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Two decades of research on antibiotic resistance genes in global wastewater treatment systems: Critical knowledge gaps and pathways toward integrated risk management

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Abstract: Wastewater treatment plants serve as both barriers against and gateways for the environmental dissemination of antibiotic resistance genes (ARGs), yet the scientific community's collective research priorities over the past two decades have shaped – and been shaped by – critical knowledge gaps that now constrain effective risk management. Through systematic analysis of 2,894 publications (2004–2024) from the Web of Science Core Collection, this study identifies three fundamental imbalances that undermine progress toward resistance control. First, a source-research misalignment: livestock wastewater, responsible for >50% of global antibiotic consumption, accounts for merely 5.55% of research attention, while municipal wastewater – with lower selective pressure – dominates at 68.98%, leaving the most significant resistance reservoir critically understudied. Second, a mechanism-application disconnect: fundamental research on horizontal gene transfer predominantly utilizes hospital-derived clinical isolates, yet hospital wastewater research constitutes only 25.47% of publications, limiting the translational value of mechanistic insights. Third, a risk-investment mismatch: although "super resistance genes" (*mcr-1*, *bla*NDM) with demonstrated clinical impact have emerged as post-2020 research hotspots, targeted control technologies remain in early development, with 78% of treatment studies focusing on conventional processes that are inadequate for high-risk gene elimination. China leads global output (1,184 articles, 40.9%) but exhibits lower average citations (24.1), suggesting a scale-depth trade-off. Temporal analysis reveals three paradigm shifts—from mechanism cognition (2004–2007), through technical standardization (2008–2013), to risk management (2014–2024) – with current emphasis on "pollution," "surveillance," and "generation" signaling integration of resistome research with intelligent early-warning technologies. Addressing these imbalances requires: (1) rebalancing research investment toward livestock-associated resistance through targeted funding mechanisms; (2) developing translational frameworks that connect mechanistic insights (e.g., MGE-mediated transfer) to source-specific intervention design; and (3) prioritizing technology development for high-risk gene elimination (*tet*, *mcr-1*, *bla*NDM) over incremental optimization of conventional processes. This analysis transforms bibliometric patterns into actionable scientific priorities, providing a roadmap for transitioning from reactive monitoring to proactive resistance control

Introduction

Since Alexander Fleming, a British microbiologist, discovered and successfully isolated penicillin in 1928, antibiotics have been applied in human medicine, livestock breeding, and agricultural production for over ninety years. However, the widespread and often excessive use of antibiotics has led to their persistent residues in environmental matrices, creating an ecological selection pressure that drives the evolution and dissemination of antibiotic-resistant bacteria (ARB) and ultimately results in the continuous accumulation and spread of antibiotic resistance genes (ARGs) in the environment (Malayil et al. 2022, Murray et al. 2022). Unlike chemical pollutants, ARGs, as an emerging class of biological

contaminants, exhibit significant environmental persistence and biological activity. They can propagate across different bacterial strains, environmental compartments, and even organisms via horizontal gene transfer (HGT) through mobile genetic elements (MGEs) such as integrons and transposons (Ben et al. 2017, Jian et al. 2021). Their expression directly confers resistance to bacteria, substantially elevating the risk of clinical treatment failure (Chen et al. 2013, Rizzo et al. 2014). The World Health Organization has unequivocally stated that infections caused by multidrug-resistant pathogens pose a major threat to global public health security (Murray et al. 2022).

In this context, wastewater treatment plants (WWTPs) have been confirmed to act both as sinks for ARGs and as

secondary emission sources disseminating them into the natural environment (Michael-Kordatou et al. 2018). The high organic load, complex microbial communities, and biofilm microenvironments within activated sludge systems provide ideal conditions for the horizontal gene transfer of ARGs (Zhao et al. 2025). Therefore, a thorough understanding of the distribution characteristics, transmission mechanisms, and control strategies of ARGs in wastewater treatment systems has become a critical research topic in the field of environmental microbiological safety.

Bibliometrics, a core method of scientometrics since its formal introduction by Pritchard in 1969, has demonstrated a powerful capacity for constructing knowledge maps across multiple disciplines. This method systematically deconstructs the knowledge architecture, paradigm shifts, and interdisciplinary intersections of academic literature based on authoritative databases and statistical analysis (Qian et al. 2015, Parida et al. 2021, Magro et al. 2020, Cadore et al. 2020). It has been successfully applied to research on emerging pollutants such as perfluorinated compounds and microplastics (Chen et al. 2022, Zhong et al. 2023, Zheng et al. 2019). However, systematic bibliometric analyses specifically focusing on ARG research in wastewater remain relatively scarce. Existing reviews predominantly concentrate on specific technological advancements, lacking a comprehensive, macro-scale revelation of the imbalances in research power dynamics and the evolutionary patterns of research hotspots within this field (Wen et al. 2024, Zheng et al. 2018, Tierno et al. 2017). Critical questions remain unanswered: Is there a structural imbalance in research investment across different wastewater sources (municipal, hospital, livestock)? How has research shifted over time? These issues lack quantitative evidence derived from knowledge mapping.

Addressing this gap, the present study conducts a systematic bibliometric and visual analysis of global research on ARGs in wastewater treatment systems. Utilizing 2,894 publications indexed in the Web of Science Core Collection from 2004 to 2024 as the data source, and employing VOSviewer and CiteSpace for analysis, this study aims to: (1) analyze annual publication trends and characteristics of highly cited papers to delineate the field's development trajectory; (2) identify core countries, institutions, and journals to reveal the landscape of research forces; and (3) track the migration trajectory of research hotspots and identify structural imbalances through keyword burst detection, timezone mapping, and cluster analysis.

This investigation is expected to provide a systematic knowledge framework for future ARG-related research and offer a reference for risk management and technological innovation concerning ARGs in wastewater treatment systems.

Materials and Methods

Visualization Tools

This study employs VOSviewer (version 1.6.20) and CiteSpace (version 6.3.R1) to conduct a bibliometric analysis and visual presentation of the retrieved data. VOSviewer, developed by the team of van Eck and Waltman at Leiden University in the Netherlands, excels in network construction and multi-dimensional visualization of large-scale literature data, effectively revealing the co-occurrence structures and

clustering characteristics of research themes (Jiang et al. 2022). CiteSpace focuses on detecting the knowledge evolution trajectory and research frontiers within a scientific domain. Through citation network analysis, keyword co-occurrence, and timezone mapping, it identifies pivotal node literature, core research forces, and evolutionary trends of hotspots (Xu et al. 2022, Zare et al. 2022). The combined use of these two tools provides complementary insights, enabling a systematic presentation of the knowledge structure and evolutionary patterns of this field.

