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Advancing antimicrobial resistance mitigation through artificial intelligence: A critical review and new perspectives on integrated monitoring and smart quaternary treatments of urban wastewater

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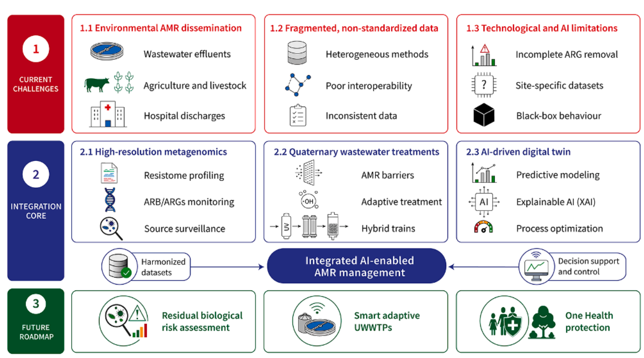
HIGHLIGHTS

- Quaternary treatments of wastewater play a key role in mitigating AMR dissemination.
- Fragmented and non-standardized datasets limit reliable AMR-risk assessment.
- AI can support quaternary treatment optimization and resistome dynamics interpretation.
- A roadmap for next-generation UWWTPs in the One Health perspective is proposed.
- Multidisciplinary collaboration is needed to fully exploit the integration of AI.

GRAPHICAL ABSTRACT



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ABSTRACT

Urban wastewater treatment plants (UWWTPs) play a key role in protecting environmental quality and public health by reducing pollutant discharges into receiving ecosystems. In this context, antimicrobial resistance (AMR) mitigation is emerging as a priority to limit the environmental dissemination of antibiotic resistant bacteria (ARB), antibiotic resistance genes (ARGs), and mobile genetic elements (MGEs). However, conventional UWWTP configurations are not designed to specifically control these biological determinants. Recent regulatory frameworks, within a One Health perspective, require monitoring of micropollutants that threaten water quality and public health, along with the progressive implementation of advanced treatment steps ("quaternary treatments") to remove them. Compliance with more stringent requirements, combined with the need for sustainable treatment processes, poses new challenges while creating opportunities for technological innovation. To support progress in the quaternary treatment of wastewater, the integration of advanced technologies with robust monitoring, modeling, and control systems is essential. This review critically examines recent advances in the design, management, and optimization of quaternary treatment processes through artificial intelligence (AI)

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algorithms, with specific emphasis on AMR mitigation. Quaternary treatments, including advanced oxidation processes (AOPs), membrane filtration, adsorption, and hybrid treatments, are discussed as promising barriers against AMR dissemination. AI models are identified as powerful tools for developing smart, adaptive, and efficient design, monitoring, and control systems for these technologies. Nevertheless, further research is needed to optimize model performance and practical feasibility in UWWTPs specifically oriented toward AMR mitigation. Key strengths, limitations, and research priorities are highlighted to support the development of next-generation smart wastewater treatment systems.

Lists of abbreviations

List of abbreviations of AI modeling terms

AdaBoost	Adaptive Boosting
ANN	Artificial Neural Networks
AUROC	Area Under the Receiver Operating Characteristic Curve
CatBoost	Categorical Boosting
CNN	Convolutional Neural Network
CV	Cross-validation
DL	Deep Learning
FIA	Feature Importance Analysis
GBDT	Gradient Boosting Decision Tree
LASSO	Least Absolute Shrinkage and Selection Operator
LightGBM	Light Gradient Boosting Machine
LIME	Local Interpretable Model-agnostic Explanations
LSTM	Long Short-Term Memory
MAE	Mean Absolute Error
MAPE	Mean Absolute Percentage Error
ML	Machine Learning
MLP	MultiLayer Perceptron
MLR	Multiple Linear Regression
MORF	Multi-target Regression Random Forest
RBF	Radial Basis Function
RF	Random Forest
RFE	Recursive Feature Elimination
RMSE	Root Mean Square Error
RSM	Response Surface Methodology
SHAP	SHapley Additive exPlanations
SVM	Support Vector Machines
SVR	Support Vector Regression
XAI	Explainable AI
XGBoost	eXtreme Gradient Boosting

List of abbreviations of AMR terms

AMR	Antimicrobial Resistance
ARB	Antibiotic Resistant Bacteria
ARG	Antibiotic Resistance Gene
dPCR	digital PCR
eARG	extracellular ARG
eDNA	extracellular DNA
HGT	Horizontal Gene Transfer
iARG	intracellular ARG
qPCR	quantitative PCR
MGE	Mobile Genetic Elements
PCR	Polymerase Chain Reaction
VBNC	Viable But Non-Culturable
WBE	Wastewater-Based Epidemiology

1. Introduction

The emergence and dissemination of antimicrobial resistance (AMR) has escalated into a critical global public health challenge, directly responsible for an estimated 4.95 million deaths in 2019 alone (Murray et al., 2022). Projections indicate that by 2050, AMR could kill up to 10 million people per year, imposing a cumulative global economic burden of 100 trillion USD and ranking AMR among the top 10 global public health threats (O'Neill, 2016). In addition to naturally occurring resistance, acquired AMR in pathogens is particularly problematic because it reduces the effectiveness of antimicrobial therapies, leading to infections that are more difficult to treat, increased mortality, prolonged disease courses, and rising healthcare costs (Ventola, 2015; WHO, 2025). This global crisis is further aggravated by the rapid spread of last-resort antibiotic resistance genes, such as *blaNDM, mcr*, and *tet(X)*, which compromise the efficacy of final therapeutic barriers against multidrug-resistant infections such as carbapenems, polymyxins, and tigecycline (Wu et al., 2026).

The spread of AMR is largely driven by the excessive and inappropriate use of antibiotics in human and veterinary medicine (Rooklidge, 2004). Although transmission occurs predominantly through human–human interactions, both within and outside healthcare settings, increasing evidence highlights the critical role of environmental compartments in the persistence and dissemination of resistance (Salam et al., 2023). Humans, animals, and environmental matrices are interconnected reservoirs that continuously exchange and spread resistant microorganisms and genes. Among the major environmental dissemination pathways, wastewater and sludge from urban wastewater treatment plants (UWWTPs), together with natural fertilizers and livestock activities, represent key entry routes of AMR into the environment (Krzemiński et al., 2020). Because up to 90% of administered antibiotics may be excreted unmetabolized by humans and livestock, raw wastewater contains pharmaceutical residues and microorganisms, making UWWTPs critical anthropogenic hotspots for AMR dissemination (Pinto Jimenez et al., 2023). Within these plants, secondary biological treatments, such as the well-known activated sludge process, are characterized by high microbial densities and nutrient-rich conditions, which create an ideal environment for the emergence and spread of resistance (Moura et al., 2012). These biological tanks function as “incubators”, in which sub-inhibitory concentrations of antibiotics exert selective pressure and promote horizontal gene transfer (HGT) via mobile genetic elements (MGEs) such as plasmids, transposons, and integrons (Rizzo et al., 2013).

The critical vulnerability lies in the fact that conventional UWWTPs rely mainly on primary and secondary treatments and are designed to target organic matter and macronutrients, resulting in insufficient efficiency toward bioactive micropollutants such as antibiotic resistance genes (ARGs) and bacteria (ARB) (Khan et al., 2020). Consequently, treated effluents frequently discharge significant loads of resistant bacteria and determinants into aquatic ecosystems (Kumar et al., 2020). To mitigate this risk, the additional implementation of advanced treatment technologies is increasingly recognized as an environmental and public health necessity (Werkneh and Islam, 2023). Among available alternatives, disinfection, advanced oxidation processes (AOPs), membrane filtration systems, adsorption, and hybrid systems have emerged as effective options to ensure sufficient DNA damage and prevent the environmental release of resistance determinants (Pant et al., 2022). However, the performance of these technologies remains highly variable and strongly dependent on operational conditions, environmental factors, wastewater characteristics (e.g., pH, organic load, solid content), and specific gene persistence (Pan et al., 2024).

The complexity of these so-called "quaternary treatments", based on physical, biological, or chemical processes, as well as combinations of them, demands a significant effort toward advanced monitoring, optimization, and control systems aimed at improving treatment performance. In this context, artificial intelligence (AI) modeling, particularly machine learning (ML) and deep learning (DL) algorithms, has proven to be an effective tool for addressing the complexity and nonlinear behavior typically associated with water and wastewater treatment processes (Cairone et al., 2024a; Yue et al., 2025). AI is attracting increasing interest in wastewater treatment, as demonstrated by the rapid increase in the number of related papers published over the years (Fig. 1). Currently, 3088 documents in the Scopus database match the TITLE-ABS-KEY search string «("artificial intelligence" OR "machine learning" OR "deep learning") AND ("wastewater treatment" OR "waste water treatment")». A notable increase in publications has been observed in recent years, with 2675 documents published since 2020, accounting for about 87% of the total (Fig. 1a).

More specifically, a network was generated using VOSviewer software (ver. 1.6.20, Leiden University, The Netherlands), including all keywords and applying the full counting method (Fig. 1b). After thesaurus-based refinement, 6782 keywords were identified; 122 met the minimum occurrence threshold of ten, and these were selected for visualization and cluster analysis. The analysis reveals a limited presence of antimicrobial resistance-related keywords. Specifically, the keywords "metagenomics", "antibiotic resistance genes", "wastewater-based epidemiology", and "antibiotics" appeared among the 122 selected keywords, with occurrences of 20, 14, 14, and 10, respectively. These findings highlight that, despite the rapidly growing interest in AI applications for wastewater treatment and monitoring, the specific integration of AI with wastewater genomics and AMR mitigation remains relatively underexplored. This aspect was further confirmed by a more topic-specific TITLE-ABS-KEY search in the Scopus database using the following string: «("artificial intelligence" OR "machine learning" OR "deep learning") AND ("wastewater" OR "waste water") AND ("antimicrobial resistance" OR "AMR" OR "antibiotic resistant bacteria" OR "ARB" OR "antibiotic resistance gene" OR "ARG" OR "horizontal gene transfer" OR "HGT" OR "mobile genetic elements" OR "MGE" OR "genetics")». This search resulted in a total of 142 documents, including a limited number of 94 research articles, thereby confirming the relatively limited research efforts devoted to this integration so far.

Nevertheless, substantial advances have been made recently in the analytical characterization of ARB and ARGs. The advent of environmental genomics and culture-independent shotgun metagenomics has revolutionized our ability to map these complex resistomes, uncovering both established and latent antimicrobial determinants that were previously undetectable (Beltrán De Heredia et al., 2025). In parallel, AI has emerged as an effective tool to further advance this field, as the integration of massive genomic datasets with AI/ML/DL offers unprecedented opportunities to precisely identify ARB and ARGs and predict their dissemination pathways across environmental matrices (Baker et al., 2023; Behling et al., 2023; Scaglione et al., 2026).

Similarly, integrating AI-driven monitoring, optimization, and control systems with advanced treatment technologies has been demonstrated to improve process performance and management (Liu et al., 2025). This review critically examines recent advances in these fields, highlighting the current state-of-the-art and underlining their advantages and limitations. Considering the benefits of these approaches alone, the full integration of AI-enhanced environmental genomics and AI-driven monitoring, optimization, and control systems into quaternary wastewater treatments may support the development of next-generation UWWTPs. Existing reviews have focused either on the application of AI to AMR-related challenges, including the detection and classification of ARB and ARGs, as well as the prediction of resistance patterns, to enhance AMR surveillance, drug discovery and decision-making (Jangid et al., 2026; Latif et al., 2026), or on the broader role of AI in next-generation wastewater treatment plants as a cutting-edge tool for supporting process monitoring, optimization and control (Li et al., 2024b; Yang et al., 2025; Zhao et al., 2020). However, to date, to the best of the authors' knowledge, a comprehensive analysis integrating AI-enhanced environmental genomics and AI-driven monitoring, optimization, and control systems into quaternary wastewater treatment has not yet been provided. This combined perspective represents the main contribution of the present work. To address this gap, a potential pathway to develop an AI-enabled framework for advancing AMR monitoring and mitigation in quaternary wastewater treatment is presented, highlighting new perspectives, potential benefits, and current limitations. Such a framework may be of particular interest in light of the new EU Urban Wastewater Treatment Directive (UWWTD) 2024/3019, which strengthens the role of wastewater management in protecting both the environment and public health through the progressive implementation of quaternary treatments for micropollutant removal and strategies for monitoring public health-related parameters, including AMR.

2. Detection and attenuation of ARGs in UWWTPs

2.1. Measurement of the wastewater resistome

The environmental resistome has gained increasing attention as a critical component in the emergence, selection, and dissemination of AMR. UWWTPs, acting as convergence points for various microbial communities, viruses, and residual antimicrobials, provide a comprehensive matrix at a regional scale, and characterizing their resistome is a fundamental first step to address this threat. In general, the main objectives of qualitative and quantitative resistome characterization are to (i) quantify ARG abundance, (ii) assess ARG diversity, and (iii) identify the range of microbial hosts carrying ARGs (Begmatov et al., 2024). While microbiological monitoring traditionally relied on culture-dependent methods and colony-forming units, the reality is that only a very small fraction of environmental bacteria is cultivable, e.g., about 4.8% in activated sludge from wastewater treatment plants (Zheng et al., 2024). In the case of treated/disinfected wastewater effluents, even more cells enter a viable but non-culturable (VBNC) state, causing over 99% of viable *Escherichia coli* and *Enterococcus* to be non-culturable (Tang and Lau, 2022). The need to include VBNC cells in AMR investigations has driven a shift toward culture-independent molecular techniques, including polymerase chain reaction (PCR) and metagenomic analyses (Miłobedzka et al., 2022).

