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中国疾病预防控制中心周报

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18-24 NOVEMBER

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WORLD ANTIBIOTIC AWARENESS WEEK ISSUE

Preplanned Studies

Occurrence and Removal of Antibiotic Resistance in Nationwide Hospital Wastewater Deciphered by Metagenomics Approach — China, 2018–2022 1023

Methods and Applications

Effect of CeO₂ Nanoparticles on the Spread of Antibiotic Resistance in a Reclaimed Water-Soil-Radish System — Shenzhen City, Guangdong Province, China, April 2023 1029

Commentary

Mitigating Antibiotic Resistance Emissions in the Pharmaceutical Industry: Global Governance and Available Techniques 1038

Notifiable Infectious Diseases Reports

Reported Cases and Deaths of National Notifiable Infectious Diseases — China, September 2023 1045



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This week's issue was organized by Guest Editor Yongning Wu.

Preplanned Studies

Occurrence and Removal of Antibiotic Resistance in Nationwide Hospital Wastewater Deciphered by Metagenomics Approach — China, 2018–2022

Jin Huang^{1,2,&}; Zhongyu Wang^{1,&}; Zihan Chen¹; Hebin Liang¹; Xiaoyan Li^{1,2}; Bing Li^{1,#}

Summary

What is already known about this topic?

Hospital wastewater represents a significant conduit for the dissemination of antibiotic resistance genes (ARGs), including those that are of clinical importance. Yet, the variation in antibiotic resistance on a national level, which can be influenced by regional differences, has not been thoroughly elucidated.

What is added by this report?

Uniform methodologies were employed to analyze data from various provincial-level administrative divisions in China, aiming to investigate the patterns of antibiotic resistance across these regions. This analysis illuminated the location preferences of clinically ARGs on plasmids. Additionally, our study uncovered regional variations in the presumed bacterial hosts of ARGs at the genus level.

What are the implications for public health practice?

This investigation has shed light on the nationwide prevalence of ARGs in hospital wastewater in China, uncovering the significant ecological and public health risks associated with the disposal of such effluents.

Antibiotic resistance has emerged as a significant threat to global public health. The excessive use of antibiotics is known to hasten the spread of antibiotic resistance. The selective pressure exerted by antibiotics is exceptionally high in acute care hospitals, where an estimated 20%–30% of inpatients in Europe are administered antibiotics during their hospitalization (1). Hospital wastewater typically contains higher concentrations of antibiotics and antimicrobials than municipal wastewater (2–4), making it a critical reservoir for antibiotic resistant bacteria (ARB) and a conduit for the proliferation of these resistant strains (2,5). The occurrence of pathogens resistant to "last-resort" antibiotics in hospital settings, combined with the nosocomial transmission of such resistant mechanisms, represents a profound risk to patient

health and the surrounding environment (4).

In this study, 78 metagenomic datasets of hospital wastewater from six Chinese provincial-level administrative divisions (PLADs) were either obtained from the National Center for Biotechnology Information (NCBI) database or generated through direct collection and sequencing. Employing standardized methods, the research set out to analyze data from diverse regions within China to explore regional variations in antibiotic resistance. The investigation revealed that numerous clinically ARGs are carried on plasmids. Notably, the study identified regional differences in the potential bacterial hosts of ARGs at the genus level. Additionally, it was found that the existing hospital wastewater treatment systems are insufficient in effectively removing antibiotic resistance, further emphasizing the substantial ecological and health hazards associated with hospital wastewater discharge.

Metagenomic datasets representing hospital wastewater in China, spanning the years 2018 to 2022, were retrieved from the NCBI database. Additionally, we collected both influent and disinfected effluent samples from a general hospital in Shenzhen City, Guangdong Province, China. The samples underwent metagenomic sequencing on the Illumina NovaSeq 6000 platform, facilitated by Novogene (Tianjin, China), utilizing a paired-end 150 bp sequencing approach. Comprehensive details of the public datasets are presented in Supplementary Table S1 (available in <https://weekly.chinacdc.cn/>). To quantify the abundance of ARGs in each sample, we employed the standardized ARGs-OAP pipeline in conjunction with the SARG database (Version 3.2, <https://smile.hku.hk/ARGs/Indexing>), conforming to the suggested parameters and thresholds (6–7).

Quality assessment of the raw paired-end reads was conducted using Fastp (Version 0.22.0, <https://github.com/OpenGene/fastp>), ensuring that only reads of quality score above 20 were retained for subsequent analysis. These high-quality reads from each sample

were individually assembled into contigs with MegaHit (Version 1.2.9, <https://github.com/voutcn/megahit>). Predictions of open reading frames (ORFs) were executed using Prodigal (Version 2.6.3, <http://prodigal.ornl.gov/>). We utilized Platon in accuracy mode to differentiate putative plasmid-derived contigs from chromosomal contigs, ensuring meticulous discrimination. Subsequent alignment of ORFs on these assembled sequences against the SARG database was performed (8). For contigs presumed to be of chromosomal origin as determined by Platon, taxonomic classification was executed using Kaiju (Version 1.9.2, <https://bioinformatics-centre.github.io/kaiju/>). The relative abundance of ARG-carrying contigs (ACCs) was calculated in accordance with methodologies previously described in the literature (9).

This study identified a total of 1,899 ARG subtypes across 28 different ARG types within the wastewater samples analyzed (Figure 1A). Principal Coordinate Analysis (PCoA) based on the prevalence of ARG subtypes demonstrated distinct clustering of samples from Hebei, Guangdong, and Hunan provinces (Figure 1B). Conversely, in Sichuan Province, samples from the same hospital exhibited more similar ARG profiles. A significant positive correlation was observed between the aggregate abundance of ARGs and the presence of ARG-harboring plasmids (Figure 2B). A diversity of ARGs localized on plasmids was observed, with several subtypes such as *aadA*, *qacEΔ1*, *cmlA5*, *sul1*, *catB3*, *ereA*, OXA-10, and OXA-1 frequently co-occurring. Notably, plasmids routinely carried numerous clinically significant ARGs, including CTX-M-3, KPC-2, NDM-6, and the *vanM* gene cluster (Figure 2C). At the phylum level, Pseudomonadota was consistently the most prevalent ARG host across all PLADs (Figure 3A). However, at the genus level, variations in ARG genomic landscape were identified across different regions (Figure 3B). Pathogenic genera such as *Escherichia*, *Acinetobacter*, *Aeromonas*, *Pseudomonas*, *Klebsiella*, and *Enterococcus* were associated with a higher diversity of ARGs when compared to non-pathogenic genera.

DISCUSSION

In this study, the prevalence of antibiotic resistance in hospital wastewater across a national scope was evaluated using standardized methodologies that employed both read-based and assembly-based metagenomic analysis. This examination uncovered

distinctions in the profiles of ARGs, with geographical location and hospital type significantly impacting the distribution of these ARGs (Supplementary Figure S1, available in <https://weekly.chinacdc.cn/>). Although there was variance in the overall abundance of ARGs across different regions, the predominant categories of ARGs, including those conveying resistance to aminoglycosides, beta-lactams, macrolide-lincosamide-streptogramin (MLS), sulfonamides, and tetracyclines, remained consistent across PLADs (10). It is likely that the observed geographical influence can be attributed to regional differences in antibiotic prescription trends. Similarly, the type of hospital appeared to directly affect the variation in antibiotic use (3).

Mobile genetic elements (MGEs) are well-documented vehicles for the transference of ARGs, facilitating their propagation (11). This study hones in on plasmids, a critical type of MGE capable of transferring ARGs across diverse phylogenetic boundaries. Our analysis identified a significant positive correlation between the prevalence of ARGs and the presence of plasmids harboring these genes, underscoring plasmids' role in the mobilization of ARGs. The distribution of ARGs on plasmids was particularly telling, as numerous ARGs with clinical significance were found to be plasmid-borne, highlighting the considerable risk of their transference among bacterial species. Corroborating previous research, our study also detected the *vanM* gene cluster (4), which imparts resistance to vancomycin, on plasmids. Initially discovered in an *Enterococcus faecium* isolate from Shanghai Municipality, China, in 2006, *vanM* has been prevalent in the region since then (12–13). The detection of *vanM* in hospital wastewater on a national scale indicates widespread dissemination and points to plasmids as a likely contributor to this trend. Our findings demonstrate the pervasive presence of clinically significant ARGs on plasmids, suggesting that plasmids play a pivotal role in the dissemination of ARGs.

Upon further investigation into the ARG genetic context, we discovered that Pseudomonadota consistently emerged as the principal ARG carrier at the phylum level, regardless of the geographic PLADs. At the genus level, however, we observed pronounced regional variations in the ARG genetic context. Notably, the genus *thauera* exhibited a significantly higher ARG carrier abundance in Hebei Province compared to other PLADs, whereas the genus *Acinetobacter* was more abundantly identified as an ARG carrier in Gansu Province ($P < 0.05$). Additionally,

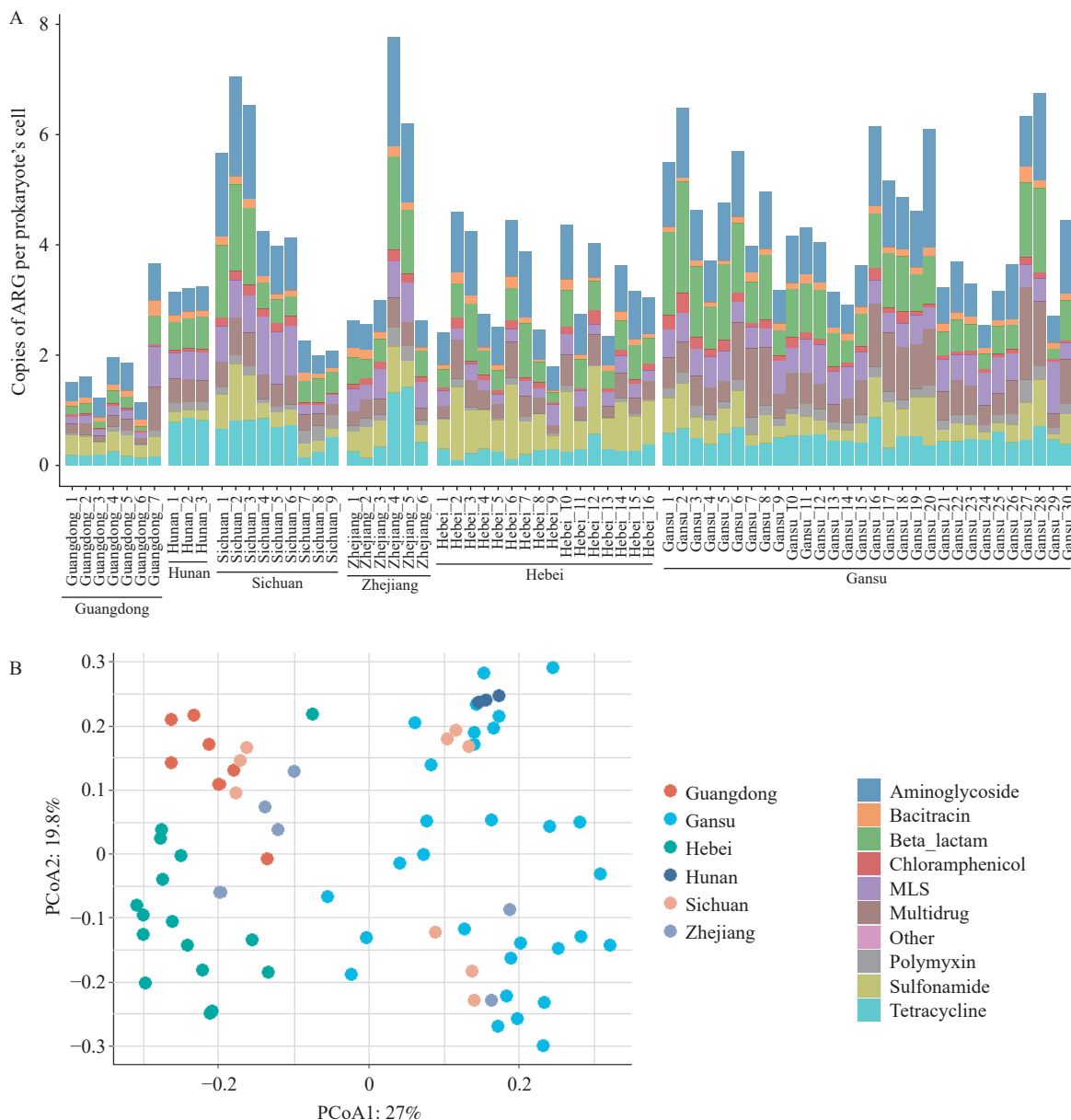


FIGURE 1. The occurrence of ARGs in untreated hospital wastewater. (A) The abundance of ARGs in untreated hospital wastewater sampling from different provinces. (B) PCoA analysis based on Bray-Curtis distance of ARG subtypes' abundance.

