ASP

自然科学学报 Chinese Journal of Natural Science, Vol. 1, No. 1
http://dx.doi.org/10.52810/CJNS.2024.002

REVIEW

抗生素抗性基因的传播机制及对策

韩爱萍[1,*]

[1] 北京工商大学，轻工科学与工程学院，北京 100048

学术编辑： Shenglun Yi；**收稿日期：** 2024-03-26；**录用日期：** 2024-04-17；**发布日期：** 2024-04-26

***通讯作者：** ✉韩爱萍，h18131135342@163.com

文章引用
韩爱萍. 抗生素抗性基因的传播机制及对策. 自然科学学报, 2024, **1**(1): 16–25.

Citation
Han, A. (2024). Transmission Mechanism and Countermeasures of Antibiotic Resistance Genes. Chinese Journal of Natural Science, 1(1), 16–25.

摘　要

抗生素的出现极大地便利了生活，但由于滥用抗生素，全球抗药性的扩散对公共卫生构成了严重威胁。用于治疗和预防的抗生素正在全球范围内得到越来越广泛的使用。耐药菌株的数量在增加，越来越多的耐药基因正在出现。这将给自然环境、人类生产和生活带来更大的潜在危害。本文简要介绍了污水处理中抗生素抗性基因 (ARGs) 传播的现状。此外，总结了基因编辑、全基因组测序 (WGS) 和人工智能 (AI) 在降低或阻止抗生素抗性扩散方面的应用，并探讨和展望了几种污水处理过程的工程可行性。这为找到能减少抗微生物药物抗性基因的出现，从根源上抑制抗生素抗性基因的传播，并最大限度地维护生活环境和保护公共健康安全的方法提供了参考。

关键词: 抗生素抗性基因，传播机制，对策，基因编辑，人工智能

Transmission Mechanism and Countermeasures of Antibiotic Resistance Genes

Aiping Han[1,*]

[1]School of Light Industry Science and Engineering, Beijing Technology and Business University, Beijing 100048, China

Academic Editor: Shenglun Yi; **Submitted:** 2024-03-26; **Accepted:** 2024-04-17; **Published:** 2024-04-26

***Correspondence Author:** ✉Aiping Han, h18131135342@163.com

Abstract

The emergence of antibiotics has brought increased convenience to livelihoods; however, public health is at serious threat as the abuse of antibiotics has produced the spread of antimicrobial resistance worldwide. Antibiotics, which are applied for treatment and prevention, are increasingly used worldwide. The number of drug-resistant bacteria is increasing, and an increasing number of drug-resistant genes are appearing. This will bring increased hidden dangers to the natural environment, human production, and life. This paper briefly introduces the current situation of ARG transmission in wastewater treatment. In addition, it summarizes the applications of gene editing, whole genome sequencing (WGS) and artificial intelligence (AI) in reducing or preventing the spread of antibiotic resistance and discusses and prospects the engineering feasibility of several wastewater treatment processes. This provides a reference for finding methods that can reduce the emergence of antimicrobial resistance genes, inhibit the spread of antimicrobial resistance genes from the root and maximize the maintenance of the living environment and the protection of public health and safety.

Keywords: Antibiotic-resistant genes, transmission mechanism, countermeasures, gene editing, artificial intelligence

1 引言

抗微生物药物耐药性（AMR）目前正在全球范围内传播，而抗生素抗性基因（ARG）的出现严重威胁着人类的生活环境，甚至可能引发公共卫生危机[1]。目前，每年因抗生素抗性感染死亡的人数多达70万[2]。抗生素的滥用和过度使用导致了越来越多的病原体对抗生素产生抗性。通过染色体突变和水平基因转移（HGT）从其他细菌或环境中获取遗传物质是获取AMR的主要途径。后者在细菌群落的进化和生态学中发挥了重要作用。目前主要的抗性基因转移机制包括：加速与双酚类似物的结合转移、噬菌体转导、质粒偶联和整合子介导的转移。研究发现，噬菌体是唯一与核心ARG丰度关联的可移动元素，这表明在市政污水处理厂接收水环境中，转导可能在市政污水处理厂接收水环境中ARG的传播中起主导作用。在污水处理过程中，由于污水中耐药细菌的数量和种类众多，处理过程复杂，使得该过程成为控制AMR的关键[3]。许多ARGs在膜反应器[4]和反渗透（RO）污水处理过程中被传播。另一方面ARGs能够通过偶联机制进行耐药基因的生物膜转移[5]。其相关感染主要包括非生物表面和宿主组织生物膜感染。生物膜群落中药物耐药机制的关键是生物膜的多细胞性质[6]。