Quantitative PCR (qPCR) remains the primary method for targeted monitoring in environmental and epidemiological studies, owing to its high sensitivity and wide dynamic range. It enables the tracking of specific ARGs across different compartments and provides a useful proxy for assessing potential transmission from the environment to humans (Miłobedzka et al., 2022). However, qPCR is inherently limited to predefined targets and is susceptible to quantification bias arising from primer mismatches and matrix inhibition, where environmental contaminants can interfere with polymerase efficiency. In addition, the lack of standardized approaches for the normalization of results remains a major limitation; in fact, universal reference genes, such as the 16S rRNA gene, may not be so accurate in wastewater, making it difficult to distinguish between changes in ARG abundance and shifts in total microbial load (Bustin et al., 2009). Digital PCR (dPCR) has emerged as a high-precision alternative, offering absolute quantification without the need for standard curves (Whale et al., 2012). While dPCR is generally more resilient to inhibitors, its accuracy can still be influenced by template concentration and variability in partition volumes across thousands of micro-reactions, which may introduce systematic uncertainty

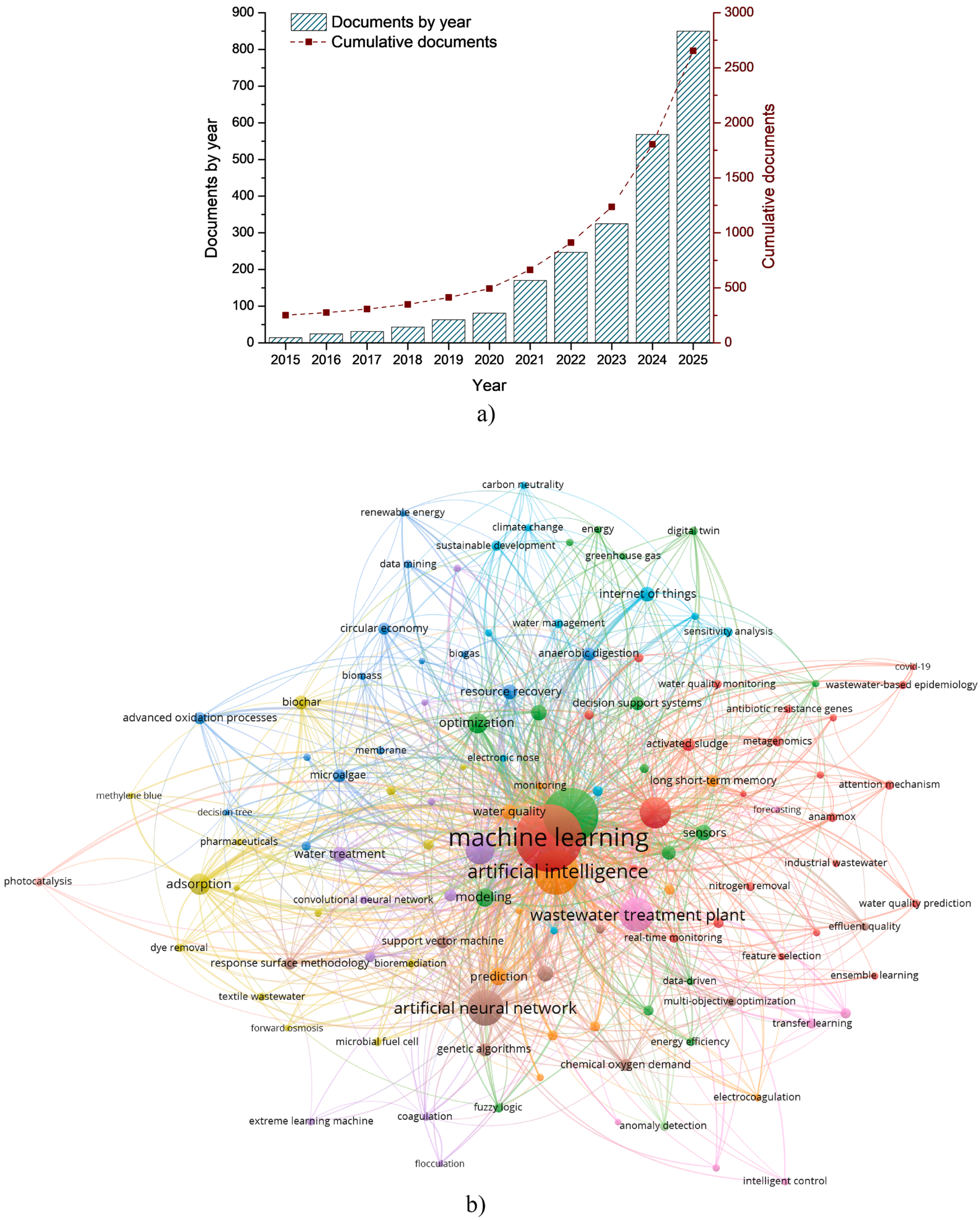

Fig. 1. Documents by year and cumulative number of documents in the Scopus database matching the TITLE-ABS-KEY search string «("artificial intelligence" OR "machine learning" OR "deep learning") AND ("wastewater treatment" OR "waste water treatment")» over the period 2015–2025 (a) and keyword co-occurrence network showing the main thematic clusters and co-occurrence patterns in the analyzed research domain (b).

(Whale et al., 2012).

For non-targeted, comprehensive resistome profiling, shotgun metagenomics represents the most powerful tool currently available, enabling the identification of hundreds of ARGs and their taxonomic hosts through metagenome-assembled genomes (Quince et al., 2017). Nevertheless, metagenomics is affected by a fundamental sensitivity limitation, whereby sequencing depth often fails to capture rare but clinically relevant ARGs present at trace levels (Miłobedzka et al., 2022). Moreover, unlike qPCR, which enables both absolute quantification (e.g., gene copy number per unit of sample) and relative normalization (e.g., to 16S rRNA gene copies), metagenomic sequencing data are compositional and expressed as relative abundances, which can introduce spurious correlations and bias interpretation if not properly accounted for (Quince et al., 2017).

The most critical challenge in resistome characterization lies in the lack of methodological standardization, as results are strongly influenced by user expertise, repeatability, reproducibility, and trade-offs between throughput, resolution, and costs. Although the choice of analytical approaches should be aligned with specific monitoring objectives, the growing volume of data generated by different research groups, using distinct sampling strategies, DNA extraction kits, sequencing platforms, and bioinformatic pipelines, has made cross-study comparisons increasingly uncertain (Zhu et al., 2025). In the absence of harmonized guidelines, individual studies often remain isolated snapshots, limiting the ability to extract consistent and transferable insights from the vast amount of data generated over the past decade (Miłobedzka et al., 2022). This fragmentation ultimately constrains researchers' capacity to translate resistome characterization into effective control and mitigation strategies.

2.2. Quaternary treatments to advance AMR mitigation in UWWTPs

While conventional wastewater treatment processes are often insufficient to achieve substantial AMR mitigation, the complexity of the resistome itself represents an additional challenge (Drane et al., 2024; Yu et al., 2023). Antibiotic resistance in wastewater is not associated with a single target but is distributed among viable ARB, intracellular ARGs (iARGs) and extracellular ARGs (eARGs), and MGEs, each contributing differently to the persistence and dissemination of resistance. Consequently, effective AMR mitigation requires treatment strategies capable of targeting multiple biological compartments. The conceptual framework in Fig. 2 summarizes these key genetic and cellular components contributing to AMR risk in urban wastewater and their potential reduction through advanced quaternary treatment processes. Drug-resistant donor cells may contain iARGs and, upon cell damage, death, or lysis, release these genes as extracellular DNA (eDNA), which may persist as a residual reservoir of ARGs for transformation. MGEs may further promote the mobilization and horizontal transfer of genetic elements between microorganisms. Accordingly, Fig. 2 distinguishes three complementary mechanisms through which quaternary treatments can reduce HGT-related risk: (i) ARB inactivation, (ii) ARG removal, degradation or functional deactivation, and (iii) MGE reduction. In this way, the combined actions of advanced quaternary treatment processes can significantly reduce the overall biological risk associated with AMR dissemination.

Because these mechanisms are not equally addressed by different treatment technologies, advanced quaternary processes rely on distinct physicochemical and oxidative pathways to reduce specific components of AMR risk. The main technologies currently investigated for this purpose are summarized in Table 1, highlighting strengths and

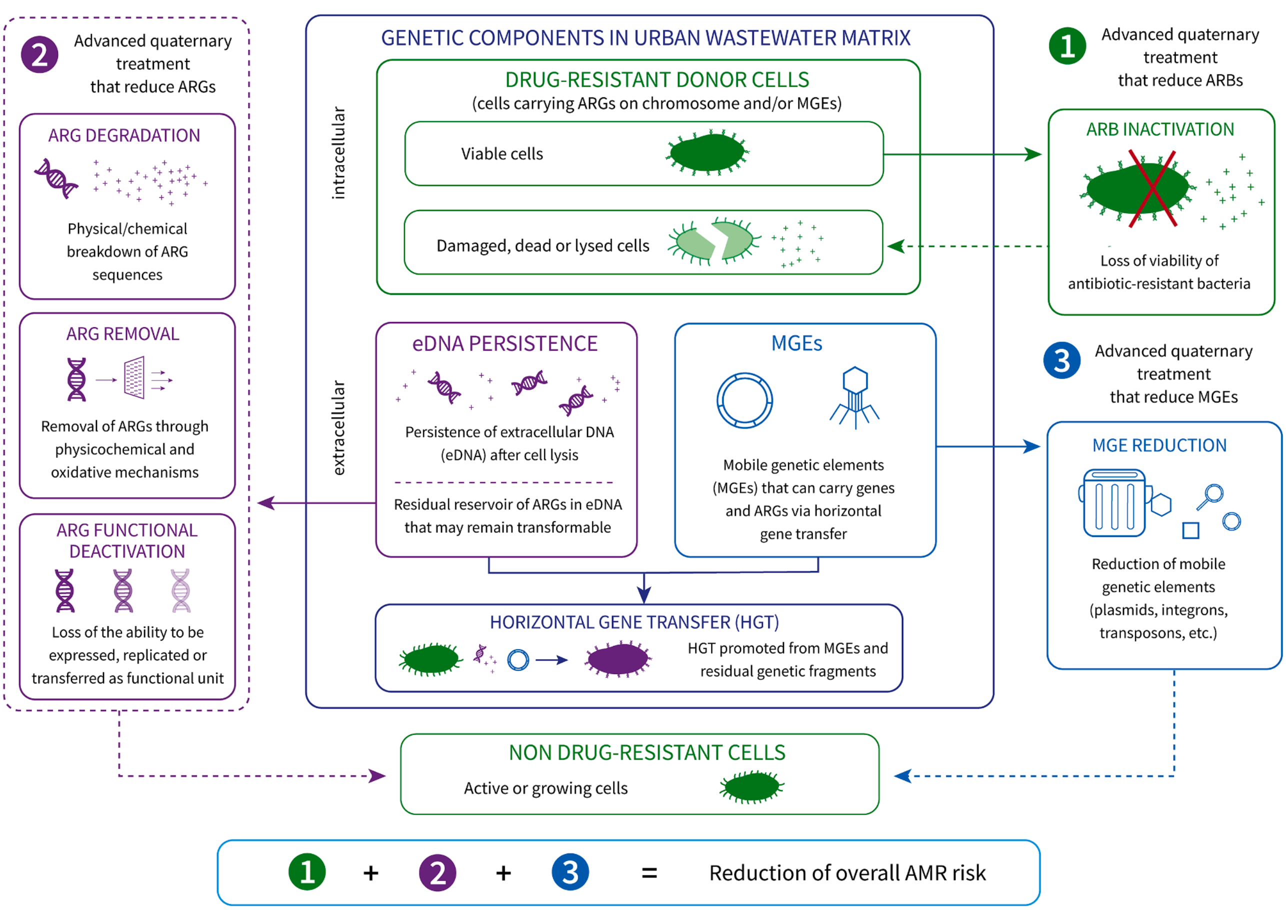

Fig. 2. Conceptual scheme of main factors affecting the reduction of HGT-risk and thus the overall AMR risk. The following combination is needed: (1) ARB inactivation, (2) ARG degradation, removal or functional deactivation, (3) limited eDNA persistence and MGE reduction.

Table 1
Overview of the disinfection processes and principal quaternary treatment technologies investigated for AMR mitigation; main mechanisms, attenuation efficiency, advantages, and operational limitations are also addressed for each treatment category.

Technology	Main mechanisms	ARB attenuation	ARG attenuation	Key strengths	Limitations
Disinfection technologies	Cl_2: cell membrane oxidation and nucleic acid damage UV: formation of pyrimidine dimers blocking DNA replication	High: >4 logs reduction for most ARB	Low: <1 to 2 logs. High Cl_2 doses (160 mg/L) and UV (200–400 mJ/cm^2) required for 3 logs removal	Well-established; very reliable for bacterial inactivation (ARB)	Formation of toxic DBPs (Cl_2); risk of photoreactivation repair (UV); stimulation of HGT at sub-lethal doses.
AOPs	O_3: cell lysis and direct molecular oxidation Generation of reactive oxygen species (•OH, SO_4•$^-$, H$^+$) causing intracellular oxidative stress and non-selective degradation of DNA	Very high: up to 7 logs reduction	Moderate to high: 1.1 to 6.0 logs	Robust against priority pathogens; can target persistent ARGs like *blaTEM* and MGEs like *intI1*	High costs; pH-dependency (pH~3 for Fenton); radical scavenging; eDNA release after lysis may remain more resistant than intact host cells
Membrane systems	Physical size exclusion; biomass retention; charge neutralization, and adsorption onto flocs (with pre-coagulation)	Very high: 6–7 logs	Moderate to high: 1–3 orders higher than CAS. >5.2 logs with pre-coagulation	Effective physical barrier; long SRT favors specialized microbial communities that degrade antibiotics	Membrane fouling; high energy demand (NF/RO); cell-free ARGs and those in viruses/phages can still persist (UF/NF); risk transferred to sludge/retentate
Adsorption processes	Physicochemical interactions: micropore filling, hydrophobic interactions, and π–π bonding. Physical retention of hosts within the sorbent bed	Moderate to high: >3 logs reduction	Moderate: ~1 log for GAC	Scalable and cost-effective; simultaneous removal of antibiotics and genetic material	Sorbent saturation/regeneration needs; biofilm growth can turn the bed into an AMR reservoir; risk of re-releasing ARGs
Hybrid treatment trains	Synergistic multi- barrier combining oxidation, physical separation, and biological degradation	High across multiple microbial targets	ARG fragmentation is combined with retention of residues and degradation	Multi-target attenuation; oxidation by-products are reduced	Operational complexity; high investment; potential for eDNA release during cellular membrane lysis

Abbreviations: CAS = Conventional activated sludge; DBP = Disinfection by-products; eDNA = Extracellular DNA; GAC = Granular activated carbon; NF = Nanofiltration; RO = Reverse osmosis; SRT = Sludge Retention Time; UF = Ultrafiltration.

limitations. A detailed explanation of each treatment category is presented and discussed in the next sections.

Although conventional disinfection processes (i.e. chlorination and UV) are not considered quaternary treatments in the regulatory sense, they are included in this analysis as a baseline/reference barrier because they are commonly implemented in UWWTP treatment trains and can affect ARB and ARGs, thereby influencing overall AMR mitigation.

2.2.1. Disinfection technologies

Chlorination (Cl_2, ClO_2) primarily targets microorganisms, leading to permeabilization of cellular membranes, loss of cell integrity, and disruption of metabolic functions (Pant et al., 2022). This antimicrobial action also involves oxidation of cell membranes and damage to enzymatic proteins and nucleic acids (Stefaniak et al., 2025). Although chlorination is highly effective at inactivating ARB, often achieving reductions > 4 logs, its ability to remove ARGs is substantially lower and varies by gene type (Khan et al., 2025; Yuan et al., 2015; Zhang et al., 2015). Most studies report ARG removals of <1.5 logs, thus requiring extreme Cl_2 dosages (up to 160 mg/L) to reach 3 logs reductions (Yuan et al., 2015; Zhang et al., 2015; Zhuang et al., 2015). Critical limitations include the formation of toxic and carcinogenic disinfection by-products (DBPs), such as chloroform and trihalomethanes, and the potential to stimulate HGT at sub-lethal doses by increasing cell permeability without fully degrading the DNA (Yu et al., 2023; Yuan et al., 2015).

