Infection and Drug Resistance

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ORIGINAL RESEARCH

Detection and Analysis of Drug and Disinfectant Resistance Genes in the Sewage of a Center for Disease Control and Prevention

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Purpose: Sewage is a significant reservoir for drug and disinfectant resistance genes and a medium for dissemination. This study aimed to evaluate the presence of drug and disinfectant resistance genes in the sewage of a Center for Disease Control and Prevention (CDC) and to assess the risks of their dissemination.

Methods: Sewage from a CDC in Hangzhou was collected, filtered, and enriched, and its microorganisms were cultured. The isolated bacteria were identified, and the minimum inhibitory concentration (MIC) was determined. The drug and disinfectant resistance genes in the sewage and bacteria were detected through polymerase chain reaction amplification.

Results: Three kinds of bacteria were isolated from the sewage sample. The MIC for *Sphingomonas* and *Staphylococcus xylosus* against chlorine-containing disinfectants was 250 mg/L, whereas the MIC for *Bacillus firmus* was 500 mg/L. The β-lactam resistance gene *TEM* and the disinfectant resistance gene *qacA* were positive in the bacteria, whereas the β-lactam resistance genes *TEM, SHV*, and *VIM-1*, the tetracycline resistance gene *tetM*, the aminoglycoside resistance genes *aac(6')/aph(2')* and *aph3'-III*, and the disinfectant resistance genes *qacA, qacE*, and *qacEΔ1* were positive in the sewage.

Conclusion: Drug and disinfectant resistance genes were found in the sewage of a CDC and were associated with bacteria. Thus, optimizing the monitoring and treatment of sewage is crucial.

Keywords: center for disease control and prevention, sewage, drug resistance gene, disinfectant resistance gene

Introduction

Chlorine-based disinfectants, quaternary ammonium compounds, aldehydes, and other disinfection methods are essential for controlling the spread of pathogenic microorganisms and infectious diseases. However, these measures increase the tolerance of some microorganisms to disinfectants, which results in strains carrying disinfectant resistance genes[1] propagating through water circulation. Sewage is a significant reservoir for drug and disinfectant resistance genes and a medium for dissemination. In recent years, due to the contamination of water bodies with drugs and disinfectants, associated resistance genes have been detected in various water environments, including municipal water,[2] urban sewage,[3] hospital wastewater,[4] and constructed wetlands,[5] posing a potential threat to public health.

In the current study, we analyzed the sewage from a Center for Disease Control and Prevention (CDC) in Hangzhou to detect and assess the contamination levels of drug and disinfectant resistance genes and to provide a basis for improving wastewater treatment strategies. This study aimed to evaluate the presence of drug and disinfectant resistance genes in the sewage of a CDC and to assess the risks of their dissemination.

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Materials and Methods

Sample Source

A 10-liter sewage sample was collected from a CDC in Hangzhou, Zhejiang Province before it was discharged into the municipal sewage system.

Reagents and Instruments

For wastewater enrichment, S-PAK sterile membranes (Pall Corporation, USA) and a stainless-steel multi-unit filtration system (Huankai Microbial Pty Ltd., China) were used. Bacterial identification was performed using the matrix-assisted laser desorption ionization-time of flight mass spectrometry (MALDI-TOF MS) microbial identification system (bioMérieux, France). The molecular experiments involved an automatic nucleic acid extractor (BioPerfectus Technologies Pty Ltd., China), a polymerase chain reaction (PCR) thermal cycler (QIAGEN, Germany), an electrophoresis system (BIO-RAD, USA), and a chemiluminescent gel imaging system (ProteinSimple, USA).

DNA extraction kits were sourced from BioPerfectus Technologies Pte Ltd., China, PCR reagents were purchased from Takara Bio Inc., Japan, and primers were synthesized by Sangon Biotech (Shanghai) Co., Ltd., China.