Note: Samples including Sichuan_1, Sichuan_2, and Sichuan_3 were taken from a general hospital while Sichuan_4, Sichuan_5, Sichuan_6 were taken from a hospital of traditional Chinese medicines, and Sichuan_7, Sichuan_8, Sichuan_9 were taken from a hospital of stomatology. Samples including Zhejiang_1, Zhejiang_2 and Zhejiang_3 were taken from an eye hospital and Zhejiang_4, Zhejiang_5, Zhejiang_6 were taken from a general hospital. Abbreviation: MLS=macrolide-lincosamide-streptogramin.

our findings indicated that pathogenic genera harbored a greater diversity of ARGs compared to their non-pathogenic counterparts (Figure 3C). Complementing these observations, investigation of metagenomic draft genomes from hospital wastewater environments revealed that approximately 60% of the identified potential pathogens (14). Among the *E.coli* resistant to erythromycin, ampicillin, or trimethoprim/

sulfamethoxazole isolated from the same fecal specimen of children, pathogenic isolates were found to be approximately as twice as non-pathogenic isolates (15). This result might be related to the clinical usage of antibiotics against pathogens, as previously confirmed that hospital-specific antibiotic usage was generally associated with increased hospital antibiotic susceptibility (16).

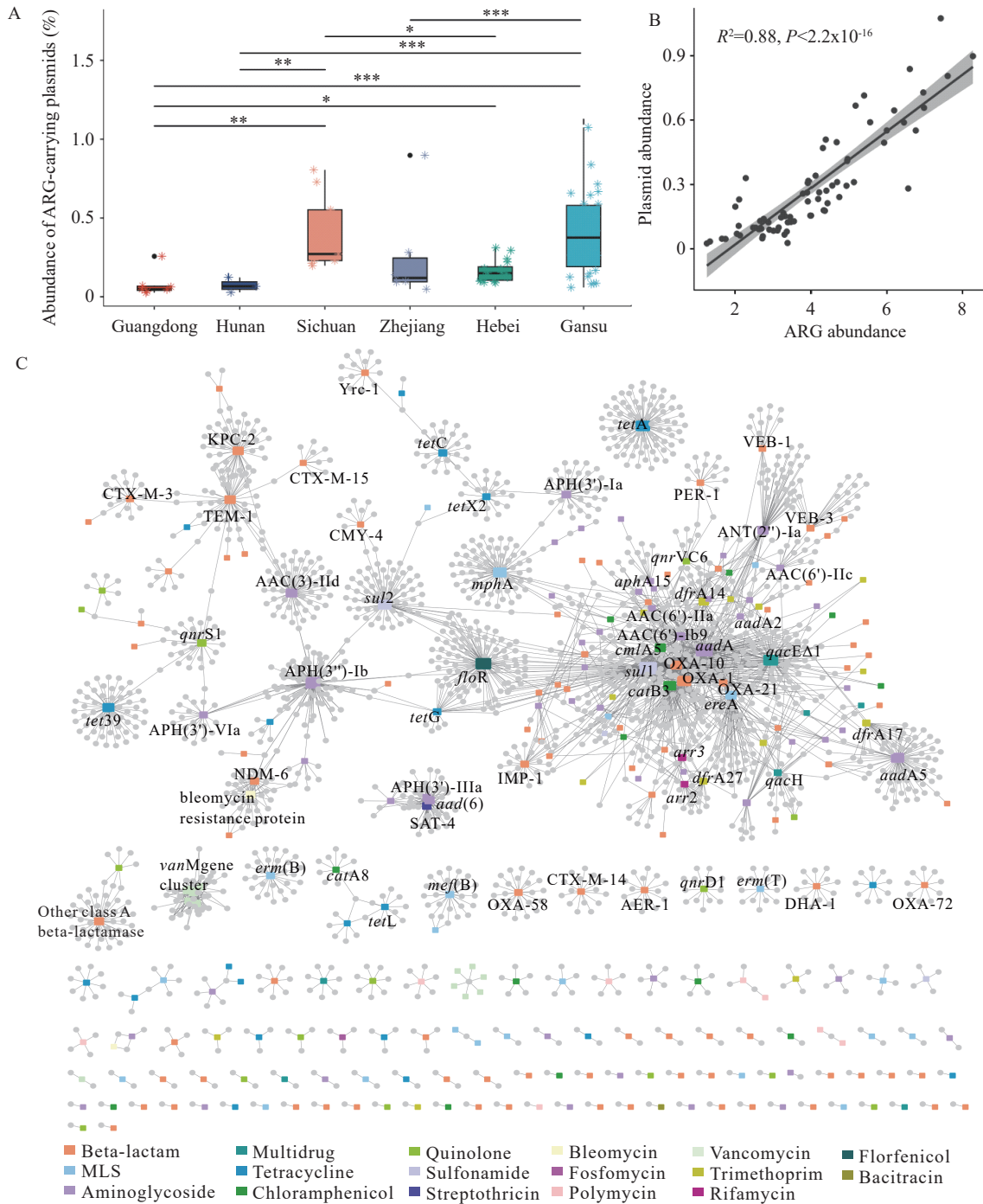


FIGURE 2. The profile of ARGs located on plasmids. (A) The abundance of ARG-carrying plasmids in different provinces. (B) The Pearson correlation between the total abundance of ARGs and the abundance of ARG-carrying plasmids. (C) The ARG-carrying pattern on the putative plasmid contigs.

Note: The ARGs with more than 10 connections to the plasmid contigs were labeled in the figure.

Abbreviation: MLS=macrolide-lincosamide-streptogramin; ATGs=antibiotic resistance genes.

* $P<0.05$;

** $P<0.01$;

*** $P<0.001$.

Wastewater treatment processes are not always effective at eradicating ARGs. Notably, while ARGs were found to be significantly reduced in the

wastewater from a general hospital in Zhejiang Province, the total abundance of ARGs in the treated wastewater of eye hospitals actually increased, rather

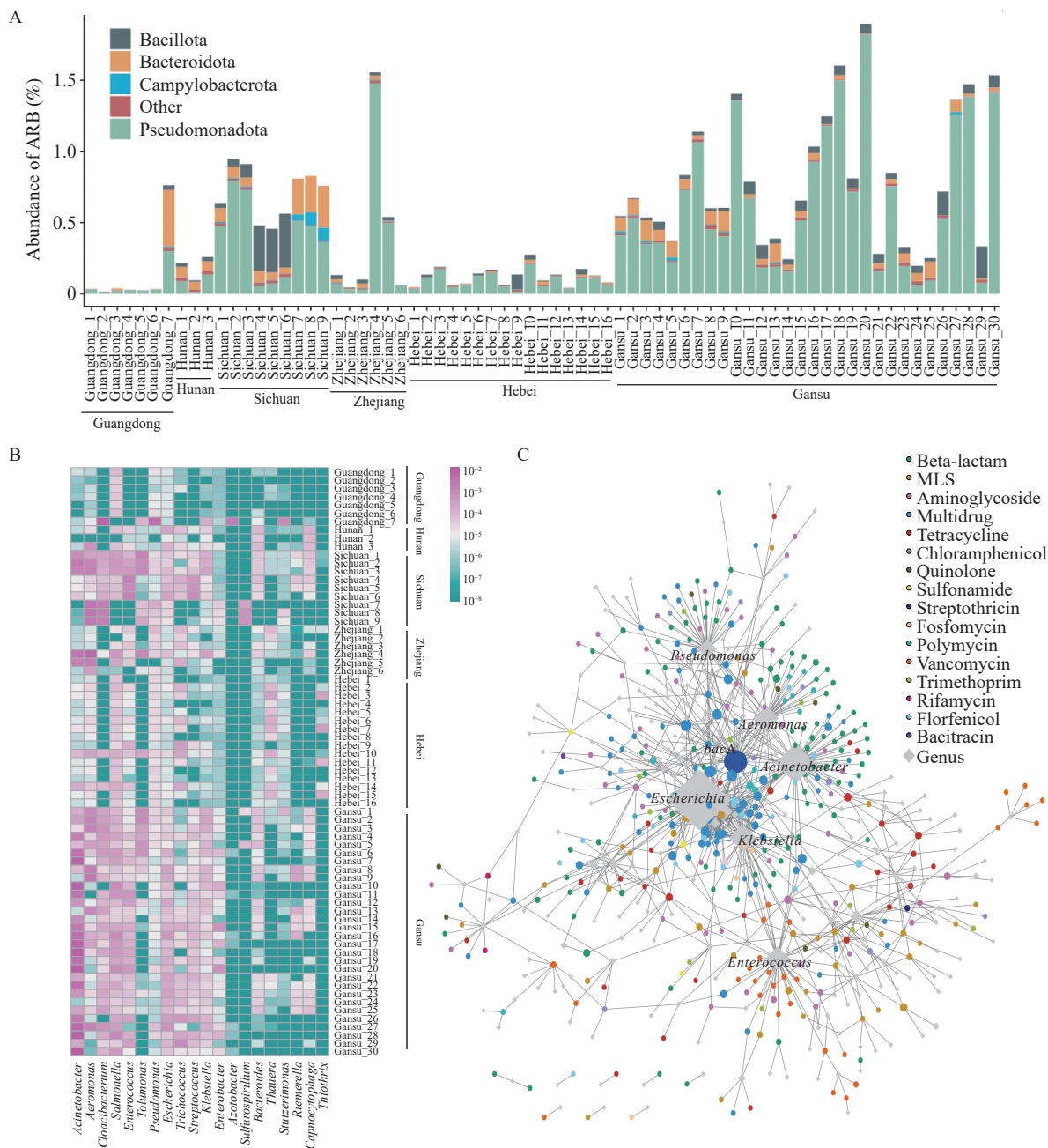


FIGURE 3. The genetic context of ARGs. (A) The genetic context of ARGs at phylum level. (B) The genetic context of ARGs at genus level. (C) The ARG-carrying patterns of different genera. Abbreviation: MLS=macrolide-lincosamide-streptogramin; ATGs=antibiotic resistance genes.

than decreased (Supplementary Figure S2, available in <https://weekly.chinacdc.cn/>). This suggests that the treatment methods implemented failed to effectively eradicate bacteria harboring multiple ARGs. Prior research has demonstrated variable responses of ARGs to treatment processes, with certain genes such as *ermB* and *tetW* showing reductions while others, including *bla_{TEM}*, *sul1*, and *qnrS*, have been found to increase post-treatment (2,17). Zhu et al. conducted an investigation into antibiotic resistance in three hospital

wastewater treatment systems and reported that chlorination, typically the final treatment stage before wastewater discharge, was largely ineffective at significantly reducing most ARGs (14). However, compared to relative abundance, absolute quantification of ARGs is more significant for evaluating the environmental implications of ARGs and the effectiveness of treatment technologies. Further investigation based on the absolute abundance of ARGs is desired to explore the treatment efficiency for

reducing antibiotic resistance. Furthermore, analysis of the genetic context of ARGs revealed an increased relative abundance of pathogenic genera in treated water (Supplementary Figure S2B). Pathogenic antibiotic-resistance bacteria (ARB) such as *Aeromonas*, *Pseudomonas*, *Salmonella*, and *Klebsiella* exhibited higher prevalence in treated wastewaters (Supplementary Figure S2B). There is mounting evidence suggesting that wastewater treatment plants may actually foster an environment that supports the enrichment and proliferation of ARB and that these facilities are not adequately removing multidrug-resistant pathogens (14,18). Collectively, these findings suggest that treated wastewater could have an elevated presence of pathogenic genera that carry multiple ARGs, posing a potential risk to public health.

This investigation provides valuable insights into antibiotic resistance within hospital wastewater across China, illuminating the considerable impact of geographical location and hospital categorization on the distribution of ARGs. In addition, this study reveals a preference for certain clinically significant ARGs to be located on plasmids, as well as regional variations in the genetic context of ARGs at the genus level. The findings also demonstrate that existing wastewater treatment facilities in hospitals are inadequate in effectively eliminating antibiotic resistance. This research highlights the urgent need for enhanced management and treatment of hospital wastewater to reduce the potential spread of antibiotic resistance emanating from healthcare facilities.