Ultraviolet (UV) radiation, particularly at 254 nm, directly targets microbial DNA and RNA, inducing the formation of pyrimidine dimers (e.g., cyclobutane pyrimidine dimers) that physically block replication and transcription (Chang et al., 2017; Pan et al., 2026; Stefaniak et al., 2025). Conventional UV doses (20–50 mJ/cm^2) are efficient for ARB inactivation (>4 logs) but typically achieve minimal ARG removal (<1 log) (Khan et al., 2025; Zhuang et al., 2015). In fact, substantial ARG degradation requires significantly higher fluences, ranging from 200 to 400 mJ/cm^2 (McKinney and Pruden, 2012). The main limitations of UV treatment include the lack of a protective residual effect, which increases the risk of downstream regrowth or secondary contamination, and the capacity of surviving bacteria to initiate DNA repair mechanisms (photoreactivation and dark repair) to restore genetic functionality after exposure to UV radiation (Guo et al., 2009; Khan et al., 2025; Zhang et al., 2015). Recent studies emphasize the need to explore alternative UV wavelengths to overcome the limited efficacy of conventional UV irradiation at 254 nm (UV254) (Pan et al., 2026). In particular, UV irradiation at 222 nm (UV222)-based systems have been evaluated for their potential to remove disinfection residual bacteria (Gao et al., 2025). Findings indicate that UV222 generally exhibits higher inactivation efficacy compared to UV254, while composite systems, such as UV222/H_2O_2 and UV222/peroxymonosulfate, further accelerate the inactivation rates through the synergistic action of free radicals and oxidants. However, even when residual bacteria are reduced to safe thresholds, high UV222 fluences (300–500 mJ/cm^2) are still required to effectively inhibit post-treatment regrowth (Gao et al., 2025). Additionally, ultraviolet light-emitting diode (UV-LED) with a peak emission of 280 nm has recently emerged as a robust alternative for the on-site inactivation of ARB in hospital effluents (Azuma et al., 2024). This technology offers superior energy efficiency and the flexibility to combine multiple wavelengths in advanced reactor designs. The performance of the UV-LED-mediated disinfection is comparable to conventional mercury lamp, achieving 99.9% ARB inactivation within 3 min and ARG log removals between 0.1 and 1.3, with no statistically significant difference between the two UV sources (Azuma et al., 2024).

In addition to conventional options such as chlorination and UV irradiation, ozonation represents an alternative for the disinfection of treated wastewater effluents and may also contribute to AMR mitigation. However, its relatively high capital and operating costs, together with the potential formation of by-products, have generally limited its application when microbial inactivation is the sole treatment objective. Consequently, ozonation is more commonly implemented when disinfection is combined with the oxidative removal of organic micropollutants (Gulde et al., 2021). For example, Switzerland is among the leading countries in the full-scale implementation of ozonation for organic micropollutant removal from municipal wastewater effluents, with treatment systems designed to achieve an overall elimination efficiency of at least 80% (Bourgin et al., 2018; Gunten et al., 2025). Accordingly, ozonation is discussed more comprehensively in the AOPs section of this review (Section 2.2.2), where its combined roles in microbial inactivation and the oxidation of organic compounds can be addressed in an integrated manner.

2.2.2. Advanced oxidation processes

Ozonation (O_3) relies on both direct molecular oxidation and indirect radical pathways (•OH) to disrupt the bacterial structure, leading to cell lysis and the release of intracellular components (Cataldo, 2006; Iakovides et al., 2019; Stefaniak et al., 2025). Although ozone effectively inactivates many priority pathogens, its impact on ARGs is often inconsistent and does not always scale linearly with the applied dose, with reported removals generally ranging between 0.6 and 2 logs (Czekalski et al., 2016; Zheng et al., 2017; Zhuang et al., 2015). Additional constraints for large-scale implementation include high energy and capital costs for ozone generation, the formation of toxic by-products like bromates and aldehydes, and the risk of releasing eDNA into the effluent after bacterial cell lysis, potentially facilitating a subsequent transformation in downstream environments despite the successful microbial inactivation by ozone (Stefaniak et al., 2025; Yang et al., 2019; Zheng et al., 2017). Recent research has focused on novel ozone-based configurations to overcome these limitations. For instance, microbubble ozonation has emerged as an effective technique to increase ozone stability and enhance ARG degradation in hospital wastewater (Hsiao et al., 2023). Compared to traditional bubbling methods, this setup allows for deeper oxidative action and better dosage control, achieving an average ARG removal efficiency of 3.82 ± 0.67 logs at an ozone concentration × time (CT) value of 171 mg O_3 L^{-1} min (20 min response time). Finally, the integration of ozone-based advanced treatments, such as O_3/UV and O_3/UV/H_2O_2, has demonstrated superior performance, deactivating >99.9% of ARB within 10 min of treatment (Azuma et al., 2022). These hybrid configurations leverage synergistic radical generation to achieve higher levels of both microbial inactivation and gene degradation (further details on AOPs are provided in the following section).

Fenton and Photo-Fenton processes are based on the catalytic decomposition of H_2O_2 by iron ions, which generate highly reactive, non-selective hydroxyl radicals (•OH) with a high standard oxidation potential of 2.80 V capable of damaging cellular structures and nucleic acids (Bautista et al., 2008; Zhang et al., 2016). In the context of antibiotic resistance, these processes are particularly effective in ARB reduction (up to 7 logs) as they can induce intracellular oxidative stress, as both H_2O_2 and Fe^{2+}may diffuse across bacterial membranes and trigger internal radical formation, which leads to irreversible damage to DNA and effective inactivation of priority pathogens, such as *K. pneumoniae* and *P. aeruginosa* (Hou et al., 2019; Vilela et al., 2021). Achievable reductions for ARGs range from 1.1 to 6.0 logs, often surpassing the performance of conventional disinfection methods like chlorination or UV alone (Hou et al., 2019; Werkneh and Islam, 2023; Zhang et al., 2016). However, despite these promising results, most studies to date have been conducted at the laboratory scale using simulated matrices, e.g., pharmaceutical wastewater, limiting the direct transferability of the findings to full-scale UWWTPs (Hou et al., 2019).

In addition, traditional homogeneous Fenton processes have the drawback of requiring acidic conditions (typically pH ≈ 3) to avoid iron precipitation and the production of iron-based precipitates or residues (Bautista et al., 2008; Hou et al., 2019; Zhang et al., 2016), leading to the further need to neutralize pH before the wastewater is discharged into receiving water bodies. To overcome this operational barrier, recent research has successfully implemented solar photo-Fenton at neutral pH using strategies such as solar simulator chamber and intermittent iron dosing (Vilela et al., 2021) or the use of heterogeneous catalysts, including metal-organic frameworks (MOFs) and graphitic carbon nitride, which facilitate visible-light activation and catalyst recovery (Li et al., 2024a). However, a significant challenge remains: while ARB are efficiently inactivated, complete degradation of ARGs, particularly in the form of eDNA, requires higher oxidant doses (>300 mg H_2O_2 L^{-1}) or longer contact times (> 2 h) to minimize the risk of HGT from residual genetic fragments (Hou et al., 2019; Li et al., 2024a; Yang et al., 2024).

The UV photolysis of hydrogen peroxide (UV/H_2O_2) process is another homogeneous AOP in which UV irradiation photolyzes H_2O_2 to generate hydroxyl radicals (•OH) (Zhang et al., 2016). This treatment is highly effective against microorganisms and can achieve complete inactivation of antibiotic-resistant *E. coli,* although only under relatively long contact times (up to 240 min) (Ferro et al., 2017). However, its ability to degrade ARGs is considerably lower. Studies have shown that even after complete inactivation of bacterial hosts, ARGs such as *blaTEM* may persist in the treated effluent DNA (Ferro et al., 2017). Process efficiency is strongly influenced by the water matrix and operating parameters, with optimal ARG removal (2.8–3.5 log) observed under acidic conditions (pH around 3.5) and specific H_2O_2 dosages of 0.01 M (Zhang et al., 2016). Additional limitations include the potential for radical scavenging at excessive H_2O_2 doses and higher operational costs compared to conventional chlorination (Zhang et al., 2016).

Heterogeneous photocatalysis utilizes semiconductor materials, most commonly titanium dioxide (TiO_2) and graphitic carbon nitride ($g{-}C_3N_4$), which generate reactive oxygen species (ROS) after light activation (Chen et al., 2022; Yang et al., 2024). These ROS, including hydroxyl radicals (•OH), superoxide radicals (•O_2^-), and photogenerated holes (H^+), can effectively damage bacterial cells and genetic material. Recent advances in visible-light-activated catalysts have achieved rapid disinfection kinetics, with reductions of ARB up to 7 logs within 30 min (Yang et al., 2024). Furthermore, integrating photocatalysts into ultrafiltration membranes creates photocatalytic ultrafiltration systems (PUF) that combine physical separation with oxidative degradation of the resistome (Li et al., 2024a) (see Section 2.2.5). Despite these capabilities, extracellular ARGs remain more resistant to ROS attack than their bacterial hosts, limiting complete resistome removal (Chen et al., 2022). Wastewater constituents such as phosphates may also reduce process efficiency by blocking catalyst active sites and interfering with oxidative pathways (Chen et al., 2022).

Lastly, UV-activated persulfate (UV/PS) is an emerging AOP that generates both sulfate radicals (SO_4•$^-$) and hydroxyl radicals, providing strong oxidation capacity and selectivity across a broader pH range compared to many conventional AOPs (Zhou et al., 2020). This process has demonstrated high efficiency in wastewater disinfection, achieving up to 99.9% inactivation of quinolone-resistant bacteria in 10 min and reducing total ARG abundance by approximately 3.84 logs (Zhou et al., 2020). A distinct advantage of UV/PS is its ability to effectively remove MGEs like *intI1*, thereby reducing the risk of HGT in receiving water bodies (Zhou et al., 2020). Metagenomic sequencing has further revealed that UV/PS can induce favorable shifts in microbial community composition, more effectively reducing UV-resistant and potentially pathogenic taxa such as *Proteobacteria* and *Actinobacteria*, than UV treatment alone (Zhou et al., 2020).

2.2.3. Membrane systems

Membrane bioreactors (MBR), which combine biological removal processes with membrane filtration, typically microfiltration (MF) or ultrafiltration (UF), have emerged as effective barriers against AMR (Le et al., 2018; Li et al., 2019a; Nnadozie et al., 2017). One of the main advantages of AMR mitigation lies in the typical use of membranes with pores (0.01–0.4 μm) that are substantially smaller than bacterial cells (>0.5 μm, in general), allowing ARB reductions of up to 6–7 logs (Le et al., 2018; Wang et al., 2020). In addition, MBRs generally achieve ARG removals one to three orders of magnitude higher than conventional activated sludge (CAS) processes (Li et al., 2019a; Lin et al., 2021). This improved performance is often linked to the longer sludge retention time (SRT), which favors the development of more diverse microbial communities capable of degrading persistent antibiotics while also enhancing ARG adsorption onto the sludge flocs retained by the membrane (Li et al., 2019a; Nnadozie et al., 2017; Wang et al., 2020).

Despite their advantages, conventional MF and UF-based MBRs are still unable to fully remove cell-free ARGs, and those associated with the smallest biological particles, such as viruses and bacteriophages (Wang et al., 2020). Several studies report that although ARB are almost completely retained, measurable ARG concentrations, up to 10^3 copies/mL, can persist in the permeate, maintaining the potential for environmental dissemination (Le et al., 2018). To address this limitation, integrated systems combining pre-coagulation (e.g., with iron salts) and membrane filtration have been developed. In these systems, negatively charged DNA fragments are captured through charge neutralization and adsorption onto iron flocs, which are subsequently retained by the membrane, enabling eARG removals above 5.2 logs (Li et al., 2019b). Similarly, advanced nanofiltration (NF) and reverse osmosis (RO) can achieve even higher retention of common ARGs (4.98–9.52 logs) due to their denser membrane structures and different transport mechanisms (Lan et al., 2019). However, while membrane systems are currently among the most effective physical barriers, their broader implementation is still constrained by high energy demand, severe membrane fouling, and the management of concentrated retentate streams requiring further treatment (Nasrollahi et al., 2022).

2.2.4. Adsorption processes

Adsorption onto carbon-based materials, particularly granular activated carbon (GAC) and powdered activated carbon (PAC), has emerged as a scalable and relatively cost-effective quaternary treatment strategy for removing organic micropollutants, including antibiotic residues and associated resistance determinants (Zheng et al., 2025). These materials remove contaminants through a combination of physicochemical interactions, including micropore filling, hydrophobic interactions, hydrogen bonding, and π–π interactions (Zheng et al., 2025). PAC is particularly attractive because of the rapid adsorption kinetics, driven by its high surface-to-volume ratio, especially when super-fine PAC particles smaller than 10 μm are used (Merkler et al., 2025). It is frequently integrated into hybrid systems such as PAC-ultrafiltration (see 2.2.5), which combine in situ adsorption with membrane separation to improve microbial and chemical removal efficiency.

Conversely, GAC is commonly used in fixed-bed filters, often operating as bioactivated carbon (BAC), where adsorption is combined with biological degradation. In the context of antibiotic resistance, GAC acts as a dual-function barrier by combining physical retention of ARB with adsorption of extracellular genetic material (Mailler et al., 2024; Yang et al., 2019). Optimized GAC filters, characterized by balanced distributions of micropores (70–75%) and *meso*/macropores (25–30%), have achieved pharmaceutical removals above 80% and approximately 1 log reduction of ARGs (Mailler et al., 2024). In addition, modified GAC materials co-loaded with metals such as Ag, Fe, or Mg demonstrated dual abilities: selective enrichment of antibiotic-degrading functional genes involved in microbial degradation and the reduction of the overall ARG abundance in treated effluents (Zheng et al., 2025).

Biochar is increasingly being explored as a more sustainable and lower-cost alternative to activated carbon. Produced by biomass pyrolysis, usually exploiting waste residues, biochar possesses a highly porous structure capable of adsorbing antibiotics (Phan et al., 2026; Wu et al., 2020). Functionalized biochar, including β-cyclodextrin-modified materials, has demonstrated improved simultaneous removal of heavy metals, azo dyes, and ARGs (Wu et al., 2020).

More generally, adsorption-based processes face several inherent limitations related to long-term operation. Active adsorption sites become progressively saturated, requiring the regeneration or replacement of the adsorbent, which increases operational costs (Yang et al., 2019; Zheng et al., 2025). At the same time, biofilm growth in the filling medium may favor the enrichment of resistant microbial communities, including persistent taxa such as *Firmicutes* and *Proteobacteria* that can tolerate high localized concentrations of adsorbed antibiotics and their transformation products (Sompornpailin et al., 2024; Zheng et al., 2025). Continuous accumulation of antibiotics within the adsorbent may create selective pressure that transforms the filter itself into a reservoir of AMR, potentially leading to the re-release of ARGs and ARB into the treated effluent (Phan et al., 2026). Lastly, spent adsorbents must be carefully disposed of to avoid reintroducing concentrated resistance determinants into the environment (Zheng et al., 2025).

2.2.5. Hybrid treatment trains

Recent studies on quaternary wastewater treatment have highlighted the importance of exploring hybrid systems to improve overall sustainability and performance (Pasciucco et al., 2025). In particular, hybrid treatment trains combine physical, chemical, and/or biological processes into synergistic multi-barrier systems that have been identified as promising solutions. One representative example is ozonation followed by GAC filtration: ozone oxidizes contaminants and inactivates pathogens, while GAC, often operating as BAC, adsorbs oxidation by-products and supports further biological degradation of residual organic matter (Yang et al., 2019). This combination has demonstrated improved performance (with ARG removal > 2.5 logs) compared to standalone GAC processes for ARG attenuation and total organic carbon removal (Yang et al., 2019).