Methods

Bacterial Enrichment from Sewage

The 10-liter sewage sample was filtered through 0.45 μm pore size S-PAK sterile membranes using the stainless-steel multi-unit filtration system, with the bacteria retained on the membranes for enrichment. Filters were replaced as soon as they became saturated. A nucleic acid elution buffer was prepared by mixing 100 mL of distilled water with 3 g of beef extract. Once the membranes were immersed in the elution buffer and mixed via shaking, 0.5 mL of the elution solution was removed in a clean room and inoculated onto blood agar plates. The sample was evenly spread using an L-shaped rod and incubated at 37 °C for 24 h to isolate bacteria from the sewage. The remaining elution solution was divided into three 50 mL centrifuge tubes and centrifuged at 10,000 × *g* for 10 min, with both acceleration and deceleration set to 5. Subsequently, the supernatant was discarded, and 10 mL of phosphate buffered saline (PBS) was added to dissolve the pellet. The solution was centrifuged under the same conditions, the supernatant was discarded, and 2 mL of PBS was added to each tube to dissolve the contents, which were then combined into a single tube and stored at –80 °C for future analysis.

Isolation and Identification of Bacteria and Determination of Minimum Inhibitory Concentration (MIC)

After incubation, a colony growth was observed on the blood agar plates. Characteristic, morphologically distinct single colonies were removed using an inoculation loop and inoculated onto fresh blood agar plates before incubation at 37 °C for 24 h. Subsequently, well-grown colonies were selected and identified using the MALDI-TOF MS microbial identification system. Next, MIC values for the identified bacteria were determined following the nutrient broth dilution method proposed in Section 2.1.8.4 of the "Technical Standard for Disinfection (2002 edition)".[6] The disinfectant diluted with sterile hard water to concentrations of 100, 250, 500, 1000, and 2000 mg/L. Subsequently, 2.5 mL of the disinfectant solution was added to tubes containing 2.5 mL of double-strength nutrient broth. After thorough mixing, 0.1 mL of bacterial suspension (approximately 10^8 CFU/mL) was inoculated into each disinfectant-containing broth tube. The tubes were incubated at 37°C for 48 hours. The standard strain *Escherichia coli* (8099) was used as a reference. A positive control group (nutrient broth without disinfectant) and a negative control group (nutrient broth without bacterial inoculation) were included. The minimal inhibitory concentration (MIC) was determined as the highest dilution of disinfectant that showed no bacterial growth in the test groups.

DNA Extraction from Sewage and Bacteria

Bacterial suspensions were prepared in physiological saline from the identified colonies. For each strain, 200 μL of the bacterial suspension and 200 μL of the enriched wastewater were added to a DNA extraction kit. The kit was placed in an automatic nucleic acid extractor to obtain the DNA, which was used as the template for PCR amplification.

PCR Amplification and Detection

PCR amplification was conducted using a PCR thermal cycler with One Step PrimeScript™ RT-PCR Kit (Perfect Real Time) (code: RR064A) containing 12.5 μL of 2× One Step RT-PCR Buffer III, 0.5 μL of TaKaRa Ex Taq HS, 0.5 μL of PrimeScript RT Enzyme Mix II, 0.5 μL each of both forward and reverse primers, 5.5 μL of sample solution, and 5 μL of ddH_2O. The mixture was placed in 8-well strips, centrifuged, and loaded into the PCR cycler. The PCR amplification conditions were 95 °C for 2 min for initial denaturation, followed by 35 cycles of 95 °C for 30s for denaturation, 56 °C for 30s for annealing, and 72 °C for 1 min for extension, concluded with a final extension at 72 °C for 5 min. The amplified products were subsequently employed for gel electrophoresis.

This study targeted the amplification and detection of β-lactam resistance genes *CTX-M, TEM, SHV*, and *VIM-1*; tetracycline resistance gene *tetM*; aminoglycoside resistance genes *aph3'-III* and *aac(6')/aph(2')*; macrolide resistance genes *ermA* and *ermC*; and seven disinfectant resistance genes *qacA, qacC/D, qacE, qacEΔ1, qacF, qacH*, and *smr*. The primer sequences for the target genes are listed in Table 1.