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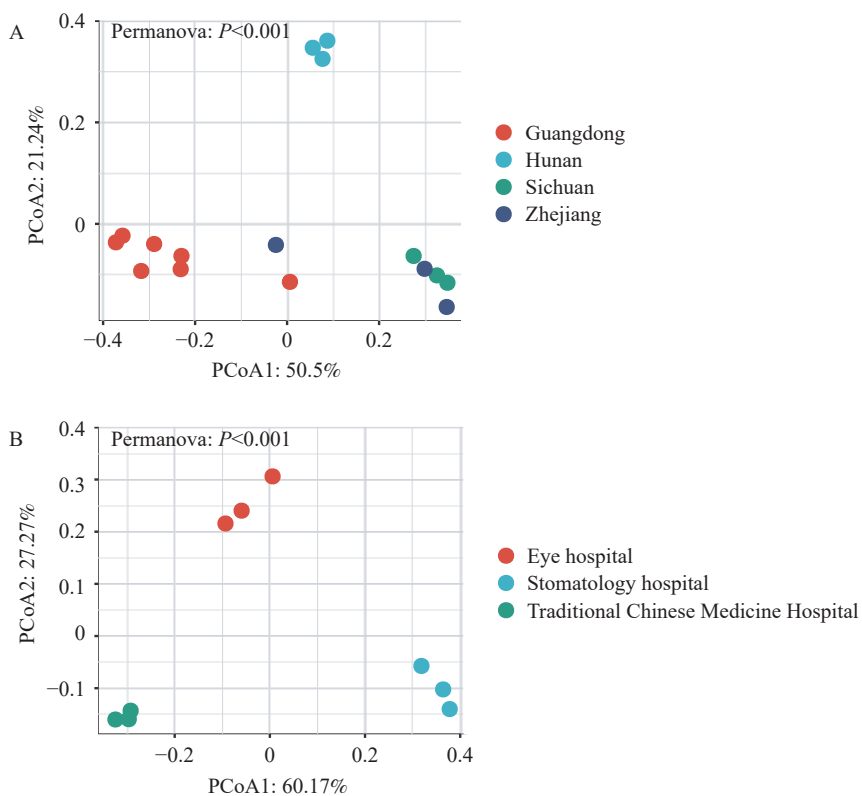
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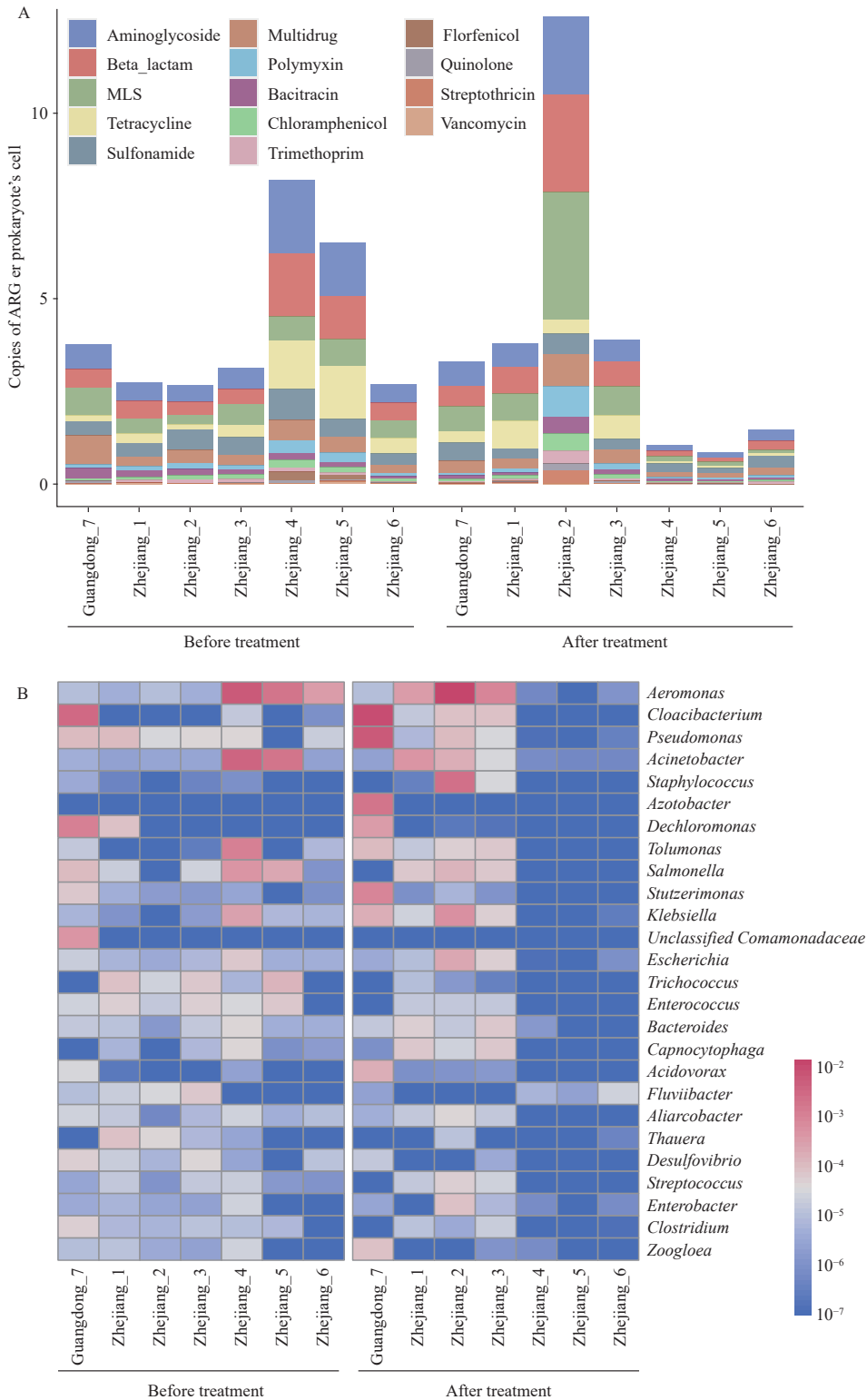
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SUPPLEMENTARY MATERIAL



SUPPLEMENTARY FIGURE S1. The influence of geographical position and types of hospitals on ARG profiles based on PCoA analysis. (A) Analysis based on Bray-Curtis distance of ARG subtypes' abundance from different general hospitals. (B) Analysis based on Bray-Curtis distance of ARG subtypes' abundance from different types of hospitals.



SUPPLEMENTARY FIGURE S2. The comparison of antibiotic resistance before and after treatment. (A) The abundance of ARGs before and after treatment. The ARG types whose average abundance ≥ 0.01 copies per cell were presented. (B) The relative abundance of ARG-carrying genera (average relative abundance $\geq 0.1\%$).

Note: The treatment for samples of Zhejiang_1, Zhejiang_2, and Zhejiang_3 was activated sludge process. The treatment for samples of Zhejiang_4, Zhejiang_5, and Zhejiang_6 was chlorination disinfection. The treatment for the sample of Guangdong_7 was UV disinfection.

Abbreviation: MLS=macrolide-lincosamide-streptogramin; ARGs=antibiotic resistance genes.

SUPPLEMENTARY TABLE S1. The detailed information of metagenomic datasets.

NCBI Project ID	Province	ID	Data size (Mb)	Note
PRJNA989503	Gansu	Gansu_1	3397.5	before treatment
		Gansu_2	3159.9	before treatment
		Gansu_3	3550.7	before treatment
		Gansu_4	3201.8	before treatment
		Gansu_5	3168.3	before treatment
		Gansu_6	3727.5	before treatment
		Gansu_7	3806.4	before treatment
		Gansu_8	3315.4	before treatment
		Gansu_9	3268.8	before treatment
		Gansu_10	3240.5	before treatment
		Gansu_11	3775.9	before treatment
		Gansu_12	3215.4	before treatment
		Gansu_13	3286.0	before treatment
		Gansu_14	3378.2	before treatment
		Gansu_15	3245.6	before treatment
		Gansu_16	3448.7	before treatment
		Gansu_17	3204.3	before treatment
		Gansu_18	3878.9	before treatment
		Gansu_19	3260.1	before treatment
		Gansu_20	3324.4	before treatment
		Gansu_21	3423.6	before treatment
		Gansu_22	3320.3	before treatment
		Gansu_23	3210.2	before treatment
		Gansu_24	3319.3	before treatment
		Gansu_25	3278.4	before treatment
		Gansu_26	3296.9	before treatment
		Gansu_27	3266.7	before treatment
		Gansu_28	3160.5	before treatment
		Gansu_29	3321.8	before treatment
		Gansu_30	4105.5	before treatment
PRJNA876047	Hebei	Hebei_1	2653.3	before treatment
		Hebei_2	2792.5	before treatment
		Hebei_3	2970.6	before treatment
		Hebei_4	2833.8	before treatment
		Hebei_5	2656.2	before treatment
		Hebei_6	2724.7	before treatment
		Hebei_7	2748.3	before treatment
		Hebei_8	2772.1	before treatment
		Hebei_9	2733.4	before treatment
		Hebei_10	2741.5	before treatment
		Hebei_11	2253.3	before treatment
		Hebei_12	2766.4	before treatment
		Hebei_13	2767.6	before treatment
		Hebei_14	3057.4	before treatment
		Hebei_15	2692.4	before treatment
		Hebei_16	2763.2	before treatment

TABLE S1. (Continued)

NCBI Project ID	Province	ID	Data size (Mb)	Note
PRJNA862690	Hunan	Hunan_1	2550.5	before treatment
		Hunan_2	2472.8	before treatment
		Hunan_3	2633.3	before treatment
PRJNA723368	Sichuan	Sichuan_1	4090.7	before treatment
		Sichuan_2	4154.7	before treatment
		Sichuan_3	4178.7	before treatment
		Sichuan_4	4086.4	before treatment
		Sichuan_5	4448.0	before treatment
		Sichuan_6	4203.4	before treatment
		Sichuan_7	4414.1	before treatment
		Sichuan_8	5001.2	before treatment
		Sichuan_9	4602.3	before treatment
PRJNA612238	Guangdong	Guangdong_1	2231.2	before treatment
		Guangdong_2	2361.1	before treatment
		Guangdong_3	2302.8	before treatment
		Guangdong_4	2645.6	before treatment
		Guangdong_5	2251.6	before treatment
		Guangdong_6	2759.7	before treatment
		Guangdong_7	9748.5	before treatment
This study		Guangdong_7	10086.4	after treatment
PRJNA770854	Zhejiang	Zhejiang_1	3051.5	before treatment
		Zhejiang_2	7300.7	before treatment
		Zhejiang_3	4646.1	before treatment
		Zhejiang_4	3712.8	before treatment
		Zhejiang_5	3571.9	before treatment
		Zhejiang_6	4548.1	before treatment
		Zhejiang_1	3910.9	after treatment
		Zhejiang_2	3711.8	after treatment
		Zhejiang_3	4037.6	after treatment
		Zhejiang_4	3537.1	after treatment
		Zhejiang_5	3245.9	after treatment
		Zhejiang_6	3293.7	after treatment

Methods and Applications

Effect of CeO₂ Nanoparticles on the Spread of Antibiotic Resistance in a Reclaimed Water-Soil-Radish System — Shenzhen City, Guangdong Province, China, April 2023

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ABSTRACT

Introduction: The use of reclaimed water (RW) for irrigation in agricultural practices raises concerns regarding the dissemination of antibiotic resistance genes (ARGs) from soils to edible crops. The effectiveness of nanoparticles (NPs) in reducing antibiotic resistance in vegetables irrigated with RW remains largely unexplored.

Methods: To investigate the effects, we conducted pot experiments in which radishes were planted in soil amended with CeO₂ NPs using various application techniques. The abundance of ARGs was characterized using high-throughput quantitative PCR (HT-qPCR). Concurrently, we utilized 16S ribosomal RNA (rRNA) gene sequencing to evaluate the microbial community structure of both the rhizosphere soil and the endophytic compartment within the radishes. Employing bioinformatics analysis, we probed the potential mechanisms by which NPs influence the resistome within the reclaimed water-soil-radish system.

Results: Following the application of CeO₂ NPs, there was a noticeable reduction in both the number and concentration of ARG genotypes in the rhizosphere soil, as well as within the radish. Concurrently, CeO₂ NPs appeared to mitigate the propagation of ARGs within the reclaimed water-soil-radish system. The ability of CeO₂ NPs to modulate the resistome is linked to alterations in microbial community structure. Soil treatment with NPs emerged as the most effective strategy for curbing the spread of ARGs.

Discussion: This finding provides a theoretical foundation for the development of nano-agricultural technologies aimed at controlling the proliferation of ARGs.

By 2050, it is projected that water withdrawals for irrigation will increase by approximately 10%. Utilizing reclaimed water (RW) for irrigation may provide effective strategies to alleviate pressure on groundwater resources (1). While numerous sterilization processes exist to eliminate pathogens and antibiotic-resistant bacteria (ARBs) in RW, antibiotic resistance genes (ARGs) possess a range of mechanisms, such as dormancy and mutations in ARBs, that enable their persistence (2). ARGs and ARBs have the potential to be transferred and reaccumulated in various plant tissues, including those that are edible, thereby entering the food chain. This poses a significant health risk, particularly when these plants are consumed raw.

The dissemination of ARGs in soil-plant systems through RW irrigation is influenced by two primary factors: the colonization of ARBs and the horizontal gene transfer (HGT) of ARGs to indigenous bacteria within these systems. Notably, nanoparticles (NPs) possess the capability to not only alter microbial community structures, thereby affecting the colonization of ARBs (3), but also to potentially inhibit the HGT of ARGs (4). Furthermore, NPs can enhance the efficiency of agricultural resource utilization and more effectively manage environmental challenges, paving the way for a forthcoming nano-agriculture revolution (5). Despite these promising attributes, the role of NPs in reducing ARG dissemination within soil-plant systems during RW irrigation has yet to be fully elucidated.

This study sought to examine the effects of CeO₂ NP exposure on radish seedlings grown in soil irrigated with RW. Various application methods were employed — adding NPs to reclaimed water, NP seed dressing, and direct soil mixing with NPs. It was found that, generally, radish plant growth can be significantly

enhanced by the presence of CeO₂ nanomaterials at concentrations up to 100 ppm (5), which was the predetermined upper limit for this investigation. Following 45 days of irrigation with RW, radishes were harvested and assessed.