本文对预防和应对抗性基因传播的措施进行了回顾，并讨论了几种污水处理工艺的工程可行性。

2 对策

2.1 基因编辑

传统的消毒方法在控制污水处理系统和环境中的耐药病原体方面存在困难[7,8]。基因编辑是一种高效、有针对性且简单的工具，通过针对基因失活来降低环境病原体的风险。吴洁等人利用气单胞菌作为模型，证实基因编辑能够精准识别和选择抗抗生素基因，有效抑制了细菌的耐药性，且不影响细菌的正常表型及其降解污染物的能力[9]。Madoka等人利用这项技术确定了白蘑菇对唑类药物的抗药性，由甾醇去甲基酶（Cyp51A）介导，并发现Cyp51A参与了白蘑菇对唑类药物的抗药性。这项发现阐明了Cyp51A对唑类药物的抗药性机制，同时也推动了新药的开发[10]。

CRISPR-Cas基因编辑技术是一种高度便捷和高效的前沿基因编辑技术，可以修改目标基因的特定DNA[7,11]。耐甲氧西林金黄色葡萄球菌（MRSA）对青霉素、甲氧西林和苯唑西林β-内酰胺类抗生素具有抗药性。研究者

设计了两种针对甲氧西林抗性基因上不同位点的 CRISPR-dCas9 系统，以抑制 MRSA 中的抗生素抗性。

质粒和噬菌体是在 CRISPR-Cas 系统中常用的传递载体[12]。Dong 等人利用 CRISPR-Cas9 系统构建了一个针对 mcr-1 的靶向基因，mcr-1 是位于质粒上的一种可水平转移的肠粘菌耐药基因，并且构建了一个共轭质粒。结果表明，结合利用质粒不仅清除了耐药质粒，而且使接受细胞对 mcr-1 产生了免疫[13]。Wang 等人将质粒 p MBLcas9-sg RNA 转入大肠杆菌的两个菌株中，并测试了 mcr-1 的清除效率。结果表明，mcr-1 耐药基因可以有效地被消除[14]。通过提高质粒传递效率，可以克服 CRISPR-Cas 系统在临床应用中的局限性[15]。一些研究明确了粪肠球菌的信息素反应质粒（PRP）可以促进供体和受体细菌的聚集，提高信息素反应载体的偶联效率，并且由受体细菌分泌的信息素诱导蛋白质粘附素的合成[16]。

噬菌体感染宿主细菌的能力强；可以携带大的 DNA 片段，并且包裹在噬菌体蛋白中的核酸不易被降解。由于这些能力，噬菌体已经成为了当前研究人员首选的传递工具[17]，因此，它们成为了研究人员当前首选的传递工具[18]。Zhang 等人开发了一种高效的基因组编辑技术，不依赖于原位邻近基序（PAM），可以在任何地点使用。这项技术已得到改进，以克服 CRISPR/Cas9 系统在存在 PAM 独立序列时无法直接编辑基因组位点的缺陷[19]。Geng 等人利用生物信息学和 CRISPR/Cas12a 方法研究了运动酵母并鉴定出与氨苄西林耐药性相关的基因[20]。这为探索其他 ARGs 奠定了基础。

2.2 全基因组测序

全基因组测序（WGS）可以将质粒相关的耐药决定因素与选定的病原体联系起来，为抗微生物耐药性（AMR）的快速特征分析提供了强大的工具。WGS 被用来监测污水中的抗药性基因，以确保处理后的污水可以安全地排放到环境中。Micaela 等人通过全基因组测序和抗生素敏感度测试，对从污水处理厂的进水和出水中分离出的两组不同的碳青霉烯类耐药菌进行了特征分析。结果显示，在潜在的环境分离物中未检测到耐药基因，而潜在的病原菌株表现出广泛的耐药基因多样性[21]。Easler 等人对来自八个不同污水处理设施的产 β-内酰胺酶（ESBL）大肠杆菌进行序列化，以确定最丰富的耐药基因，并通过 WGS 识别出耐抗生素的表型[22]。全基因组测序还可以帮助确定潜在的耐药基因的来源菌株。Jiang 等人利用全基因组测序方法对从污水处理厂分离出的多重耐药的 *Citrobacter frederii* R17 进行了测序，发现该菌株可能是耐药基因的潜在来源。该菌株编码总共有 13 个耐药基因，对 8 个不同的抗生素组都有耐药性[23]。通过对大量具有地理代表性的分离物进行测序，可以对 AMR 流行病学的时间和地理变化进行量化[24]，并且可以改善本地 AMR 克隆传播的误导性挑战。

