四川大学学报（医学版） 2026，57（4）：1212－1220
J Sichuan Univ（Med Sci） DOI: 10.12182/20261360301

在线全文

CRISPR/Cas系统在临床耐药菌快速检测中的应用研究进展*

宋 珍[1]，李 惠[1]，赵亚楠[1]，李 敏[2△]

1. 上海交通大学医学院附属瑞金医院 医学技术学院（上海 200025）；2. 上海交通大学医学院附属仁济医院 检验科（上海 200127）

【摘要】 抗菌药物的滥用使得抗微生物药物耐药性成为危害全球公共健康的重大威胁之一，如何高效地实现耐药菌和耐药基因的便捷快速检测是控制感染扩散、维护人类健康的关键举措。然而传统的检测方法（如分离培养、PCR等）在检测速度、成本效益、便捷性等方面存在明显局限，越来越难以满足当下的临床检测需求。在此背景下，CRISPR/Cas系统作为一种新兴的分子诊断技术，为该领域提供了创新解决方案。本文详细介绍CRISPR/Cas系统发展现状及其在临床耐药菌快速检测中应用研究进展，系统阐述了多种基于CRISPR/Cas系统研发的检测平台（如SHERLOCK、DETECTR、HOLMES等）的工作原理和效能，并重点突出CRISPR/Cas系统在检测领域的灵敏、特异、高效、实时、便携和低成本等优势。此外，本文还探讨了CRISPR/Cas系统面临的挑战，包括对临床样本直接检测、多重检测、检测成本、临床验证和标准化等问题。最后，本文展望了未来的发展方向，强调聚焦技术创新与临床转化使得CRISPR/Cas系统成为耐药菌防控的高效工具。

【关键词】 CRISPR/Cas系统 耐药菌 快速检测 核酸靶标 综述

Research Progress on the Application of CRISPR/Cas System in Rapid Testing of Drug-Resistant Bacteria in Clinical Practice

Song Zhen[1], *Li Hui*[1], *Zhao Yanan*[1], *Li Min*[2△]. 1. *College of Health Science and Technology, Ruijin Hospital, Shanghai Jiao Tong University School of Medicine, Shanghai 200025, China*; 2. *Department of Laboratory Medicine, Renji Hospital, Shanghai Jiao Tong University School of Medicine, Shanghai 200127, China*

△ Corresponding author

This work was supported by the National Natural Science Foundation of China (No. 82302538).

[**Abstract**] The misuse of antimicrobial agents has made antimicrobial resistance one of the major threats to global public health. Efficient and rapid detection of drug-resistant bacteria and resistance genes is crucial for controlling infection spread and safeguarding human health. However, traditional detection methods (such as culture isolation, polymerase chain reaction, etc.) exhibit significant limitations in detection speed, cost-effectiveness, and convenience, increasingly failing to meet current clinical testing demands. In this context, the CRISPR/Cas system, as an emerging molecular diagnostic technology, offers innovative solutions for this field. This review details the developmental status of CRISPR/Cas systems and their research progress in rapid clinical detection of drug-resistant bacteria. It systematically explains the working principles and efficacy of various CRISPR/Cas-based detection platforms (e.g., SHERLOCK, DETECTR, HOLMES), highlighting the system's advantages in sensitivity, specificity, efficiency, real-time capability, portability, and low cost. Additionally, the article discusses challenges faced by CRISPR/Cas systems, including direct clinical sample detection, multiplex testing, cost reduction, clinical validation, and standardization. Finally, it outlines future development directions, emphasizing technological innovation and clinical translation to establish CRISPR/Cas systems as efficient tools for drug-resistant bacteria prevention and control.

[**Key words**] CRISPR/Cas system Drug-resistant bacteria Rapid detection Nucleic acid targets Review

抗微生物药物耐药性（antimicrobial resistance，AMR）是全球人类健康、现代医学和社会可持续发展所面临的最大威胁之一[1-2]，由耐药菌引起的感染会导致严重的全球疾病负担。有研究显示，2019年全球估计有495万人的死亡与AMR相关，其中因AMR直接死亡的高达127万人[3]。WHO发布的《2024年细菌类重点病原体目录》里就包含了耐碳青霉烯酶的肺炎克雷伯菌、耐第三代头孢菌素大肠埃希菌、耐碳青霉烯酶的鲍曼不动杆菌、耐利福平的结核分枝杆菌等多种临床常见耐药菌[4]。如何应对AMR带来的挑战是当今医疗卫生部门需要面对的一大难题。

* 国家自然科学基金（No. 82302538）资助

△ 通信作者

出版日期：2026-07-20

目前应对AMR的主要举措有健全感染防控体系、及时诊断治疗、监测流行趋势和研发新的抗菌药物、诊断方法和预防工具[4]。准确快速检测出耐药菌是感染防控的第一步，也是控制AMR扩散的关键步骤，因为这意味着医生可以更快地锁定合适的抗菌药物，从而改善患者的治疗结果，降低药物不良事件的风险，减少病原体传播的可能性[5]。现如今在临床微生物实验室普遍用于耐药菌检测的实验方法有：传统的手工药敏检测（K-B纸片扩散法、肉汤稀释法等）、全自动药敏检测系统、质谱检测、基于聚合酶链式反应（polymerase chain reaction, PCR）的分子检测技术等。传统的手工法和全自动药敏检测系统往往依赖于病原菌的培养，耗时至少36 h甚至更长，而且药物种类不够全面；质谱检测耗时短，但只能检测特定的酶（如β -内酰胺酶或碳青霉烯酶等），检测范围较狭窄[6]；基于PCR的分子检测技术虽然检测速度快，耗时1 ~ 4 h不等，但设备的购买和维护依然昂贵[5]。

规律成簇间隔短回文序列（clustered regularly interspaced short-palindromic repeat, CRISPR）/CRISPR相关核酸内切酶（CRISPR-associated endonuclease, Cas）系统作为一种基因编辑技术，还具有DNase/RNase特性，在病原体分子诊断方面展现出卓越的灵敏度、特异性、快速性和便携性[7]，这类方法有望克服传统耐药菌检测方法的局限，助力耐药菌的快速检测。