A comprehensive evaluation was carried out on the abundance of ARGs and bacterial community diversity in the rhizosphere soil, roots, and leaves of the plants. This was achieved through high-throughput quantitative PCR (HT-qPCR) and 16S ribosomal RNA (rRNA) gene amplicon sequencing. This study provides initial insight into the influence and underlying processes by which NPs might modulate the proliferation of ARGs in RW irrigation systems. Furthermore, these findings are intended to lay a theoretical foundation for the development and refinement of nanotechnologies aimed at preventing and mitigating the spread of the agricultural resistome.

METHODS

Microcosm Design and Sample Collection

Radishes (*Raphanus sativus* L. var. radculus pers) were cultivated in a plant seedling substrate soil (Nord Agri, China). Four types of treatments were designed, including non-nanoparticle treatment (CK group), reclaimed water added with 100 ppm CeO₂ NPs treatment (WT group), seed dressing by 100 ppm

CeO₂ NPs treatment (SD group) and soil mixed with 100 ppm CeO₂ treatment (ST group) (Figure 1). The reclaimed water, sourced from the Futian Wastewater Treatment Plant (WWTP) in Shenzhen, was used to maintain soil moisture levels with applications every two days. After 45 days, radish plants were harvested, including samples from the rhizosphere soil, roots, and leaves. The dried radish tissue samples were then digested using pure HNO₃ through a microwave digestion system. Subsequently, total cerium (Ce) content was quantified using inductively coupled plasma-mass spectrometry (ICP-MS).

DNA Extraction and HT-qPCR

The radish shoot, root, and rhizosphere soil were individually weighed and placed into PowerBead tubes for DNA extraction, as per the manufacturer's protocol. We utilized the HT-qPCR method to examine the diversity and quantify the abundance of ARGs and mobile genetic elements (MGEs) (6). A suite of 296 primer pairs was utilized to profile the antibiotic resistome. We established a cycle threshold (CT) of 31 as the cutoff for detection (7). The relative gene copy number was calculated using the equation: relative gene copy number = $10^{(31-CT)/(10/3)}$, in which CT represents the results from the quantitative PCR assay. To determine bacterial abundance, we quantified absolute 16S rRNA gene copy numbers using standard

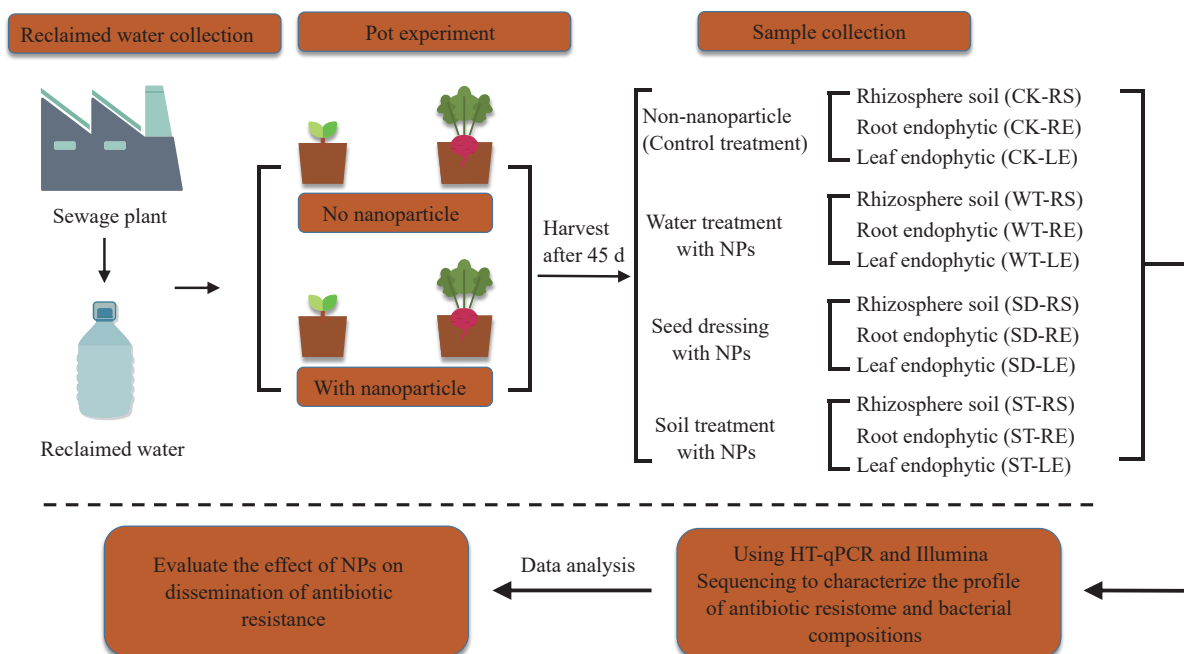


FIGURE 1. Schematic workflow depicting experimental design, methods, and objective of this study. Abbreviation: NPs=nanoparticles; HT-qPCR=high-throughput quantitative PCR.

curves. Measurements were conducted using a Bio-rad CFX (Biorad, USA) and a Takara Taq PCR kit (Takara, Japan).

Bacterial 16S rRNA Gene Sequencing and Bioinformatic Processing

To characterize the structures and compositions of bacterial communities, the V4–V5 hypervariable region of the 16S rRNA gene was amplified using primers 515F (GTGCCAGCMGCCGCGG) and 907R (CCGTCAATCMTTTRAGTTT) (8). Resulting amplicons underwent purification and were quantified before being pooled for sequencing. High-throughput sequencing was then conducted on an Illumina HiSeq 2500 platform at Novogene in Guangzhou City, Guangdong Province, China.

To ensure the quality of downstream analyses, raw paired-end reads were filtered to eliminate those containing three or more ambiguous nucleotides. We also removed reads with a low average quality score (<20) and short reads (<100 nt), including those associated with barcodes, to generate clean concatenated reads that spanned the complete V4–V5 region of the 16S rRNA gene. High-quality sequences were subsequently trimmed and demultiplexed using the Quantitative Insights Into Microbial Ecology (QIIME1) pipeline (9). Operational taxonomic units (OTUs) were identified employing the unoise3 algorithm, which is a k-mer-based approach for sequence identification and error correction. OTUs derived from chloroplasts and mitochondria (approximately 1%), as well as singleton OTUs, were removed from the final OTU dataset (10). The Greengenes database was utilized for taxonomic classification with a confidence threshold of 90%.

Statistical Analysis

Statistical analyses were executed utilizing SPSS 20 (SPSS Inc., Chicago, USA), with a significance threshold set at $P < 0.05$ for all tests. Principal Coordinate Analysis (PCoA) was conducted to ascertain the global distribution patterns of ARGs and bacteria across the samples, applying the Bray-Curtis dissimilarity metric, facilitated by Canoco version 5.0. The significance of differences in microbial community compositions between treatment groups was assessed using Adonis, implemented in the R environment utilizing the “vegan” package (11). Additionally, heatmaps were generated in R employing the “pheatmap” package to visually represent the data (12).

The relationships between ARGs and MGEs patterns were determined using ordinary least squares (OLS) regression models (13). Moreover, the Mantel test was applied for correlation analysis, leveraging the capabilities of R Studio in conjunction with the “vegan” package.

RESULTS

Composition of ARGs in Reclaimed Water-Soil-Plant System

The enumeration of ARGs and MGEs varied across samples, with a range of 28 to 38 detected in each (Figure 2A). Notably, the quantity of ARGs present in plant roots and leaves was less than that found in the corresponding rhizosphere soil. Furthermore, when comparing treatments, it was observed that seed dressing and soil application of CeO₂ NPs notably reduced the abundance of ARGs within the rhizosphere soil, in contrast to treatments using reclaimed water containing CeO₂ NPs.

The concentration of ARGs in the soil, which ranged from 5.21×10^9 to 7.74×10^9 copies/g soil, was approximately an order of magnitude higher than that observed in the phyllosphere, with values spanning from 5.04×10^8 to 7.31×10^8 copies/g plant tissue (Figure 2B). Moreover, the concentration of ARGs in root endophytes (ranging from 1.25×10^9 to 2.36×10^9 copies/g plant) was significantly lower than the concentration found in rhizosphere soil.

To ensure that variations in the native bacterial populations did not confound our results, we normalized the abundance of ARGs to the number of bacterial cells, quantified as 16S rDNA copy numbers (Supplementary Table S1, available in <https://weekly.chinacdc.cn/>). Given that the current average number of 16S rRNA genes per bacterial cell is approximately 4.1, the normalized ARG copy numbers varied from 0.10 to 0.35 copies per bacterial cell. The application of NPs had a discernible impact on the normalized abundance of ARGs, mirroring the effect seen with the absolute ARG copy numbers, with the exception of the rhizosphere soil subjected to the WT treatment (Supplementary Table S1).

Composition of MGEs in reclaimed water-soil-plant system

The abundance of mobile genetic elements (MGEs) exhibited considerable variability, spanning more than an order of magnitude, with counts ranging from

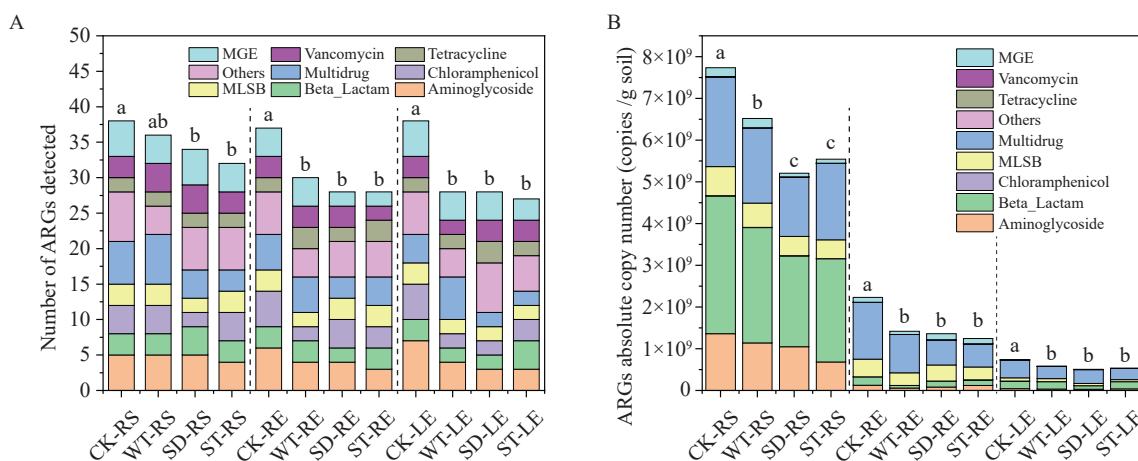


FIGURE 2. The number and absolute abundances of ARGs genotypes. (A) Number of ARGs genotypes detected in different samples. (B) Absolute abundances of ARGs expressed as copies per g solid.

Abbreviation: ARGs=antibiotic resistance genes; MGE=mobile genetic element; MLSB= macrolide-lincosamide-streptogramin B.

1.79×10^7 to 1.21×10^8 copies per gram of solid matter in the phyllosphere, and from 8.57×10^8 to 5.54×10^9 copies per gram of solid matter in the soil (Figure 2B). Similarly, reflecting the observed decline in the abundance of ARGs, the introduction of NPs was associated with a consequent reduction in the prevalence of MGEs within the water-soil-plant continuum.

Distribution of ARGs in Reclaimed Water-Soil-Plant System

If ARGs are identified solely in the roots or leaves of radishes when irrigated with reclaimed water, these ARGs are considered transmissible within the reclaimed water-soil-radish pathway. As depicted in Figure 3A, there are 30 ARGs capable of moving from reclaimed water to the leaves of radishes under the control condition. Relative to the control condition, we observed a reduction of 30.0%, 43.4%, and 40.0% in the number of transmissible ARGs from reclaimed water to radish leaves when NP applications in reclaimed water, seed dressing, and soil mixing interventions were implemented, respectively (Figure 3B–D).

After the application of NPs, the majority of ARGs exhibited a decreasing trend in abundance. The *aadA2* gene, associated with aminoglycoside resistance, exhibited the most substantial reduction, with a 48% decrease in the ST-RE group as shown in Figure 3E. Conversely, there was an upward trend observed in the abundance of vancomycin resistance genes, tetracycline resistance genes, and *mexB*, which is linked to

multidrug resistance. Two ARGs, *pbrT* and *qepA*, showed a significant increase in the radish root when subjected to seed dressing and soil treatments. Following the NPs treatments, the abundance of three MGEs (*Tn403*, *TrbC*, and *IS256*) showed a significant decrease, particularly in the WT-RS, WT-LE, and SD-LE treatments.

Microbial Community Assembly

Upon merging overlapped read pairs and conducting quality filtering, we obtained a total of 12,412 high-quality sequences from all the samples. These sequences were subsequently denoised, yielding 1,219 OTUs. We organized the microbial communities into 17 phyla by using the 16S rRNA database (GreenGene, gg_13_5 version). Principal coordinate analysis (PCoA) at the genus level indicated distinct separation of soil, root, and leaf sample communities along the PC1, PC2, and PC3 axes, which accounted for 30.5%, 22.6%, and 5.3% of the variation, respectively (Figure 4A). The PCoA further revealed pronounced clustering of bacterial communities that correlated with the application of different NPs in the root and leaf samples. This finding suggests that NP application significantly influences the overall composition of bacterial communities in both roots and leaves.