More recently, PUF systems, which integrate photocatalysts with UF membranes, have been introduced as a promising hybrid solution. In detail, these systems incorporate visible-light-responsive catalysts, such as TiO_2 or MOFs, directly into the membrane matrix (Li et al., 2024a). As a result, PUF systems combine physical size exclusion of bacterial hosts with oxidative degradation of residual genetic material through the generation of reactive oxygen species within the membrane pores. While PUF membranes have demonstrated ARG removal efficiencies exceeding 91.8%, a secondary challenge remains: lysis of bacteria on the membrane surface may release eARGs, which may be more resistant to oxidative attack than their original hosts (Li et al., 2024a).

Lastly, hybrid PAC-UF systems have shown strong performance, achieving >3 logs reductions of bacterial pathogens and substantial retention of most ARGs, bringing them to levels below the detection limit of the applied method (Merkler et al., 2025). In these systems, PAC adsorbs dissolved micropollutants and eDNA fragments, while the UF membrane acts as a physical barrier retaining both ARB and saturated PAC particles (Merkler et al., 2025). The addition of coagulants, such as ferric chloride, can further improve gene removal by promoting aggregation and capture of DNA-containing particles (Merkler et al., 2025).

2.2.6. Comparative analysis of ARG removal efficiency across disinfection processes and quaternary treatment technologies

When considering the broader landscape of available technologies, no single treatment consistently outperforms all others. To provide a comparative overview of current performance, Fig. 3 summarizes the minimum and maximum ARG removal values extracted from the studies discussed in the preceding sections.

Studies were included when treatment performance was evaluated using real wastewater matrices, including municipal secondary effluents and hospital or pharmaceutical wastewater. For each study, the lowest and highest reported log-removal values across the investigated ARG targets were extracted and grouped by treatment category. Differences in analytical methods, operational conditions (such as dosages and contact times), and wastewater characteristics across the cited studies are detailed in Table S1 of the Supplementary Materials.

In particular, the compiled studies target a broad spectrum of ARGs covering key clinical and environmental resistance classes. These include sulfonamides (e.g., *sul1, sul2, sul3*) and trimethoprim

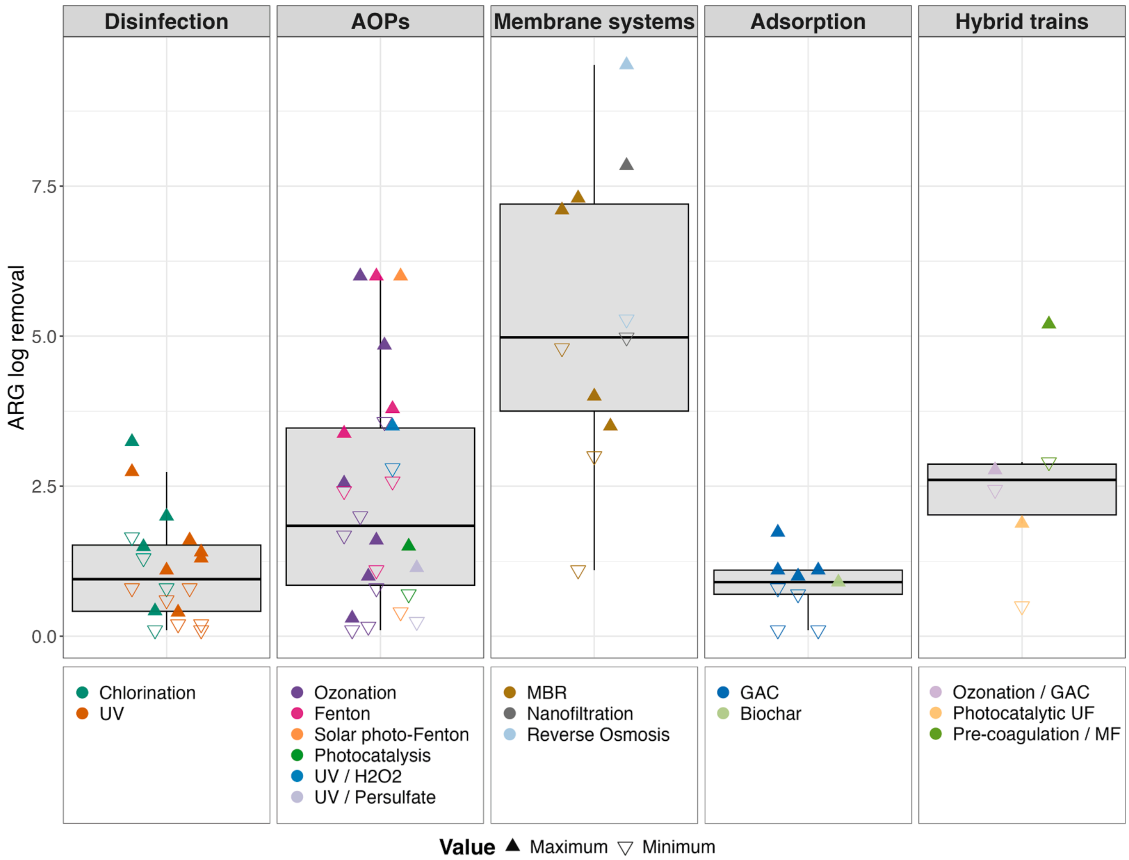

Fig. 3. Distribution of reported log removal values of ARGs across disinfection processes and major categories of quaternary wastewater treatments. Individual data points represent minimum (▽) and maximum (▲) values extracted from the literature for each treatment, while boxplots summarize the overall variability within each category. The line within each box represents the median value.

determinants (*dfrA, dfrA1*), alongside a wide variety of tetracycline resistance genes (*tetA, tetM, tetW, tetO, tetG, tetX*). The targeted β-lactams encompass both extended-spectrum β-lactamases and highly relevant carbapenemases (*blaTEM, blaCTX-M, blaNDM-1, blaKPC, blaOXA, blaIMP, blaGES*). Additional targets include macrolide resistance markers (*ermA, ermB, ermF, ereA, ereB*), aminoglycosides (*aadA1*), and glycopeptides such as the vancomycin resistance gene *vanA*. Some studies also monitor resistance to last-resort antibiotics like colistin (*mcr*-1), fluoroquinolone determinants (*qnrA/B/D/S, qepA*), and specific chromosomal mutations associated with resistance (*gyrA/B, parC/E*). Some focus is directed toward multidrug resistance and efflux pump systems, represented by genes such as *cfr*, the *acrA/acrB* complex, and the determinants *emrD*-1 and *sugE*, which are frequently detected in complex matrices like hospital wastewater. Finally, to assess potential horizontal gene transfer and resistance to quaternary ammonium disinfectants, common markers such as class 1 and 2 integrons (*intI1, intI2*), transposase *tnpA*, and the resistance genes *qacEΔ1* and *qacF/H* are included.

Although Fig. 3 provides an overview of the reported removal ranges, direct comparisons among technologies should be interpreted cautiously. Current studies differ substantially in target ARGs, wastewater matrix composition, operational conditions and treatment design, preventing robust benchmarking of treatment performance. Rather than maximizing ARG removal alone, technology selection should increasingly consider which component of AMR risk is being targeted. In fact, AMR dissemination is driven by multiple interacting components, including viable ARB, iARGs and eARGs, and MGEs, and different technologies preferentially act on different parts of this pathway (Fig. 2). For this reason, bacterial inactivation or a reduction in ARG abundance does not necessarily demonstrate the loss of gene functionality or a reduction in HGT potential.

From a practical perspective, treatment selection should therefore be guided by the primary treatment objective rather than by reported log removals alone. Conventional disinfection is highly effective when rapid ARB inactivation is the main goal, whereas AOPs offer greater potential for degradation of eDNA and MGEs. Membrane systems provide excellent physical containment of bacteria and genetic material but transfer part of the AMR burden to concentrated retentate or sludge streams requiring further management. Adsorption processes primarily contribute to the indirect mitigation of AMR by removing antibiotics and residual contaminants, thereby reducing selective pressure, rather than by directly removing genetic materials. On the other hand, hybrid treatment trains combine complementary mechanisms and are therefore expected to provide the most comprehensive reduction of AMR risk. The wide performance variability observed within each treatment category further reflects the biological heterogeneity of the resistome itself. For example, membrane systems may achieve near-complete retention of cell-associated ARGs while exhibiting substantially lower removal of eDNA or smaller genetic fragments, resulting in markedly different reported efficiencies depending on the selected targets. Similar technology-dependent variability is observed for disinfection and AOPs, where removal strongly depends on the persistence of individual ARGs, wastewater composition, and process operating conditions.

The available evidence consequently supports the adoption of tailored multi-barrier treatment trains rather than reliance on a universally effective stand-alone process.

3. Artificial intelligence in quaternary treatments

Quaternary treatment technologies can achieve substantial removals for many target compounds, but their performance remains highly sensitive to wastewater matrix variability, operating conditions, and multi-objective trade-offs involving contaminant removal, energy consumption, operating costs, and by-product formation. In this context, AI-driven tools are emerging as enabling technologies for adaptive quaternary treatment, leveraging their ability to predict process performance, thereby supporting design, monitoring, optimization, and control strategies (Table 2). This section analyzes the main applications of AI for enhancing the performance of quaternary treatment processes, including predictive modeling, advanced monitoring, process understanding, optimization and control, and smart design strategies.

The studies reviewed in this section demonstrate that AI is already contributing to addressing several methodological challenges relevant to quaternary wastewater treatment by enabling accurate performance prediction, surrogate monitoring, process optimization, interpretable feature analysis, and accelerated process and material design. However, much of the available evidence concerns heavy metals, per- and polyfluoroalkyl substances (PFAS), pharmaceuticals, and other micropollutants rather than direct AMR-related endpoints. Accordingly, this section draws on these studies to identify transferable AI capabilities and provides a critical synthesis of the current state of the art, the main unresolved challenges and the key requirements for translating existing knowledge into AMR-oriented treatment strategies.

3.1. AI in predictive modeling

One of the most established roles of AI in wastewater treatment is predicting process performance under complex and highly variable operating conditions. In quaternary treatment applications, AI has demonstrated high accuracy in predicting micropollutant removal efficiencies, substantially reducing experimental workload. Traditional mathematical/mechanistic methods are often constrained by simplified assumptions and complex equations, whereas ML models can capture strong nonlinear relationships among collected data (Cairone et al., 2024a). For example, artificial neural network (ANN)-based models have achieved very high predictive performance in metal adsorption studies, with reported R^2 values up to 0.9974 for copper removal (Bhagat et al., 2021). Similarly, Taiwo and Musonge (2026) implemented AI models to predict adsorption-based removal efficiencies for heavy metals such as zinc and lead. Wang et al. (2026) showed that properly tuned ML models achieved a nearly sixfold improvement in predictive performance of antimony adsorption by MOFs, with R^2 increasing from 0.13 to 0.76 when relevant chemical features were included as input variables.

An analogous predictive role has emerged in membrane-based quaternary treatments. For technologies such as forward osmosis (FO) and NF, AI offers a data-driven approach to address the classical trade-off between permeability and selectivity (Gao et al., 2026). ML models can predict membrane rejection efficiency, water flux, and other process indicators by incorporating both membrane properties and molecular descriptors of target contaminants. Feature engineering is especially useful in this context, since descriptors such as molecular weight and McGowan volume help identify the most relevant micropollutant properties for predicting rejection behavior and, ultimately, for designing membranes with pore structures better suited to specific wastewater matrices (Zhu et al., 2023b). In the study by Yogarathinam et al. (2024), AI models achieved very high accuracy for predicting the removal efficiency of micropollutants through nanopore-sized membranes (R^2 up to 0.965). Likewise, hybrid models combining support vector regression (SVR) with metaheuristic optimization methods, such as the Firefly Algorithm, have achieved strong predictive performance, with correlation coefficients (R) up to 0.970 for predicting micropollutant rejection rate in FO membrane systems (Aldrees et al., 2024).

In the case of AOPs, predictive modeling can play a crucial role, as treatment performance is highly sensitive to fluctuations in influent wastewater characteristics and operating conditions. Since the experimental quantification of trace organic contaminants and the determination of degradation kinetics require laborious, expensive analyses based on chromatography and tandem mass spectrometry (e.g., LC-MS/MS or GC–MS/MS), AI represents a promising alternative for estimating treatment performance starting from surrogate variables. For example, fluorescence excitation-emission matrix (EEM) data have been successfully coupled with multi-target regression random forest (MORF) models to accurately predict (R^2 up to 0.95) pollutant degradation in municipal secondary effluent (Yang et al., 2024). In particular, in this latter study, AI models improved predictive accuracy compared to conventional linear regression (by about 0.15 in R^2), thereby confirming AI algorithms' ability to capture process performance under conditions that are

Table 2
Summary of the main applications, expected benefits, and key challenges associated with the integration of AI/ML models in quaternary wastewater treatments.

Application	Short description	Benefits	Challenges
Process performance prediction	Data-driven models can capture complex and non-linear relationships among wastewater characteristics, operating conditions, process configuration, and treatment performance	AI/ML models can accurately predict process efficiency without requiring the explicit formulation of complex mechanistic equations or simplifying assumptions	Predictive accuracy strongly depends on the availability of large, representative, and high-quality datasets. Robust data collection, storage, preprocessing, and analytics workflows are required
Real-time monitoring and control	When integrated with online sensors, Internet of Things (IoT) devices, and supervisory control systems, AI can enable continuous monitoring, early-warning capabilities, and adaptive process control	AI-driven monitoring and control can reduce reliance on offline analyses, fixed thresholds, and operator experience, allowing more proactive management of process anomalies	Real-time implementation requires reliable sensors, robust data pipelines, and adequate maintenance. Sensor fouling, calibration drift, noisy signals, model inaccuracies, and complex wastewater matrices may compromise measurement reliability and control actions
Process optimization	Predictive models can be coupled with optimization algorithms to identify suitable operating conditions and support more informed process management	AI-based optimization can enhance treatment efficiency by improving micropollutant removal, while reducing energy demand, chemical consumption, and experimental burden	AI-based optimization may perform poorly when applied to operating conditions, water matrices, or treatment configurations that are not adequately represented in the training dataset
Support for process understanding	Explainable AI techniques can improve the interpretability of black-box models by identifying influential input variables and clarifying their contribution to model predictions	Improved interpretability can support hypothesis generation, reveal relevant process patterns, and facilitate the integration of data-driven insights with mechanistic knowledge	Interpretability methods provide model-based explanations rather than direct causal evidence. Expert assessment and experimental validation remain essential to avoid misleading mechanistic interpretations
Advanced process and technology design	AI/ML models can accelerate the screening, comparison, and design of treatment technologies by capturing relationships among material properties, operating conditions, wastewater characteristics, and treatment performance	AI-driven design can reduce trial-and-error experimentation and support the rational development of next-generation quaternary treatment technologies, including membranes, adsorbents, catalysts, advanced oxidation systems, and hybrid configurations	AI-driven design remains limited by data availability and transferability across treatment systems and wastewater matrices. Experimental validation remains essential before real-world implementation

challenging to describe using traditional methods.

These studies demonstrate that AI can accurately predict the performance of complex quaternary treatment processes. However, available models have been developed for conventional micropollutants, heavy metals, or treatment indicators, and therefore do not directly demonstrate their effectiveness for AMR control. Their transfer to AMR mitigation requires model retraining and validation using specific outputs such as ARB inactivation, iARG and eARG removal, MGE abundance, and residual transfer potential.