本文主要介绍了CRISPR/Cas系统的功能和检测效用，特别是其在耐药菌和耐药基因检测中的重要应用，包括目前已开发的主要检测平台、检测优势、挑战和未来方向，为控制和减少细菌耐药性的扩大提供了参考。

1　CRISPR/Cas系统简述

CRISPR序列是一组存在于细菌和古细菌中的适应性免疫机制，其免疫过程经历适应、表达和干扰三个阶段。在适应阶段通过摄取来自入侵噬菌体的一段前间隔序列（protospacer）整合到自身的CRISPR 序列中形成间隔序列（spacer），获取“免疫记忆”；当再次遇到携带同源间隔序列的噬菌体时进入表达阶段，转录CRISPR阵列并加工为成熟的CRISPR RNA（crRNA），产生特异性靶向噬菌体外源DNA的向导RNA（guide RNA, gRNA）；在干扰阶段，gRNA引导Cas蛋白识别并切割互补的外源噬菌体DNA，发挥免疫应答作用[8]。Jinek等[9]由此获得灵感，于2012年首次实现了 CRISPR/Cas9 系统的体外结合并切割靶标DNA的特定位点；张峰团队[10]在2013年首次将CRISPR/Cas9系统应用于小鼠和人类细胞，完成体内基因组位点的精确切割，从而为CRISPR /Cas用于核酸检测奠定了机制基础。利用人工设计的crRNA序列取代细菌捕获的外源DNA片段，使其与目标病原体序列（靶标）完全互补，Cas蛋白在crRNA引导下识别并切割靶标成为将CRISPR/Cas系统研发为分子诊断工具的突破口。

CRISPR/Cas系统的结构由CRISPR基因座（DNA序列）和Cas蛋白共同组成，前者包含位于CRISPR阵列上游的前导序列、在CRISPR阵列中规律间隔排列的重复序列和间隔序列，后者包含核心相应蛋白及辅助蛋白。CRISPR/Cas 系统根据其Cas蛋白的组成差异和效应器中蛋白组合序列可分为两类，第一类为Ⅰ、Ⅲ、Ⅳ型，第二类为Ⅱ、Ⅴ、Ⅵ型，两者的区别在于前者的效应器是由多个Cas蛋白组成的复合体，后者效应器是单个有多结合域的Cas 蛋白，目前在耐药菌检测方面广泛应用的Cas9（Ⅱ型）、Cas12（Ⅴ型）、Cas13（Ⅵ型）、Cas14（Ⅴ型）都属于第二类[11-12]。

2　CRISPR/Cas系统在耐药菌检测中的基本原理

CRISPR/Cas系统在分子诊断中的应用主要基于其核酸靶向识别和切割能力。不同类型的Cas效应蛋白具有不同的核酸切割活性。表1列出了不同CRISPR/Cas系统

表 1　不同CRISPR/Cas系统的结构和特点

Table 1　Structures and features of different CRISPR-Cas system

Cas effector	Cas9	Cas12a	Cas12b	Cas13	Cas14
Type	Type Ⅱ	Type Ⅴ	Type Ⅴ	Type Ⅵ	Type Ⅴ
Spacer length	18-24 nt	18-25 nt	18-20 nt	22-30 nt	20-40 nt
Functional domain	HNH & RuvC	RuvC	RuvC	HEPN	RuvC
Guide RNA	tracrRNA-crRNA	crRNA	tracrRNA-crRNA	crRNA	tracrRNA-crRNA
PAM/PFS sequence	NGG	TTTN	TTN	Non-G	Not required
Cleavage type	Cis-cleavage	Cis-cleavage & Trans-cleavage	Cis-cleavage & Trans-cleavage	Cis-cleavage & Trans-cleavage	Cis-cleavage & Trans-cleavage
Cleavage pattern	Blunt end cut	Single staggered cut	Single staggered cut	Multiple cleavage sites	Single staggered cut
Target substrate	dsDNA	ssDNA, dsDNA	dsDNA	ssRNA	ssDNA
Collateral cleavage	No	Yes, ssDNA	Yes, ssDNA	Yes, ssRNA	Yes, ssDNA
Reference	[9]	[13]	[14]	[15]	[12]

PAM: protospacer adjacent motif; PFS: protospacer flanking site.

在分子诊断应用中的重要结构和特点。

2.1 基于与靶标DNA结合的检测

CRISPR/Cas9系统: Cas9蛋白包含两个主要的核酸酶结构域: 切割非互补DNA链的RuvC结构域和切割互补DNA链的HNH结构域。Cas9/sgRNA复合体通过识别dsDNA上的特定基序(protospacer-adjacent motif, PAM)与靶向dsDNA结合, 从而启动Cas9蛋白的酶切[16]。但它由于不具备信号放大功能, 因此往往需要与核酸扩增技术相结合, 比如滚环扩增(rolling circle amplification, RCA)、PCR等实现靶标DNA的检测[17-18]。有研究人员已经开发了基于CRISPR/Cas9系统的快速检测空气中耐甲氧西林金黄色葡萄球菌(methicillin- resistant *Staphylococcus aureus*, MRSA)和耐万古霉素肠球菌(vancomycin-resistant *enterococcus*, VRE)的方法[19], 还有研究人员将CRISPR/Cas9与下一代测序(next-generation sequencing, NGS)技术相结合, 开发一种低丰度耐药菌检测技术(finding low abundance sequences by hybridization-next generation sequencing, FLASH-NGS), 可检测出临床样本中的MRSA、VRE和多种耐药基因如*vanA*、*dfrC*、*ermA*和*ermC*等[20]。

dCas9是Cas9的一种衍生物, 其不具备酶切能力, 但仍能特异性识别并结合靶标dsDNA, 以dCas9/sgRNA复合体为靶向DNA探针, 结合免疫学方法或荧光原位杂交技术(fluorescence in situ hybridization, FISH)可检测MRSA[21-22]。