2.3 人工智能

人工智能（AI）是一种具有多种可能性的革命性方法。此外，它以高效率、低成本和高成功率加速了抗药性基因的探索和预测。人工智能可以用来及时检测与细菌感染相关的疾病，减轻抗生素在治疗过程中的应用，减少医疗废物排入水环境的抗药性基因含量。Li 等人开发了一个基于随机森林的应变蛋白模型（CRP），可以准确区分下呼吸道的细菌和病毒感染[25]。Xu 等人开发了一个深度学习模型来区分 HIV/结核病共感染和 HIV 单独感染[26]。

可以使用深度学习来预测抗药性基因[27]。Espinoza 等人开发了基于优化 Clairvoyance 新特征算法的 CoHEC 模型，这是一个层次化的综合分类模型。它正确预测了以前未曾观察到的化合物的主要作用机制，净化对其精度没有影响，并且正确识别了具有新的 MOA 的抗生素，达罗巴丁[28]。这种方法为探索未知的抗生素及其主要作用机制提供了新的路径。Lu 等人将人工智能和拉曼光谱结合，开发了基于长短期记忆（LSTM）的拉曼平台，以在基因型和表型水平上预测微生物和抗药性基因[29]。该方法提高了准确性，并可以减少抗生素的滥用。

此外，AI 方法可以抵抗生物膜并防止药物抵抗。一些研究者预测，基于无监督的机器学习，源自非生物杀虫精油的化学成分可以用作多种菌株（包括假单胞菌、金黄色葡萄球菌和表皮链球菌）的生物膜产生的调节器，以

表 1. 不同方法对抗菌药物耐药性的研究结果

Table1. Search results of different methods in antimicrobial resistance

方法	主要作用	参考文献
基因编辑	靶向耐药基因抑制抗生素耐药性	[32-40]
全基因组测序	AMR 的快速表征和监测以及传输机制的推断	[23, 41-53]
人工智能	提高临床治疗效率，加快 ARG 预测和新药开发	[54-63]

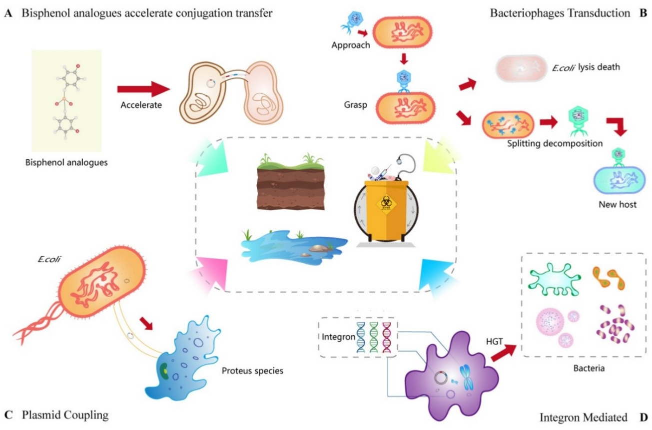

图 1. 双酚类似物、噬菌体、质粒和整合子对 ARG 的传递机制

Figure1. Transmission mechanism of ARG by bisphenol analogues, bacteriophages, plasmids and integrons.

防止药物抵抗[30,31]。越来越多的研究人员投入到使用上述方法的研究中，积极为公共环境发现新的抗药性基因，探索其作用机制，并找到新的替代品。表 1 列出了一些基于基因编辑、全基因组测序和 AI 的抗药性基因研究结果。

3 工业应用和前景

水环境加速了微生物中 ARGs 的传播速度。反硝化是污水处理中的一个重要过程，主要基于生化硝化和反硝化。在不同的氮去除途径中，发现微生物与 ARGs 之间存在着共线关系。特别是，反硝化路径的差异对微生物群落和 ARGs 的传播模式有一定的影响[64,65]。阻断 ARGs 与其宿主及社区中敏感微生物之间的连接水平和基因转移是减少污水处理厂 (WWTPs) 中 ARGs 传播的关键。此外，还可以通过改变污水处理厂的不同运营参数来改变微生物结构，从而减少 ARGs。