3.2. AI in real-time monitoring

AI can be effectively implemented to develop surrogate monitoring tools and real-time sensing frameworks, making them suitable also for the quaternary treatment of wastewater. Monitoring micropollutants at environmentally relevant concentrations remains one of the most demanding aspects of wastewater treatment. Conventional analytical techniques, such as LC-MS/MS, are sensitive and reliable, but costly and time-consuming. Contrarily, AI algorithms enable the use of indirect yet rapidly and often economically measurable signals as surrogates for treatment performance, thereby paving the way for real-time monitoring strategies (Yang et al., 2024). This concept has been demonstrated in AOP monitoring, where coupling fluorescence EEM spectroscopy with MORF models provided faster prediction of pollutant removal during the UV/H_2O_2 process (Yang et al., 2024). Rather than waiting for offline chemical analysis, UWWTP operators can use AI tools to dynamically track process performance in (almost) real-time.

More broadly, AI is also enhancing the performance of advanced sensors for wastewater quality monitoring. In fact, integrating nanotechnology with DL is creating new opportunities for ultra-sensitive contaminant detection. For instance, the combination of carbon quantum dots with a powerful combination of convolutional neural networks (CNN) and Long Short-Term Memory (LSTM) networks (the CNN-LSTM architecture) has enabled simultaneous detection of multiple contaminants (including heavy metals, pharmaceuticals, and pesticides) at subnanomolar levels, corresponding to a 50/100-fold improvement over conventional sensors (Jena et al., 2025). In parallel, AI-supported Laser-Induced Raman and Fluorescence Spectroscopy has demonstrated strong potential for online monitoring of micropollutants in wastewater (Post et al., 2022). When coupled with CNN, these systems enabled the detection of several compounds in UWWTP effluents, including pharmaceuticals such as carbamazepine, naproxen, and tryptophan, at concentrations of a few μg/L.

These applications demonstrate that AI can convert rapidly measurable optical or sensor-based signals into surrogate indicators of treatment performance. Nevertheless, direct online measurement of ARB, ARGs, and MGEs is not yet routinely available, and surrogate relationships may vary among wastewater matrices and UWWTPs. Transfer to AMR monitoring will therefore require the calibration of online signals against standardized microbiological and genomic measurements, followed by external validation under variable full-scale operating conditions.

3.3. AI in process optimization and control

Beyond performance prediction, AI can support process optimization and control strategies, reducing reliance on sequential, time-consuming experiments.

Ebere Enyoh et al. (2023) demonstrated the effectiveness of AI models, particularly ANN, in predicting ciprofloxacin removal efficiency through adsorption processes, consistently outperforming the statistical approach of Response Surface Methodology (RSM). In this study, the developed predictive models, combined with a desirability function approach, enabled the identification of the optimal operating conditions, including temperature, pH, and ionic strength, aimed at maximizing removal efficiency.

In membrane processes, AI-assisted optimization supports both system design and operational management, aiming at maximizing micropollutant rejection while maintaining substantial permeate flux and minimizing energy demand. The integration of ML models with metaheuristic optimization algorithms holds great potential in this context. This approach enables the prediction of process behavior and supports the identification of optimal operating conditions and membrane selection to enhance treatment performance (Aldrees et al., 2024; Viet et al., 2022). ML models are also increasingly being explored to predict membrane fouling trends, achieving performance comparable to, and in some cases surpassing that of, conventional models. Membrane fouling dynamics are inherently complex and are strongly influenced by feed characteristics, membrane properties, and operating conditions. While conventional modeling methods often fail to capture the intricate interactions between these factors, AI algorithms have proven effective for this purpose. These advantages of AI in modeling membrane fouling support the development of predictive maintenance strategies and membrane cleaning procedures, thereby contributing to extending membrane lifespan and reducing operating costs (Cairone et al., 2024c, 2024b).

In AOPs, operational control is particularly challenging because oxidant demand and reaction pathways are highly sensitive to wastewater characteristics. In this context, AI models can support the optimization of key process variables, such as the optimal oxidant dose, by using readily measurable parameters such as pH, dissolved organic carbon (DOC), and alkalinity (Cha et al., 2024, 2021). This approach enables more precise adjustment of operating parameters and improved control of oxidation conditions, underscoring the promising potential of AI for optimizing quaternary treatment processes.

More broadly, implementing AI-driven monitoring, optimization, and control systems for quaternary wastewater treatment can enhance operational resilience by enabling the early detection of abnormal conditions, facilitating timely corrective actions, and improving overall process reliability and stability.

Available evidence indicates that AI can identify operating conditions that improve contaminant removal while reducing chemical consumption, energy demand and experimental effort. However, the analyzed optimization frameworks do not include AMR-specific risk endpoints and may overlook unintended effects, such as ARG release or enhanced HGT under sub-lethal treatment conditions. AMR-oriented optimization should therefore incorporate constraints related to ARB viability, functional ARG persistence, MGEs, HGT potential, and residual antibiotic selective pressure, rather than relying solely on conventional removal efficiency.

3.4. AI in process understanding and mechanistic interpretation

AI modeling may support the understanding of treatment processes by providing valuable insights that should be further validated through experimental tests. Although early applications of AI in environmental engineering were often criticized for being “black-box” approaches, recent advancements in explainable AI (XAI) have substantially enhanced model interpretability, facilitating a better understanding of the reasoning behind model outputs. XAI methods, such as SHapley Additive exPlanations (SHAP) and Local Interpretable Model-agnostic Explanations (LIME), are designed to improve the transparency of ML models by providing: (i) global interpretability, which explains the model’s overall decision logic, and (ii) local interpretability, which focuses on explaining specific predictions made by the model. Therefore, the implementation of XAI techniques allows the identification of the dominant input variables that influence model outputs. In quaternary wastewater treatment, this capability supports the verification of model consistency with technical knowledge and physicochemical factors, while also facilitating the formulation of hypotheses about process performance dynamics.

Many studies have demonstrated the potential of XAI methods to identify the variables that predominantly drive process modeling, thereby providing valuable insights for an in-depth understanding. For example, XAI-based interpretations have revealed that pH is one of the most influential parameters for predicting micropollutant removal, including PFAS and pharmaceuticals, across different treatment processes, such as ion exchange, NF, and adsorption (Karbassiyazdi et al., 2022; Wang et al., 2024). Similarly, XAI has been implemented to identify key factors influencing the predictions of adsorption capacity of heavy metals on ion exchange or chelate resins (Xu et al., 2025a). From a water process engineering perspective, this potential of XAI methods to provide insights on the key variables influencing data-driven modeling may support mechanistic understanding, thereby also offering guidance for process optimization and smart technology design (see Section 3.5).

XAI methods have improved the interpretation of data-driven models by identifying the variables most strongly associated with treatment performance. However, feature importance and model explanations describe statistical relationships and should not be interpreted as direct evidence of causality. Their transfer to AMR mitigation therefore requires experimental and microbiological validation to determine whether the identified variables actually govern AMR-related processes, including ARG persistence, host association, cellular damage, or HGT.

3.5. AI in smart process and technology design

AI tools, particularly XAI techniques, play an important role in helping engineers to design materials and configurations for the quaternary wastewater treatment. Recent studies have demonstrated the effectiveness of AI in screening advanced adsorbents, including MOFs, hydrogels, biochar, and functionalized sorbents for micropollutant removal (Shu et al., 2026; Yuan et al., 2024; Zhang et al., 2024). For example, Warren-Vega et al. (2025) proposed ANN models to study the adsorption mechanisms of Cu(II) onto different adsorbents. Their results suggested that the adsorption process may be governed by a combination of adsorbent properties, including porous texture and surface chemistry. These insights can help tailor the design of adsorbents with enhanced properties, thereby improving efficiency in wastewater treatment processes. By rapidly screening large combinations of structural and chemical descriptors, AI tools can substantially reduce the experimental burden required for material discovery and optimization. This is highly relevant in the development of next-generation adsorbents, where the number of potentially useful formulations is too large to be explored solely through conventional laboratory testing. Recent studies have also demonstrated the feasibility of integrating experimental design and ML to design sustainable Fenton processes for advanced wastewater treatment (Pervez et al., 2026). Similarly, AI-assisted design offers a promising approach to guide the development of next-generation membranes with tailored separation performance by moving beyond conventional trial-and-error approaches (Cao et al., 2024; Ignacz et al., 2025; Liang et al., 2025; Pervez et al., 2023). ML models can thus be used to predict treatment performance and identify the physicochemical and structural features that control permeability, selectivity, separation mechanisms, and fouling behavior, thereby supporting an optimized membrane design.

These studies demonstrate that AI can accelerate the screening and design of adsorbents, membranes, catalysts and treatment configurations by linking material properties with process performance. Nevertheless, most available material databases and predictive targets are not specifically designed around AMR-related mechanisms. Transfer to AMR mitigation requires the inclusion of descriptors associated with bacterial adhesion and inactivation, DNA adsorption and degradation, extracellular ARG retention, membrane-microorganism interactions, and the prevention of resistance enrichment within treatment materials.

4. AI-driven monitoring and optimization of quaternary wastewater treatments for AMR mitigation

As discussed in Section 3, the integration of AI/ML into predictive modeling, process optimization, control, and advanced monitoring systems can improve the performance of quaternary treatments, including the enhancement of AMR mitigation. In this context, integrating AI with metagenomic analysis represents a promising advancement for monitoring antibiotic resistance throughout wastewater treatment. This approach has the potential to improve the understanding and prediction of resistome dynamics within wastewater treatment systems, ultimately providing valuable insights to enhance treatment performance. This section explores the multifaceted applications of AI models (briefly summarized in Table 3 and in-depth explained in the following subsections), organized into three primary areas: (i) wastewater resistome profiling; (ii) prediction of AMR dynamics; and (iii) optimization of quaternary treatment for AMR mitigation.

4.1. AI applications in wastewater resistome profiling

Recent studies show that AI-driven approaches are moving beyond simple detection of genetic markers toward more predictive, high-resolution surveillance of complex resistomes in diverse urban and environmental matrices. For instance, Arango-Argoty et al. (2018) highlight the potential of DL models to outperform conventional bioinformatics approaches, achieving high precision (> 0.97) and recall (> 0.90) in ARG prediction.

Complementing these approaches, Lund et al. (2025) applied a Random Forest (RF) model to predict horizontal ARG transfer between bacteria. In this study, the authors explored the factors governing HGT across nearly one million bacterial genomes and >20,000 metagenomes, including water and wastewater microbiomes. Their results identify wastewater as a key environment facilitating the exchange of ARGs across phylogenetically distant hosts, with particularly strong signals associated with aminoglycoside acetyltransferases. In fact, despite inherent biological constraints such as genetic incompatibility, the high ecological connectivity of wastewater systems appears to promote gene transfer, with the model achieving robust predictive accuracy (performance metric AUROC = 0.873).

From a broader perspective, Arnold et al. (2025) emphasize the potential of AI-driven wastewater monitoring within a One Health framework to better capture the complex interactions between human, environmental, and microbial systems. As reviewed by Arnold et al. (2025), advanced ensemble methods such as XGBoost and RF have been successfully applied to demonstrate that genomic data across varying bacterial populations can be leveraged to identify novel resistance patterns before they eventually emerge and become relevant in clinical settings. A critical advantage of these AI-driven workflows is the implementation of feature selection strategies, which are essential to prevent introducing noise into models when handling high-dimensional data, such as sequence data, thereby reducing the risks of overfitting to the training data and poor generalization to novel sequences (Arnold et al., 2025).

4.2. AI-enabled prediction and interpretation of AMR dynamics in wastewater treatment

Recent studies have proposed ML approaches to monitor and predict the spread of ARGs in wastewater treatment systems, while also supporting their mechanistic interpretation (Alexa et al., 2026; Lu et al., 2025). These approaches are particularly valuable for handling high-dimensional metagenomic datasets and for integrating multiple layers of information, including microbial community composition, the chemical exposome, and UWWTP operational conditions.

An early demonstration of this potential was provided by Sun et al. (2021), who explored the feasibility of using RF models to predict the

Table 3
Summary of metagenomics studies, model performance metrics, and strategic applications of AI/ML models for wastewater AMR characterization and mitigation.

Application	Input variables	Output	Model/ Algorithm	Model development and evaluation	Performance metrics	Main strengths	Reported limitations	Reference
HGT prediction	12 genomic, ecological, taxonomic, and ARG-related features.	Probability of horizontal ARG transfer between bacterial hosts	RF	Data retrieved from public repositories; 70/30 split; default hyperparameters; 8 models were developed: one trained on HGT representing all analyzed ARG classes and seven for specific resistance mechanism; FIA was performed	AUROC = 0.873; Mean sensitivity = 0.806; Mean specificity = 0.785	Integrates data from ~1 million bacterial genomes with co-occurrence patterns from >20,000 metagenomic samples to predict the HGT of ARGs between bacterial hosts and reveal insights into how genetic and ecological factors govern the spread of ARGs	Additional factors influencing the successful HGT of ARGs will likely be discovered as the genomic and metagenomic repositories become more comprehensive; Using Gram staining as a proxy for cell envelope structure may have reduced model performance in predicting the HGT of ARGs for some taxa	Lund et al. (2025)
ARG abundance prediction in activated sludge	11 abundant genera, 29 (opportunistic) pathogens, 3 indicator bacteria and 7 nitrifiers	Abundance of 22 selected ARGs	RF	Data collected from previously published studies; 60/40 split; 5-fold CV; trial-and-error process to determine the best set of hyperparameters; variable importance evaluation; the best performing model was applied to a full-scale plant not included in the development dataset	R^2 up to 0.718 (test)	Identifies taxa strongly associated with ARG abundance and potential hosts	RF regression may underestimate high values and overestimate low values; wastewater characteristics and operational parameters could be included as input variables	Sun et al. (2021)
Resistome–exposome analysis	Resistome: 80 targeted ARGs and 12 MGEs; Exposome: antibiotics, biocides, non-steroidal anti-inflammatory drugs, and heavy metals; Metadata: physicochemical properties and climatic parameters	Variable most affected by treatment (RF) and factors associated with relative abundance of ARGs (LASSO)	RF (impact of UWWTPs on the resistome and exposome) and LASSO (impact of environmental factors on ARGs)	Data from 96 samples collected during four campaigns at three UWWTPs; a leave-one-week-out sensitivity analysis assessed the potential effect of the small dataset	RF mean accuracy: 69.2–94.2%	Provides valuable insights into the associations between environmental factors and ARGs in special settings	Limited sampling frequency; values below detection limits prevented distinction between very low abundance and gene absence from wastewater samples	Alexa et al. (2026)
Predicting ARG and MGE dynamics in biological nitrogen removal	For MGEs predictions features included 9 environmental factors, 3 sludge characteristics and 3 microbial community characteristics. For ARGs predictions, the MGEs variation efficiency was additionally included	Changes in ARG and MGE abundance	CatBoost, RF, XGBoost, ANN	Data collected from previously published studies; 80/20 split; hyperparameter optimization via Optuna; external validation using 16 independent data points from previously published literature; FIA and SHAP analysis were conducted	ARGs: R^2 = 0.843 (CatBoost, test set) MGEs: R^2 = 0.708 (RF, test set)	Provides a platform with a graphical user interface that supports the assessment of ARGs transmission risks and the decision-making in BNR systems	Lack of experimental work to validate the model; Heterogeneous ARG/MGE quantification methods and target types may affect predictions; potentially relevant features were omitted because of inconsistent data reporting	Lu et al. (2025)
Identifying key factors influencing conjugative ARG transfer under low doses of free chlorine	Free chlorine, anion, cations and dissolved organic matter concentrations, exposure mode, pH	ARG conjugative transfer frequency	Linear/RBF/ polynomial SVM, RF, MLP	189 experimental data points; 75/25 split; 5-fold CV; SHAP and tipping points analysis were performed	R^2 = 0.975; RMSE = 0.012; MAE = 0.009; Accuracy = 0.882; Variance = 0.002 (Linear SVM, test set)	Provides insights into the impact of low-dose chlorine on ARGs in the reclaimed water reuse process, supporting a more comprehensive AMR assessment framework	Limited dataset size; further investigation is warranted to develop a more comprehensive predictive framework for ARGs dissemination under complex environmental conditions	Xu et al. (2025b)