Data Sources and Search Strategies

This study utilized the Web of Science Core Collection as the data source, with the retrieval time span set from January 1, 2004, to May 1, 2024. The retrieval strategy employed a topic search, with the specific search query formulated as: TS = ("antibiotic resistance gene") AND TS = ("wastewater treatment"). The document types were limited to Articles and Reviews, yielding a total of 2,894 relevant publications. The full records and cited references of all these publications were exported in plain text format, serving as the foundational dataset for subsequent analyses.

To further investigate the distribution characteristics of ARG research across different wastewater sources, a secondary classification was performed on the 2,894 publications. Based on an analysis of abstracts and keywords, the publications were categorized into four groups: municipal wastewater, hospital wastewater, livestock breeding wastewater, and other types of wastewaters. This classification aims to reveal the disparities in research attention paid to ARG issues originating from different pollution sources, thereby providing data to underpin the identification of a "structural imbalance" in the research landscape.

Results

Publication Trends and Highly Cited Articles:

A Two-Decade Analysis

Annual Publication Trends

Annual publication output and citation frequency of scholarly literature serve as core indicators for assessing the developmental trajectory of a scientific field (Liu et al. 2021, Zhang et al. 2022). Since 2004, both the annual number of publications and citation frequency in global research on ARGs in wastewater have exhibited a sustained and rapid growth trend, indicating increasingly widespread academic attention to this field (Figure 1a).

Based on publication trends and key milestone events, the research trajectory of ARGs in wastewater can be delineated into four phases.

The Initial Exploration Phase (2004–2010): During this period, annual publications numbered fewer than 50, and research lacked a systematic framework, primarily focusing on the ecological effects of clinical antibiotic residues. ARG detection relied mainly on traditional culture-based methods and conventional polymerase chain reaction (PCR), with research efforts concentrated predominantly in the medical field (Schwartz et al. 2006, Silva et al. 2006).

The Policy-Driven Phase (2011–2014): The World Health Organization's global call to combat antibiotic resistance in 2011 and the release of the Global Action Plan on Antimicrobial

Resistance in 2014 catalyzed an initial paradigm shift in research (Grimbs et al. 2017, Millet et al. 2011). The research scope expanded from clinical settings to environmental matrices, PCR detection techniques became standardized, and research focus gradually shifted toward point-source pollution represented by wastewater treatment plants, although the publication growth rate remained relatively modest (Rizzo et al. 2013, Michael et al. 2013).

The Deepening Breakthrough Phase (2015–2019): Multidisciplinary integration propelled the annual publication growth rate to 15%. Key advancements manifested in three dimensions: First, the characterization of ARG environmental distribution systematically revealed the abundance profiles of ARGs in environments such as wastewater treatment plants and livestock farms (Guo et al. 2017, Ma et al. 2021). Second, methodological innovations in detection technology, particularly the application of metagenomics, transcended the target limitations of PCR and enabled comprehensive profiling of ARGs (Zhu et al. 2023, Zhao et al. 2018). Third, theoretical advancements in transmission mechanisms systematically elucidated horizontal gene transfer and biofilm-mediated effects (Zhang et al. 2024, Yang et al. 2019).

The Exponential Growth Phase (2020–2024): Research entered a rapid development trajectory, with publication volume increasing exponentially. Research foci exhibited three major shifts: from single-compartment investigations to quantification of multi-source contributions, with systematic assessments of ARG input fluxes from hospital, agricultural, and municipal wastewater (Yang et al. 2024, Zhang et al. 2024); from optimization of conventional treatment processes to exploration of emerging technologies, with cutting-edge approaches such as CRISPR gene editing and single-molecule tracking being applied to ARG mitigation (Wang et al. 2025, Brown et al. 2024); and from phenomenological descriptions to mechanistic elucidations, with continuous deepening of research on ARG transmission pathways and driving factors (Liang et al. 2022, Madhogaria et al. 2024, Zheng et al. 2017).

However, with the diversification of research foci, internal imbalances have become increasingly prominent. As shown in Figure 1(b), global research on ARG pollution across different wastewater matrices exhibits significant uneven distribution. The latest bibliometric data indicate that municipal wastewater treatment plants, as a hotspot for ARG research, account for 68.98% of related publications, far exceeding the proportions for medical wastewater (25.47%) and livestock wastewater (5.55%). This pronounced gradient disparity in scientific research output reveals a critical blind spot in environmental governance: although agriculture and animal husbandry represent the primary sectors for antibiotic use, research investment into the migration and transformation mechanisms of ARGs in livestock wastewater and agricultural environments remains notably insufficient. This situation serves as a warning that future research should establish a comprehensive “human-animal-environment” monitoring network to address the lag in risk assessment of agricultural-source antibiotic resistance pollution.

China ranks first globally in this research domain in both total publications (1,184 articles) and total citations (28,476 citations), underscoring its central position in the field of ARGs in wastewater research.

Core Themes of Highly Cited Literature

Highly cited literature reflects the foundational knowledge and core concerns of a research field. Table 1 summarizes the top 10 most frequently cited studies. Concerning environmental distribution and fate, Zhang et al. (2015) revealed the multi-source input, transport, transformation, and dynamic behavior of antibiotics in the environment through multimedia modeling and first proposed a potential correlation between antibiotic discharge and ARG enrichment. Baquero et al. (2008) comprehensively reviewed the distribution, sources, and transmission mechanisms of antibiotics and ARGs in aquatic environments from a macro perspective, explicitly identifying water systems as key reservoirs for resistance dissemination.