短程硝化反硝化（SCND）过程是一种低能耗、高过程稳定性保留的良好氮去除过程[66]。Yang 等人使用短程硝化-反硝化（SCND）过程模拟测序批处理反应器（SBR）中的污水处理，并发现该过程可以有效控制 ARG 污染，但相关研究不足，需要进行进一步的系统工作[67]。Wang 等人提出了一种 SCND 同时去除常规污染物和 ARG 的新策略，在实验室规模和中试规模上进行了初步验证。然而，关于 ARG 的传播及其在水-污泥界面的迁移模式的研究还很少。根据 ARG 减少过程中相应的微生物群落变化，建立了具有反馈调节的动态减少模型[68]。此外，由于长期的抗生素压力导致反硝化细菌数量显著减少，对 SCND 过程中 ARG 的扩散的研究很少。SCND 过程的操作因素对 ARG 动态和微生物群落的影响也缺乏相应的探索。

厌氧氨氧化（Anammox）是一种新型的氮去除过程，已成功应用于许多高氨氮废水的生物氮去除项目。其低能耗在实现污水处理的碳中和方面具有广阔的前景[69]。目前，大多数研究者只研究了单一抗生素对厌氧氨氧

化过程的影响，而对不同抗生素对厌氧氨氧化过程的影响，特别是在含氮废水中存在多种抗生素联合感染的情况下，尚未进行系统研究。其次，不同抗生素对厌氧氨氧化过程的抑制效应及其潜在机制尚未进行比较和阐明。

新兴技术的出现和发展为短程硝化反硝化（SCND）的实际应用和厌氧氨氧化过程应用范围的扩大带来了希望。通过测序技术发现新的反硝化细菌，扩大反硝化细菌库，推动 SCND 的研究和工业应用。例如，Lee 等人使用测序技术研究了较少研究的 Geobacterium 属的反硝化能力，发现 *Geobacterium subtilis* KCTC 3922T 具有反硝化活性[70]。Tao 等人利用基因编辑技术对两种反硝化细菌进行了突变，建立了一个框架标记无突变体系统，并成功探索了它们对低 pH 值的抗性和在污染环境中占据主导地位的能力[71]。这为我们在污水处理过程中提出了一个新的想法——寻找携带 ARGs 的优势菌群，对其进行基因编辑，引导其产生突变，探索其在 ARGs 传播中的地位。

机器学习在污水处理中具有实际应用前景。它不仅可以检测水质和优化操作条件，还可以模拟污水处理过程和预测污水处理器的性能。Oluyemi 等人成功地使用适应性神经模糊推理系统（ANFIS）预测了影响污水处理期间硝化的参数，预测结果与 Spearman 相关系数一致[72]。Xu 等人使用人工神经网络模型研究了四种不同抗生素对厌氧氨氧化系统的关键抑制浓度，并准确预测了厌氧氨氧化系统中的氮去除过程[62]。

此外，微藻-细菌颗粒污泥法（MBGS）有望成为一种可持续的污水处理方法。然而，目前该过程仍处于实验阶段，无法评估这个过程的工程和经济可行性。此外，这项技术还有很多问题需要紧急解决，如如何确保 MBGS 的水产量在光代谢和暗代谢的交替过程中保持稳定，以及如何生产出适合大规模应用的 MBGS。

4 结论

抗生素的出现和使用虽然为人们的生活、生产和医疗治疗带来了更大的便利，但也推动了抗生素耐药性的出现和传播。本文主要回顾总结了几种有效减少 ARGs 传播的理论方法，并对几种污水处理工艺的工程可行性进行了讨论和展望。

基因编辑技术自出现以来一直存在争议。特别是 CRISPR-Cas 基因编辑，有望成为对抗抗生素耐药性的强大工具。然而，这项技术的局限性是显而易见的。例如，在使用基于 CRISPR-Cas9 的 RNA 靶向基因编辑技术时，应仔细研究所有可能的缺失影响。人工智能领域的持续进步为探索和预测 ARG 提供了新的方法。随着全基因组测序（WGS）的成本降低和使用频率以及数字医疗的普及度增加，将产生大量的数字数据，为 AI 的应用提供了宏大的数据库。同时，AI 的进步为将 WGS 产生的 AMR 数据转化为临床实践提供了有效的帮助[73]。

在污水处理过程中，我们当前应扩大对 ARB 和 ARG 传播机制和影响因素的研究，并基于耐药性的传播机制，结合关键的技术参数开发针对性的新的污水处理过程，以控制耐药基因的传播，并优化还在实验室阶段的处理技术的运行条件，以提高对 ARB 和 ARG 的减少效果。新技术的出现、进步和组合，为应对抗生素耐药性挑战和开发新的污水处理技术提供了新的思路。

创新说明

（1）综述了污水处理中抗生素抗性基因 (ARGs) 传播的最新研究现状。

（2）本文总结了基因编辑、全基因组测序 (WGS) 和人工智能 (AI) 在降低或阻止抗生素抗性扩散方面的应用。

（3）探讨和展望了几种污水处理过程的工程学方法抑制抗生素抗性基因的传播可行性。

参考文献

[1] Xu C, Kong L, Liao Y, et al. Mini-review: antibiotic-resistant Escherichia coli from farm animal-associated sources [J]. Antibiotics, 2022, 11(11): 1535-1552.