2.2 基于"旁侧剪切"活性的检测

CRISPR/Cas12系统: 由一个单独的RuvC结构域组成, 但具有顺式和反式切割的活性。CRISPR/Cas12a在crRNA的介导下[23], CRISPR/Cas12b在crRNA和tracr RNA(或单独融合sgRNA)共同介导下[24-25], 能特异性识别靶标上的PAM远端位点并切割ds DNA和ssDNA序列(对dsDNA特异性更高[16]), 同时非特异性切割周围的非靶标ssDNA报告分子。通过引入荧光标记的ssDNA报告分子, 当其被激活的Cas12切割时, 荧光信号会被释放, 从而实现对靶病原体核酸的检测。Shin等[26]在2024年报道了基于PCR偶联CRISPR/Cas12a的荧光测定法检测携带bla_{NDM-1}的大肠埃希菌, 其检测限达2.7 CFU/mL, 较常规凝胶电泳PCR灵敏度高出100倍。

CRISPR/Cas14系统: 是CRISPR/Cas系统最小的蛋白, 只有400 ~ 700个氨基酸, 是同类核酸酶体积的一半。CRISPR-Cas14有CRISPR-Cas12类似的切割活性, 但与dsDNA相比, 对靶向识别切割ssDNA的特异性更高, 并且不依赖于PAM序列的激活。此外, Cas14的靶标识别会触发ssDNA分子的非特异性切割, 这种活性可以实现高保真的单核苷酸多态性基因分型[12, 16]。Lai等[27]人开发了一种基于Cas14的可视化即时检测系统(Cas14VIDet), 可检测幽门螺杆菌对左氧氟沙星的耐药基因*GyrA*, 其检测灵敏度接近单个细菌菌落水平(10^0 CFU/mL)。

CRISPR/Cas13系统: 与Cas9、Cas12、Cas14检测DNA型核酸不同的是, Cas13仅能特异性识别并切割RNA。Cas13同样具有顺式和反式切割的活性, Cas13/sgRNA在识别特异性的靶向RNA序列后一方面靶标序列远端酶切, 另一方面引发邻近标记的RNA序列的非特异性切割释放信号, 从而达到病原体核酸检测的目的[28]。张峰团队[29]基于CRISPR-Cas13开发的SHERLOCK检测平台能够区分具有两种不同耐药基因的肺炎克雷伯菌的临床分离株, 即耐碳青霉烯酶的肺炎克雷伯菌(*Klebsiella pneumoniae* carbapenemase, KPC)和产新德里金属β -内酰胺酶1的肺炎克雷伯菌(New Delhi Metallo-β-lactamase-1-producing *Klebsiella pneumonia*, NDM-1)。

3 基于CRISPR/Cas系统的耐药菌检测平台

CRISPR技术在病原体诊断领域发展迅速, 已涌现出多种创新的诊断平台, 在检测耐药菌或耐药基因方面表现出较大潜力。表2 详细列出了不同类型的CRISPR /Cas的核酸检测平台及其特征。图1展示SHERLOCK、DETECTR和HOLMES在耐药菌检测中的基本原理。

3.1 SHERLOCK (Specific High-sensitivity Enzymatic Reporter Unlocking)

SHERLOCK被称为特异性高灵敏度酶切报告系统, 是由张峰[29]团队在2017年开发的基于CRISPR/Cas13与等温扩增技术相结合的分子检测平台, 其检测流程为将样品中纯化的DNA进行重组酶聚合酶扩增(recombinase polymerase amplification, RPA)扩增, T7 RNA聚合酶将扩增的DNA转录为RNA, 通过Cas13介导的旁侧RNA切割释放报告信号来检测靶RNA。检测的靶标主要有病毒RNA、细菌mRNA。一年后又在原有的平台上进行技术升级, 将Cas13与Csm6(一种CRISPR系统辅助因子)结合, 构建了SHERLOCK v2定量检测平台, 该平台支持多路和横向流式条上的视觉/比色检测, 更灵敏更快速更便捷, 可同时用Cas13、Cas12检测靶标RNA和DNA。通过SHERLOCK v2平台, 研究人员检测到了低至aM浓度范围的铜绿假单胞菌酰基转移酶基因和金黄色葡萄球菌耐热核酸酶基因[15]。SHERLOCK检测平台适合于高灵敏度即时检测和多重检测, 但依赖预扩增, 会增加检测步骤和样品污染的风险, 另外对样本纯度要求较高, 遇到临床复杂

表 2　不同类型的CRISPR /Cas的核酸检测工具及其特点

Table 2　Different Types of CRISPR/Cas-based nucleic acid detection tools and their characteristics