Understanding the specific members of a community that drive bacterial population shifts is crucial. As indicated in Supplementary Tables S2–S4 (available in <https://weekly.chinacdc.cn/>), the genus *Pseudomonas* plays a pivotal role in the bacterial

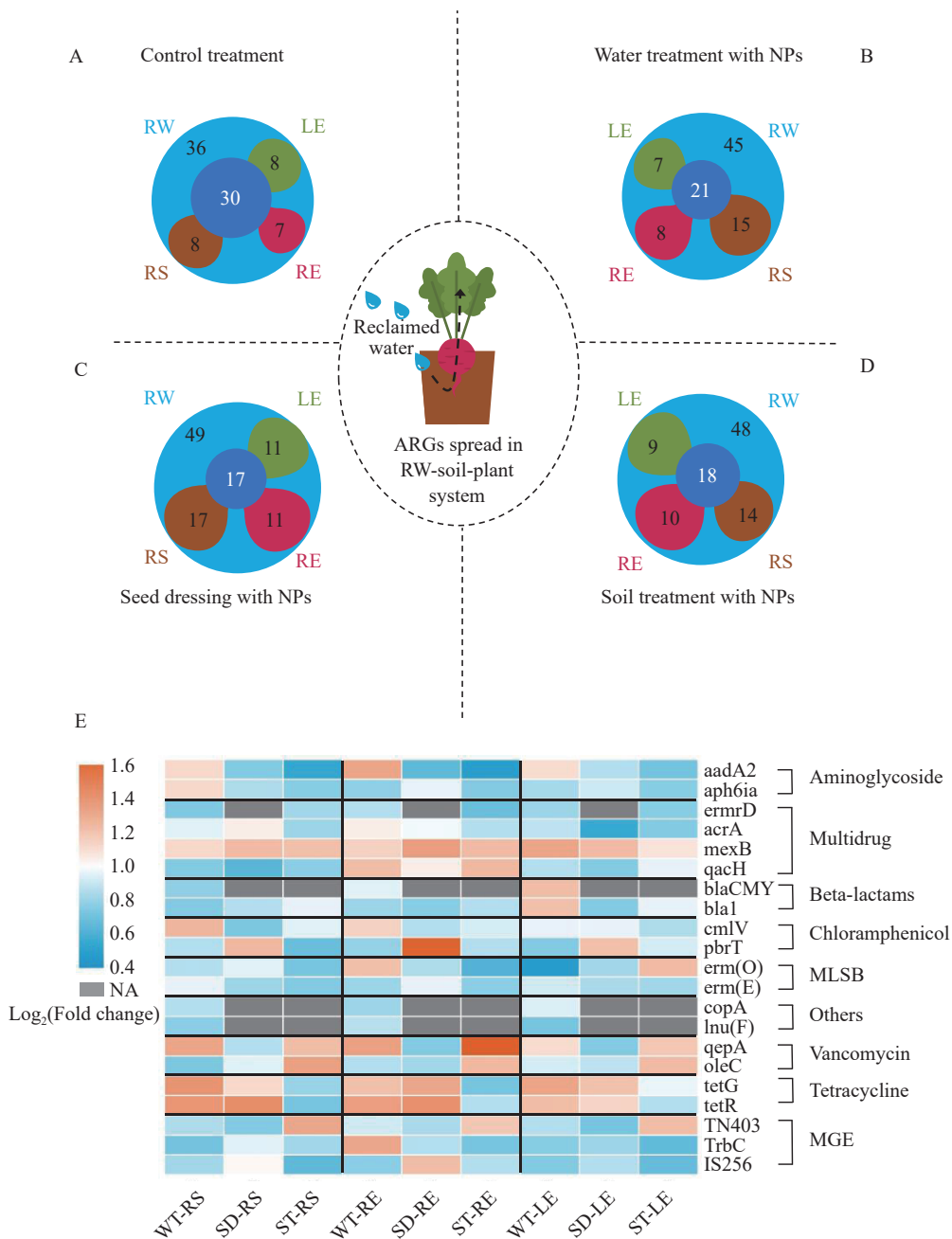


FIGURE 3. Venn diagrams depicting the dissemination of ARGs from reclaimed water to radish seedlings. (A) control treatment, (B) water treatment, (C) seed dressing, and (D) soil treatment with NPs. (E) The fold change of the main 21 ARGs and MGEs which can spread from reclaimed water to radish.

Note: The outermost light blue circle represents the number of ARGs detected in reclaimed water, while the inner brown, green, and red circles represent the number of ARGs detected in soil, leaf endophytic, and root endophytic proportions, respectively. The dark blue circle in the middle represents the number of ARGs that can propagate in the reclaimed water-soil-radish system.

Abbreviation: NPs=nanoparticles; ARGs=antibiotic resistance genes; RW=reclaimed water; MGE=mobile genetic element; MLSB=macrolide-lincosamide-streptogramin B.

community dynamics of rhizosphere soil, as well as radish roots and leaves. Intriguingly, *Pseudomonas* was the taxonomic group that exhibited a significant decline across various NPs application methods in both

soil and radish samples (Supplementary Figure S1, available in <https://weekly.chinacdc.cn/>). This reduction can likely be attributed to the pronounced antimicrobial properties of NPs and their ability to

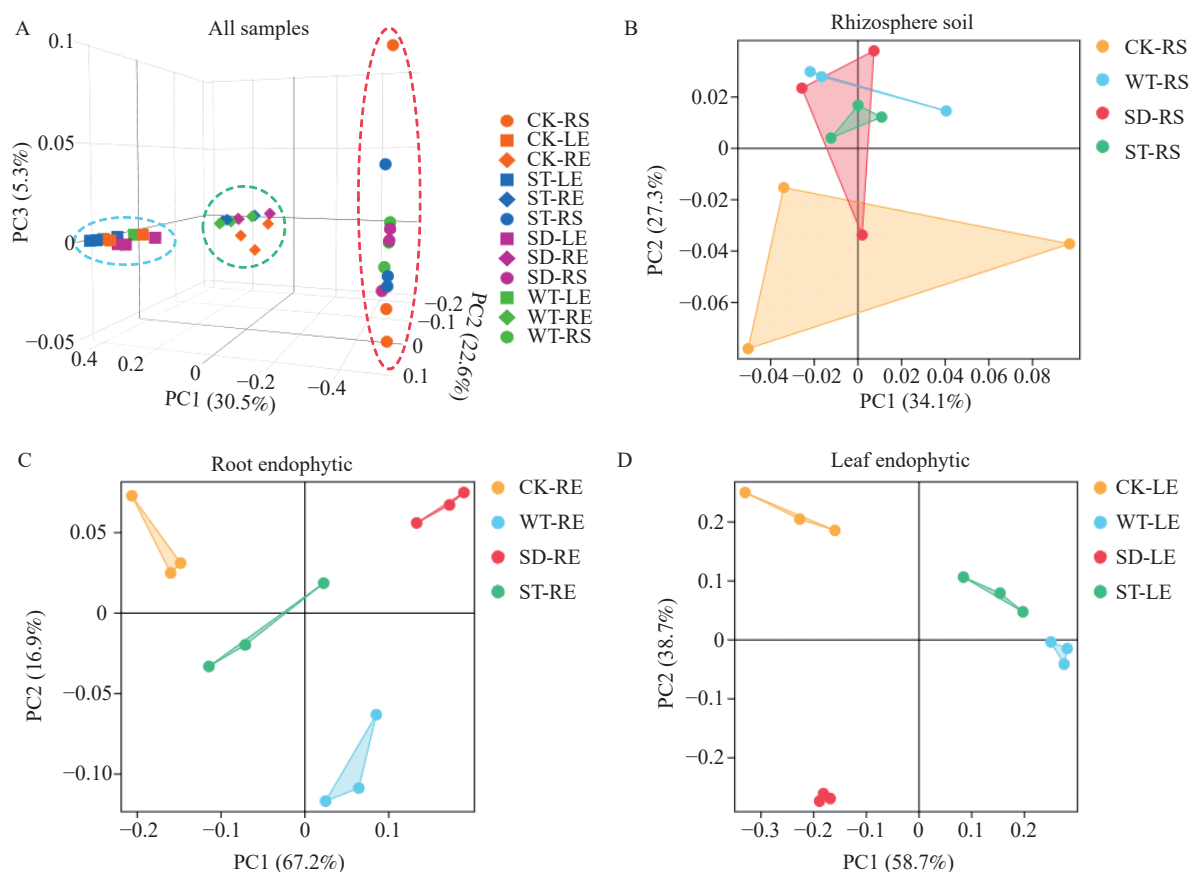


FIGURE 4. Principal coordinate analysis (PCoA) based on genus level communities profile (using Bray-Curtis distance). (A) overall, (B) rhizosphere soil, and (C) root and (D) leaf endophytic proportions of radishes.

inhibit biofilm formation. It appears that NPs may reduce the competitive edge of certain bacteria, forcing them to relinquish their ecological niches and leading to an alteration in the structure of the bacterial community.

Bacterial diversity and MGEs accounting for antibiotic resistome

The absolute abundance of MGEs was found to be linearly and positively correlated with the absolute abundance of ARGs ($R^2=0.64$, $P<0.001$) (Figure 5A). This indicates that the variation within the antibiotic resistome of reclaimed water-soil-plant systems is closely associated with MGEs. Figure 5B–D demonstrates that Procrustes analysis, utilizing Bray-Curtis dissimilarity metrics, confirmed a significant correlation between the abundance of ARGs and bacterial diversity within reclaimed water-soil-plant systems ($P<0.01$). Considering the observed community variations attributed to NP application (Figure 4), we postulate that NPs could potentially reduce the risk of ARG transmission in these systems

by fostering a more beneficial community structure. As illustrated in Figure 6, microbial communities primarily facilitate the transmission of ARGs targeting beta-lactam, aminoglycoside, and tetracycline antibiotics, with considerable statistical significance ($P<0.01$, $R>0.8$). MGEs are chiefly associated with the dissemination of ARGs against aminoglycoside, multidrug, and macrolide-lincosamide-streptogramin B (MLS_B) antibiotics. The regulation of aminoglycoside ARGs appears to be governed not only by bacterial communities but also by MGEs. Specifically, the *aadA2* gene, associated with aminoglycoside resistance, exhibited the most pronounced decrease following silver nanoparticle treatment (Figure 3E). This suggests that the reduction may result from the combined influence of microbial community structure and horizontal gene transfer mechanisms. Conversely, the tetracycline resistance genes *tetG* and *tetR* increased under WT and SD conditions, leading us to conjecture that microbial communities play a vital role in this dynamic. Overall, the findings indicate that modifications in the resistome of reclaimed water-soil-plant systems are determined by the interplay of

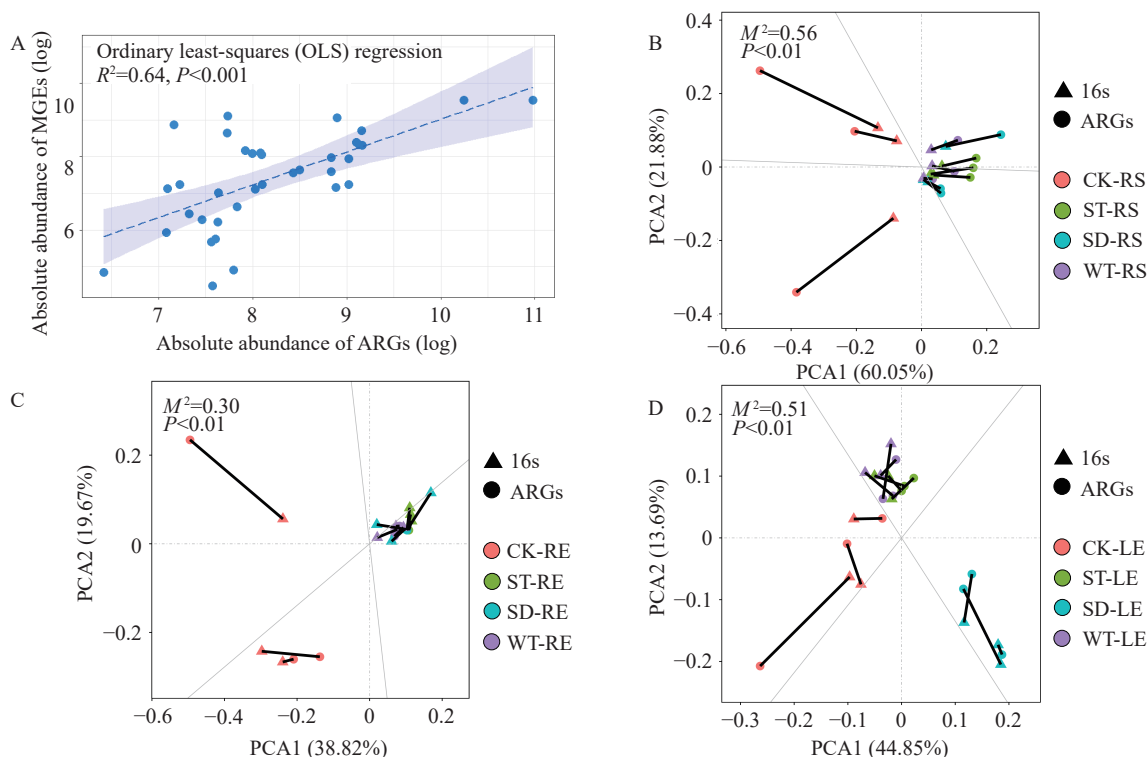


FIGURE 5. Correlation analysis of ARGs with MGEs and bacterial community. (A) Ordinary least squares (OLS) regression showing the relationship between total absolute abundance of ARGs and MGEs. (B–D) Procrustes analysis depicts the correlation between ARG content and bacterial community in different samples (B, Rhizosphere soil; C, Root endophytic; D, Leaf endophytic).