[2] Balcazar J. How do bacteriophages promote antibiotic resistance in the environment? [J]. Clinical Microbiology And Infection, 2018, 24(5): 447-449.

[3] Mutuku C, Gazdag Z, Melegh S. Occurrence of antibiotics and bacterial resistance genes in wastewater: resistance mechanisms and antimicrobial resistance control approaches [J]. World Journal Of Microbiology & Biotechnology, 2022, 38(9): 152-179.

[4] Yang D, Wang J, Qiu Z, et al. Horizontal transfer of antibiotic resistance genes in a membrane bioreactor [J]. Journal of Biotechnology, 2013, 167(4): 441-417.

[5] Sharma D, Misba L, Khan A. Antibiotics versus biofilm: an emerging battleground in microbial communities [J]. Antimicrobial Resistance and Infection Control, 2019, 8: 76-86.

[6] Sugimoto S, Okuda K, Miyakawa R, et al. Imaging of bacterial multicellular behaviour in biofilms in liquid by atmospheric scanning electron microscopy [J]. Scientific Reports, 2016, 6: 25889-25902.

[7] Cesare A, Fontaneto D, Doppelbauer J, et al. Fitness and recovery of bacterial communities and antibiotic resistance genes in urban wastewaters exposed to classical disinfection treatments [J]. Environmental Science & Technology, 2016, 50(18): 10153-10161.

[8] Zhang S, Wang Y, Lu J, et al. Chlorine disinfection facilitates natural transformation through ROS-mediated oxidative stress[J]. The ISME Journal, 2021, 15(10): 2969-2985.

[9] Wu J, Liu D, Li H, et al. Controlling pathogenic risks of water treatment biotechnologies at the source by genetic editing means [J]. Environmental Microbiology, 2021, 23(12): 7578-7590.

[10] Tateno M, Umeyama T, Inukai T, et al. Examination of Cyp51A-resistance in aspergillus ientulus using CRISPR/Cas9 genome editing [J]. Medical Mycology, 2022, 63(2): 27-35.

[11] Zhang S, Wang Y, Lu J, et al. Chlorine disinfection facilitates natural transformation through ROS-mediated oxidative stress [J]. ISME Journal, 2021, 15(10): 2969-2985.

[12] Tao S, Chen H, Li N, et al. The application of the CRISPR-Cas system in antibiotic resistance [J]. Infection and Drug Resistance, 2022, 15: 4155-4168.

[13] Dong H, Xiang H, Mu D, et al. Exploiting a conjugative CRISPR/Cas9 system to eliminate plasmid harbouring the mcr-1 gene from Escherichia coli [J]. International Journal of Antimicrobial Agents, 2019, 53(1): 1-8.

[14] Wang P, He D, Li B, et al. Eliminating mcr-1-harbouring plasmids in clinical isolates using the CRISPR/Cas9 system [J]. Journal of Antimicrobial Chemotherapy, 2019, 74(9): 9-16.

[15] Chen G, Cheng D, Chen B. Development of CRISPR technology and its application in bone and cartilage tissue engineering [J]. Nan Fang Yi Ke Da Xue Xue Bao, 2019, 39(12): 1515-1520.

[16] Zou J, Tang Z, Yan J, et al. Dissemination of linezolid resistance through sex pheromone plasmid transfer inEnterococcus faecalis [J]. Frontiers in Microbiology, 2020, 11: 1185-1196.

[17] Borges A, Castro B, GOVINDARAJAN S, et al. Bacterial alginate regulators and phage homologs repress CRISPR-Cas immunity [J]. Nature Microbiology, 2020, 5(5): 679-687.

[18] Fage C, Lemire N, Moineau S. Delivery of CRISPR-Cas systems using phage-based vectors [J]. Current Opinion in Biotechnology, 2021, 68: 174-180.

[19] Zhang H, Cheng Q, Liu A, et al. A novel and efficient method for bacteria genome editing employing both CRISPR/Cas9 and an antibiotic resistance cassette [J]. Frontiers in Microbiology, 2017, 8: 812-823.