Table 1 Primer Sequence and Amplification Product Size of Drug Resistance and Disinfectant Resistance Genes

No.	Gene	Primer	Sequence (5′→3′)	Product Length (bp)
1	*ermA*	*ermA-F*	AAGCGGTAAACCCCTCTGA	190
		ermA-R	TTCGCAAATCCCTTCTCAAC	
2	*aph3'-III*	*aph3'-III-F*	GCCGATGTGGATTGCGAAAA	292
		aph3'-III-R	GCTTGATCCCCAGTAAGTCA	
3	*aac(6')-aph(2')*	*aac(6')-aph(2')-F*	CCAAGAGCAATAAGGGCATA	220
		aac(6')-aph(2')-R	CACTATCATAACCACTACCG	
4	*tetM*	*tetM-F*	AGTGGAGCGATTACAGAA	158
		tetM-R	CATATGTCCTGGCGTGTCTA	
5	*qacC/D*	*qacC/D-F*	ATAAGTACTGAAGTTATTGGAAGT	286
		qacC/D-R	TTCCGAAAATGTTTAACGAAACTA	
6	*CTX-M*	*CTX-M-F*	TTTGCGATGTGCAGTACCAGTAA	876
		CTX-M-R	CGATATCGTTGGTGGTGCCATA	
7	*TEM*	*TEM-F*	CATTTCCGTGTCGCCCTTATTC	800
		TEM-R	CGTTCATCCATAGTTGCCTGAC	
8	*SHV*	*SHV-F*	AGCCGCTTGAGCAAATTAAAC	713
		SHV-R	ATCCCGCAGATAAATCACCAC	
9	*VIM-1*	*VIM-1-F*	AGTGGTGAGTATCCGACAG	260
		VIM-1-R	ATGAAAGTGCGTGGAGAC	
10	*qacEΔ1*	*qacEΔ1-F*	AATCCATCCCTGTCGGTGTT	175
		qacEΔ1-R	CGCAGCGACTTCCACGATGGGGAT	
11	*qacF*	*qacF-F*	GTCGTCGCAACTTCCGCACTG	229
		qacF-R	TGCCAACGAACGCCCACA	
12	*qacE*	*qacE-F*	ATAAGCAACACCGACAGGG	145
		qacE-R	GGCGAAGTAATCGCAACAT	
13	*ermC*	*ermC-F*	AATCGTCAATTCCTGCATGT	229
		ermC-R	TAATCGTGGAATACGGGTTTG	
14	*qacA*	*qacA-F*	GCTGCATTTATGACAATGTTTG	629
		qacA-R	AATCCCACCTACTAAAGCAG	
15	*qacH*	*qacH-F*	CAATAGTCAGTGAAGTAATAGGCAGTG	295
		qacH-R	TGTGATGATCCGAATGTGTTT	
16	*smr*	smr-F	GCCATAAGTACTGAAGTTATTGGA	195
		smr-R	GACTACGGTTGTTAAGACTAAACCT	

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Table 2 Bacterial Minimal Inhibitory Concentration in the Sewage

Inhibitory Concentration (mg/L)	*Sphingomonas*	*Staphylococcus xylosus*	*Bacillus firmus*	Reference Strain	Positive Control	Negative Control
100	+	+	+	+	+	–
250	–	–	+	–	+	–
500	–	–	–	–	+	–
1000	–	–	–	–	+	–
2000	–	–	–	–	+	–

Notes: "+"indicates turbid bacterial suspension, suggesting positive results, whereas "-"indicates clear bacterial suspension, suggesting negative results.

Results

Identification of Bacterial Strains in Sewage

Bacteria isolated and cultured from the sewage of the CDC were identified using the MALDI-TOF MS microbial identification system. The identified strains were *Sphingomonas, Staphylococcus xylosus*, and *Bacillus firmus*.

MIC Results

The MIC for *Sphingomonas* and *Staphylococcus xylosus* against chlorine-containing disinfectants was 250 mg/L, consistent with the standard strain *Escherichia coli* (8099). In contrast, the MIC for *Bacillus firmus* was 500 mg/L, higher than that of the standard strain. (Table 2).

Bacterial Gene Detection Results

PCR amplification results for drug and disinfectant resistance genes in the bacteria isolated from the sewage of the CDC indicated that *Sphingomonas* and *Bacillus firmus* tested positive for the β-lactamase resistance gene *TEM*, whereas *Staphylococcus xylosus* tested positive for the disinfectant resistance gene *qacA* (Figures 1–3).