Abbreviation: ARGs=antibiotic resistance genes; MGEs=mobile genetic elements; 16s=16S ribosomal RNA.

bacterial communities and horizontal gene transfer, working in concert to shape antibiotic resistance patterns.

DISCUSSION

The utilization of NPs significantly impacted the prevalence of ARGs within reclaimed water (RW)-soil-plant systems, as demonstrated by a marked reduction in the absolute copy number of ARGs ($P<0.05$). This phenomenon may be explained by two potential mechanisms: (1) NP application modified the structure of the bacterial community, and (2) NP application suppressed the horizontal gene transfer of ARGs.

The resistome exhibited a significant correlation with the structures of bacterial communities (7). Our research findings indicate that different NP application methods can elicit distinct alterations in the microbial community structure of radishes, which suggests that these methods may influence the crop's resistome in varied ways. Intriguingly, our results also suggest that the modulation of bacterial communities by NPs tends to decrease the abundance of ARGs within plant

microbiomes. This phenomenon may be attributable to the suppressive effects of NPs on biofilm formation, which serves as a nexus for ARG transmission. Furthermore, NPs may also impede the HGT of ARGs. HGT constitutes a primary pathway for the dissemination of ARGs in the environment, involving the exchange of genetic material among different bacterial species. Studies have shown that NPs can obstruct the transfer of plasmids — small, circular DNA molecules capable of carrying ARGs. This obstruction has the potential to thwart the inter-species propagation of ARGs, thereby curtailing their prevalence.

Alterations in the structure of endophytic bacterial communities may be linked to the varied levels of CeO₂ NP uptake by plants, a process influenced by the differing NP application techniques (Supplementary Figure S2, available in <https://weekly.chinacdc.cn/>). These results suggest that the method of NP administration can cause fluctuating NP absorption rates in plants. When compared to WT and SD treatments, the ST demonstrated a more pronounced inhibitory effect on the transmission of ARGs. This

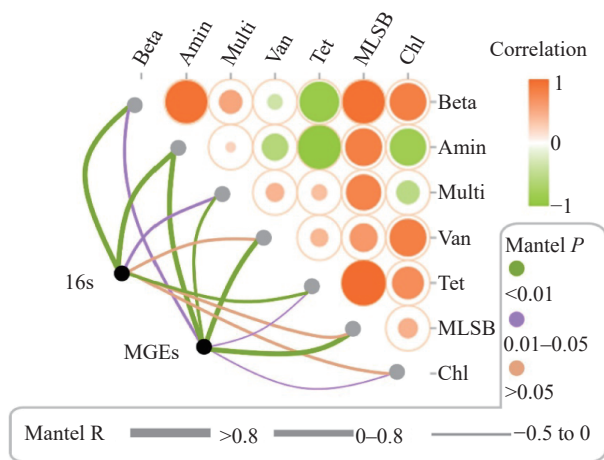


FIGURE 6. Pairwise comparisons of ARGs.

Note: 16s (OTUs) and MGEs were related to each ARG by partial (geographic distance–corrected) Mantel tests. Edge width corresponds to Mantel's R statistic for the corresponding distance correlations, and edge color denotes the statistical significance.

Abbreviation: Beta=beta-lactams; Amin=aminoglycoside; Multi=multidrug; Van=vancomycin; Tet=tetracycline; MLSB=macrolide-lincosamide-streptogramin B; Chl=chloramphenicol.

effect is likely due to the more efficient NP uptake by radishes in the ST treatment. Given that plants predominantly absorb nutrients via their roots from deeper soil layers, it can be inferred that the more homogeneously integrated CeO₂ NPs in the ST-treated soil facilitated their uptake by the radishes. Previous studies have shown that different NP concentrations can induce varying levels of bacterial community modifications. Consistent with this, our prior research has established that varying NP concentrations lead to different impacts on ARG dissemination. Therefore, it is hypothesized that variations in NP uptake by radishes, which arise from the use of diverse NP application methods, primarily drive resistome changes within the radish. Our earlier work has demonstrated that NPs can enhance the development of the root exodermis barrier, hindering the absorption of exogenous bacteria via water transport pathways (14). Lignin content is commonly used as a marker for root lignification, indirectly indicating the maturity of the root's extracellular barrier (15). However, we detected no substantial changes in radish root lignin levels following CeO₂ NP addition (Supplementary Figure S3). Although CeO₂ NPs did not promote radish growth (Supplementary Figure S4, available in <https://weekly.chinacdc.cn/>), the literature highlights their significant potential in reducing vegetable wilt diseases. Consequently, the

utilization of CeO₂ NPs in reclaimed water irrigation emerges as a promising strategy for controlling diseases in vegetable crops.

In conclusion, our research indicates that the utilization of NPs can significantly diminish the prevalence of ARGs in reclaimed water-soil-plant systems. The observed reduction in ARG abundance can be linked to the unique characteristics of NPs, which modify the composition of the bacterial community and suppress the horizontal transfer of ARGs. Our findings suggest that soil treatment with NPs (ST group) enhances plant uptake of these particles, resulting in changes to the microbial community and regulation of gene transfer. Although CeO₂ NPs may not visibly affect the phenotypical parameters of radishes, they potentially provoke metabolic changes at the subcellular level, such as increased disease resistance, among other responses. This study underscores the promising role of nanotechnology in diminishing ARGs in agricultural settings. Continued research is warranted to refine nanoparticle applications for this purpose.

Conflicts of interest: No conflicts of interest.

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SUPPLEMENTARY MATERIAL

SUPPLEMENTARY TABLE S1. 16s rRNA copies in different NPs application groups determined by qPCR absolute quantification.

Group	16s rDNA copies (10^{10} copies/g soil)	ARGs abundance/16s rDNA copies
CK-RS	3.56±1.01	0.32±0.06
WT-RS	3.06±0.89	0.28±0.05
SD-RS	4.06±1.15	0.14±0.05*
ST-RS	3.97±1.12	0.13±0.09*
CK-RE	1.04±0.29	0.35±0.04
WT-RE	1.06±0.27	0.20±0.05*
SD-RE	1.17±0.33	0.19±0.09*
ST-RE	1.20±0.34	0.10±0.12*
CK-LE	0.54±0.15	0.28±0.03
WT-LE	0.50±0.14	0.19±0.04*
SD-LE	0.49±0.14	0.20±0.09*
ST-LE	0.52±0.15	0.11±0.13*

SUPPLEMENTARY TABLE S2. Contribution of rhizosphere soil PCA.

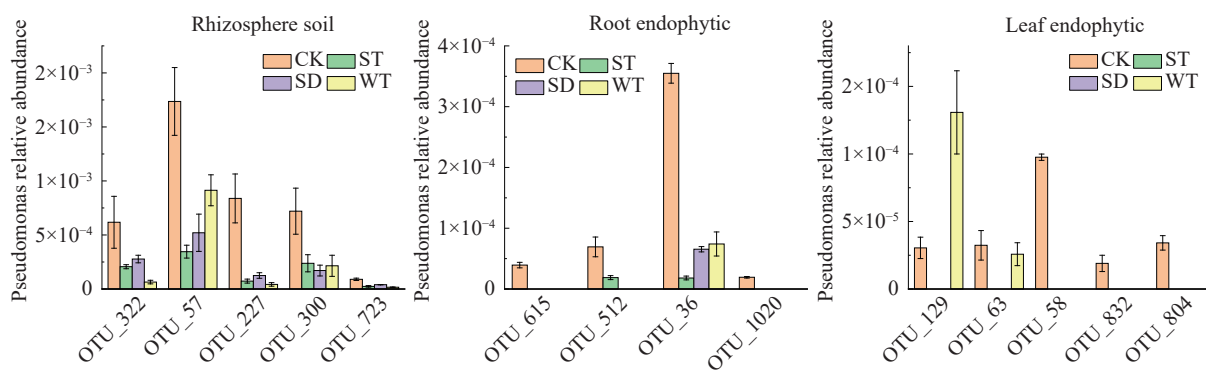
OUT	Mean decrease accuracy	Mean decrease gini	Genus
OUT_4276	2.00	0.09	<i>Comamonadaceae</i>
OUT_322	1.97	0.03	<i>Pseudomonas</i>
OUT_209	1.94	0.04	<i>Streptomyces</i>
OUT_627	1.93	0.08	<i>Enterobacteriaceae</i>
OUT_5230	1.86	0.05	<i>Azohydromonas</i>
OUT_57	1.74	0.05	<i>Pseudomonas</i>
OUT_227	1.74	0.04	<i>Pseudomonas</i>
OUT_442	1.73	0.10	<i>Myxococcales</i>
OUT_300	1.71	0.05	<i>Pseudomonas</i>
OUT_723	1.70	0.06	<i>Pseudomonas</i>

SUPPLEMENTARY TABLE S3. Contribution of root endophytic PCA.

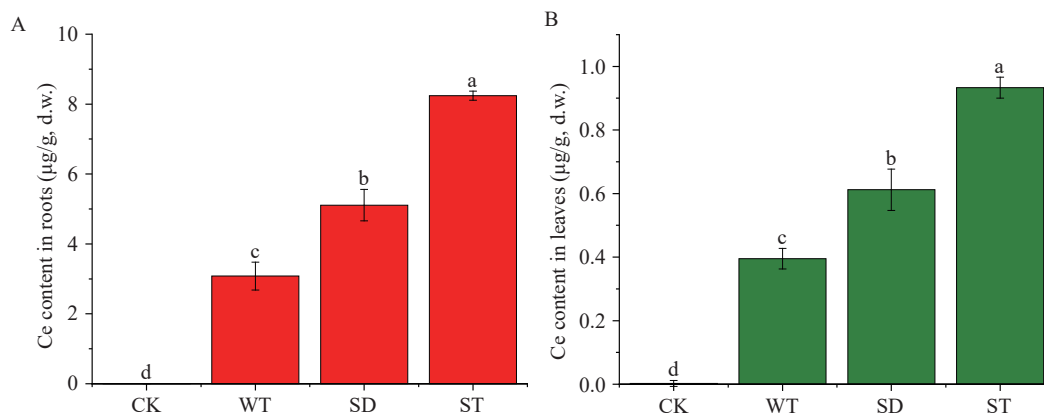
OUT	Mean decrease accuracy	Mean decrease gini	Genus
OUT_68	2.53	0.09	<i>Rhodanobacter</i>
OUT_33	2.00	0.07	<i>Burkholderia</i>
OUT_615	1.97	0.07	<i>Pseudomonas</i>
OUT_74	1.96	0.06	<i>Rhodanobacter</i>
OUT_128	1.96	0.04	<i>Rhodanobacter</i>
OUT_65	1.94	0.06	<i>Rhodanobacter</i>
OUT_512	1.89	0.04	<i>Pseudomonas</i>
OUT_20	1.87	0.08	<i>Burkholderia</i>
OUT_36	1.73	0.04	<i>Pseudomonas</i>
OUT_1020	1.73	0.03	<i>Pseudomonas</i>

SUPPLEMENTARY TABLE S4. Contribution of leaf endophytic PCA.

OUT	Mean decrease accuracy	Mean decrease gini	Genus
OUT_627	2.96	0.11	<i>Enterobacteriaceae</i>
OUT_129	2.80	0.13	<i>Pseudomonas</i>
OUT_63	2.68	0.14	<i>Pseudomonas</i>
OUT_58	2.68	0.15	<i>Pseudomonas</i>
OUT_832	2.37	0.15	<i>Pseudomonas</i>
OUT_466	2.35	0.07	<i>Enterobacteriaceae</i>
OUT_833	2.32	0.04	<i>Enterobacteriaceae</i>
OUT_3190	2.31	0.05	<i>Enterobacteriaceae</i>
OUT_804	2.84	0.07	<i>Pseudomonas</i>
OUT_104	2.21	0.04	<i>Rhodanobacter</i>

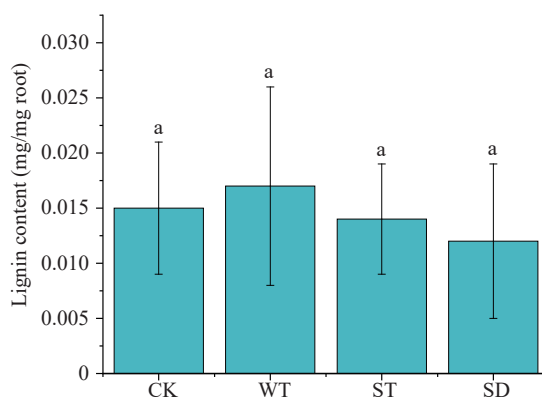
SUPPLEMENTARY FIGURE S1. The relative abundance of *Pseudomonas*.