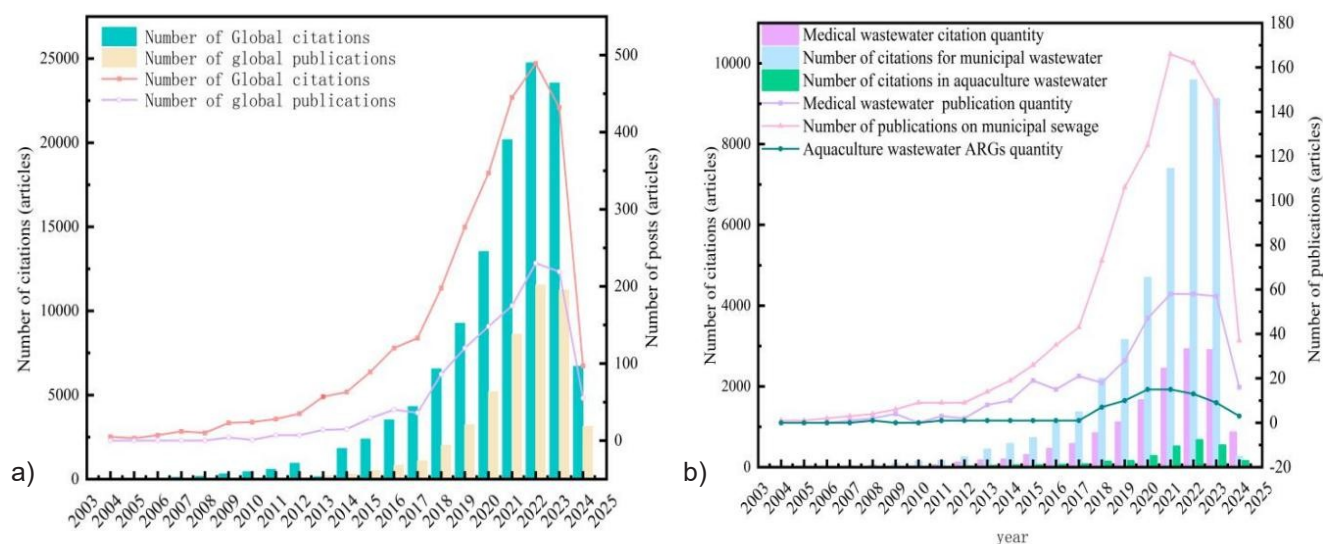


Fig 1. (a) The global and Chinese publication and citation quantities from 2004 to 2024 (b) The publication and citation quantities of ARGs in municipal wastewater, medical wastewater, and livestock farm wastewater globally.

Pruden et al. (2006), investigating a wastewater treatment plant in Colorado, USA, employed high-throughput quantitative PCR to elucidate the dynamics of ARGs during treatment processes, laying the conceptual foundation for ARGs as “emerging environmental contaminants”.

In terms of pollution source apportionment, Rodriguez-Mozaz et al. (2015) comparatively analyzed the distribution characteristics of antibiotics and ARGs in municipal wastewater and hospital wastewater from a Chinese city, finding that their input contributions to receiving water bodies significantly exceeded background levels and confirming hospital wastewater as a crucial source of riverine ARG pollution. Guo et al. (2017) employed metagenomic approaches to identify 18 major classes and 578 ARG subtypes in a full-scale wastewater treatment plant, quantifying ARG persistence and removal efficiencies and thereby providing high-resolution data for process efficacy assessment. Karkman et al. (2017) detected the dominance of sulfonamide and tetracycline ARGs in antibiotic production wastewater and found a significant correlation between the abundance of class 1 integrons (intI1) and sulfonamide resistance gene levels, revealing the critical role of mobile genetic elements in ARG dissemination.

Regarding control strategies and mechanisms, Wang et al. (2020) systematically reviewed the concentration ranges of antibiotics (10^{-3} – 10^4 µg/L) and ARGs (10^4 – 10^{12} copies/L) in municipal wastewater treatment plants, noting that the removal efficiencies of ARGs by anaerobic and aerobic treatment processes varied depending on gene type and structural configuration. Mao et al. (2015) further discovered that optimizing microbial community structure could significantly enhance ARG removal efficiency, providing a theoretical basis for bioaugmentation strategies.

In terms of journal distribution, among the aforementioned top 10 highly cited publications, *Water Research* accounts for four, while *Environmental Science & Technology* and *Science of the Total Environment* each account for two, indicating that these three journals possess core influence in

the ARG-wastewater research field. Regarding the evolution of research themes, early highly cited literature (2006–2013) primarily focused on the environmental distribution and conceptual establishment of ARGs; the mid-phase (2015–2017) concentrated on pollution source apportionment and process evaluation; and more recent studies (2018–2020) have increasingly emphasized transmission mechanisms and control strategies. This evolutionary pattern corroborates the research phase delineation presented in Section 3.1.1.

Distribution of Core Research Forces by Country, Institution, and Journal

Leading Countries by Publication Output

The number of publications indexed in SCI and their citation frequencies serve as important indicators for evaluating a country’s scientific research capacity and academic influence (Liu et al. 2021). Figure 2a illustrates the distribution of the top 20 countries by publication output in the global research field of ARGs in wastewater. Worldwide, authors contributing to this field are primarily concentrated in China, the United States, Germany, India, Spain, Canada, Poland, the United Kingdom, Portugal, and Australia. China leads globally with 1,184 publications, accounting for 40.9% of the total publications, the United States ranks second with 440 publications, Germany (153 publications), India (150 publications), and Spain (135 publications) occupy the third to fifth positions, respectively (Figure 2a).

China’s leading position in this field is driven by multiple factors (Kümmerer et al. 2009, Ren et al. 2023). First, policy guidance: sustained investment through policy documents such as the National Action Plan to Contain Antimicrobial Resistance (2016–2020, 2022–2025) has provided institutional support for ARG research. Second, problem-driven impetus: China accounts for approximately 50% of global antibiotic usage, with prominent ARG pollution issues in intensive livestock farming areas and in surrounding environments of wastewater treatment plants, catalyzing substantial applied

Table 1: Information about top 5 highly cited papers.

Rank	Journal	title	Author	Year	Citations	Country
1	Environmental science & technology	Comprehensive Evaluation of Antibiotics Emission and Fate in the River Basins of China: Source Analysis, Multimedia Modeling, and Linkage to Bacterial Resistance	Zhang Q.Q.	2015	2741	China
2	Science of the total environment	Urban wastewater treatment plants as hotspots for antibiotic resistant bacteria and genes spread into the environment: A review	Rizzo L.	2013	1571	Italy
3	Water research	Urban wastewater treatment plants as hotspots for the release of antibiotics in the environment: A review	Michael I .	2012	1417	Cyprus
4	Current opinion in biotechnolog	Antibiotics and antibiotic resistance in water environments	Baquero F.	2008	1407	Spain
5	Environmental science & technology	Antibiotic resistance genes as emerging contaminants: Studies in northern Colorado	Pruden A.	2006	1330	USA