(*continued on next page*)

Table 3 (*continued*)

Application	Input variables	Output	Model/ Algorithm	Model development and evaluation	Performance metrics	Main strengths	Reported limitations	Reference
Performance prediction in chlorine, UV, O_3, and UV/H_2O_2 processes	Initial features for ARG degradation included chemical, biological and genetic parameters. For ARG deactivation, parameters related to recipient cells and to transformation experiments were additionally included	ARG degradation, deactivation and removal efficiency	RF, GBDT, XGBoost, LightGBM, CatBoost	Data collected from previously published studies; 90/10 split; GridSearch with CV and RFE for model optimization; external validation on unseen ARGs; SHAP analysis was performed	ARG degradation: R^2 = 0.926–0.989; RMSE = 0.098–0.289 (test set) ARG deactivation: R^2 = 0.871–0.929; RMSE = 0.253–0.466 (test set)	Links qPCR-measured degradation and transformation assay-measured deactivation of multiple types of ARGs in disinfection processes to predict the ARG removal efficiency	Limited data volume; small dataset for external validation; the models did not consider the effects of wastewater quality and microorganisms	Huang et al. (2025)
Design of antimicrobial nanomaterial membranes	Physicochemical properties of five representative nanomaterials (zeta potential, contact angle, specific surface area, particle size)	ARB and ARG removal efficiency	RF, AdaBoost, Decision Tree	Experimental data complemented by data extracted from previously published studies; 75/25 split; CV and Bayesian optimization; FIA was performed	RF: R^2 = 0.8718; RMSE = 0.0093; MAE = 0.0045	Identifies the features most strongly influencing ARB/ARG removal; the top-performing membrane identified by the model was fabricated and successfully tested on raw hospital wastewater	Extending the approach to a wider range of material library will be essential for deriving more universal design rules; comprehensive evaluation under extreme operating conditions (e.g., high salinity, variable pH, elevated organic matter) will be necessary	Liu et al. (2026)
Optimizing tubular membrane separation processes to improve the removal of ARGs	Membrane properties, polymer properties, additive properties, operating conditions, water quality parameters and ARG type	ARG removal efficiency	Different ML algorithms (CatBoost, XGBoost) combined with different categorical feature encoding methods (JamesStein, OneHot, Ordinal, Helmert, Hashing and CatBoost)	Data extracted from previously published studies; 80/20 split; 5-fold CV; leave-one-out robustness assessment; SHAP analysis was performed	R^2 = 0.711; MAPE = 0.126 (JamesStein-XGBoost model)	Provides actionable ranges for transmembrane pressure (5–20 bar) to optimize ARG retention	Due to the lack of on-site or laboratory experimental verification, caution should be exercised when directly applying the research findings to actual membrane treatment processes	Tao et al. (2026)

Abbreviations: AdaBoost = Adaptive Boosting; AUROC = Area Under the Receiver Operating Characteristic Curve; BNR = biological nitrogen removal; CatBoost = Categorical Boosting; CV = cross-validation; FIA = feature importance analysis; GBDT = gradient boosted decision trees; LASSO = Least Absolute Shrinkage and Selection Operator; LightGBM = Light Gradient Boosting Machine; MAE = mean absolute error; MAPE = mean absolute percentage error; MLP = multilayer perceptron; RBF = radial basis function; RMSE = root mean squared error; RF = random forest; RFE = recursive feature elimination; SHAP = SHapley Additive exPlanations; SVM = support vector machine; XGBoost = eXtreme Gradient Boosting.

abundance of specific ARGs in activated sludge based on shotgun metagenomic data. Their work relied on a systematic analysis of 248 metagenomic datasets collected from UWWTPs across 10 countries. Three groups of bacterial genera were used as explanatory variables: “abundant bacteria”, “pathogens and indicator bacteria”, and “nitrifiers”. The RF models achieved moderate predictive performance, with R^2 up to 0.72 on the testing set. Beyond prediction, variable importance analysis provided biologically meaningful insights into potential ARG hosts. In particular, the group including (opportunistic) “pathogens and indicator bacteria” exhibited stronger positive relationships between individual genera and ARGs than “abundant bacteria” and “nitrifiers”; in particular, taxa such as *Bacteroides, Clostridium*, and *Streptococcus* appear as primary potential ARG hosts. These findings demonstrate that ML can support ARG prediction and hypothesis generation regarding the microbial drivers of ARG dissemination, thereby providing useful information for optimizing wastewater treatment processes.

Alexa et al. (2026) further investigated how the exposome, climate variables, and physicochemical factors influence ARG dynamics across urban wastewater systems. The authors collected samples during four campaigns representative of different seasons from eight UWWTPs treating influents from distinct catchments, characterized by varying combinations of urban, hospital, poultry farming, and tourist-related inputs. Resistome and exposome in UWWTP influents and effluents were profiled. Then, ML algorithms, including RF classifiers and Lasso regression, were applied to identify the ARGs and exposome components most affected by wastewater treatment, as well as the environmental factors associated with ARG occurrence. Based on ML outputs, the authors identified the “upstream gene”, i.e., the relative abundance of ARGs in the influent source of wastewater (in other words, the anthropogenic inputs like neighborhoods, agriculture, farming, hospitals, etc.), as a primary driver of effluent ARGs. The authors also highlighted associations between context-specific exposome (e.g., antibiotics, selected metals, temperature) and ARG removal. Their analysis showed that the macrolide-resistance gene *ermB*, which is among the most abundant genes in hospital and urban contexts (touristic and non-touristic), decreased in effluents from UWWTPs, indicating a consistent reduction of specific macrolide-resistance markers in the conventional wastewater treatment. Regarding the exposome, ciprofloxacin and Cu were among the compounds most reduced by UWWTPs in both touristic and non-touristic areas. These results reinforce the idea that ARG dynamics in UWWTPs cannot be explained solely on the basis of microbial composition. Rather, they emerge from the complex interplay among biological communities, chemical stressors, and operating conditions.

Recent studies have further expanded this approach by explicitly incorporating operating parameters, pollutant types and concentrations, and microbial community characteristics to predict ARG abundance in wastewater treatment systems. In this context, advanced interpretability tools have been implemented to identify the dominant factors shaping ARG patterns. Among these studies, Lu et al. (2025) investigated the effects of environmental factors and antimicrobial agent exposure on changes in the abundance of ARGs and MGEs during biological nitrogen removal (BNR), a core process in UWWTPs. The authors collected 372 experimental data points from published studies related to nitrification, denitrification, and anammox systems. They compared four ML algorithms, including Categorical Boosting (CatBoost), RF, XGBoost, and ANNs, using physicochemical parameters, environmental factors, antimicrobial concentrations, and microbial community descriptors as input variables. Among the tested models, CatBoost showed the best predictive performance for ARG abundance changes ($R^2 = 0.843$), whereas RF performed best for predicting MGE abundance changes ($R^2 = 0.708$). To improve the mechanistic interpretation of ARG and MGE spread during wastewater treatment, the authors applied XAI techniques, including SHAP and feature importance analysis (FIA). FIA revealed that changes in MGE abundance were the most influential input feature for predicting changes in ARG abundance, suggesting that horizontal gene transfer plays a key role in ARG dissemination. Similarly, SHAP analysis showed that several environmental factors, including exposure time and pollutant concentration, together with microbial community characteristics such as the abundance of *Bacteroidetes* and *Proteobacteria*, were among the most important predictors of changes in ARG and MGE abundance. These findings enabled the authors to propose strategies for controlling operational parameters in the plant, in order to maintain effective treatment performance while minimizing the risk of ARG spread. Finally, the practical relevance of the work by Lu et al. (2025) was further strengthened by the development of a graphical user interface designed to support ML-driven operational decision-making for the prediction and control of ARG spread in wastewater treatment systems.

The findings obtained from these studies highlight the growing importance of integrating XAI techniques to improve the interpretability of complex ML models and to extract actionable insights from their predictions. In fact, a major limitation of many AI-based approaches is their “black-box” nature, which can obscure the reasoning behind model outputs and hinder the interpretation of relationships among the variables governing treatment performance (Wang et al., 2023a, 2023b). In this context, XAI provides a set of methods that allow users to better understand and evaluate AI-generated outputs by making model decision processes more transparent and interpretable (Holzinger et al., 2022; Tsang and Benoit, 2023). Besides, these techniques can help identify the most relevant input features, clarify their contribution to model predictions, verify consistency with domain knowledge, and support the mechanistic validation of model behavior (Cairone et al., 2026).

4.3. AI-assisted optimization of quaternary treatment processes for AMR control

ML has also been increasingly applied to predict ARG behavior during advanced wastewater treatment processes, including disinfection, membrane filtration, and emerging material-based technologies. In this field, AI-assisted approaches can support the rational design, optimization, and control of treatment processes for ARG and ARB removal.

Xu et al. (2025b) collected 189 samples of reclaimed water produced in two UWWTPs and disinfected with low-dose chlorine. The authors implemented several ML algorithms, including RF, SVM, and multilayer perceptron (MLP) models, to analyze the effect of low-dose free chlorine (0.05–0.3 mg/L) on conjugative ARG transfer (a form of HGT) in reclaimed water and to identify the key factors influencing this transfer. The results showed that low-dose free chlorine favored conjugative ARG transfer, with 0.15 mg/L having a stronger promoting effect than free chlorine concentrations of 0.05 or 0.3 mg/L. Intermittent exposure patterns were also associated with a higher risk of ARGs dissemination. Among the tested algorithms, the SVM model achieved the best predictive performance, with $R^2 = 0.975$ and root mean squared error (RMSE) = 0.012, as evaluated using a training/test split. SHAP analysis identified bicarbonate, sodium alginate, nitrate, and humic acid as key drivers influencing ARG transfer under low-dose chlorine exposure. Importantly, the study revealed threshold values at 0.03 mg/L free chlorine, 20 mg/L bicarbonate, and 2.25 mg/L sodium alginate, beyond which the promotion of ARG transfer increased markedly. This finding is particularly relevant because it suggests that suboptimal disinfection conditions may unintentionally enhance ARG dissemination rather than suppress it. To improve the model’s generalizability, the authors emphasize the need to expand the training dataset by incorporating data from diverse geographical regions and continuously monitored reclaimed water systems. Moreover, they highlight the importance of developing a more comprehensive predictive framework for ARG dissemination under complex environmental conditions, integrating biological factors, hydrological dynamics, and environmental variability, such as rainfall.

Further recent studies addressed a key challenge in ARG removal via disinfection, concerning the distinction of molecular damage from true functional deactivation of resistance determinants. Specifically, Huang et al. (2025) developed an ML-based framework using qPCR results as proxies to estimate transformation assay measurements for different ARG types and during various disinfection methods: free available chlorine (FAC), UV254, O_3, and UV/H_2O_2. Data were collected from previously published studies on ARG degradation and deactivation, totaling 1341 data points for degradation and 491 data points for deactivation. The authors tested five nonlinear ML algorithms, including RF, gradient boosting decision tree (GBDT), XGBoost, light gradient boosting machine (LightGBM), and CatBoost, to predict ARG degradation rates and ARG deactivation rates. The models achieved high predictive performance, with $R^2 > 0.926$ and RMSE < 0.289 for ARG degradation, as well as $R^2 > 0.871$ and RMSE < 0.466 for ARG deactivation. These models substantially outperformed traditional linear regression approaches, such as multiple linear regression (MLR), which showed limited predictive accuracy. The authors also developed a tandem prediction strategy combining ARG degradation and deactivation models to estimate ARG removal efficiency under given disinfection conditions. This strategy achieved good predictive performance, with $R^2 > 0.828$ and RMSE < 0.411. This framework was further translated into an online platform, named “Aquatic Chemistry with Artificial Intelligence” (ACAI, http://www.ac-ai.tech/ACAI/OP/), to facilitate broader application by providing users with access to the developed ML models for rapid evaluation of ARG removal efficiency. Despite these high predictive accuracies, the framework is constrained by the homogeneity of the training data, which may not fully capture ARG behavior, as the effects of wastewater quality and microorganisms can significantly alter disinfection dynamics and the fate of resistance genes. SHAP analysis was implemented to assess the importance of input features in predicting ARG degradation and deactivation, thereby supporting the identification of factors that exert the greatest influence on specific treatment processes. For example, pH was identified as more important for the FAC process than for the other processes, reflecting the sensitivity of FAC disinfection efficiency to solution acidity or alkalinity. Finally, the authors identified O_3 and UV/H_2O_2 as more effective processes for the degradation and deactivation of the tested ARG types compared with FAC and UV254 (Huang et al., 2025).

The latest research has proposed ML to guide the design and optimization of novel wastewater treatment technologies. In particular, Liu et al. (2026) proposed an ML-supported strategy to design high-performance filtration membranes based on iron sulfide nanomaterials for the simultaneous removal of ARB and ARGs from hospital wastewater. Rather than relying on broad empirical screening, the authors synthesized a targeted set of iron sulfide nanocrystals with different crystalline phases and interfacial properties, aiming to establish clear structure-property-function relationships for the simultaneous inactivation of ARB and ARGs. Among the tested materials, a pyrite-based nanocrystal emerged as the most effective candidate, showing strong activity toward both iARG disruption and eARG degradation. To identify the physicochemical features governing antimicrobial performance, the authors integrated experimental data with a curated literature dataset including both laboratory simulations and real environmental water samples, and applied ML models, including RF, Adaptive Boosting (AdaBoost), and Decision Tree regressors. The RF model achieved the best predictive performance, with $R^2 = 0.8718$, RMSE $= 0.0093$, and MAE $= 0.0045$. FIA revealed that interfacial properties, particularly zeta potential and surface hydrophobicity, were the dominant factors controlling ARB and ARG removal. By contrast, conventional descriptors such as particle size and specific surface area played a secondary role. The analysis indicated that near-neutral surface charge and increased hydrophobicity favored interactions with bacterial membranes and DNA promoting ARB/ARG removal, whereas highly negative and hydrophilic surfaces reduced treatment efficiency. ML results were further supported by microbiological assays, spectroscopic analyses, and molecular simulations, which helped clarify the mechanisms responsible for iARG degradation and eDNA binding. The optimized membrane, characterized by moderately negative surface charge and enhanced hydrophobicity, was then applied to actual hospital wastewater treatment, achieving broad-spectrum iARG and eARG removal ($> 97\%$ and $> 90\%$, respectively). Even over five operational cycles, the filtration membrane maintained stable hydraulic performance (80–85 % of its initial rate) and durable ARB removal efficiency. Although iron sulfides are naturally abundant minerals with relatively low material costs, suggesting favorable economics for large-scale production, their practical implementation in full-scale UWWTPs remains to be demonstrated. Beyond material synthesis, key challenges include long-term membrane stability, fouling resistance, regeneration requirements, and performance under highly variable wastewater matrices (e.g., fluctuations in salinity, pH, and organic matter content).