Note: Different letters represent significant differences between the treatment means ($P < 0.05$), $n = 3$.



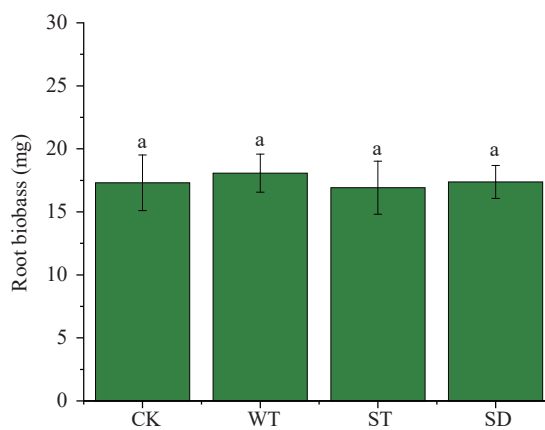
SUPPLEMENTARY FIGURE S2. The Ce content in radish roots and leaves.

Note: Different letters represent significant differences between the treatment means ($P < 0.05$), $n = 4$.



SUPPLEMENTARY FIGURE S3. The lignin content in radish roots.

Note: Different letters represent significant differences between the treatment means ($P < 0.05$), $n = 4$.



SUPPLEMENTARY FIGURE S4. Root biomass of radish.

Note: Different letters represent significant differences between the treatment means ($P < 0.05$), $n = 4$.

Commentary

Mitigating Antibiotic Resistance Emissions in the Pharmaceutical Industry: Global Governance and Available Techniques

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We highlight the paramount importance of pretreatment processes preceding the biological treatment of antibiotic manufacturing wastewater as a strategy to curb the escalation of antibiotic resistance. In an effort to curtail the advancement of antimicrobial resistance (AMR) in antibiotic production in China, a comprehensive array of technical and regulatory initiatives have been implemented. These measures offer valuable insights that could inform and enhance global AMR containment strategies. Looking ahead, there is a pressing need to form an interdisciplinary team tasked with refining antibiotic emission standards and tailoring environmental engineering practices for the pharmaceutical sector.

Characteristics of the AMR Crisis in the Pharmaceutical Industry

Antibiotic contamination is a pervasive environmental issue, largely stemming from the excretions of humans and livestock (urine and feces) as well as the effluent from antibiotic production processes. The pharmaceutical sector is notably significant in this regard, as its contributions to antibiotic pollution greatly exceed those of other sources. It is a critical nexus for the proliferation of antibiotic resistance (Figure 1). The production of active pharmaceutical ingredient (API) for fermentative antibiotics generally involves microbial fermentation, with typical concentrations expressed in milligrams per kilogram (mg/kg). Treating the resulting wastewater requires a biological process, given its high organic matter content. However, residual antibiotics in the wastewater can disrupt the microbial communities and promote an increase in antibiotic resistance genes (ARGs) within biological treatment systems (1–3). Consequently, this leads to the release of both antibiotics and ARGs into the surrounding environment. Adopting a One Health approach, effectively curbing AMR emissions from the pharmaceutical industry could lessen the environmental selection pressure on ARGs. In concert

with reducing their transfer to humans and animals, this strategy offers a considerable advantage.

Trends in the Management of Antimicrobial Emissions from Antibiotic Production Facilities Globally

Intergovernmental organizations are increasingly focused on the control of AMR stemming from pharmaceutical sector activities. In August 2013, the United Nations Environment Programme (UNEP) and the World Health Organization (WHO) teamed up to hold a symposium titled “Green Procurement for Pharmaceutical Manufacturing” in Bonn, Germany. The objective of this forum was to explore the viability and possible breadth of guidelines for environmentally responsible procurement practices within the healthcare field. During this gathering, attendees recognized an urgent need to broaden the conventional approach to evaluating pharmaceuticals and medical products. Traditionally, clinical decision-making relies on evidence-based standards, but the environmental impact of these products demands a wider view. A more comprehensive approach, factoring in a full range of environmental repercussions and life-cycle assessments, was proposed. Moving forward, it is expected that global procurement in the health sector will increasingly scrutinize pharmaceutical companies' responses to the AMR crisis. It will underscore the importance of choosing products that not only meet clinical requirements but are also designed and manufactured with environmental sustainability in mind (4).

In 2020, the WHO, in partnership with the Food and Agriculture Organization of the United Nations (FAO) and the World Organization for Animal Health (WOAH), published a significant report entitled “Technical brief on water, sanitation, hygiene and wastewater management to prevent infections and reduce the spread of antimicrobial resistance” (5). A prominent section within this brief focused on the “Manufacturing of antimicrobials”, highlighting the

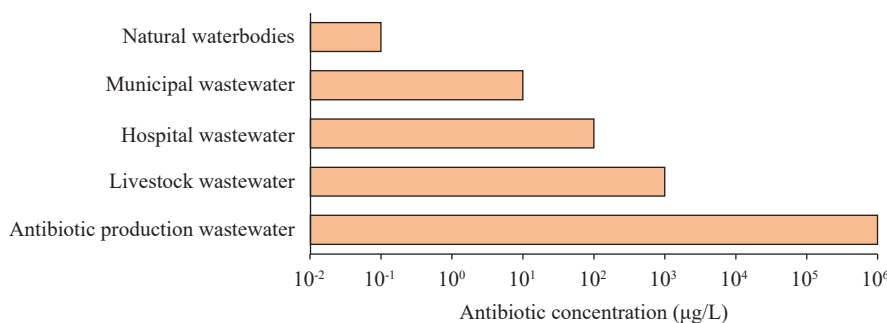


FIGURE 1. Antibiotic pollution in the environment.

Note: This figure was modified from Larsson et al. (2021) (12), depicting a simplification of the typical ranges of antibiotic concentrations in various environmental media.

urgent need for pollution control measures in the production of antimicrobials and API. These measures are imperative for mitigating the global threat of AMR that arises from manufacturing processes. The WHO is currently developing new guidance titled “WHO Guidance on waste and wastewater management in pharmaceutical manufacturing with emphasis on antimicrobial production”, which will set forth pivotal criteria in this domain.

The pharmaceutical sector and its international supply chain have acknowledged the AMR risks associated with antibiotic production processes. The AMR Industry Alliance (6) is a collaboration of biotech, diagnostics, generics, and research-based pharmaceutical organizations, dedicated to driving and tracking industry advancements in combating AMR. In 2018, the AMR Industry Alliance’s set of antibiotic manufacturing discharge targets, based on Predicted No-Effect Concentrations (PNECs), was embraced by the Pharmaceutical Supply Chain Initiative (PSCI) (7). These targets offer a scientific framework for establishing environmentally safe concentrations of antibiotics released from manufacturing sites (8–9). More recently, in 2022, the AMR Industry Alliance, in conjunction with the British Standards Institution, unveiled the “Antibiotic Manufacturing Standard: Minimizing the risk of developing antibiotic resistance and aquatic ecotoxicity in the environment resulting from the manufacturing of human antibiotics” (10). This new standard specifies requirements aimed at diminishing the progression of AMR and mitigating associated ecological risks in surface waters due to antibiotic manufacturing activities.

It is important to recognize that the antibiotic manufacturing discharge benchmarks established by the AMR Industry Alliance are predicated on PNECs of antibiotics. These PNEC values are obtained through modeling techniques that specifically target

pathogenic bacteria found in existing databases (11). While the current methodology provides a foundational approach, the veracity of the model would benefit from additional validation via empirical field studies. Moreover, although the PNECs are tailored to aquatic environments, there exists a significant challenge in extrapolating these effect levels to solid or semi-solid matrices (12–13). This limitation underscores the need for further research to adapt PNEC values for comprehensive environmental assessments.

Techniques for Removing Antibacterial Potency from Antibiotic Production Wastewater

The prevalent approach to managing wastewater from antibiotic manufacturing typically involves biological treatment systems due to its characterization as high-concentration organic wastewater (Figure 2). Biological treatment is a favored option in pharmaceutical operations as it can effectively reduce the levels of conventional pollutants such as chemical oxygen demand (COD), at a relatively low cost. However, antibiotics that persist in the wastewater also enter these biological treatment systems, imposing a selective pressure on the bacterial communities therein. This can lead to the discharge of both antibiotics and ARGs into the environment (14–15). To prevent excessive antibiotics from entering both the biological wastewater treatment system and the surrounding ecosystem, it is crucial to implement pretreatment strategies aimed at eliminating the antibacterial potency of the untreated antibiotic production wastewater. Therefore, the potential for physico-chemical pretreatment processes in dealing with these wastewaters warrants further investigation.

In this study, we have synthesized a detailed review

of existing techniques and their integration for the removal of antibacterial potency from antibiotic production wastewater (Table 1). Physical processes, including coagulation, sedimentation, and adsorption using activated charcoal or alternative materials, as well as chemical oxidation methods, are characterized by low efficiency and selectivity for antibiotic removal. As a result, the expenses tied to these processes are relatively substantial. The hydrolysis of antibiotics can be enhanced by optimizing the pH, additionally heating the wastewater, or adding solid base catalysts (16–19). Employing semi-empirical prediction tools based on frontier molecular orbital (FMO) theory, correlations were established between the enhanced hydrolysis efficiency and the energy gap (ΔE) between the lowest unoccupied and highest occupied molecular orbitals (E_{LUMO} and E_{HOMO}). In this regard, enhanced hydrolysis is suggested for treating wastewater containing antibiotics such as tetracycline, oxytetracycline, penicillin V, erythromycin, spiramycin, streptomycin, chloramphenicol, vancomycin, bacitracin, and colistin (17). Enhanced hydrolysis demonstrates exceptional efficacy due to its selective destruction of antibiotic functional groups responsible for their potency. Notably, this approach has been successfully implemented in full-scale pharmaceutical wastewater treatment facilities in China (Figure 3) (20–24). The similar hydrolysis-based method has also been adapted for the treatment of solid waste from fermentative antibiotic production, which offers notable implications for the disposal of antibiotic fermentation residues (13,25). For wastewater that contains oil, employing yeast as a biological treatment strategy is favorable, as it prevents the emergence of bacterial antibiotic resistance (26–27). Moreover, advanced treatment strategies,

such as synchronized oxidation-adsorption (SOA) (28), ozonation, and advanced oxidation processes (AOPs) using UV light with photosensitizers or Fenton's reagent, should be considered as final safeguards before discharge. Membrane technology, such as reverse osmosis, particularly for water recycling, exhibits strong efficacy in mitigating antibacterial potency and ARGs in treated effluent, although it is cost-prohibitive (3). Presently, the optimized strategy for the treatment of antibiotic production wastewater involves a combination of biological treatment, pretreatment, and advanced treatment, with the goal of preventing the release of antibiotics and ARGs. This integrated method is strategically designed to comply with wastewater (Figure 2). Additionally, certain higher-cost techniques, such as mechanical vapor recompression (MVR), have been applied within the industry with satisfactory outcomes.

Management of AMR in Antibiotic Production in China

In China, numerous successful instances of AMR control within antibiotic production sectors provide instructive benchmarks for implementing global AMR containment strategies (29). From the standpoint of engineering applications, the efficacy of enhanced hydrolysis pretreatment has been convincingly established through a range of full-scale implementations by pharmaceutical corporations across the country. Importantly, this method has been incorporated into and recommended by the WHO's technical brief, and has been endorsed by the standards of the Ministry of Ecology and Environment of China (Table 2). The China Pharmaceutical Enterprises Association (CPEA) has documented additional full-

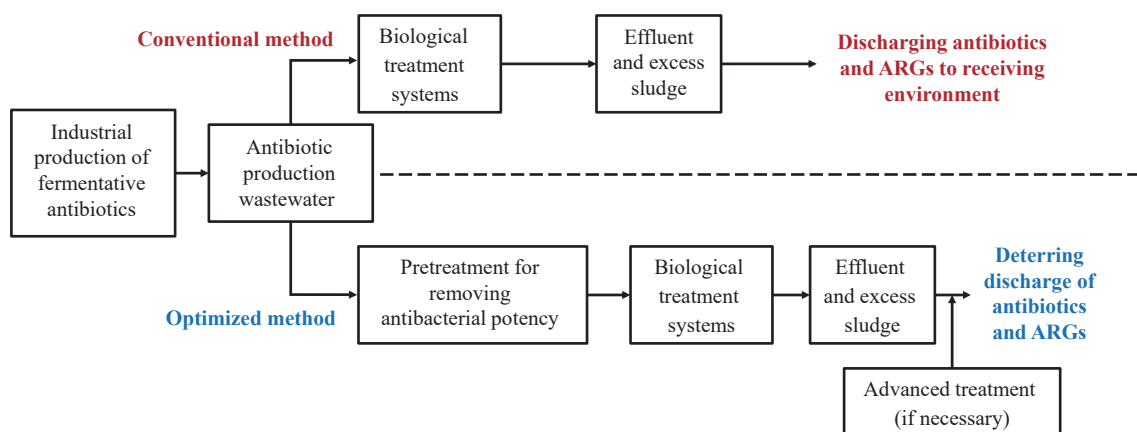


FIGURE 2. Control strategies of antibiotics and antibiotic resistance genes (ARGs) from wastewater of antibiotic manufacturing.

TABLE 1. Available techniques and technique integration for removing antibacterial potency from antibiotic production wastewater.