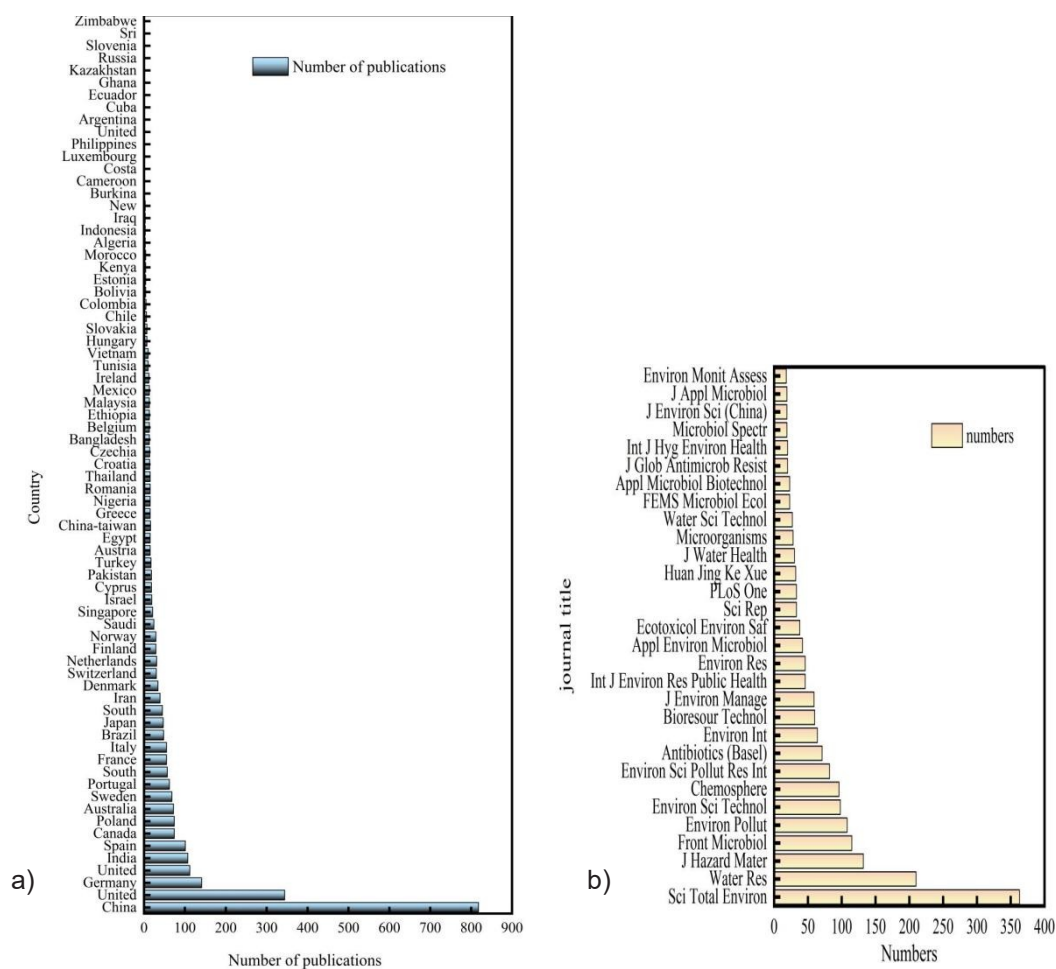


Fig 2. (a) Number of national publications (b)Number of journal publications.

research (Zhang et al. 2015). Third, technological advancement: declining costs of metagenomic sequencing have enabled large-scale environmental surveys.

Notably, despite China’s leading publication output, its average citations per publication (24.1) are lower than those of the United States (32.5) and Germany (29.8), indicating room for improvement in research “depth” and “impact” (based on Web of Science data statistics). However, in terms of total citations, China far surpasses all other countries with 28,476 citations, underscoring its substantial overall contribution to the field despite the dilution effect commonly associated with large publication volumes.

Regarding country-specific research themes (based on keyword co-occurrence analysis), Chinese research predominantly focuses on process optimization and pollution status surveys, such as evaluating ARG removal efficiencies of different treatment technologies. In contrast, the United States and European countries demonstrate stronger influence in elucidating transmission mechanisms and health risk assessment, including mediation mechanisms of mobile genetic elements and associations between ARGs and clinical resistance (Cacace et al. 2019, Garner et al. 2024). This configuration—characterized by a pattern of “leadership in scale” coexisting with a “need to catch up in depth,” along with differentiated distribution of research themes—provides

a foundation for future complementary advantages through international collaboration.

Core Research Institutions

Based on an analysis of publication output by research institutions in the global field of ARGs in wastewater (Table 2), the Chinese Academy of Sciences ranks first worldwide with 245 publications, surpassing the second-ranked institution by 116.8%, underscoring its central position in this research domain. Following sequentially are the University of Chinese Academy of Sciences (113 publications), the Research Center for Eco-Environmental Sciences (101 publications), Tsinghua University (76 publications), and Harbin Institute of Technology (70 publications). Among the top 10 institutions by publication output, Chinese research institutions occupy seven positions, forming a comprehensive research cluster centered around institutions affiliated with the Chinese Academy of Sciences, spanning fundamental research, technological innovation, and engineering practice.

The dominant position of Chinese research institutions in this field benefits from their well-defined division of labor and collaborative framework. The Research Center for Eco-Environmental Sciences focuses on environmental behavior and fate analysis of ARGs, Tsinghua University possesses advantages in technological innovations such as membrane

Table 2: Top 10 Institutions in Global and Chinese Research on ARGs in Wastewater from 2004 to 2024 by Number of Published Papers

Top 10 global contributors	Publication volume	Top 10 contributors in China	Publication volume
Chinese academy of sciences	245	Chinese academy of sciences	245
University of chinese academy of sciences	113	University of chinese academy of sciences	113
Research center for eco environmental sciences	101	Research center for eco environmental sciences	101
Tsinghua university	76	Tsinghua university	76
Harbin institute of technology	70	Harbin institute of technology	70
Centre national de la recherche scientifique cnrs	56	Tongji university	55
Tongji university	55	Zhejiang university	53
Universidade catolica portuguesa	55	Institute of Urban Environment, Chinese Academy of Sciences	50
Zhejiang university	53	Ministry of agriculture rural affairs	50
Indian institute of technology system iit system	50	University of hong kong	44

separation, and Harbin Institute of Technology has developed distinctive strengths in engineering practice areas including disinfection process optimization. This complete chain of “fundamental research—technological development—engineering application” provides systematic support for China’s continued production of high-quality research outputs in the ARG-wastewater field.

Highly Influential Authors

Table 3 lists the top eight scholars by publication output in the global research field of ARGs in wastewater, whose research directions encompass the core dimensions of this field. Su et al. (2017) applied metagenomics to urban sewage in China, revealing 381 widely shared resistance genes with seasonal variation, and suggesting that the core resistome is associated with human gut microbiota and may spread via sewage disposal. Manaia et al. (2018) focused on tracking methodologies for ARB and ARGs in municipal wastewater treatment plants, providing a theoretical foundation for establishing standardized monitoring approaches. As a corresponding author, Pruden et al. (2024), through metagenomic sequencing of 12 wastewater treatment plants across six continents, revealed significant inter-gene variability in ARG removal efficiencies, with total ARG abundance and clinically relevant ARGs being higher in Asian wastewater compared to Europe and North America. Yang et al. (2023), as the corresponding author, elucidated the inhibitory effects of high organic loads and oxytetracycline concentrations on nitrifying bacteria, demonstrating that adjusting process parameters could reduce relative ARG abundance by 63.2%. Liu et al. (2023) systematically evaluated the inactivation of ARB and inhibition of plasmid-mediated horizontal gene transfer by peroxydisulfate. The research of these scholars collectively constitutes the core knowledge contribution network of this field.