[20] Geng B, Huang X, Wu Y, et al. Identification and characterization of genes related to mmpicillin antibiotic resistance in zymomonas mobilis [J]. Antibiotics, 2022, 11(11): 1476-1488.

[21] Oliveira M, Leonardo I C, Nunes M, et al. Environmental and pathogenic carbapenem resistant bacteria isolated from a wastewater treatment plant harbour distinct antibiotic resistance mechanisms[J]. Antibiotics, 2021, 10(9):

1118.

[22] Easler M, Cheney C, Johnson D, et al. Resistome characterization of extended-spectrum beta-lactamase (ESBL)-producing Escherichia coli isolated from wastewater treatment utilities in Oregon [J]. Journal Of Water And Health, 2022, 20(4): 670-679.

[23] Jiang X, Cui X, Liu W, et al. Genetic characterization of a novel sequence type of multidrug-resistant Citrobacter freundii strain recovered from wastewater treatment plant [J]. Infection and Drug Resistance, 2019, 12: 2775-2779.

[24] Ingle D, Levine M, Kotloff K, et al. Dynamics of antimicrobial resistance in intestinal Escherichia coli from children in community settings in South Asia and sub-Saharan Africa [J]. Nature Microbiology, 2018, 3(9): 1063-1073.

[25] Li Y, Van C, Boers S, et al. The diagnostic value of nasal microbiota and clinical parameters in a multi-parametric prediction model to differentiate bacterial versus viral infections in lower respiratory tract infections [J]. PLoS One, 2022, 17(4): 267140-267160.

[26] Xu S, Yuan H. A three-methylation-driven gene-based deep learning model for tuberculosis diagnosis in patients with and without human immunodeficiency virus co-infection [J]. Microbiology And Immunology, 2022, 66(6): 317-323.

[27] Sunuwar J, Azad R. Identification of novel antimicrobial resistance genes using machine learning, homology modeling, and molecular docking [J]. Microorganisms, 2022, 10(11): 2102-2117.

[28] Schlessinger A, Espinoza J, Dupont C, et al. Predicting antimicrobial mechanism-of-action from transcriptomes: A generalizable explainable artificial intelligence approach [J]. Plos Computational Biology, 2021, 17(3): 1008857-1008882.

[29] Lu J, Chen J, Huang L, et al. Rapid identification of species, antimicrobial-resistance genotypes and phenotypes of gram-positive cocci using long short-term memory raman spectra methods [J]. Advanced Intelligent Systems, 2023, 10(1): 235-245.

[30] Artini M, Patsilinakos A, Papa R, et al. Antimicrobial and antibiofilm activity and machine learning classification analysis of essential oils from different mediterranean plants against pseudomonas aeruginosa [J]. Molecules, 2018, 23(2): 482-495.

[31] Patsilinakos A, Artini M, Papa R, et al. Machine learning analyses on data including essential oil chemical composition and in vitro experimental antibiofilm activities against staphylococcus species [J]. Molecules, 2019, 24(5): 890-916.

[32] Freudenberg R, Wittemeier L, Einhaus A, et al. The spermidine synthase gene SPD1: a novel auxotrophic marker for chlamydomonas reinhardtii designed by gene editing [J]. Cells, 2022, 11(5): 837-854.

[33] Gratacap R, Regan T, Dehler C, et al. Efficient CRISPR/Cas9 genome editing in a salmonid fish cell line using a lentivirus delivery system [J]. BMC Biotechnology, 2020, 20(1): 35-44.

[34] Kim M, Kim H, Jeong D, et al. Cytosine base editor-mediated multiplex genome editing to accelerate discovery of novel antibiotics in bacillus subtilis and paenibacillus polymyxa [J]. Frontiers in Microbiology, 2021, 12: 691839-691851.

[35] Minh-duy P, Peters K, Fraga L, et al. Plasmid-mediated ciprofloxacin resistance imparts a selective advantage on Escherichia coli ST131 [J]. Antimicrobial agents and chemotherapy, 2022, 66(1): 2146-2157.

[36] Ruotsalainen P, Penttinen R, Mattila S, et al. Midbiotics: conjugative plasmids for genetic engineering of natural gut flora [J]. Gut Microbes, 2019, 10(6): 643-653.

[37] Wu Z, Huang Y, Chao W, et al. Reversal of carbapenem-resistance in Shewanella algae by CRISPR/Cas9 genome editing [J]. Journal of Advanced Research, 2019, 18: 61-69.