Cas Effectors	Method	Amplification	Readout	Multiplexing	Targeted types	Targeted Pathogens	Time	Sensitivity	Samples	Reference
CRISPR/Cas9	Au-IDE coupled with CRISPR/Cas9	RCA	Electrochemical	No	dsDNA	*S. aureus*	1.5 h	7 CFU/mL	Food	[17]
CRISPR/Cas9	CN-TAR	RCA	Fluorescence	Yes	dsDNA	MRSA, VRE	0.5 h	1.40 copies/μL (MRSA) 1.13 copies/μL (VRE)	Airborne	[19]
CRISPR/Cas9	FLASH	PCR	NGS	Yes	dsDNA	antimicrobia-resistance genes		1.9 aM	Cultured bacterial isolates/ clinical samples	[20]
CRISPR/Cas9	CUT-LAMP	LAMP	Fluorescence	Yes	dsDNA	*Salmonella*	–	100 copies	Bacterial cultures	[30]
CRISPR/dCas9	FISH	–	Fluorescence	No	dsDNA	*S. aureus*	0.5 h	10 CFU/mL	Clinical isolates	[22]
CRISPR/dCas9	dCLISA	HCR	Colorimetric	No	dsDNA	MRSA	1 h	8.5 CFU/mL	Clinical isolates	[21]
CRISPR/Cas12a	CRISPR-MTB	RPA	Flourescence	No	dsDNA	*M.tuberculosis*	< 2 h	50 CFU/mL	Clinical samples	[31]
CRISPR/Cas12a	PCR-coupled CRISPR/Cas12a	PCR	Fluorescence	No	dsDNA	bla_{NDM}	1 h	2.7×10^0 CFU/mL	Bacterial cultures/ food	[26]
CRISPR/Cas12a	SRCA-CRISPR/ Cas12a	SRCA	Electrochemical	No	dsDNA	*S. aureus*	< 2 h	3 CFU/mL	Pure cultures	[32]
CRISPR/Cas12a	DropCRISPR	LAMP	Fluorescence	No	dsDNA	*Salmonella typhimurium*	< 1.5 h	10^2 CFU/mL	Pure cultures	[33]
CRISPR/Cas12a	RCCS	RPA	Flourescence, lateral flow assay	No	dsDNA	bla_{KPC}, bla_{NDM}	50 min	10 fg/μL, 1 ng/μL	Clinical isolates	[34]
CRISPR/Cas12a	dual RPA combined with CRISPR/Cas12	RPA	Fluorescence, blue light irradiation, ultraviolet irradiation, lateral flow assay	No	dsDNA	*rcs A*, *KPC*	< 1 h	1-10 pg/μL(*rcsA*), 1×10^2 pg/μL(*KPC*)	Clinical isolates	[35]
CRISPR/Cas12a	CRISPR-LbCas12a	RPA	Fluorescence	Yes	dsDNA	*KPC, NDM, OXA*	0.5 h	aM	Blood samples	[36]
CRISPR/Cas12a	Cas12a/Guide RNA	LAMP	Flourescence, lateral flow assay	No	dsDNA	*nuc*, *mecA*	< 1.5 h	4×10^3fg/μL	Clinical isolates	[37]
CRISPR/Cas12a	E-Si-CRISPR	–	electrochemical	No	dsDNA	MRSA	< 1.5 h	3.5 fM	Human serum	[38]
CRISPR/Cas13a	SHERLOCK	RPA, RT-RPA	Flourescence, lateral flow assay	No	DNA, RNA	*KPC, NDM-1*	< 1 h	2 aM	Clinical isolates	[29]
CRISPR/Cas12, Cas13, Csm6	SHERLOCK v2	RPA, RT-RPA	Flouresccence, lateral flow assay	Yes	DNA, RNA	*P. aeruginosa* acyltransferase gene, *S. aureus* thermonuclease gene	–	aM	Clinical isolates	[15]
CRISPR/Cas13a	SHIELD	RAA	Lateral flow assay	Yes	RNA	*H.pylori*, clarithromycin resistance mutations	< 1.5 h	50 copies/μL	Saliva samples	[39]
CRISPR/Cas14	Cas14VIDet	PCR	Fluorescence	No	ssDNA	*H. pylori* resistance genes	10 min	10^0 CFU/mL	Gastric tissues	[27]

CRISPR: clustered regularly interspaced short-palindromic repeat; Cas:CRISPR-associated endonuclease; RCA:rolling circle amplification; CN-TAR:copy number-targeted assembly and recombination; FLASH: inding low abundance sequences by hybridization; LAMP: loop-mediated isothermal amplification; CUT-LAMP: contamination-free LAMP; PCR: polymerase chain reaction; NGS: next-generation sequencing; FISH: fluorescence in situ hybridization; dCLISA: digital chemi luminescence lmmuno sorbent assay; HCR: hybridization chain reaction; RPA: recombinase polymerase amplification; SRCA-CRISPR/Cas12a: saltatory rolling circle amplificationcombined with CRISPR/Cas12a system;DropCRISPR: droplet-digital LAMP-CRISPR/Cas12a assay platform; RCCS: RPA-coupled CRISPR/Cas12a system; SHERLOCK: specific high-sensitivity enzymatic reporter unlocking; SHERLOCK v2: SHERLOCK version 2; RT-RPA: reverse transcription recombinase polymerase amplification; RAA: recombinase aid amplification; SHIELD: sensitive *H. pylori* identified easy-read dual detection; Cas14VIDet: Cas14-based visual instant detection.

样本可能需额外处理。

3.2　DETECTR (DNA Endonuclease Targeted CRISPR Trans Reporter)

2018年, Chen等[13]提出了另一种CRISPR/ Cas检测平台, 命名为DNA内切酶靶向的CRISPR反式报告基因。该检测平台将CRISPR/Cas12与RPA等温扩增技术相结合, 通过Cas12依赖crRNA识别病原核酸并降解相邻DNA探针释放荧光团, 借助荧光计检测稳定而强烈的荧光信号。RPA不仅避免对复杂和昂贵设备的需求, 还可富集目标序列提高检测灵敏度。Chen等[13]利用DETECTR检测并区分HPV16和HPV18, 并推测原则上可以扩展到快速检测任何感兴趣的DNA序列。基于DETECTR检测平台, Li等[40]实现20 min内可视化检测MRSA, 多个研究团队实现了肠杆菌目细菌耐药基因(*KPC*、*NDM*和*OXA*)的快