Sewage Gene Detection Results

PCR amplification results for drug and disinfectant resistance genes in the sewage of the CDC revealed the presence of the aminoglycoside resistance genes *aph3'-III* and *aac(6')/aph(2')*, the tetracycline resistance gene *tetM*, the β-lactamase resistance genes *TEM, SHV*, and *VIM-1*, and the disinfectant resistance genes *qacEΔ1, qacE*, and *qacA*. Conversely, the β-lactam resistance gene *CTX-M*, the macrolide resistance genes *ermA* and *ermC*, and the disinfectant resistance genes *qacC/D, qacF, qacH*, and *smr* were not detected in the sewage (Figure 4).

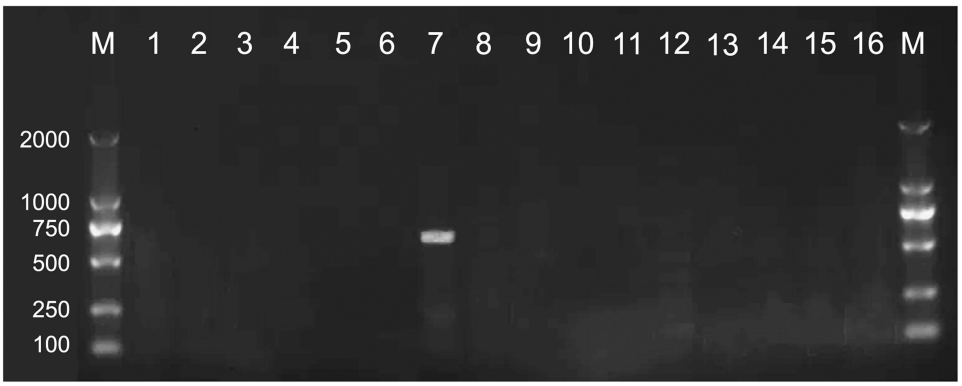

Figure 1 Electrophoresis of PCR amplification products of drug resistance and disinfectant resistance genes in *Sphingosine*.

Figure 2 Electrophoresis of PCR amplification products of drug resistance and disinfectant resistance genes in *Bacillus fortis*.

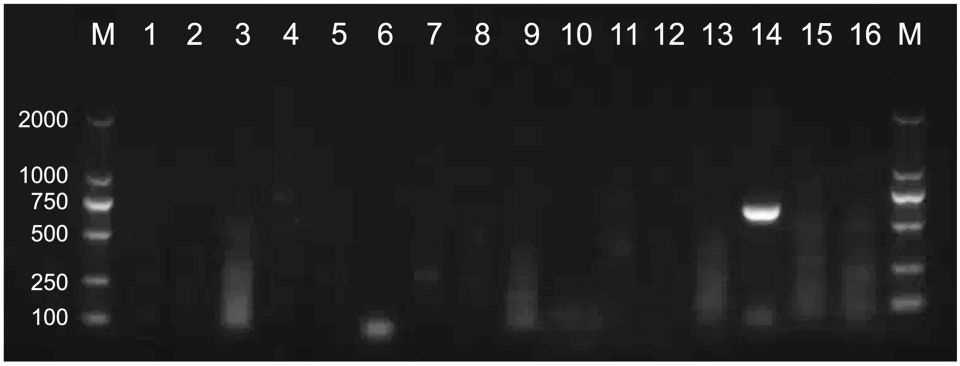

Figure 3 Electrophoresis of PCR amplification products of drug resistance and disinfectant resistance genes in *Staphylococcus xylosus*.
Notes: M represents DNA Marker; 1 denotes *ermA*; 2 denotes *aph3′-III*; 3 denotes *aac(6′)/aph(2′)*; 4 denotes *tetM*; 5 denotes *qacC/D*; 6 denotes *CTX-M*; 7 denotes *TEM*; 8 denotes *SHV*; 9 denotes *VIM-1*; 10 denotes *qacEΔ1*; 11 denotes *qacF*; 12 denotes *qacE*; 13 denotes *ermC*; 14 denotes *qacA*; 15 denotes *qacH*; 16 denotes *smr*.