Major Publishing Journals

The distribution of journals publishing research on ARGs in global wastewater systems is presented in Figure 2b. *Science of the Total Environment* ranks first with 363 publications (accounting for 12.5%), emphasizing the investigation of ARG distribution, dissemination, and removal mechanisms from an environmental systems perspective. *Water Research* ranks second with 210 publications (7.25%), focusing on the behavioral mechanisms of ARGs in aquatic environments and process optimization. *Journal of Hazardous Materials* ranks third with 132 publications (4.56%), with a primary focus on targeted treatment technologies for ARGs in high-risk scenarios such as pharmaceutical and livestock wastewater.

From the perspective of journal functional differentiation, ARG research has formed a complete knowledge chain encompassing “mechanisms—technologies—risks.” *Frontiers in Microbiology* (115 publications, 3.97%) elucidates ARG hosts and horizontal transfer mechanisms from a microbial ecology perspective, *Environmental Pollution* (108 publications, 3.73%) emphasizes ARG distribution characteristics and ecological risk assessment, *Environmental Science & Technology* (98 publications, 3.39%) advances treatment technology innovation, and *Chemosphere* (96 publications, 3.32%) primarily evaluates ARG removal efficiencies through laboratory-scale simulation studies. The differentiated positioning of these journals collectively promotes the multidimensional development of ARG research.

Regarding the journal distribution of highly cited literature (Table 1), *Water Research* accounts for four publications, while *Environmental Science & Technology* and *Science of the Total Environment* each account for two, further confirming the core influence of these three journals in the ARG-wastewater research field.

Table 3: Top 8 authors with the highest number of publications on ARGs in wastewater worldwide from 2004 to 2024 by Number of Published Papers

Author	Number of publications	Title	Total citations	Average citations
Su J.Q	65	Metagenomics of urban sewage identifies an extensively shared antibiotic resistome in China	212	78.22
Manaia C. M.	52	Antibiotic resistance in wastewater treatment plants: Tackling the black box	5853	112.56
Zhang T.	42	Plasmid Metagenome Reveals High Levels of Antibiotic Resistance Genes and Mobile Genetic Elements in Activated Sludge	3807	90.64
Yang M.	39	Unveiling the threshold values of organic and oxytetracycline loadings for nitrification recovery of a full-scale pharmaceutical wastewater treatment system	1840	47.18
Pruden A.	37	Antibiotic resistance genes as emerging contaminants: Studies in northern Colorado	4614	124.7
Harnisz M.	34	Antibiotic resistant <i>Escherichia coli</i> in hospital and municipal sewage and their emission to the environment	1511	44.44
Liu Y.	33	Removal of antibiotic resistant bacteria, genes and inhibition of plasmid-mediated horizontal transfer by peroxymonosulfate: Efficiency and mechanisms	1325	40.15
Schwartz T.	33	Real-time PCR detection of <i>Pseudomonas aeruginosa</i> in clinical and municipal wastewater and genotyping of the ciprofloxacin-resistant isolates	5420	164.24

3.3 Evolution of Research Hotspots and Analysis of Structural Imbalance

Analyzing research hotspots requires not only revealing the temporal migration trajectory of academic attention but also discerning its spatial imbalance across different wastewater types. This section systematically examines the evolutionary patterns of research foci in ARG-wastewater studies over the past two decades through keyword burst detection, timezone mapping, and cluster analysis, while elucidating the structural mismatch between “hot topics” and “cold sources.”

Keyword Burst Analysis

Keyword burst refers to the phenomenon where the frequency of a specific term appearing in academic literature exhibits a concentrated surge within a particular time frame. This indicator reflects the intensity of research attention and the prominence of research themes, with longer burst durations indicating more sustained academic interest in that direction.

Bibliometric analysis based on CiteSpace reveals significant temporal heterogeneity and thematic evolutionary characteristics in keyword bursts within ARG-wastewater research from 2004 to 2024 (Table 4). Among these, “antimicrobial resistance” (2004–2015), “tetracycline resistance” (2007–2018), and “*Escherichia coli*” (2004–2016) represent the three research threads with the longest durations and highest burst intensities.

“Tetracycline resistance” emerged as the most representative long-term hotspot in this field, with a burst intensity of 20.42 and a duration of 12 years (2007–2018). The formation of this phenomenon is driven by multiple factors. First, massive usage—global annual tetracycline production reached 5,000 tons by 2015, with widespread applications in livestock breeding, agricultural production, and clinical

treatment (Kümmerer et al. 2009). Second, environmental persistence—tetracycline antibiotics exhibit soil half-lives of up to 180 days, and their corresponding resistance genes show removal efficiencies of only 20%–40% in wastewater treatment systems. Third, co-selection mechanisms—tetracycline resistance genes are frequently co-localized with heavy metal resistance genes on plasmids, enabling synergistic enrichment under metal contamination (Ren et al. 2023).

Notably, despite the prolonged prominence of tetracycline resistance research, its primary source—livestock breeding wastewater—accounts for merely 5.55% of overall studies in this field (Figure 1b). This pronounced mismatch between “hot topics” and “cold sources” profoundly reveals the structural imbalance characterizing current ARG research. The decline in this hotspot’s prominence after 2018 does not signify problem resolution but rather reflects a shift in research focus toward “super resistance genes” with higher clinical risk and greater transmission capacity (such as bla_{NDM-1}, mcr-1), exemplifying a “risk-oriented escalation” of research priorities.

Escherichia coli, serving as an indicator organism, sustained research attention for 13 years (2004–2016) with a burst intensity of 18.18. This bacterium’s 20-minute division cycle enables rapid characterization of resistance phenotypes, with approximately 40% of clinical isolates carrying multidrug resistance plasmids (Yin et al. 2025), establishing it as a key model strain for investigating environmental transmission of ARGs.

