022). Without urgent action, this number could rise to 10 million annually by 2050 (O'Neill, 2016).

Leading ARB Pathogens and Their Impact

The six leading ARB pathogens (*Escherichia coli, Staphylococcus aureus, Klebsiella pneumoniae, Streptococcus pneumoniae, Acinetobacter baumannii,* and *Pseudomonas aeruginosa*) account for over 71% of AMR-related deaths (Murray et al., 2022). The acronym ESKAPE highlights these pathogens as critical nosocomial threats due to their multidrug resistance.

The WHO prioritises ARB pathogens into three categories based on their health impact:

1. **Critical Priority**: Carbapenem-resistant *A. baumannii, P. aeruginosa,* and Enterobacteriaceae.

2. **High Priority**: Methicillin-resistant and vancomycin-resistant *S. aureus,* fluoroquinolone-resistant *Campylobacter* spp., and cephalosporin-resistant *Neisseria gonorrhoeae.*

3. **Medium Priority**: Penicillin-resistant *S. pneumoniae* and ampicillin-resistant *Haemophilus influenzae* (Davies & Simeon, 2017; WHO, 2021).

The European Antimicrobial Resistance Surveillance Network (EARS-Net) emphasises monitoring pathogens such as *E. coli, K. pneumoniae,* and *S. aureus* for targeted interventions (Cristea, 2016; ECDC, 2020).

Mitigation Strategies and Future Directions

Efforts to combat AMR must address its complex and multifactorial drivers. Key approaches include:

- Reducing antibiotic overuse through stewardship programs.

- Enhancing public awareness of AMR risks.

- Investing in advanced wastewater treatment technologies to limit ARG and ARB dissemination.

- Strengthening global surveillance systems for early detection of resistance trends.

- Developing alternative therapeutics to address critical resistance threats.

Recent studies underscore the importance of balancing treatment duration with resistance outcomes. For example, Mo et al. (2023) demonstrated that shorter antibiotic courses may mitigate or exacerbate resistance depending on bacterial and antibiotic-specific characteristics.

Current Global Status of ARB and ARG Occurrences

The global distribution of antimicrobial-resistant bacteria (ARB) and antimicrobial resistance genes (ARGs) has become a critical public health concern (WHO, 2022). The potential transfer of ARB and ARGs to humans threatens the effectiveness of antimicrobial therapies. This issue is further exacerbated by the pollution of freshwater systems, particularly from wastewater treatment plants (WWTPs), which were historically overlooked as pollution sources. Increasing bacterial resistance, alongside the declining efficacy of prophylaxis, underscores the need for a deeper investigation into the role of WWTPs in the infection cycle. Studies conducted globally highlight the occurrence of antibiotics, ARBs, and ARGs in WWTP matrices, emphasising their central role in the propagation of antimicrobial resistance.

Recent literature, including reviews by Wang et al. (2020), Noor et al. (2021), Uluseker et al. (2021), Zhuang et al. (2021), Gao et al. (2022), and Wang et al. (2022), provides valuable insights into WWTP efficacy across diverse geographical regions. These reviews indicate a persistent presence of antibiotics, ARBs, and ARGs in WWTP effluents, with negligible reductions in concentration from influents. Factors such as lipophilicity and hydrophobicity of antibiotics contribute to their persistence during physicochemical and biological treatments. Additionally, treatments may release free genetic materials, posing significant risks of horizontal gene transfer (HGT) to downstream populations. The aerosolisation of ARGs, as noted, can further facilitate their evasion from treatment processes, exacerbating their environmental spread.