Figure 4 Electrophoresis results of PCR amplification products for drug and disinfectant resistance genes in the sewage of the CDC.
Notes: M represents DNA Marker; 1 denotes *ermA*; 2 denotes *aph3′-III*; 3 denotes *aac(6′)/aph(2′)*; 4 denotes *tetM*; 5 denotes *qacC/D*; 6 denotes *CTX-M*; 7 denotes *TEM*; 8 denotes *SHV*; 9 denotes *VIM-1*; 10 denotes *qacEΔ1*; 11 denotes *qacF*; 12 denotes *qacE*; 13 denotes *ermC*; 14 denotes *qacA*; 15 denotes *qacH*; and 16 denotes *smr*.

Discussion

Drug-resistant infections are one of the most significant global public health challenges. Carriers of drug-resistance genes, such as plasmids, transposons, and integrons, facilitate the spread of these genes among bacteria through mechanisms such as transformation, transduction, and conjugation.[7] Mobile genetic elements carrying resistance genes can persist in the environment for prolonged periods even after the death of resistant bacteria.[8] This explains why more resistance genes were detected in the sewage samples than in the isolated bacteria in this study.

Previous studies have confirmed the presence of resistance genes in hospital wastewater, such as the hospital-isolated pathogenic bacteria from sewage, tested for seven antibiotic resistance genes namely *ampC, tetO, tetW, sull, sul2, qnrD*, and *qnrS* in Urumqi. The results highlighted a detection rate ranging between 66.7% and 100.0% in the corresponding resistant pathogens.[9] Similarly, in a tertiary hospital in Changchun, Jilin Province, sewage tests revealed carbapenem-resistant *Klebsiella pneumoniae* as the dominant species among carbapenem-resistant isolates. Additionally, the antibiotic-resistance gene *blaKPC* was detected.[10] Alternatively, in a study conducted in southeastern Brazil, β-lactamase-encoding genes *blaTEM* and *blaKPC* as well as tetracycline resistance genes *tetD, tetM*, and *tetA* were detected in hospital wastewater.[11] To date, no studies have detected drug-resistance genes in the sewage of CDCs. In this study, the β-lactam resistance genes *TEM, SHV*, and *VIM-1*; tetracycline resistance gene *tetM*; and aminoglycoside resistance genes *aac(6')/aph(2')* and *aph3'-III* tested positive in the sewage of a CDC. Although CDCs do not directly treat patients or administer drugs, they handle microbiological testing from various sources, such as pathogen detection during infectious disease outbreaks and microbial tracing in hospital infection investigations. Some CDCs even maintain biobanks for their regions. Therefore, the presence of resistance genes in the sewage of CDCs warrants attention as a crucial factor in mitigating the spread of drug-resistance genes.

Disinfection has gained substantial public attention following the outbreak of coronavirus disease (COVID-19), and it has become more prevalent than ever as a tool for controlling infectious diseases and hospital infections. However, prolonged or frequent disinfectant exposure can lead to resistance through gene mutations, phenotypic adaptation, and horizontal gene transfer under selective pressure.[12,13] This presents a serious challenge to both infectious disease control and hospital infection management. During the COVID-19 pandemic, Hu et al[14] analyzed water and soil samples from approximately 40 designated hospitals in Wuhan; using ultra-high-performance liquid chromatography-tandem mass spectrometry and metagenomics, they found that disinfection compounds imposed considerable selective pressure on microbial resistance in urban environments. This pressure, coupled with close interactions between efflux pump genes and mobile genetic elements, exacerbated the development of drug and disinfectant resistance genes in the environment. Additionally, in samples from the First Hospital of Lanzhou University, a high prevalence of disinfectant resistance genes, particularly *qacE, qacEΔ1*, and *qacEΔ1-SUL1*, was found in carbapenem-resistant Enterobacteriaceae.[15] Similarly, in this study, disinfectant resistance genes *qacA, qacE*, and *qacEΔ1* were detected in the sewage of the CDC. Therefore, it is essential to standardize disinfection practices and regulate disinfectant use carefully. While ensuring environmental protection, disinfectants should be applied at concentrations exceeding the minimum bactericidal concentration to achieve bacterial eradication rather than mere inhibition. This approach will help to mitigate the emergence of disinfectant resistance genes.