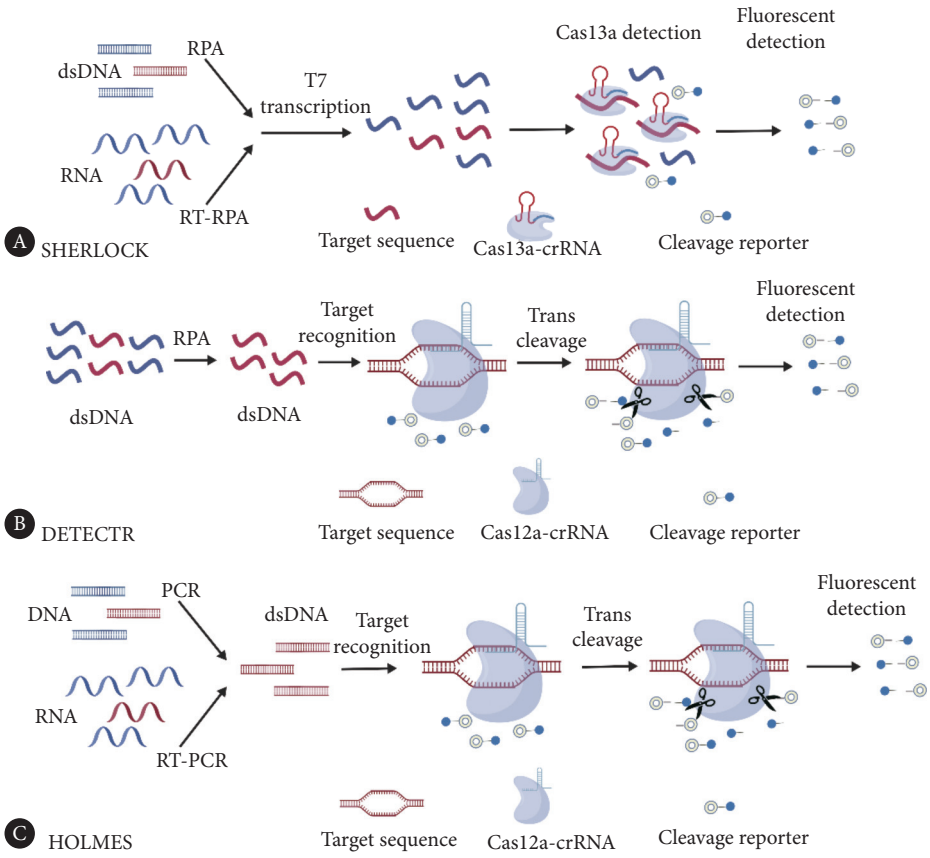


图 1 SHERLOCK、DETECTR和HOLMES在耐药菌检测中的基本原理（采用生物医学通用绘图平台制图[41]）

Fig 1 The basic principles of SHERLOCK, DETECTR, and HOLMES in drug-resistant bacteria detection (Created with BioGDP.com[41])

A, SHERLOCK Detection Assay. The SHERLOCK assay begins with preamplification of the target DNA or RNA input. The amplified sequences are subsequently transcribed into RNA using T7 polymerase. Detection occurs via Cas13a-crRNA, which upon activation cleaves fluorescent reporter RNA probes, generating a measurable signal. B, DETECTR Detection Assay. The DETECTR assay begins with preamplification of the target DNA input (or reverse-transcribed RNA). The amplified DNA is then detected by Cas12a-crRNA, upon target binding which activates collateral cleavage of fluorescent single-stranded DNA (ssDNA) reporters, generating a measurable signal. C, HOLMES Detection Assay. The HOLMES assay initiates with PCR (or RT-PCR) of target DNA or reverse-transcribed RNA. The amplified DNA is then recognized by Cas12a protein guided by target-specific crRNA. Upon target binding, activated Cas12a exhibits collateral cleavage activity, indiscriminately degrading fluorescent single-stranded DNA (ssDNA) reporters to produce a detectable signal.

速检测[34-36]。DETECTR具有与SHERLOCK相当的高灵敏度，适用于粗提样本（如粪便、唾液），抗干扰能力较强可用于DNA病原体检测、环境或食品微生物污染监测。

3.3 HOLMES (one-Hour Low-cost Multipurpose highly Efficient System)

2018年王金教授团队研发了HOLMES检测系统，这是一种将PCR与CRISPR/Cas12a系统结合的快速现场检测平台[42]，这个检测方法弥补了当时SHERLOCK系统在检测DNA序列时必须进行DNA到RNA体外转录的不便捷，可用于检测靶标RNA和DNA。然而，PCR扩增的需要昂贵的设备，并且增加一个额外的、耗时的步骤。2019年团队将HOLMES检测系统升级为HOLMES v2，将恒温扩增技术（loop-mediated isothermal amplification, LAMP）与CRISPR/Cas12b系统结合，利用Cas12b蛋白对目标双链或单链DNA模板进行识别，然后利用Cas12b的伴随剪切活性对单链DNA荧光探针进行降解，定量检测目标核酸，如日本脑炎病毒，灵敏度可达10^{-17}M[24]。Cao等[37]研究人员在HOLMES检测系统的基础上，设计特异性靶向耐热核酸酶（*nuc*）和*mecA*基因的单链向导RNA（sgRNA），构建了LAMP-CRISPR /Cas12a检测体系，快速鉴定金黄色葡萄球菌和MRSA，总检测时间为80 ~ 85 min，特异性和灵敏度可达100%。HOLMES类似DETECTR，且升级后的HOLMES v2仅需普通恒温设备，检测速度快灵敏度高，虽然多重检测能力有限，但适合基层医疗机构或应急响应。

3.4 其他耐药菌检测技术

Suea-Ngam等[38]在2021年提出了一种利用银金属化的免扩增电化学联合CRISPR/Cas12a生物传感器技术（silver-enhanced E-CRISPR biosensor, E-Si-CRISPR）来检测MRSA，检测限和定量限分别为3.5f M和10 f M，线性度超过5个数量级（ 10 fM ~ 0.1 nM），通过对gRNA前间隔序

列的简单改造就可以实现对不同靶标的调整。

Yan等[39]在2024年研发一种将重组酶介导扩增(recombinase aid amplification, RAA)和CRISPR/Cas13a结合的技术,通过侧向流试纸条检测幽门螺杆菌和克拉霉素耐药突变,该技术命名为幽门螺杆菌易读双重检测法(sensitive *H. pylori* easy-read dual detection, SHIELD)。检测限可达50 copies/μL,与qPCR比,阳性预测值可达100%(菌种鉴定)和84.6%(耐药检测)。

Mao等[43]人在2025年开发出一种将CRISPR/Cas9与NGS联合用于检测废水样品中低丰度的抗生素耐药基因(antibiotic resistance genes, ARGs)。CRISPR/Cas9富集文库制备过程中的靶向ARGs,与常规NGS方法相比,CRISPR - NGS方法将ARGs的检测限从10^{-4}降至10^{-5}(以qPCR相对丰度计),并成功在废水样品中检测到临床上重要的*KPC*耐药基因。