Available technologies	Description	Advantage and disadvantage
Pretreatment techniques (16–19,26,27,32)		
Enhanced hydrolysis	Pretreatment method used for production wastewater of fermentative antibiotics (for example, tetracyclines and macrolides). It can selectively remove antibiotics from wastewater by eliminating their active antibacterial groups. The removal of antibiotics could reach 99%, and the reduction of AMR discharge is about 80%.	Selective hydrolysis of functional groups of antibiotics with low cost and decrease of inhibition on biological treatment and dissemination of ARGs in the environment.
Biological technique using yeast	Pretreatment method used for oil-containing antibiotic production wastewater. Oil residue removal rate was 61.4%–74.2% in full-scale operations, and oil is the substrate for the fermentation production of antibiotics. No ARG from bacteria produced since yeast play the role in the biological treatment.	Avoiding the emergence of ARGs in bacteria during biological treatment.
Coagulation, sedimentation and adsorption-based techniques	Traditional pretreatment methods used for wastewater with high content of suspended solid. Some kinds of antibiotics can be partially removed, while the removal is very limited. For example, ozone oxidation and Fenton oxidation. Doses of 1.2 mg O ₃ per mg of initial OTC permitted 92% OTC removal from OTC production wastewater (OTC, 702 mg/L).	Low removal of antibacterial potency.
Oxidation-based techniques		High cost and low selectivity for antibacterial potency removal.
Advanced treatment (3,28)		
Oxidation-based techniques	For example, SOA, ozone oxidation, Fenton oxidation, electrochemical oxidation.	End protection before discharge. High cost.
Membrane separation	For example, ultrafiltration, reverse osmosis.	Good removal of antibacterial potency and ARGs in effluent. High cost.
Technique integration (5,20–24,33)		
Pretreatment +biological treatment	The effluent needs to be discharged to a municipal wastewater treatment plant.	With the pretreatment such as enhanced hydrolysis, the antibacterial potency could be removed.
Pretreatment + biological treatment + advanced treatment	It can meet the standards for direct emission.	With the pretreatment such as enhanced hydrolysis, the antibacterial potency could be removed.
MVR + harmless of solid waste and waste gas	Maximizing the recovery of water from wastewater using vapor.	No wastewater discharge. High cost. Disposal of solid waste is difficult.

Abbreviation: ARGs=antibiotic resistance genes; AMR=antimicrobial resistance; OTC=oxytetracycline; SOA=synchronized oxidation-adsorption; MVR=mechanical vapor recompression.

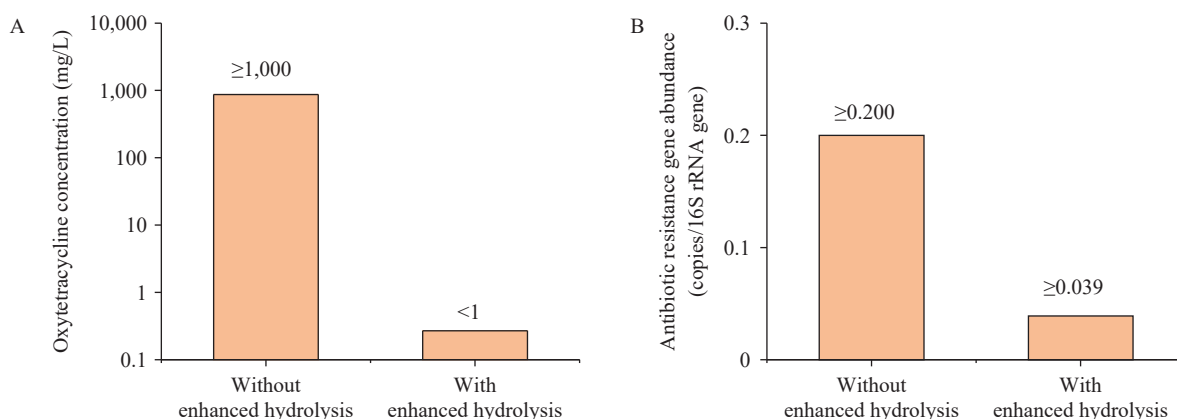


FIGURE 3. Reduction of discharge of antibiotics (A) and antibiotic resistance genes (B) from biological oxytetracycline production wastewater treatment system with enhanced hydrolysis pretreatment.

Note: This figure was modified from Yi et al. (2017) (24).

scale applications in its “Compilation of Excellent Cases in the Pharmaceutical Industry for Environment, Health, and Safety (EHS),” covering the period from

2019 to 2021.

In recent years, from an environmental management perspective, Chinese government agencies and relevant

industry associations have invested considerable effort into the establishment of standards and the advancement of technology (Table 2). The China Pharmaceutical Industry Association (CPIA) published the “Blue Book of Pharmaceutical Industry” (ISBN 9787520170635) in 2020, which includes a chapter by the Research Center for Eco-Environmental Sciences, Chinese Academy of Sciences, on “Synergistic Control of Antibiotics, Resistance Genes, and Conventional Pollutants in Pharmaceutical Wastewater.” The “Pharmaceutical Industry EHS Guide (2020)” — issued by the CPEA — recommended API management strategies and related Predicted No-Effect Concentrations (PNECs).

Additionally, the CPIA released three standards (TPIAC00001-2021, TPIAC00002-2021, and TPIAC00003-2021) aimed at determining levels of erythromycin, cephalosporin, and penicillin in antibiotic fermentation residue, raw fertilizer materials, crops, and associated environments. This trio of standards marks China’s inaugural effort to standardize the determination of antibiotics in antibiotic fermentation residue. More significantly, the Ministry

of Ecology and Environment of China introduced the “Guideline on Available Techniques of Pollution Prevention and Control for the Pharmaceutical Industry (Fermentation, Chemical Synthesis, Extraction) and Preparation Categories” (HJ 1305-2023). This authoritative guideline compiles a range of available techniques, such as enhanced hydrolysis pretreatment for API removal, and sophisticated end-protection treatments. It stands as a critical benchmark for regulating pollution, including antibiotics, within the pharmaceutical industry.

In 2023, at the PSCI China Supplier Conference in Chengdu, a poll was conducted to assess the implementation of AMR control measures by pharmaceutical companies. Out of 78 Chinese supplier representatives who participated, 42 (representing 54%) voted in favor. This outcome suggests that while the notion of mitigating AMR in antibiotic manufacturing is widely recognized, there remains a significant need for concrete action. It is apparent that enhancing awareness and promoting the dissemination of current progress and available technologies in the industry are crucial steps toward bolstering the fight

TABLE 2. Some typical successful cases of AMR control from antibiotic production wastewater in antibiotic manufacturing in China.

Areas	Description	Source or impacts
Engineering application	Pretreatment (enhanced hydrolysis), biological treatment and advanced treatment (low-cost SOA technology) were successfully applied in full-scale pharmaceutical wastewater treatment plants in Hebei Province and Jiangsu Province in China.	Pretreatment of antibiotic production wastewater to remove antibacterial potency is the best way to control the development of ARGs, as well as the above successful cases, has been subsumed in “Technical brief on water, sanitation, hygiene and wastewater management to prevent infections and reduce the spread of antimicrobial resistance” (2020) [ISBN (WHO) 978-92-4-000641-6] and Ministry of Ecology and Environment of China standard (HJ 1305-2023).
	Technique integration including the pretreatment (e.g., electrochemical oxidation, iron-carbon microelectrode, MVR), biological treatment, advanced treatment (e.g., ozone oxidation) or zero emission (e.g., MVR+ special membrane) were applied in some plants in China.	Some cases were included in the Compilation of Excellent Cases Pharmaceutical Industry Environment, Health and Safety (EHS) (2019–2021) by CPEA (http://www.cpema.org/).
Environmental management	A chapter of “Synergistic control of antibiotics, resistance genes and conventional pollutants in pharmaceutical wastewater”. API management and PNECs in pharmaceutical wastewater. Three standards for determining erythromycin, cephalosporin and penicillin in antibiotic fermentation residue, raw fertilizer material, crop, and related environments. A series of available techniques including the enhanced hydrolysis pretreatment for API removal were summarized in this standard, which is an important criterion on pollution (including antibiotics) control in pharmaceutical industry.	“Bluebook of pharmaceutical Industry” (ISBN 9787520170635) was published by CPIA in 2020. Pharmaceutical Industry Environment, Health and Safety (EHS) Guide (2020) by CPEA (http://www.cpema.org/). CPIA (TPIAC00001-2021, TPIAC00002-2021, and TPIAC00003-2021). Standard by Ministry of Ecology and Environment of China: “Guideline on available techniques of pollution prevention and control for pharmaceutical industry (fermentation, chemical synthesis, extraction) and preparation categories” (HJ 1305-2023).

Abbreviation: CPEA=China Pharmaceutical Enterprises Association; CPIA=China Pharmaceutical Industry Association; SOA=synchronized oxidation-adsorption, MVR=mechanical vapor recompression, API=active pharmaceutical ingredient, PNECs=Predicted No-Effect Concentrations, ARGs=antibiotic resistance genes.

against AMR.

Apart from China, India is also a leading exporter of antibiotics worldwide. The country is progressively implementing responsible manufacturing practices and placing greater demands on corporate compliance. All bulk-drug API manufacturing facilities are designated as “grossly polluting industries” and must comply with Zero Liquid Discharge standards (30). In 2019, the Indian government introduced legislation proposing discharge limits for antibiotics in wastewater emanating from production sites into surrounding water bodies. However, the definitive legislation published in 2021 ultimately did not incorporate these discharge limits (31).

Growing concerns about AMR in the pharmaceutical industry necessitate immediate, strategic actions. The development of an AMR management system tailored to Chinese conditions, along with increased research and advancement of risk control technologies, are imperative for fostering sustainable industry practices. Looking ahead, there are critical areas that require heightened focus and commitment.

First and foremost, concerted efforts should be directed towards the long-term risk identification, evaluation, and mitigation of AMR stemming from the disposal of antibiotic production wastewater and fermentation residues. Additionally, comprehensive guidelines and a strategic roadmap, encompassing systematic AMR management standards, must be crafted collaboratively by industry stakeholders and cross-sectoral entities. Furthermore, the industrial adoption of cost-effective and efficient methods represents a key solution for curbing the spread of AMR attributable to antibiotic manufacturing. Prioritizing the reduction of antibiotics and ARGs emissions from the pharmaceutical sector into the environment remains of paramount importance.

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Notifiable Infectious Diseases Reports

Reported Cases and Deaths of National Notifiable Infectious Diseases — China, September 2023*

Diseases	Cases	Deaths
Plague	0	0
Cholera	9	0
SARS-CoV	0	0
Acquired immune deficiency syndrome [†]	5,121	1,693
Hepatitis	147,758	177
Hepatitis A	1,038	0
Hepatitis B	123,495	20
Hepatitis C	20,022	157
Hepatitis D	15	0
Hepatitis E	2,550	0
Other hepatitis	638	0
Poliomyelitis	0	0
Human infection with H5N1 virus	0	0
Measles	78	0
Epidemic hemorrhagic fever	198	1
Rabies	8	7
Japanese encephalitis	62	1
Dengue	6,494	1
Anthrax	61	1
Dysentery	3,658	0
Tuberculosis	61,859	324
Typhoid fever and paratyphoid fever	596	0
Meningococcal meningitis	5	0
Pertussis	4,517	0
Diphtheria	0	0
Neonatal tetanus	1	0
Scarlet fever	1,546	0
Brucellosis	5,987	2
Gonorrhea	10,147	0
Syphilis	55,767	5
Leptospirosis	71	0
Schistosomiasis	3	0
Malaria	193	1
Human infection with H7N9 virus	0	0
Monkey pox [§]	80	0
Influenza	168,963	0
Mumps	10,867	0

Continued

Diseases	Cases	Deaths
Rubella	72	0
Acute hemorrhagic conjunctivitis	125,264	0
Leprosy	23	0
Typhus	227	0
Kala azar	26	0
Echinococcosis	367	0
Filariasis	0	0
Infectious diarrhea [¶]	102,559	0
Hand, foot and mouth disease	166,980	0
Total	879,567	2,213

* According to the National Bureau of Disease Control and Prevention, not included coronavirus disease 2019 (COVID-19).

† The number of deaths of acquired immune deficiency syndrome (AIDS) is the number of all-cause deaths reported in the month by cumulative reported AIDS patients.

§ Since September 20, 2023, Monkey pox was included in the management of Class B infectious diseases.

¶ Infectious diarrhea excludes cholera, dysentery, typhoid fever and paratyphoid fever.

The number of cases and cause-specific deaths refer to data recorded in National Notifiable Disease Reporting System in China, which includes both clinically-diagnosed cases and laboratory-confirmed cases. Only reported cases of the 31 provincial-level administrative divisions in Chinese mainland are included in the table, whereas data of Hong Kong Special Administrative Region, Macau Special Administrative Region, and Taiwan, China are not included. Monthly statistics are calculated without annual verification, which were usually conducted in February of the next year for de-duplication and verification of reported cases in annual statistics. Therefore, 12-month cases could not be added together directly to calculate the cumulative cases because the individual information might be verified via National Notifiable Disease Reporting System according to information verification or field investigations by local CDCs.

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