In this study, the sewage from the CDC was disinfected using chlorine-containing disinfectants. The MIC of these disinfectants was 250 mg/L for *Sphingomonas* and *Staphylococcus xylosus* and as high as 500 mg/L for *Bacillus firmus*. This indicates that some bacteria have already developed resistance to these disinfectants. Previous research has shown that clinical isolates of carbapenem-resistant *Enterobacteriaceae* often carry high proportions of disinfectant resistance genes. Some strains also resist iodine and 84 disinfectants, with MICs for chlorine disinfectants reaching 500 mg/L.[16] In our study, the simultaneous presence of drug and disinfectant resistance genes was not observed in *Sphingomonas, Staphylococcus xylosus*, or *Bacillus firmus*, suggesting the absence of cross-resistance. However, other untested resistance and disinfectant resistance genes may still be present. Consequently, regular monitoring of pathogenic microorganisms and their resistance genes in sewage is crucial. Wastewater treatment should not only comply with the requirements in the national standard GB18466-2005 "Discharge Standards for Water Pollutants in Medical Institutions"[17] but also be optimized to minimize the emergence of drug and disinfectant resistance genes. Gene editing technologies can accurately target and inactivate antibiotic resistance genes through base editing, thus significantly suppressing bacterial resistance.[18] Azuma et al[19] have shown that ozone treatment effectively inactivated drug resistance bacteria and removed antibiotic resistance genes from wastewater. Additionally, aggregation-induced emission photosensitizer[20] and UV-activated persulfate[21] have also demonstrated effective removal of drug-resistance bacteria and genes, although the removal effectiveness of these methods requires further investigation.

Conclusion

The sewage from CDCs contains bacteria carrying drug and disinfectant resistance genes, possibly originating from either vertical transmission during daily operations or the wastewater treatment process. This highlights the need for stricter disinfection and wastewater treatment practices, including a comprehensive evaluation of disinfectant types, dosages, and application methods. Furthermore, the mechanisms underlying microbial resistance to disinfectants remain to be fully elucidated. More in-depth research into these resistance mechanisms will support the development of new disinfectants with novel mechanisms of action. Further research can be conducted to expand the sample size, analyze the sewage and bacteria carrying resistance genes as well as disinfectant resistance genes in different regions of CDC, and further explore the impact of various disinfectant types and dosages on the resistance genes and disinfectant resistance genes in the samples based on the research findings.

Data Sharing Statement

Data will be made available on request.

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Disclosure

The authors report no conflicts of interest in this work.