CRISPR/Cas技术凭借其高特异性、灵敏度和可编辑性,已成为耐药菌及耐药基因检测的重要研发方向。目前已有多种创新平台被开发,主要结合等温扩增技术(如RPA、LAMP)或PCR,通过Cas蛋白(如Cas12、Cas13、Cas9)的靶向切割与信号放大实现快速、精准检测,这将彻底变革耐药菌检测领域,未来有望成为抗击抗生素耐药性的核心工具之一。

4　CRISPR/Cas系统在耐药菌检测中的优势

4.1　高特异性与灵敏度

CRISPR系统通过gRNA精确识别靶核酸序列,显著降低了非特异性结合,许多Cas蛋白(Cas9、Cas12、Cas13、Cas14等)结合信号放大和荧光[39]、电位[38]、比色[44]、侧向流[45]等技术,极大提高了检测的准确性和灵敏度。尤其是成熟的检测平台,比如DETECTR、HOLMES v2、SHERLOCK v2等[13, 15, 24],可以检测到浓度低至aM水平的病原核酸,其特异性与PCR相当,但对设备、操作人员技术、环境等要求非常低[13, 15, 24]。一些新技术在这方面也不逊色,例如Peng等[46]在2023年开发一种纳米筛装置,允许从血浆样品中快速纯化和高浓度分离MRSA,将纳米筛和RPA和CRISPR/Cas检测方法相结合,提高临床样本的检测限至100 CFU/mL; Yang等[34]在2024年将RPA偶联的CRISPR/Cas12a,构建重组酶聚合酶扩增联合CRISPR/Cas12a的检测平台(RPA-coupled CRISPR/Cas12a system, RCCS),成功检测到aM水平的*KPC*和pM水平的*NDM*耐药基因,并100%准确检测出临床分离的肺炎克雷伯菌和大肠埃希菌。

4.2　检测速度与实时监测

在抗菌药物滥用的时代背景下,AMR已演变为对全球公共健康重大威胁。因此,建立高效快速的耐药菌检测对于控制疾病进展或感染的传播非常重要[47]。研究人员将CRISPR/Cas系统与多种信号读取方式如荧光、测序、侧向流等技术结合,研发出基于CRISPR的快速检测结核病的方法(CRISPR-based assay for Mycobacterium tuberculosis complex, CRISPR-MTB)在2 h内实现结核分枝杆菌复合体的快速检测[31]; FLASH-NGS适用于对耐药菌进行大规模、快速的实时分子流行病学监测[20]; 基于CRISPR/Cas13a系统联合侧向流技术实现40 min内肉眼读取多黏菌素耐药基因*mcr-1*的检测结果[48]; 这些新技术将耐药菌的检测时间从传统方法的数天缩短至数十分钟到几小时,实现了近乎实时的监测,为临床精准用药和公共卫生监测提供了强大的技术支撑。

4.3　多重检测与未知突变筛查

CRISPR/Cas系统可以通过设计不同的gRNA同时检测多种病原体或同一病原体的不同位点。与PCR等传统核酸检测技术相比,CRISPR/Cas系统在应对临床未知病原体感染和复杂耐药情况时,其多重检测和未知突变筛查功能在速度和简便性方面展现出显著优势。例如SHERLOCK v2采用四种正交Cas酶,实现一次实验中对四个目标(包括突变位点)同步检测,具备单碱基分辨率,适用于快速突变筛查[15]; 有研究人员研发一种基于CRISPR/Cas9联合NGS的结核菌检测技术(finding low abundance sequences by hybridization-tuberculosis, FLASH-TB)实现直接从痰标本中检测结核分枝杆菌合并关键耐药基因,提升临床检测效率[49]; 还有研究人员将CRISPR/Cas13与液滴微阵列结合,设计出细菌核酸多重微阵列(bacteria-combinatorial arrayed reactions for multiplexed evaluation of nucleic acids, bCARMEN)检测系统,可实现52种常见临床菌和几种耐药基因的同时检测,为快速的现场多重细菌病原体鉴定和抗生素敏感性测试开辟了可能性[50]。从而使患者得到及时有效的治疗,也能为医院和社区面对可能存在的病原体扩散危险时,实施防控措施争取时间。

4.4　便携化与低成本设备

许多CRISPR/Cas诊断平台设计的核心理念之一就是排除复杂昂贵的仪器设备,达到在资源有限的环境中实施快速检测目的,例如便携式纸基或微流控等设备,这对于疫情暴发、现场监测和床旁检测(point-of-care, POC)等场景的即时诊断至关重要。COVID-19 的暴发引发全球公共卫生危机时,多个研究团队将CRISPR/Cas12a与逆转录-重组酶聚合酶扩增(reverse transcription recombinase polymerase amplification, RT-RPA)或纳米颗

粒或逆转录-指数扩增技术(reverse transcription-exponential amplification reaction, RTF-EXPAR)结合[51-53],研发出多种便携式的SARS-CoV-2 RNA检测方法; Mao等[54]设计研发一种结合CRISPR/Cas12a 和LAMP的便携式生物传感器,可通过侧流试纸和荧光信号对废水中的耐药基因*ermB*进行可视化检测,实现现场低成本检测,灵敏度可达2.75×10^{3}copies/μL,这为有众多污水处理厂的大城市或农村地区实现现场实时监测废水中的耐药基因提供了一种新思路。

5 CRISPR/Cas系统在耐药菌检测中的挑战和未来展望

CRISPR/Cas系统因为具有特异性的靶标识别和顺式/反式切割活性,使它在准确性、便携性、效率和成本方面优于临床目前的传统检测手段,这意味着它在未来的耐药菌检测领域将展现出巨大潜力,然而,该技术在实际应用中仍面临多重挑战,未来发展需聚焦技术创新与临床转化。

5.1 临床样本直接检测能力还需优化

临床样本(如血液、尿液、痰液等)通常包含多种抑制剂和背景核酸,背景的干扰物质一方面可能抑制Cas酶活性,导致假阴性结果,另一方面仍可能出现Cas酶脱靶切割风险,产生假阳性信号[55]。面对这种情况,能利用人工智能优化CRISPR/Cas系统,提升其对复杂临床样本的直接检测能力。