Within the broader framework of AI-assisted process optimization, Tao et al. (2026) developed ML models to correlate polymer properties, membrane characteristics, additive parameters, and operating conditions with ARG removal efficiency in wastewater treatment using tubular membranes. The dataset was extracted from previously published literature and included 130 data points, covering 9 different tubular membranes and 31 ARGs. Among the tested algorithms, the JamesStein-XGBoost model showed the best performance, with $R^2 = 0.711$ and MAPE $= 0.126$ on the test set. SHAP analysis indicated that ARG subtype, influent concentration, feed characteristics such as pH, phosphate concentration and total organic carbon (TOC) content, and operating parameters such as transmembrane pressure (TMP) were the main contributors to ARG removal efficiency. Based on SHAP analysis, the authors identified suitable ranges for key input features, providing a specific basis for selecting preferred membrane materials and operating conditions to optimize ARG removal. For example, moderate TMP values (5–20 bar) were suggested to enhance ARG retention. These findings illustrate the potential of interpretable ML to generate actionable operational insights to advance wastewater treatment technologies. However, the authors emphasize the need for on-site or laboratory experimental validation before these data-driven strategies can be reliably implemented in full-scale engineering practice.

The analyzed studies indicate that AI modeling is becoming an increasingly valuable tool for studying and controlling antibiotic resistance in wastewater systems, including quaternary treatment processes. Its value lies not only in its predictive performance but also in the ability to integrate heterogeneous datasets, reveal nonlinear relationships, identify key biological and chemical drivers, and translate complex interactions into decision-support frameworks (Fig. 4). In this context, ML is increasingly emerging as a practical instrument for the design, optimization, and control of wastewater treatment strategies aimed at mitigating AMR dissemination.

5. Current challenges, future perspectives, and roadmap for integrating AI in quaternary wastewater treatment to enhance AMR mitigation

Following the adoption of the revised EU UWWTD 2024/3019, UWWTPs are expected to play an increasingly strategic role in AMR mitigation within a One Health surveillance framework. In this context, WBE may represent a key surveillance tool, enabling integrated, population-level epidemiological monitoring and supporting the early detection of emerging trends before they are fully captured by clinical surveillance systems (Corpuz et al., 2020; Foladori et al., 2024). This section discusses the key scientific, technological, and operational challenges that must be addressed to successfully implement AI-driven monitoring, optimization, and control systems for quaternary wastewater treatment to enhance AMR mitigation. Special emphasis is placed on big data analytics, comprehensive technology assessment, and AI-driven smart monitoring and control.

5.1. *Strategic advances driven by big data analytics*

The progressive implementation of advanced treatment stages,

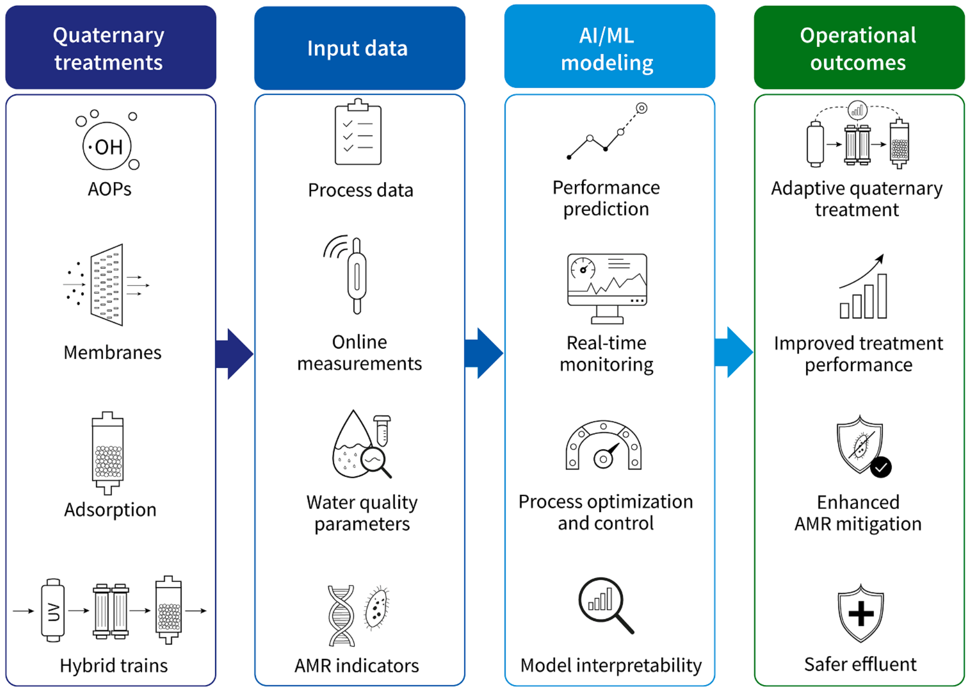

Fig. 4. Integration of AI models to advance quaternary wastewater treatments.

together with the online and real-time monitoring of wastewater parameters relevant to public health, is expected to generate large volumes of data on wastewater quality, treatment efficiency, and AMR-related indicators. The key challenge will therefore not be limited to improving ARG and ARB removal, but will also involve transforming monitoring data into actionable insights for process optimization and public-health decision-making. This aspect is particularly relevant because monitoring data, when properly processed and interpreted, can substantially support the improvement of treatment processes aimed at both micropollutant removal and AMR control.

Regular monitoring of AMR loads is essential from epidemiological and engineering perspectives. From an epidemiological perspective, AMR monitoring in urban UWWTPs can provide information on the resistance burden within served populations, complementing clinical surveillance and supporting early detection of emerging resistance patterns. From an engineering perspective, it enables evaluation of treatment performance and identification of technologies, operating conditions, and process configurations that are more effective at reducing AMR dissemination. However, the value of these monitoring efforts will strongly depend on the ability to extract meaningful insights from the collected data. This potential often remains untapped in current UWWTP practice, where large amounts of data are collected, archived, and rarely converted into operational knowledge, thus becoming "data graveyards" rather than "data mines" (Cairone et al., 2024a). This underutilization is mainly due to the lack of reliable analytical tools, limited interoperability among monitoring platforms, and gaps in operators' knowledge, which hinder rapid and efficient data processing, analysis, and interpretation. Therefore, future efforts should focus not only on increasing the amount of data collected but also on improving data quality, harmonization, accessibility, and interpretability, as well as on the speed at which these actions can be performed.

A further limitation is that current AMR studies often differ in sampling strategies, sample preservation procedures, DNA extraction protocols, sequencing depth, target genes or bacteria, normalization methods, and bioinformatic workflows. As a result, cross-study comparisons remain challenging, and translating individual findings into generalizable design or operational criteria is still limited. Recognizing these limitations, several international initiatives have recently been launched to harmonize wastewater-based AMR surveillance. In Europe, the implementation of Article 17 of the UWWTD 2024/3019 is being supported by collaborative initiatives such as EU-WISH and EU-JAMRAI 2, which are working toward standardized surveillance targets, sampling procedures, analytical methods, quality assurance protocols, and shared reporting frameworks for wastewater AMR monitoring (Hock et al., 2026). A key priority is the definition of a shared core panel of surveillance targets for treated effluents and receiving waters, prioritizing resistance determinants of human or animal health relevance that are sufficiently prevalent to ensure consistent detection across sites and interpreted alongside general fecal indicators such as *E. coli* (Hock et al., 2026). For treatment-performance assessment, this core panel should include quantitative targets measurable by qPCR or dPCR, ranging from selected resistant bacteria to clinically relevant ARGs. However, as the relevant predictors, response variables, temporal resolution and modeling strategy depend on the intended application, the harmonization of AMR monitoring should not be interpreted as requiring a single universal dataset structure or analytical workflow. Instead, the objective should be to establish a comprehensive and transparent monitoring framework that promotes interoperability across laboratories and regions, while retaining sufficient flexibility to accommodate different treatment technologies, wastewater matrices, monitoring objectives and modeling applications. The recommendations presented below should therefore be regarded as a framework for supporting the standardized collection, documentation and integration of data for AI-enabled AMR monitoring.

A fundamental step in constructing datasets for AI applications supporting AMR mitigation in quaternary wastewater treatment is the clear definition of the AMR-related endpoints to be monitored and modelled. Depending on the application, these endpoints may include the absolute or normalized abundance of ARGs, the ARB concentration, the MGE occurrence and abundance, treatment-specific log-reduction values, the functional inactivation of ARGs, the transformation or conjugation potential, and composite indicators of AMR risk. When normalized endpoints are reported, the normalization procedure, reference quantity and associated assumptions should be explicitly documented. Each endpoint should be accompanied by sufficient methodological and quality-control information to support interpretation, reproducibility, and comparison across studies. Relevant metadata may include sample-processing procedures, DNA extraction methods, primers and analytical assays, sequencing platform and depth, reference database and version, bioinformatic pipeline, software implemented, measurement units, normalization method, analytical approach, number of biological and technical replicates, field and laboratory blanks, limits of detection and quantification (LOD and LOQ) and quality-control flags. When surrogate endpoints or indirect sensor measurements are used, their relationship with appropriate microbiological, molecular, or functional reference methods should be established for the intended application and wastewater matrix. The domain of validity of the surrogate measurement should also be reported to avoid extrapolation beyond the conditions under which it was evaluated.

In addition to AMR-specific measurements, datasets should describe the sampling and treatment contexts in which the data were generated. Essential information may include the treatment configuration, sampling location, sampling date and time, sampling frequency, sampling mode, sample volume, preservation method, transport conditions, storage temperature and duration, and the time elapsed between sample collection and analysis. Relevant catchment characteristics should also be documented, such as the relative contribution of domestic, hospital, industrial, agricultural or other wastewater sources, the served population and the presence of combined sewer systems. These contextual data may be complemented by application-specific predictor variables. Depending on the modelling objective, these may include: (i) routinely measured wastewater characteristics corresponding to the same sample, sampling period or treatment unit used for AMR analysis, such as temperature, pH, conductivity, turbidity, total suspended solids, chemical or biochemical oxygen demand, and nutrient concentrations; (ii) treatment-specific operating parameters such as hydraulic retention time, sludge retention time, dissolved oxygen concentration, oxidation–reduction potential, oxidant or disinfectant dose, contact time, UV fluence, membrane flux, transmembrane pressure, backwashing conditions, adsorbent dose; and (iii) where available and relevant, microbial-community descriptors, ARG and MGE profiles, taxonomic and functional features, and other metagenomic variables. Variables should be temporally and spatially aligned with the corresponding AMR endpoint, and any aggregation, interpolation or missing data treatment should be explicitly documented.

A structured metadata framework is essential for determining whether datasets produced by different studies, treatment plants, or monitoring frameworks are sufficiently comparable to be combined or jointly analyzed. Rather than requiring the immediate creation of a single centralized dataset, harmonization could support interoperable databases or distributed data infrastructures based on common data models, standardized units and quality criteria. Such an approach would facilitate the progressive development of comprehensive and regularly updated AMR data resources while preserving information on data provenance, methodological differences, access conditions, and suitability for specific AI applications.

5.2. Moving toward comprehensive evaluation of quaternary treatment technology performance

Conventional disinfection and quaternary treatment technologies, including AOPs, membrane filtration, adsorption, and hybrid configurations, have demonstrated potential for AMR mitigation. However, their reported performance varies widely depending on wastewater matrix composition, target genes, operating conditions, and analytical methods. Future research should therefore focus on improving treatment efficiency and defining the specific conditions under which each technology is most effective and sustainable.

Most available studies quantify AMR mitigation in terms of percentage removal or log reduction of selected ARGs and ARB. Although these metrics are useful for comparing treatment efficiency, they do not necessarily reflect the residual biological risk associated with treated effluents. For example, a reduction in targeted ARG abundance does not necessarily imply complete loss of gene functionality, elimination of eDNA, or removal of viable resistant hosts. Likewise, treatment processes that effectively inactivate ARB may still promote the release of ARGs, while sub-lethal oxidative or disinfectant exposure may increase cell permeability and potentially enhance HGT. Future assessment frameworks should therefore integrate multiple dimensions of AMR risk, including ARG persistence, host association, MGE occurrence, transformation potential, and residual antibiotic selective pressure. This shift would allow quaternary treatments to be evaluated not only as micropollutant removal steps, but also as risk-management barriers against AMR dissemination. In addition, broader evaluations of AMR mitigation technologies should combine ARG and ARB removal efficiencies with sustainability performance indicators. Processes optimized only for ARG removal may entail undesirable trade-offs, such as increased formation of oxidation by-products, higher energy consumption, greater carbon footprint, increased operating costs, or accumulation of resistance determinants in secondary waste streams, such as spent adsorbents, membrane concentrates, or sludge. Therefore, future technology assessment should adopt a multi-criteria perspective that jointly considers AMR risk reduction, micropollutant removal, ecotoxicological impacts, resource consumption, operational robustness, and economic feasibility. In this perspective, AI-supported multi-objective optimization could help identify operational windows that maximize public-health and environmental benefits while minimizing unintended burdens.

5.3. Developing smart monitoring and control systems for adaptive quaternary treatment

Pioneering studies have demonstrated the potential of AI/ML to support ARG/ARB prediction and to model quaternary wastewater treatment performance for AMR mitigation. These approaches are particularly promising because AMR dynamics in wastewater are complex and influenced by interactions among microbial communities, chemical stressors, treatment conditions, and environmental variability. However, many AI models used in wastewater treatment are currently trained on relatively small and site-specific datasets, limiting their transferability across UWWTPs, climate conditions, wastewater characteristics, and treatment configurations. This limitation is particularly relevant for AMR mitigation, since resistome composition is shaped by local antibiotic consumption patterns, hospital discharges, industrial inputs, livestock and agricultural activities, seasonal variability, and plant-specific operating conditions. Efforts should therefore be directed toward developing large, comprehensive, high-quality, representative, and harmonized training datasets that combine ARG/ARB concentrations and removal efficiencies with metadata describing sampling context, wastewater characteristics, operational parameters, treatment configuration, microbial community composition, and analytical workflows. In parallel, model testing and validation across different UWWTPs should become a standard requirement for assessing the robustness, generalizability, and practical applicability of predictive AI models.

With the rapid advances in AI/ML algorithms, predictive models can reach high levels of accuracy. However, their practical value may be constrained by their inherent black-box nature, particularly when model outputs are intended to support engineering decisions or risk-management strategies. Explainable AI techniques represent a key approach to overcoming this limitation, as they can identify the variables that most strongly influence predictions and clarify how these variables affect model outputs. Nevertheless, explainability alone should not be interpreted as causal evidence. Future efforts should move toward explainable and physics-informed AI models (Di Bella et al., 2026). By integrating ML approaches with essential physicochemical, biological, and genomic knowledge, including kinetic descriptions, mass-transfer relationships, oxidative degradation mechanisms, microbial interactions, and horizontal gene transfer dynamics, these models could provide more reliable, generalizable, and scientifically grounded predictions. The choice of modeling strategy should be guided by the scientific or operational objective, the size and structure of the available dataset, the type of AMR-related output, the temporal and spatial resolution of the data, the expected complexity and nonlinearity of the underlying relationships, and the required level of model interpretability. Different modeling approaches may be appropriate depending on whether the objective is to predict quantitative AMR indicators, classify samples or treatment conditions into predefined categories, identify temporal trends or anomalous events, or support early warning and process control applications. No single algorithm can therefore be recommended across all applications. Model selection should instead be based on the characteristics of the data and the intended use of the model results.