During the 2020–2024 period, research focus significantly shifted toward ecological risk prevention and control, with three interconnected emerging hotspots: “pollution” (burst intensity 18.76), “surveillance” (burst intensity 15.32), and “generation” (burst intensity 12.45), marking the deep

integration of environmental resistomics and intelligent monitoring technologies. The “pollution” theme concentrates on elucidating pollution mechanisms and innovating monitoring technologies. Yan et al. (2024) employed multi-omics approaches to reveal co-evolutionary patterns of ARGs and metal resistance genes in sewage-irrigated paddy fields. Soni et al. (2024) developed a microfluidic chip-based rapid capture system for resistant bacteria and established a transmission risk index based on mobile genetic element abundance. The emergence of the “surveillance” direction signifies a strategic shift in research emphasis from “passive response” to “active early warning.” Dong et al. (2024), through 18-month dynamic monitoring of wastewater systems, revealed spatiotemporal distribution patterns of ARGs, with source apportionment models indicating that 78.4% of ARG host bacteria originate from the human gut microbiome. Gholipour et al. (2023) systematically outlined four major bottlenecks confronting current wastewater surveillance efforts. Addressing these challenges, the World Health Organization launched an antibiotic resistance gene source tracing initiative in 2023, proposing a three-tier technical framework comprising metagenomic census, culturomics-based host resolution, and mobile genetic element risk assessment. The emergence of the “generation” hotspot stems from fundamental inquiries into the environmental behavior mechanisms of antibiotic resistance genes. Bengtsson-Palme et al. (2021) modeled resistance emergence, suggesting the environment plays a minor role in most scenarios, yet large uncertainties demand

more quantitative data for targeted interventions. Madhogaria et al. (2024) proposed an “adsorption-resistance coupling” theory, demonstrating that biosorption processes drive adaptive evolution of ARGs by altering antibiotic bioavailability.

3.3.2. Time-Zone View Analysis

While Section 3.1.1 delineated the research trajectory into four phases based on publication trends and policy milestones, keyword time-zone mapping offers a complementary perspective centered on the evolution of research content itself. As revealed below, this content-based analysis condenses the trajectory into three phases, reflecting the intrinsic cognitive progression of the field from fundamental mechanisms to technical standardization and ultimately to systematic risk management. These two frameworks are not contradictory but rather illuminate different facets of the field’s development—the former capturing external drivers, the latter capturing internal intellectual shifts.

Compared to keyword burst analysis, time-zone mapping provides a more intuitive visualization of keyword occurrence frequency and temporal distribution characteristics, facilitating the tracking of dynamic evolutionary trajectories of research hotspots (Figure 3). The time-zone map constructed in this study reveals a clear phased progression in the evolution of research hotspots within ARG-wastewater studies.

Phase I (2004–2007): Mechanism Cognition Stage. Research hotspots predominantly centered on “antibiotic resistance,” “bacteria,” and *Escherichia coli*. During this

Table 4: Keywords with the Strongest Citation Bursts

Keywords	Year	Strength	Begin- End	2004~2024
escherichia coli	2004	18.18	2004-2016	
Antibiotic resistance	2004	15.73	2004-2015	
identification	2004	7.13	2004-2012	
tetracycline resistance	2007	20.42	2007-2018	
determinants	2007	7.61	2007-2016	
bacteria	2004	9.47	2008-2016	
sewage treatmentplants	2009	9.9	2009-2018	
sewage	2009	7.23	2009-2016	
enterobacteriaceae	2009	6.17	2009-2017	
real time pcr	2009	5.73	2009-2016	
solid phase extraction	2009	5.48	2009-2015	
activated sludge	2006	6.94	2010-2014	
strains	2011	12.61	2011-2016	
south china	2011	6.07	2011-2018	
integrons	2009	7.17	2012-2018	
resistance	2009	5.16	2012-2014	
molecular characterization	2013	6.55	2013-2016	
municipal waste water	2012	5.15	2014-2019	
sewage treatment plant	2008	6.23	2015-2019	
point source	2015	5.43	2015-2018	
china	2014	8.42	2017-2018	
receiving river	2017	7.41	2017-2018	
pollution	2020	6.19	2020-2022	
surveillance	2021	5.63	2021-2024	
generation	2021	5.5	2022-2024	

phase, studies primarily investigated plasmid-mediated resistance gene transmission mechanisms, revealing plasmids as core vectors for resistance dissemination (Szczepanowski et al. 2004, Tennstedt et al. 2005). Foundational research encompassed detection methodologies for resistant bacteria and resistance mechanisms, while preliminary assessments of treatment process efficacy compared ARG removal efficiencies across different technologies (Garcia et al. 2007).

Phase II (2008–2013): Technical Standardization Stage. Research hotspots expanded to include “wastewater,” “activated sludge,” “gene,” “fate,” and “effluent.” During this period, researchers focused on transmission mechanisms linking ARGs and mobile genetic elements (Zhai et al. 2016, Zhang et al. 2011). Significant methodological standardization advances occurred, with applications of PCR, quantitative PCR, and metagenomic technologies substantially enhancing monitoring accuracy (Diehl et al. 2010, Börjesson et al. 2009). Concurrently, integrated treatment strategies began receiving attention (Yang et al. 2013).

Phase III (2014–2024): Risk Management and Control Stage. Research hotspots further expanded to encompass “microbial community,” “pharmaceutical,” “environment,” “tetracyclines,” “removal,” and “degradation.” This phase focused on developing and evaluating novel wastewater treatment technologies, driving the paradigm shift from “single-pollutant removal” toward “comprehensive resistance prevention and control” (Zarei-Baygi et al. 2020, Li et al. 2021). Multimedium transmission pathways and host evolutionary characteristics of ARG environmental dissemination were systematically elucidated, with ecological risks associated with pharmaceutical wastewater and sludge reuse attracting increasing attention (Qiao et al. 2025, Cachetas et al. 2024).

The important conclusions, core viewpoints, and breakthrough progress of the above literature have been summarized in Table 4.

3.3. Keyword Cluster Analysis

Keyword cluster analysis further reveals the internal structure and associative characteristics of research themes. This study identified three major clusters (Figure 4), corresponding to the three core dimensions of ARG research. By correlating cluster themes with the current research status across different wastewater types, the “imbalance landscape” of the contemporary research landscape can be clearly delineated.

Cluster 1 (Red) encompasses keywords including “mechanisms,” “bacteria,” “bacterial community,” *Escherichia*

coli, “genes,” “diversity,” “tetracycline resistance,” “metagenomic analysis,” “antimicrobial resistance,” “disinfection,” and “prevalence.” This cluster focuses on fundamental research into ARG transmission mechanisms and microbial community evolution. Within wastewater treatment plants, plasmid-mediated horizontal gene transfer constitutes a crucial mechanism for ARG dissemination, with mobile genetic elements such as integrons and transposons playing pivotal roles (Yin et al. 2022, Gillings et al. 2015). Reductions in bacterial community diversity may potentiate horizontal gene transfer of ARGs by intensifying niche competition (Ju et al. 2019). Correlation analysis with wastewater types: The fundamental mechanism research encompassed in Cluster 1 is closely associated with the complex and diverse resistance mechanisms present in hospital wastewater, which harbors clinically relevant resistant bacteria, multidrug resistance plasmids, and high abundances of MGEs. However, hospital wastewater research accounts for merely 25.47% of global ARG literature (Figure 1b). This reveals a critical imbalance: the application scenario most requiring mechanistic elucidation (hospital wastewater) receives relatively insufficient research investment, creating a disconnect between fundamental research and application contexts.