Studies also reveal that the type and abundance of ARGs in WWTP influents correlate with antibiotic usage patterns in the region. For instance, Wang et al. (2022) documented elevated ARG concentrations in WWTPs within high- and upper-middle-income regions, consistent with antibiotic consumption data from Western Europe and East Asia, which reported daily defined doses (DDD) of 3,364 million and 4,413 million units, respectively. ARGs detected in these studies include *sul*, *tet*, *erm*, *mph*, *bla*, *qnr*, *msr*, and *mex*, conferring resistance to sulfonamides, tetracyclines, β-lactams, macrolides, and

quinolones (Zhuang et al., 2021). Quinolones, for example, are mainly excreted unchanged in human waste, accumulating in sludge due to their hydrophilic properties (Hendricks & Pool, 2012). Resistance genes such as *qnrB*, *qnrD*, and *qnrS* are often propagated through HGT, as observed in China (Mao et al., 2015; Pazda et al., 2019).

Genes	WWTP	Concentration (*copies / ml*) in the influent	Concentration (*copies / ml*) in the effluent	References
ermB	WWTP1	$9x10^5$	$2x10^3$	Mao et al., 2015
	WWTP2	$1x10^6$	$2x10^5$	Mao et al., 2015
	NGWRP	$1.2x10^5$	0	Pazda et al., 2019
	WRPF	$6.31x10^5$	$1x10^2$	Kappell et al., 2018
	WWTP5 (UV)	$5.37x10^4$ *cell eqivalents / 100ml*	$3.75x10^4$ *cell eqivalents / 100ml*	Jager et al., 2018
	WWTP5 (Ozone)	$5.37x10^4$ *cell eqivalents / 100ml*	$1.01x10^3$ *cell eqivalents / 100ml*	Jager et al., 2018
	WWTP5 (UV and Ozone)	$5.37x10^4$ *cell eqivalents / 100ml*	$1.07x10^3$ *cell eqivalents / 100ml*	Jager et al., 2018
qnr	WWTP1	$7x10^4$	$1x10^3$	Mao et al., 2015
	WWTP2	$2x10^5$	$9x10^3$	Mao et al., 2015
sul	WWTP1	$3x10^7$	$5x10^5$	Mao et al., 2015
	WWTP2	$9x10^6$	$6x10^5$	Mao et al., 2015
	WWTP3	$1.19x10^8$	$4.52x10^6$	Zhang et al., 2017
	WWTP4 (UV)	$1.86x10^5$	$2.5x10^3$	Chen et al., 2020a
	WWTP4 (UV and EC)	$1.86x10^5$	$2x10^2$	Chen et al., 2020a
	WWTP5 (UV)	$1.33x10^6$ *cell equivalents / 100ml*	$9.33x10^5$ *cell eqivalents / 100ml*	Jager et al., 2018
	WWTP5 (Ozone)	$1.33x10^6$ *cell equivalents / 100ml*	$6.83x10^4$ *cell eqivalents / 100ml*	Jager et al., 2018
	WWTP5(UV and Ozone)	$1.33x10^6$ *cell equivalents / 100ml*	$5.53x10^4$ *cell eqivalents / 100ml*	Jager et al., 2018
	NGWRP	$1.55x10^5$	0	Wallmann et al., 2021
	WRP	$2x10^6$	$3x10^1$	Quach-Cu et al., 2018
	WRPF	$6.31x10^6$	$3.98x10^3$	Kappell et al., 2018
tet	WWTP1	$8x10^5$	$3x10^4$	Mao et al., 2015
	WWTP2	$2x10^6$	$3x10^5$	Mao et al., 2015
	WWTP3	$1.78x10^8$	$2.49x10^7$	Zhang et al., 2017
	WWTP4 (UV)	$3.18x10^3$	$1.2x10^3$	Chen et al., 2020a
	WWTP4 (UV and EC)	$3.18x10^3$	$1x10^2$	Chen et al., 2020a
	WRPF	$5.01x10^5$	$6.31x10^1$	Kappell et al., 2018
bla$_{SHV/TEM}$	WRP	$8x10^3$	$6x10^0$	Quach-Cu et al., 2018
	WWTP5 (UV)	$1.22x10^5$ *cell equivalents / 100ml*	$1.83x10^5$ *cell eqivalents / 100ml*	Jager et al., 2018
	WWTP5 (Ozone)	$1.22x10^5$ *cell equivalents / 100ml*	$1.1x10^4$ *cell eqivalents / 100ml*	Jager et al., 2018
	WWTP5(UV and Ozone)	$1.22x10^5$ *cell equivalents / 100ml*	$1.12x10^4$ *cell eqivalents / 100ml*	Jager et al., 2018