[38] Xu C, Liu H, Pan X, et al. Mechanisms for development of ciprofloxacin resistance in a clinical isolate of pseu-

domonas aeruginosa [J]. Frontiers in Microbiology, 2021, 11: 598291-5982919.

[39] Xu Z, Li M, Li Y, et al. Native CRISPR-Cas-mediated genome editing enables dissecting and sensitizing clinical multidrug-resistant P.aeruginosa [J]. Cell Reports, 2019: 1707-1717.

[40] Zhang N, He J, Muhammad A, et al. CRISPR/Cas9-mediated genome editing for pseudomonas fulva, a novel pseudomonas species with clinical, animal, and plant-associated isolates [J]. International Journal of Hygiene and Environmental Health, 2022, 23(10): 5443-5456.

[41] Aksomaitiene J, Novoslavskij A, Kudirkiene E, et al. Whole genome sequence-based prediction of resistance determinants in high-level multidrug-resistant campylobacter jejuni Isolates inlithuania [J]. Microorganisms, 2021, 9(1): 66-77.

[42] Fatahi-bafghi M, Naseri S, Alizehi A. Genome analysis of probiotic bacteria for antibiotic resistance genes [J]. Antonie Van Leeuwenhoek, 2022, 115(3): 375-389.

[43] Kumar M, sodhi K, Singh D. Draft genome of Serratia sp. R1 gives an insight into the antibiotic resistant genes against multiple antibiotics [J]. Molecular Biology Reports, 2022, 49(6): 4479-4484.

[44] Pouget C, Chatre C, Lavigne J, et al. Effect of antibiotic exposure on staphylococcus epidermidis responsible for catheter-related bacteremia [J]. International Journal Of Molecular Sciences, 2023, 24(2): 1547-1563.

[45] Pouget C, Chatre C, Lavigne J, et al. Effect of antibiotic exposure on staphylococcus epidermidis responsible for catheter-related bacteremia [J]. International Journal Of Molecular Sciences, 2023, 24(2): 1547-1563.

[46] Thach X, Trang L, Trieu L, et al. Whole-genome sequencing and characterization of an antibiotic resistant Neisseria meningitidis B isolate from a military unit in Vietnam [J]. Annals Of Clinical Microbiology And Antimicrobials 2019, 18(1): 16-25.

[47] Turumtay H. Whole-genome sequencing-based characteristics of Escherichia coli Rize-53 isolate from Turkey [J]. Advances in Clinical and Experimental Medicine, 2022: 152704-152710.

[48] Turumtay H, Allam M, Sandalli A, et al. Characteristics in the whole-genome sequence of Klebsiella pneumoniae ST147 from turkey [J]. Acta Ichthyologica Et Piscatoria, 2022, 69(2): 144-149.

[49] Ullmann I, Nygaard A, Benteson, et al. Whole genome sequencing and antibiotic diffusion assays, provide new insight on drug resistance in the genus Pedobacter [J]. FEMS Microbiology Ecology, 2020, 96(6): 88-99.

[50] Zhang H, Liu X, Gu Q, et al. Molecular characteristics and antibiotic resistance of Bacillus cereus from foods using whole genome sequencing [J]. Chinese Journal of Food Hygiene, 2021, (5): 529-535.

[51] Zhang S, Yang G, Jiang Y. Antibiotic and metal resistance of Stenotrophomonas maltophilia isolates from Eboling permafrost of the Tibetan Plateau [J]. Environmental Science and Pollution Research, 2022, 30: 11798–117810.

[52] Zhao G, Luo Z, Wang Y, et al. Draft genome sequencing and annotation of a low-virulence Morganella morganii strain CQ-M7, a multidrug-resistant isolate from the giant salamander in China [J]. The Journal of Global Antimicrobial Resistance, 2020, 20: 248-252.

[53] Zhou Y, Zhong Z, Hu S, et al. A survey of helicobacter pylori antibiotic-resistant genotypes and strain lineages by whole-genome sequencing in china [J]. Antimicrobial Agents and Chemotherapy, 2022, 66(6): 2188-2203.

[54] Costa K, Araujo F, Morais J, et al. Text mining for identification of biological entities related to antibiotic resistant organisms [J]. PeerJ, 2022, 10: 13351-13367.

[55] Haffiez N, Chung T, Zakaria B, et al. Exploration of machine learning algorithms for predicting the changes in abundance of antibiotic resistance genes in anaerobic digestion [J]. Science of The Total Environment, 2022, 839: 156211-156224.