例如使用AI工具(DeepHF、CRISPRon和DeepCRISPR等)结合深度学习模型(如BiLSTM)分析gRNA二级结构(如折叠自由能、发卡结构),提高gRNA特异性与活性;使用TIGER(卷积神经网络模型)分析gRNA与靶标及非靶标的错配模式,piCRISPR整合序列特征和表观遗传特征,CRISTA基于随机森林,利用GUIDE-seq数据训练,预测脱靶位点,量化脱靶概率;通过AI语言模型ProGen2训练筛选出OpenCRISPR-1,与天然SpCas9序列相似度仅45%,但编辑活性却提升20%;基于AlphaFold2预测的HEPN结构域,通过Foldseek聚类分析鉴定出新型祖先型Cas13变体(Cas13an),其体积仅为传统Cas13的1/3,更适合腺相关病毒(adeno-associated virus, AAV)载体递送[56-57]。

总之,人工智能通过优化gRNA设计、预测脱靶风险、改造Cas蛋白及简化样本处理,显著提升CRISPR/Cas平台对临床样本的直接检测能力,未来将进一步推动该技术向高效、自动化、临床实用化方向发展。

5.2 多重病原体和耐药基因的多通道检测有待提高

现有CRISPR/Cas平台为了达到好的检测效果,通常针对某一类耐药菌或单一耐药基因设计,但实际感染往往涉及多种病原体和多种耐药机制,而CRISPR/Cas多重检测平台的开发的确具有挑战性,因为在同步检测多种靶标时,gRNA设计易发生交叉反应,且信号输出通道可能相互干扰。因此将微流控、微液滴、微阵列等技术与光学传感器等超灵敏检测技术与CRISPR/Cas相结合[58-60],是构建CRISPR/Cas多重检测平台的发展方向之一。

5.3 检测成本的进一步降低

要实现CRISPR/Cas检测的广泛应用,尤其是在资源有限地区,实质性降低单次检测成本是关键。CRISPR/Cas技术在这方面还有巨大的发展潜力。例如革新样本前处理,通过开发简单的热裂解或电化学裂解等新技术[37-38],简化核酸提取流程;开发冻干Cas蛋白与缓冲液的一体化试剂片,可以在室温下长期保存,显著减少对冷链运输和−80°C超低温储存设备的需求[61];开发纸基微流控检测(如侧流层析试纸条)、智能手机图像分析等低成本结果读出方式取代传统的荧光读数的专业设备[62];构建多靶标、复用gRNA阵列和单管多重检测体系[15, 36],实现低消耗高效率的检测能力;优化Cas蛋白和gRNA的制备工艺,以及配套仪器的开发实现规模化生产。这些策略的综合应用将使CRISPR/Cas诊断技术更加经济高效,使其在临床床旁、偏远地区和突发疫情等即时诊断场景中更具可及性。

5.4 临床验证和标准化提升

不同实验室使用的CRISPR/Cas检测体系(如SHERLOCK、DETECTR)在引物设计、报告系统方面存在差异,缺乏统一验证标准[13, 29, 63]。因此,加强CRISPR/Cas诊断平台在真实临床样本中的大规模验证,积累可靠的临床数据,并制定统一的检测标准和指南,确保检测结果的准确性和可比性,有利于CRISPR/Cas检测体系临床推广应用[47]。

* * *

作者贡献声明 宋珍负责论文构思、调查研究、可视化和初稿写作,李惠负责调查研究和监督指导,赵亚楠负责数据可视化处理、提供资源和经费支持,李敏负责论文构思,项目管理、监督指导和审读编辑修改工作。所有作者已经同意将文章提交给本刊,且对将要发表的版本进行最终定稿,并同意对工作的所有方面负责。

Author Contribution Song Zhen is responsible for conceptualization, investigation, visualization, and writing--original draft. Li Hui is responsible for investigation and supervision. Zhao Yanan is responsible for visualization, resources, and funding acquisition. Li Min is responsible for conceptualization, project administration, supervision, and writing--review and editing. All authors consented to the submission of the article to the Journal. All authors approved the final version to be published and agreed to take responsibility for all aspects of the work.

利益冲突　所有作者均声明不存在利益冲突

Declaration of Conflicting Interests　All authors declare no competing interests.

参　考　文　献

[1] Miller W R, Arias C A. ESKAPE pathogens: antimicrobial resistance, epidemiology, clinical impact and therapeutics[J]. Nat Rev Microbiol, 2024, 22(10): 598-616. DOI: 10.1038/s41579-024-01054-w.

[2] Luo Q, Lu P, Chen Y, *et al*. ESKAPE in China: epidemiology and characteristics of antibiotic resistance[J]. Emerg Microbes Infect, 2024, 13(1): 2317915. DOI: 10.1080/22221751.2024.2317915.

[3] Murray C J L, Ikuta K S, Sharara F, *et al*. Global burden of bacterial antimicrobial resistance in 2019: a systematic analysis[J]. Lancet, 2022, 399(10325): 629-655. DOI: 10.1016/S0140-6736(21)02724-0.

[4] WHO Bacterial Priority Pathogens List 2024: bacterial pathogens of public health importance, to guide research, development, and strategies to prevent and control antimicrobial resistance[M]. 1st ed. Geneva: World Health Organization, 2024.

[5] Burnham C A D, Leeds J, Nordmann P, *et al*. Diagnosing antimicrobial resistance[J]. Nat Rev Microbiol, 2017, 15(11): 697-703. DOI: 10.1038/nrmicro.2017.103.

[6] Leonard H, Colodner R, Halachmi S, *et al*. Recent advances in the race to design a rapid diagnostic test for antimicrobial resistance[J]. ACS Sens, 2018, 3(11): 2202-2217. DOI: 10.1021/acssensors.8b00900.

[7] Huang T, Zhang R, Li J. CRISPR-Cas-based techniques for pathogen detection: retrospect, recent advances, and future perspectives[J]. J Adv Res, 2023, 50: 69-82. DOI: 10.1016/j.jare.2022.10.011.

[8] Barrangou R, Fremaux C, Deveau H, *et al*. CRISPR provides acquired resistance against viruses in prokaryotes[J]. Science, 2007, 315(5819): 1709-1712. DOI: 10.1126/science.1138140.