Cluster 2 (Green) encompasses keywords including “removal of tetracycline,” “degradation,” “microbial community,” “performance,” “activated sludge,” “biodegradation,” “extracellular polymeric substances,” and “sulfamethoxazole.” This cluster emphasizes treatment process efficacy evaluation and biodegradation mechanism research. Membrane-aerated biofilm reactors exhibit strong degradation capacity for sulfamethoxazole (Li et al. 2024), while disinfection processes have been systematically evaluated for their ARG removal efficiency and effects on conjugative transfer (Lin et al. 2016, Guo et al. 2015, Michael-Kordatou et al. 2017). Correlation analysis with wastewater types: The process efficacy research represented by Cluster 2 primarily serves municipal wastewater treatment needs—municipal wastewater is characterized by large volumes, well-established collection systems, and mature treatment processes. Municipal wastewater research constitutes as much as 68.98% of global ARG literature (Figure 1b). This indicates that in the municipal wastewater domain, a virtuous cycle of “process needs driving technological development” has formed, with relatively mature technologies.

Cluster 3 (Blue) encompasses keywords including “resistance genes,” “fate,” “wastewater treatment plants,” “veterinary antibiotics,” “pharmaceuticals,” “personal care products,” and “soil.” This cluster focuses on environmental fate and cross-media migration of ARGs. ARGs can disseminate across environmental compartments through pathways including bioaerosols, groundwater infiltration, and sludge migration (Chen et al. 2025), with specific bacterial genera serving as critical vectors for ARG transmission (Talat et al. 2025). Correlation analysis with wastewater types: The environmental fate concerns addressed by Cluster 3 closely align with the core risks associated with livestock wastewater—the cross-media transmission of antibiotics from livestock wastewater into soil, crops, and ultimately the food chain through manure land application represents a key proposition in ARG environmental fate research. However, livestock wastewater research accounts for merely 5.55% of global ARG

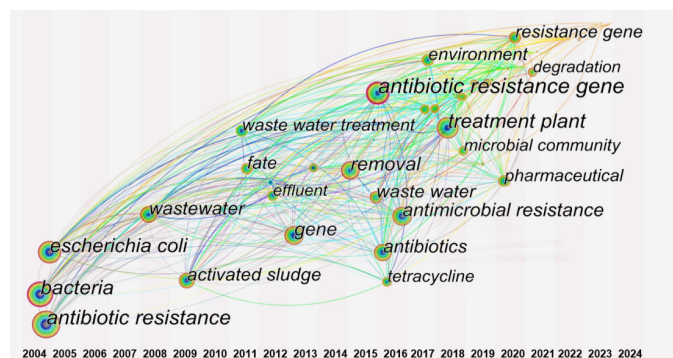


Fig 3. Time zone map of keywords in English literature during 2004~2024

Table 5: Important conclusions, core viewpoints, and breakthrough progress of literature published from 2004-2024

year	Research Focus	Key Findings	reference
2004	Isolation of erythromycin-resistant plasmids from activated sludge, characterization of plasmid pRSB101	Environmental plasmids like pRSB101 achieve cross-host spread via “functional superposition” (resistance + adaptability), likely evolving from non-resistant plant pathogens through gene capture	(Szczepanowski et al. 2004)
2005	Complete sequencing of IncP-1 α plasmid pTB11 from sewage, comparison with historic plasmid RK2	First revelation of high genetic stability and functional module conservation of IncP-1 α plasmids in WWTPs. The skeleton (Rep, Trb, Tra) is 99.9% identical to RK2, while Tn402-like integrons carry diverse resistance gene cassettes	(Tennstedt et al. 2005)
2006	Analysis of ciprofloxacin resistance mechanisms (gyrA, gyrB, parC mutations) in <i>P. aeruginosa</i> from wastewater	Environmental ciprofloxacin resistance is primarily determined by specific co-mutation patterns (e.g., gyrA Thr83Ile and parC Ser87Leu)	(Schwartz et al. 2006)
2006	Assessment of coliform antibiotic resistance and horizontal gene transfer potential in influent vs. treated water	First demonstration that treated wastewater effluent contains strains capable of horizontally transferring plasmid-mediated resistance to <i>E. coli</i> K-12. Treatment reduces total coliforms but enriches the proportion of multi-drug resistant (MDR) bacteria.	(Silva et al. 2006)
2007	Comparison of <i>E. coli</i> and <i>Enterococcus</i> resistance in two WWTPs (with/without hospital input), effect of anaerobic digestion	Hospital wastewater significantly increases initial resistance ratios in <i>Enterococcus</i> . Anaerobic digestion is confirmed to significantly reduce MDR <i>Enterococcus</i> and 2-antibiotic resistant <i>E. coli</i>	(Garcia et al. 2007)
2007	Large-scale analysis of <i>Enterococcus</i> resistance (983 strains, 42 samples), risk assessment of sludge agricultural reuse	Revealed a 49.4% MDR rate in <i>Enterococcus</i> across 14 WWTPs and quantified the reduction effect of treatment. Highlights that agricultural sludge application is a potential pathway for spreading resistant bacteria	(da Costa et al. 2007)
2010	Monitoring of 11 ARG subtypes across treatment stages in a N. China WWTP, correlation with antibiotic conc	Identified biological treatment units (e.g., anoxic ponds, aeration tanks) as key hotspots for ARG proliferation, with activated sludge processes showing a stronger effect than SBR or bio-contact oxidation	(Zhai et al. 2016)
2011	Capture of novel plasmids from activated sludge using TRACA system, metagenomic analysis of ARGs and MGEs	MGEs (e.g., Class 1 integrons, Tn3 transposons) are found to highly co-exist with ARGs, playing a key role in their capture and spread by promoting HGT	(Zhang et al. 2011)
2012	Investigation of integron-positive Enterobacteriaceae (1832 strains) prevalence, MDR phenotypes, and gene cassette diversity	Revealed a gradient distribution of integron-positive bacteria across WWTP stages. Integron-positive strains were 100% MDR, harboring 24 distinct resistance-related gene cassette arrays	(Mokracka et al. 2012)
2012	Effect of temperature (22-55°C) on tetracycline resistance genes (tet) and intl1 removal during anaerobic/aerobic digestion	High-temperature anaerobic digestion (55°C) is identified as a promising technology for eliminating resistance genes, with temperature being a critical control factor	(Diehl et al. 2010)
2012	Development of a real-time PCR (LUX™) system for quantitative detection of four ARGs (tetA, tetB, mecA, aac(6'))	First application of LUX™ fluorogenic technology for rapid, highly reproducible quantification of ARGs in wastewater environments, revealing the prevalence of tetA and aac(6')-Ie+aph(2'')	(Börjesson et al. 2009)