Another notable resistance mechanism is RNA methyltransferase *ermB*, often associated with transposons (Wallmann et al., 2021). This enzyme facilitates resistance to macrolides like erythromycin and azithromycin. β-lactam antibiotics, including penicillins and cephalosporins, are extensively used in human and veterinary medicine (Defrancesco et al., 2017). However, their instability, particularly the β-lactam ring, renders them difficult to detect in WWTPs (Pazda et al., 2019). Extended-spectrum β-lactamase (ESBL)-producing bacteria such as *E. coli* have emerged as significant multidrug-resistant (MDR) pathogens, often associated with high mortality rates (Seyedjavadi et al., 2016; Gumede et al., 2021). Resistance genes like

blaCTX-M, *blaTEM*, and *blaSHV* are key contributors to β-lactam resistance, with some variants (e.g., *blaSHV-5*) capable of hydrolyzing broad-spectrum cephalosporins (Nzima et al., 2020).

AMR in Wastewater and WWTPs

Over the past century, anthropogenic activities such as industrialization and agriculture have introduced a range of pollutants, including antibiotics, into water bodies (E. Garido et al., 2016). Wastewater treatment plants (WWTPs) were originally designed to remove physical debris, pathogens, and organic matter (Hammond et al., 2021). However, these systems often fail to eliminate emerging pollutants like antibiotics, ARB, and ARGs (Sambaza et al., 2019). The biological processes within WWTPs provide optimal conditions for bacterial growth and genetic exchange, facilitating the proliferation of ARBs and ARGs (Manaia et al., 2018; Narciso-da-Rocha et al., 2018). Key pathogens implicated in antimicrobial resistance, including *Enterococcus faecium*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Acinetobacter baumannii*, *Pseudomonas aeruginosa*, and *Enterobacter* spp., are frequently detected in these systems (Marano et al., 2021; Petrovich et al., 2020).

The sub-inhibitory concentrations of antibiotics in WWTPs promote ARG proliferation, creating novel ARB strains (Harris et al., 2013; Reygaert, 2018). Stressors such as pH fluctuations, temperature variations, heavy metals, and antibiotic residues further exacerbate this issue (Barancheshme & Munir, 2018; Bengtsson-Palme et al., 2021). These conditions have made wastewater an important target for AMR surveillance (Harris et al., 2013).

Hospital Effluents as Antibiotic Sources

Hospital effluents are major contributors to antibiotic loads in WWTPs, often containing higher concentrations of ARBs and ARGs than other urban wastewater sources (Petrovich et al., 2020). Proper pretreatment and regulation of these effluents are essential to mitigating their impact on municipal WWTPs and subsequent water bodies.

ARG and ARB Persistence and Spread

The persistence and spread of ARGs in treated wastewater result from mechanisms such as HGT and vertical gene transfer (VGT). HGT pathways enable ARG dissemination across bacterial populations, including conjugation, transformation, and transduction (Cai et al., 2021). These genetic exchanges often result in multidrug-resistant bacteria with enhanced survival and adaptability (Gemeda et al., 2021; Arias-Andres et al., 2018). Post-treatment discharge of wastewater into water bodies further propagates ARB and ARG dissemination, as documented in global studies (Bergeron et al., 2017; Manaia et al., 2018).

Groundwater, often considered a safer water source, is increasingly contaminated with ARGs due to agricultural and industrial practices (Ma et al., 2022). Surveillance efforts have linked such contamination to domestic sewage, hospital wastewater, and animal manure (Luiken et al., 2022).