Model development should follow established best practices (Zhu et al., 2023a), with particular attention to data quality and the use of a sample size appropriate to the modeling task. Common methodological pitfalls, including overfitting and data leakage, should be carefully addressed. The modeling workflow should be designed and implemented to prevent information from the validation or test data from influencing model training, feature selection, data preprocessing or hyperparameter optimization, thereby reducing the risk of overly optimistic estimates of model performance. Whenever feasible, model evaluation should be complemented by external validation using independent data, such as samples collected during subsequent monitoring campaigns or data obtained from UWWTPs not represented in the dataset used for model development. Such validation would provide stronger evidence of model generalizability and transferability across temporal, operational and site-specific conditions.

The potential of AI may be further enhanced by integrating predictive models into digital twins and adaptive control systems for quaternary treatment. In this vision, offline AMR monitoring could be progressively complemented by online and near-real-time surrogate measurements, which AI/ML models can use to estimate AMR-related risk indicators that cannot currently be measured continuously. When embedded in digital twins, such models could simulate the expected response of different treatment configurations and support operators in selecting optimal operating conditions. For example, AI-assisted monitoring and control systems could adaptively adjust process parameters, such as disinfectant dose, membrane backwashing frequency, oxidant dose and exposure time, adsorbent regeneration frequency, and so on, according to influent variability and target risk reduction. Such systems would enable quaternary treatment to move from fixed operating setpoints toward adaptive AMR-risk management. Importantly, external validation across different UWWTPs should become a standard requirement for assessing the reliability of AI-driven decision-support tools.

5.4. Toward risk-based evaluation of quaternary treatment technologies

Although the abundance of ARGs and ARB is fundamental information, assessing the residual biological risk posed by HGT dissemination requires specific model frameworks. The existing frameworks for assessing the human health risks can be divided in: (i) quantitative microbial risk assessment approaches, which are based on traditional time-consuming and expensive microbiology, have a well-defined structure but suffer from uncertainties regarding the exposure pathways and the not yet fully understood dose-response of AMR; (ii) ARG-centric hierarchical or quantitative models that rely on metagenomic alignment, and calculate a quantitative risk score based on ARG abundance and the corresponding risk levels. Regarding the latter approach, an integrated ML framework (i.e., an adaptive random forest model) was developed by Jiang et al. (2024) to rapidly quantify the relative burden of AMR using readily accessible environmental variables, without requiring dose-response information. However, consensus on the best model framework for systematically conducting health risk assessment remains lacking, and studies are limited (Chen et al., 2025). One of the main limitations in applying the risk model is the limited availability of data for predicting the dissemination process. For example, new emerging exposure routes related to wastewater include bioaerosols, microplastic carriers, and reclaimed water reuse, which still require new quantitative data and real-world observations that are currently lacking. To address this challenge, future efforts should focus on developing AI- and ML-based mining tools (Chen et al., 2025). In fact, the assessment of ARGs and ARB health risks is an interdisciplinary field that requires a large set of heterogeneous data, including environmental monitoring, bio-databases, clinical data, and human health data. These large volumes of complex information can be analyzed using algorithms such as ML and spatiotemporal analysis, which help extract meaningful patterns, identify relevant associations, and ultimately support the risk assessment. It is worth noting that the frameworks stop at health risk levels and are still unable to provide sufficient information for decision-makers. Risk assessment is thus currently based on fragmented domains (environmental, clinical, economic, etc.) and warrants a broader, more specific discussion beyond the scope of this manuscript.

5.5. The strategic role of multidisciplinary collaboration in implementing AI-driven monitoring, optimization and control for AMR mitigation in quaternary wastewater treatment

The scientific and technological priorities outlined in the previous sections require a coordinated framework in which microbiology, wastewater engineering, data science, AI, public health, and regulatory perspectives are integrated from the earliest stages of problem definition. In this context, multidisciplinary collaboration should be considered a prerequisite for translating AI-supported AMR monitoring into operationally meaningful, scientifically robust, and process-oriented wastewater management strategies (Fig. 5).

Within the multidisciplinary collaboration framework illustrated in Fig. 5, each discipline should contribute its specific expertise to support the effective implementation and operation of the proposed framework. An illustrative allocation of possible responsibilities and an associated workflow is described below. Public health and regulatory stakeholders could define the management question, the evidence required to support an alert or intervention, the applicable reporting and accountability procedures and the conditions under which an operational response may be considered. Environmental microbiologists could identify the relevant ARB, ARGs, MGEs and microbial hosts, establish sampling and analytical protocols, define quality-control requirements, and provide reference measurements for model development and performance evaluation. Wastewater engineers could relate AMR indicators to treatment performance and operating conditions, identify suitable online or rapidly measurable surrogate variables, and define the controllable parameters, engineering constraints, safe operating ranges and procedures for managing anomalous conditions for each treatment process. Data scientists could support the integration of laboratory results, sensor measurements, operational records and associated metadata within harmonized and traceable data architectures. Their contribution could also include data quality assessment, data preprocessing and the detection and documented treatment of anomalous or missing observations. In coordination with these disciplines, AI specialists could translate the agreed management objectives into prediction, anomaly-detection, or optimization tasks and develop models that are appropriately validated, sufficiently interpretable for expert review, and periodically assessed for changes in predictive performance.

These discipline-specific contributions could be connected through a staged, human-in-the-loop workflow. During operation, a predicted decrease in AMR attenuation could initially trigger automated checks of sensor status, data completeness, data quality and model performance. If the signal persists and its potential significance warrants further

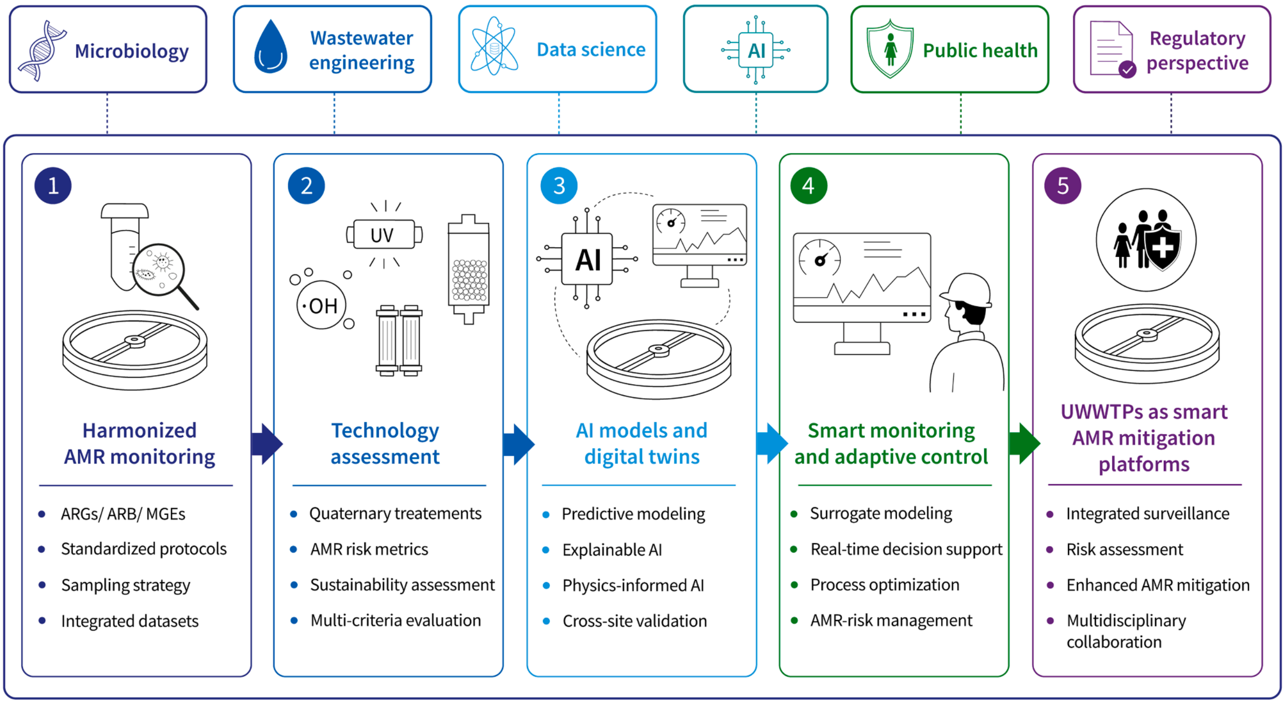

Fig. 5. Multidisciplinary approach to enhance AMR mitigation through UWWTPs.

investigation, confirmatory microbiological analysis should be undertaken before implementing a process intervention. This requirement may be adapted according to the urgency of the event, the availability and response time of the reference methods and the existence of pre-defined precautionary actions. Any operational adjustment should remain within pre-approved actions and under the responsibility of the plant operator. The effectiveness of any intervention should subsequently be evaluated using appropriate operational indicators and, where relevant, reference microbiological measurements.

AMR-related information generated in wastewater treatment systems may have different implications depending on the disciplinary perspective from which it is interpreted. For example, an increase in the relative abundance of selected ARGs in UWWTP effluents may be interpreted by microbiologists as a shift in the resistome, by wastewater engineers as a possible indication of reduced treatment performance requiring process-level investigation and optimization, and by public-health authorities as a potential environmental exposure concern. This diversity of interpretations highlights the need to co-design monitoring and decision-support objectives, including the selection of target indicators, sampling strategies, metadata processing, analytical protocols, and model outputs. Target selection for AMR surveillance requires interdisciplinary collaboration among environmental microbiologists, experts in surveillance and control of human and animal AMR, and other stakeholders. This integrated approach is essential to prevent the generation of information that, although scientifically relevant, is difficult to translate into full-scale operational decisions. For example, it can help prevent the development of AI models that are highly accurate from a predictive perspective but poorly aligned with operational decision-making needs.

In wastewater management systems aimed at mitigating AMR dissemination, AI should support expert judgment rather than replace it. Model outputs should therefore be interpreted through multidisciplinary expertise. As previously discussed, explainable AI approaches are essential to ensure that model predictions, classifications, or anomaly-detection outputs can be critically evaluated by engineers, microbiologists, and operators, and translated into technically feasible management actions. Capacity building is equally important: UWWTP operators and managers should be able to understand the meaning, limitations, and operational relevance of AMR-related indicators and AI-derived outputs, while researchers, microbiologists, and data scientists should account for the practical constraints of full-scale UWWTPs, including sampling feasibility, process variability, operating costs, data management requirements, and regulatory obligations. This mutual exchange of knowledge is essential to ensure that AI-driven monitoring, optimization, and control systems are not only scientifically advanced but also practically implementable in full-scale UWWTPs.

AI-derived outputs should be regarded as complementary decision-support information rather than as substitutes for measurements obtained using applicable reference methods. Specifically, they could complement reference monitoring by increasing monitoring frequency, enabling (almost) real-time surveillance, supporting early-warning systems, and improving the temporal and spatial resolution of AMR monitoring while optimizing analytical resources. Before AI-driven approaches are implemented in AMR monitoring, their performance should be evaluated against standardized monitoring methods using appropriate validation datasets. Future regulatory and technical guidance could progressively establish minimum requirements for data quality, model validation and continuous performance monitoring.

The successful integration of AI-driven monitoring, optimization, and control systems in quaternary wastewater treatment for AMR mitigation will therefore depend not only on technological development but also on the alignment of microbiological evidence, engineering feasibility, computational reliability, public health relevance, and regulatory acceptability. Under these conditions, UWWTPs could progressively evolve from facilities primarily designed for pollutant removal into treatment platforms embedded within environmental public health surveillance systems.

6. Conclusion

This review highlights the need to rethink urban UWWTPs from passive end-of-pipe infrastructure to active barriers, monitoring nodes, and decision-support platforms for mitigating AMR dissemination. Conventional wastewater treatment processes were not designed to specifically control ARB, ARGs, and MGEs. As a result, quaternary treatment technologies are becoming essential components of future wastewater management strategies, as required by recent regulatory frameworks.

Quaternary treatments, including AOPs, membrane-based systems, adsorption processes, and hybrid configurations, can substantially contribute to AMR mitigation and generally provide more comprehensive control of resistance determinants than conventional disinfection processes. However, their performance remains highly variable and dependent on wastewater characteristics, target resistance determinants, microbial community structure, operating conditions, and treatment configuration. This variability limits the direct transferability of results across sites and highlights the need for adaptive, technologically advanced treatment strategies. In this context, AI offers a powerful opportunity to support the next generation of wastewater treatment systems.

AI and ML models can enhance resistome profiling, predict water quality and AMR-related indicators, optimize treatment processes, detect anomalies, support real-time monitoring, and enable the development of digital twins for advanced treatment and wastewater surveillance. Additionally, AI can strengthen environmental metagenomics by supporting the interpretation of high-dimensional sequencing data, the identification of relevant resistance patterns, and the integration of microbiological, physicochemical, operational, and epidemiological information into coherent risk assessment frameworks.

Despite this potential, the implementation of AI in wastewater management for AMR mitigation remains at an early stage. Major challenges include limited availability of harmonized datasets, the lack of standardized monitoring protocols, the scarcity of externally validated AI models, and the limited interpretability of purely data-driven approaches. Future research should therefore prioritize high-quality, interoperable datasets, standardized AMR monitoring workflows, robust external validation, and the development of explainable, physics-informed AI models capable of integrating mechanistic knowledge with data-driven learning. These advances, enabled by multidisciplinary collaboration, will be essential to support predictive, interpretable, and operationally actionable decision-making.

Integrating AI, quaternary treatments, and AMR monitoring and mitigation strategies represents a promising pathway to strengthen the role of UWWTPs within the One Health framework. By integrating advanced treatment technologies, high-resolution resistome monitoring, environmental metagenomics, and intelligent decision-support systems, UWWTPs can evolve into proactive infrastructures for AMR mitigation, water safety, and public-health protection.

Declaration of generative AI use

During the preparation of this work, generative AI (ChatGPT and Notebook LM) tools were used solely for language editing to improve readability. After using these tools, AI-suggested edits were reviewed, verified, and approved by the authors to ensure accuracy, appropriateness, and academic integrity.

CRediT authorship contribution statement

Alessia Torboli: Conceptualization, Data curation, Formal analysis, Visualization, Writing – original draft, Writing – review & editing. **Stefano Cairone:** Conceptualization, Formal analysis, Visualization, Writing – original draft, Writing – review & editing. **Paolo Roccaro:** Writing – review & editing. **Paola Foladori:** Supervision, Validation, Writing – review & editing. **Vincenzo Naddeo:** Supervision, Writing – review & editing.

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

Given Prof. Dr. Paolo Roccaro's role as Editor, he had no involvement in the peer review of this article and had no access to information regarding its peer review. Full responsibility for the editorial process for this article was delegated to another journal editor. The other authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.watres.2026.126620.

Data availability

Data will be made available on request.

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