year	Research Focus	Key Findings	reference
2012	PCR-DGGE analysis of antibiotic-resistant bacteria diversity after primary and secondary treatment	Demonstrates that oxidative stress in aeration tanks and chlorination during secondary treatment exerts a strong selective pressure, enriching for specific resistant bacterial populations	(Sigala et al. 2012)
2013	Development of a structured ARG database and high-throughput pipeline for metagenomic data analysis	Established a standardized workflow for ARG detection, revealing subtypes missed by conventional PCR and eliminating amplification bias, enabling more accurate quantification and classification	(Yang et al. 2013)
2018	Lab-scale Anaerobic/Aerobic System (AAS) study on tetracycline selection pressure and int11's role in ARG fate	Tetracycline and int11 are the main factors controlling tet gene distribution. Int11 plays a crucial role in HGT, while the tetX gene shows high environmental persistence, unaffected by AAS treatment	(Gao et al. 2018)
2020	Lab-scale Anaerobic Membrane Bioreactor (AnMBR) study on the impact of antibiotics on microbial community and ARGs	Provides insights into the correlation between microbial community dynamics and ARGs in AnMBRs. Antibiotic exposure caused a transient 167x increase in effluent ARGs, correlating with shifts in the effluent microbial community, suggesting pathogen involvement	(Zarei-Baygi et al. 2020)
2021	Development of a novel magnetic anion exchange resin (MAER)-mediated UV-Fenton system for antibiotic/ARG removal	Achieves >99% degradation of three antibiotics in 30 min. The cyclic $\text{Fe}^{3+}/\text{Fe}^{2+}$ redox cycle on the MAER interface enhances electron transfer, with hydroxyl radicals ($\cdot\text{OH}$) as the primary reactive species	(Li et al. 2021)
2024	Multi-media (water, sediment, soil) sampling downstream of a sulfonamide production plant to track ARGs and hosts	A Soil depth (20 cm) effectively reduces ARG presence, but 17 high-risk ARGs can still migrate to rivers and deep soil. First report on the host diversity of the sadABC antibiotic degradation gene, suggesting environmental evolution and cross-host transmission	(Qiao et al. 2025)
2024	Global meta-analysis of 163 metagenomes (29 countries) to define a core resistome across wastewater, freshwater, and soil	Established a cross-database annotation pipeline to resolve naming ambiguities. Proposed 27 wastewater-specific ARGs as "fingerprint" biomarkers to trace wastewater discharge impacts on downstream environments	(Cachetas et al. 2024)
2024	Two-year study on Zero-Valent Iron (ZVI) enhancement of antibiotic and ARG removal in integrated anoxic-aerobic biofilm reactors (IAOBRs)	ZVI supplementation significantly reduces ARGs/MGEs by enhancing microbial metabolic activity (dehydrogenase +44.8%, ATP +45.7%) and enriching for functional bacteria that are not potential ARG hosts	(Tang et al. 2024)

literature (Figure 1b), presenting an extreme contrast with its contribution of over 50% of global antibiotic usage. This reveals the most prominent deficiency in the current research landscape: the domain with the highest risk receives the lowest research investment.

In summary, the three clusters clearly delineate the "imbalance landscape" of ARG research: Cluster 2 (municipal wastewater) demonstrates technological maturity and sufficient investment, Cluster 1 (hospital wastewater) exhibits complex mechanisms yet insufficient investment, and Cluster 3 (livestock wastewater) presents the highest risk yet

receives the lowest investment. This configuration provides unequivocal directional guidance for subsequent research resource allocation and policy formulation.

Conclusions

Based on a systematic analysis of 2,894 publications indexed in the Web of Science Core Collection from 2004 to 2024, this study elucidates the developmental trajectory and structural imbalances in global research on antibiotic resistance genes (ARGs) within wastewater treatment systems. The core conclusions are as follows:



manure-applied agricultural soils, and subsequently contaminated crops and groundwater. Furthermore, establish data-sharing protocols and risk assessment frameworks that enable integrated analysis across municipal, hospital, and livestock monitoring systems, facilitating coordinated interventions and early warning of resistance threats originating from agricultural sources.

Second, develop targeted control technologies to address “high-risk gene escalation.” Focus on high-risk genes such as *tet*, *mcr-1*, and *bla*_{NDM} by prioritizing research on coupled “anaerobic digestion + advanced oxidation” processes. Explore the feasibility of CRISPR-based gene-editing technologies and mobile genetic element-targeted inhibitors to overcome challenges in ARG removal from sludge and high-strength wastewater matrices.

Third, we call for deepening the integration of mechanistic understanding and practical applications to facilitate the transition from reactive monitoring to proactive resistance control. Priority should be given to cross-media (water, air, soil, and biota) transport dynamics and host succession patterns of ARGs, which underpin the environmental fate and dissemination of resistance. Building upon such mechanistic insights, targeted intervention technologies should be developed to eliminate high-risk genes. Emerging antibiotic removal technologies using waste-derived adsorbents (Slomkiewicz et al., 2025) represent a promising approach in this direction, yet their efficacy against ARG-hosting bacteria and mobile genetic elements requires further validation. Meanwhile, to translate technological advances into effective risk prevention, parallel efforts should be directed toward surveillance capacity. Recent advancements in watershed-scale water quality monitoring have demonstrated the value

patterns of ARGs, which underpin the environmental fate and dissemination of resistance. Building upon such mechanistic insights, targeted intervention technologies should be developed to eliminate high-risk genes. Emerging antibiotic removal technologies using waste-derived adsorbents (Slomkiewicz et al., 2025) represent a promising approach in this direction, yet their efficacy against ARG-hosting bacteria and mobile genetic elements requires further validation. Meanwhile, to translate technological advances into effective risk prevention, parallel efforts should be directed toward surveillance capacity. Recent advancements in watershed-scale water quality monitoring have demonstrated the value

First, establish a comprehensive surveillance chain to address the “structural imbalance.” Drawing upon the WHO’s “Source-Tracing Initiative for Antibiotic Resistance Genes” three-tier technical framework (metagenomic census—culturomics-based host resolution—mobile genetic element risk assessment), develop a standardized monitoring network encompassing the three major wastewater categories (municipal, hospital, and livestock). For livestock wastewater, the most critical gap in surveillance should prioritize key transmission nodes, including anaerobic storage lagoons,

of integrating real-time sensor systems with spatial modeling for early warning and source tracking (Syafrudin et al., 2025), providing a methodological framework that can be adapted to ARG surveillance across municipal, hospital, and livestock wastewater systems. Ultimately, the integration of ARGs into environmental quality standards should be promoted, thereby providing a scientific foundation for intersectoral governance of antimicrobial resistance.

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