References

1. Tong C, Hu H, Chen G, Li Z, Li A, Zhang J. Disinfectant resistance in bacteria: mechanisms, spread, and resolution strategies. *Environ Res*. 2021;195:110897. doi:10.1016/j.envres.2021.110897
2. Rajabi A, Farajzadeh D, Dehghanzadeh R, et al. Characterization of antibiotic resistance genes and bacteria in a municipal water resource recovery facility. *Water Environ Res*. 2022;94(7):e10750. doi:10.1002/wer.10750
3. Ghiglione B, Haim MS, Penzotti P, et al. Characterization of emerging pathogens carrying *blaKPC-2* gene in *IncP-6* plasmids isolated from urban sewage in Argentina. *Front Cell Infect Microbiol*. 2021;11:722536. doi:10.3389/fcimb.2021.722536
4. Xu C, Zhang Y, Hu C, et al. From disinfection to pathogenicity: occurrence, resistome risks and assembly mechanism of biocide and metal resistance genes in hospital wastewaters. *Environ Pollut*. 2024;349:123910. doi:10.1016/j.envpol.2024.123910
5. Felis E, Sochacki A, Bajkacz S, et al. Removal of selected sulfonamides and sulfonamide resistance genes from wastewater in full-scale constructed wetlands. *Sci Total Environ*. 2024;912:169195. doi:10.1016/j.scitotenv.2023.169195
6. Ministry of Health of the People's Republic of China. Technical standard for disinfection; 2002. Available from: http://www.nhc.gov.cn/wjw/gfxwj/201304/3a0121cba422455b93307f070b099cf2.shtml. Accessed April 22, 2025.
7. Berendonk TU, Manaia CM, Merlin C, et al. Tackling antibiotic resistance: the environmental framework. *Nat Rev Microbiol*. 2015;13(5):310–317. doi:10.1038/nrmicro3439
8. Li W, Zhang G. Detection and various environmental factors of antibiotic resistance gene horizontal transfer. *Environ Res*. 2022;212(B):113267. doi:10.1016/j.envres.2022.113267
9. Li C, Lu JJ, Tong YB, Liu J, Gou HG, Liu YZ. Antibiotic resistance levels of pathogens isolated from medical wastewater in Urumqi. *J Environ Occup Med*. 2016;33(8):758–762. doi:10.13213/j.cnki.jeom.2015.15664
10. Liu M, Zheng L, Zhu L, et al. Characteristics of carbapenem-resistant *Klebsiella pneumoniae* in sewage from a tertiary hospital in Jilin Province, China. *PLoS One*. 2023;18(5):e0285730. doi:10.1371/journal.pone.0285730
11. Batista MPB, Cavalcante FS, Alves Cassini ST, Pinto Schuenck R. Diversity of bacteria carrying antibiotic resistance genes in hospital raw sewage in Southeastern Brazil. *Water Sci Technol*. 2023;87(1):239–250. doi:10.2166/wst.2022.427
12. Yang K, Chen ML, Zhu D. Exposure to benzalkonium chloride disinfectants promotes antibiotic resistance in sewage sludge microbiomes. *Sci Total Environ*. 2023;867:161527. doi:10.1016/j.scitotenv.2023.161527
13. Zhang KW, Hu H, Chen G. 微生物消毒剂抗性机理. [Mechanisms of microbial resistance to disinfectants]. *Prog Biochem Biophys*. 2022;49(1):34–47.
14. Hu Z, Yang L, Liu Z, et al. Excessive disinfection aggravated the environmental prevalence of antimicrobial resistance during COVID-19 pandemic. *Sci Total Environ*. 2023;882:163598. doi:10.1016/j.scitotenv.2023.163598
15. Zhang TT, Liu ZW, Xu TF, Wu T, Chen L. 某三甲医院耐碳青霉烯类肠杆菌科细菌耐药性和耐消毒剂基因分析 [Drug resistance and disinfectant-resistance genes in *carbapenem-resistant Enterobacteriaceae* in a three-A hospital]. *Chin J Nosocomiology*. 2023;03:325–329.

16. Liu ZW, Zhang TT, Huang XF, Xu TF, Chen L. 耐碳青霉烯类肠杆菌目细菌耐消毒剂基因及四种消毒剂最低抑菌浓度检测 [Detection of disinfectant-resistant genes and minimum inhibitory concentrations of four disinfectants in *carbapenem-resistant Enterobacteriaceae]*. *Chin J Infect Control*. 2023;22(5):497–503.
17. State Environmental Protection Administration. General administration of quality supervision, inspection and quarantine. *Discharge Standard of Water Pollutants from Medical Organization: GB*; 2005:18466. Available from: https://www.mee.gov.cn/ywgz/fgbz/bz/bzwb/shjbh/swrwpfbz/200601/t20060101_69193.shtml. Accessed April 22, 2025.
18. Wu J, Liu DF, Li HH, et al. Controlling pathogenic risks of water treatment biotechnologies at the source by genetic editing means. *Environ Microbiol*. 2021;23(12):7578–7590. doi:10.1111/1462-2920.15851
19. Azuma T, Usui M, Hayashi T. Inactivation of antibiotic-resistant bacteria in wastewater by ozone-based advanced water treatment processes. *Antibiotics*. 2022;11(2):210. doi:10.3390/antibiotics11020210
20. Yang P, Huang H, Xie X. Removal of antibiotic resistant bacteria in wastewater by aggregation-induced emission photosensitizer. *Environ Pollut*. 2023;334:121738. doi:10.1016/j.envpol.2023.121738
21. Zhou CS, Wu JW, Dong LL, et al. Removal of antibiotic resistant bacteria and antibiotic resistance genes in wastewater effluent by UV-activated persulfate. *J Hazard Mater*. 2020;388:122070. doi:10.1016/j.jhazmat.2020.122070