[56] Her H, Wu Y. A pan-genome-based machine learning approach for predicting antimicrobial resistance activities of the Escherichia coli strains [J]. Bioinformatics, 2018, 34(13): 89-95.

[57] Iftikhar S, Karim A, et al. Prediction and interpretation of antibiotic-resistance genes occurrence at recreational beaches using machine learning models [J]. Journal of Environmental Management, 2023, 328: 116969-116979.

[58] Lamping F, Jack T, Ruebsamen N, et al. Development and validation of a diagnostic model for early differentiation of sepsis and non-infectious SIRS in critically ill children a data-driven approach using machine-learning algorithms [J]. BMC Pediatrics, 2018, 18: 112-123.

[59] Mahe P, Tournoud M. Predicting bacterial resistance from whole-genome sequences using k-mers and stability selection [J]. BMC Biotechnology, 2018, 19: 383-394.

[60] Rawson T, Hernandez B, WILSON R, et al. Supervised machine learning to support the diagnosis of bacterial infection in the context of COVID-19 [J]. JAC-Antimicrobal Resistance, 2021, 3(1): 2-6.

[61] Sabiha S, Anuradha S, Arya S, et al. Genome informatics and machine learning-based identification of antimicrobial resistance-encoding features and virulence attributes in Escherichia coli genomes representing globally prevalent lineages, including high-risk clonal complexes [J]. Mbio, 2022, 13(1): 3796-3813.

[62] Xu X, Liu S, Zeng M, et al. Deciphering response effect and underlying mechanism of anammox-based nitrogen removal process under exposures to different antibiotics via big data analysis [J]. Bioresource Technology, 2022, 347: 126674-126684.

[63] Yang M, Wu Y. Enhancing predictions of antimicrobial resistance of pathogens by expanding the potential resistance gene repertoire using a pan-genome-based feature selection approach [J]. BMC Biotechnology, 2022, 23(4): 131-146.

[64] Hiller C, Huebner U, Fajnorova S, et al. Antibiotic microbial resistance (AMR) removal efficiencies by conventional and advanced wastewater treatment processes: A review [J]. Science of the Total Environment, 2019, 685: 596-608.

[65] Zhang J, Buhe C, Yu D, et al. Ammonia stress reduces antibiotic efflux but enriches horizontal gene transfer of antibiotic resistance genes in anaerobic digestion[J]. Bioresource Technology, 2020, 295: 122191.

[66] Liu T, Mao Y, Shi Y, et al. Start-up and bacterial community compositions of partial nitrification in moving bed biofilm reactor [J]. Applied Microbiology And Biotechnology, 2017, 101(6): 2563-2574.

[67] SYang C, Wang L, Wang H, et al. Dynamics of antibiotic resistance genes and microbial community in shortcut nitrification-denitrification process under antibiotic stresses [J]. Environmental Science and Pollution Research, 2022, 29(31): 46848-44858.

[68] Wang L, Yang C, Yang Z, et al. Shortcut nitrification and denitrification shed light on simultaneous removal of conventional contaminants and antibiotic resistance genes (ARGs)[J]. Journal of Environmental Chemical Engineering, 2022, 10(1): 106925.

[69] Wang X, Huang J, Gao D. Effects of three storage conditions on the long-term storage and short-term reactivation performances of anammox granular sludge [J]. International Biodeterioration & Biodegradation, 2021, 164: 105310.

[70] Lee Y J, Park M K, Park G S, et al. Complete genome sequence of the thermophilic bacterium Geobacillus subterraneus KCTC 3922(T) as a potential denitrifier [J]. Journal of Biotechnology, 2017, 251: 141-144.

[71] Tao X, Zhou A, Kempher M, et al. Development of a Markerless Deletion Mutagenesis System in Nitrate-Reducing Bacterium Rhodanobacter denitrificans [J]. Applied and Environmental Microbiology, 2022, 88(14): 1-13.

[72] Awolusi O, Nasr M, Kumari S, et al. Artificial Intelligence for the Evaluation of Operational Parameters Influencing Nitrification and Nitrifiers in an Activated Sludge Process [J]. Microbial Ecology, 2016, 72(1): 49-63.

[73] Forde B, Oliveira D, Falconer C, et al. Strengths and caveats of identifying resistance genes from whole genome sequencing data [J]. Expert Review Of Anti-infective Therapy, 2022, 20(4): 533-547.

韩爱萍 北京工商大学生物工程专业硕士，研究方向为生物化工。

Aiping Han, Master's degree in Bioengineering from Beijing Technology and Business University, with a research focus on Biochemical Engineering.