Strategies to Address Antibiotic Resistance in Wastewater Treatment

Coagulation

Coagulation is a widely employed technique to eliminate colloidal particles, turbidity, color, natural organic matter, and heavy metals from water (Zainal-Abideen et al., 2012). Colloidal particles typically carry negative charges, whereas coagulants are positively charged. Upon interaction, coagulants neutralise these particles, promoting aggregation. Coagulation is often utilised as a tertiary treatment 1 in wastewater treatment plants (WWTPs) to enhance water quality and eliminate contaminants. Various organic and inorganic coagulants have been developed depending on the targeted contaminants. For instance, Jarvis et al. (2012) demonstrated that zirconium-based coagulants are more effective in reducing dissolved organic carbon in drinking water than iron-based coagulants.

Disinfection

Disinfection is crucial for eradicating pathogenic microorganisms, including bacteria, viruses, and parasites, that may cause diseases. Due to its availability and efficacy, chlorination remains the most commonly used disinfection method, though ozone and ultraviolet (UV) radiation are also employed. WWTPs often serve as hotspots for antibiotic-resistant bacteria (ARB) and antibiotic-resistance genes (ARGs), with many effluents harbouring multidrug-resistant heterotrophic bacteria 1 (Pang et al., 2016). Effective disinfection is key in disrupting ARB, ARGs, and harmful microorganisms. Advanced

oxidation processes, including ozone treatment, UV irradiation, Fenton reagents, and photocatalytic systems, have also been implemented to enhance disinfection.

Nanomaterials

Nanomaterials offer promising solutions for combating antibiotic resistance by leveraging antimicrobial nanotechnology. Two primary mechanisms underpin their effectiveness: (1) functionalised nanomaterials paired with antibiotics penetrate ARB and release toxic ions; (2) nanomaterials and antibiotics synergistically combat 1 ARGs (Aruguete et al., 2013). Aruguete et al. (2013) explored the potential of nanomaterials functionalised with antibiotics, including combinations like liposomes, dendrimers, and polymer-encased nanoparticles. Silver nanoparticles coated with polyvinylpyrrolidone were effective against Pseudomonas aeruginosa, a multidrug-resistant pathogen. Nanomaterial-based approaches thus represent a novel defence mechanism against ARB and ARGs while potentially minimising the emergence of nanomaterial-resistant organisms.

2. RECOMMENDATIONS

The increasing prevalence of antibiotic resistance necessitates innovative treatment strategies. While significant progress has been made, several gaps remain:

1. Scaling Up: Most studies are limited to laboratory or pilot-scale investigations with short durations. Large-scale tests using real-world samples are essential.

2. Risk Assessment: Quantitative assessments 1 of ARB and ARGs in WWTP effluents are necessary to establish safe thresholds for public health.

3. Operational and Environmental Factors: Factors such as pH, chlorine levels, hydraulic retention time, and environmental conditions like temperature and organic load must be considered to optimise treatment efficiency.

4. Infrastructure Modifications: Existing WWTPs should be upgraded to reduce ARBs and ARGs in effluents effectively.

5. Seasonal Variations: Long-term studies are needed to assess the prevalence of seasonal influences on ARB and ARG, particularly in systems like constructed wetlands.

6. Advanced Treatments: Research into advanced and cost-effective disinfection methods, including combined technologies, is crucial to improve ARG removal.

7. Nanomaterial Mechanisms: A greater understanding of how nanomaterials interact with microorganisms is necessary to prevent resistance while harnessing their antimicrobial potential.

8. Coagulation Optimization: Coagulants' ability to target ARGs should be further studied, given their essential role in existing treatment plants. The persistence of ARB and ARGs in wastewater underscores the urgent need for enhanced treatment technologies. Continued research will not only optimise current methods but also pave the way for novel, efficient strategies to mitigate antibiotic resistance in wastewater.

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