[9] Jinek M, Chylinski K, Fonfara I, *et al*. A programmable dual-rna–guided dna endonuclease in adaptive bacterial immunity[J]. Science, 2012, 337(6096): 816-821. DOI: 10.1126/science.1225829.

[10] Cong L, Ran F A, Cox D, *et al*. Multiplex genome engineering using CRISPR/Cas systems[J]. Science, 2013, 339(6121): 819-823. DOI: 10.1126/science.1231143.

[11] Makarova K S, Wolf Y I, Iranzo J, *et al*. Evolutionary classification of CRISPR–Cas systems: a burst of class 2 and derived variants[J]. Nat Rev Microbiol, 2020, 18(2): 67-83. DOI: 10.1038/s41579-019-0299-x.

[12] Harrington L B, Burstein D, Chen J S, *et al*. Programmed DNA destruction by miniature CRISPR-Cas14 enzymes[J]. Science, 2018, 362(6416): 839-842. DOI: 10.1126/science.aav4294.

[13] Chen J S, Ma E, Harrington L B, *et al*. CRISPR-Cas12a target binding unleashes indiscriminate single-stranded DNase activity[J]. Science, 2018, 360(6387): 436-439. DOI: 10.1126/science.aar6245.

[14] Teng F, Cui T, Feng G, *et al*. Repurposing CRISPR-Cas12b for mammalian genome engineering[J]. Cell Discov, 2018, 4: 63. DOI: 10.1038/s41421-018-0069-3.

[15] Gootenberg J S, Abudayyeh O O, Kellner M J, *et al*. Multiplexed and portable nucleic acid detection platform with Cas13, Cas12a, and Csm6[J]. Science, 2018, 360(6387): 439-444. DOI: 10.1126/science.aaq0179.

[16] van Dongen J E, Berendsen J T W, Steenbergen R D M, *et al*. Point-of-care CRISPR/Cas nucleic acid detection: Recent advances, challenges and opportunities[J]. Biosens Bioelectron, 2020, 166: 112445. DOI: 10.1016/j.bios.2020.112445.

[17] Zhen D, Zhang S, Yang A, *et al*. A supersensitive electrochemical sensor based on RCA amplification-assisted “silver chain”-linked gold interdigital electrodes and CRISPR/Cas9 for the detection of *Staphylococcus aureus* in food[J]. Food Chem, 2024, 440: 138197. DOI: 10.1016/j.foodchem.2023.138197.

[18] Wang M, Han D, Zhang J, *et al*. High-fidelity detection of DNA combining the CRISPR/Cas9 system and hairpin probe[J]. Biosens Bioelectron, 2021, 184: 113212. DOI: 10.1016/j.bios.2021.113212.

[19] Seo S B, Lee J, Choi S, *et al*. Onsite detection of airborne antibiotic-resistant bacteria via Cas9 nickase-triggered amplification reactions[J]. J Hazard Mater, 2025, 495: 138850. DOI: 10.1016/j.jhazmat.2025.138850.

[20] Quan J, Langelier C, Kuchta A, *et al*. FLASH: a next-generation CRISPR diagnostic for multiplexed detection of antimicrobial resistance sequences[J/OL]. Nucleic Acids Res, 2019, 47(14): e83. https://academic.oup.com/nar/article/47/14/e83/5490816?login=false. DOI: 10.1093/nar/gkz418.

[21] Fan X, Gao Z, Ling D, *et al*. The dCas9/crRNA linked immunological assay (dCLISA) for sensitive, accurate, and facile drug resistance gene analysis[J]. Biosens Bioelectron, 2025, 273: 117147. DOI: 10.1016/j.bios.2025.117147.

[22] Guk K, Keem J O, Hwang S G, *et al*. A facile, rapid and sensitive detection of MRSA using a CRISPR-mediated DNA FISH method, antibody-like dCas9/sgRNA complex[J]. Biosens Bioelectron, 2017, 95: 67-71. DOI: 10.1016/j.bios.2017.04.016.

[23] Yan W X, Hunnewell P, Alfonse L E, *et al*. Functionally diverse type V CRISPR-Cas systems[J]. Science, 2019, 363(6422): 88-91. DOI: 10.1126/science.aav7271.

[24] Li L, Li S, Wu N, *et al*. HOLMESv2: a CRISPR-Cas12b-assisted platform for nucleic acid detection and DNA methylation quantitation[J]. ACS synth biol, 2019, 8(10): 2228-2237. DOI: 10.1021/acssynbio.9b00209.

[25] Teng F, Guo L, Cui T, *et al*. CDetection: CRISPR-Cas12b-based DNA detection with sub-attomolar sensitivity and single-base specificity[J]. Genome Biol, 2019, 20(1): 132. DOI: 10.1186/s13059-019-1742-z.

[26] Shin J, Kim S R, Xie Z, *et al*. A CRISPR/Cas12a-based system for sensitive detection of antimicrobial-resistant genes in carbapenem-resistant enterobacterales[J]. Biosensors (Basel), 2024, 14(4): 194. DOI: 10.3390/bios14040194.

[27] Lai Y, Guo K, Zhu C, *et al*. Cas14VIDet: a visual instant method free from PAM restriction for antibiotic resistance bacteria detection[J]. Biosens Bioelectron, 2025, 268: 116884. DOI: 10.1016/j.bios.2024.116884.

[28] Shihong Gao D, Zhu X, Lu B. Development and application of sensitive, specific, and rapid CRISPR-Cas13-based diagnosis[J]. J Med Virol, 2021, 93(7): 4198-4204. DOI: 10.1002/jmv.26889.

[29] Gootenberg J S, Abudayyeh O O, Lee J W, *et al*. Nucleic acid detection with CRISPR-Cas13a/C2c2[J]. Science, 2017, 356(6336): 438-442. DOI: 10.1126/science.aam9321.

[30] Bao Y, Jiang Y, Xiong E, *et al*. CUT-LAMP: Contamination-free loop-mediated isothermal amplification based on the CRISPR/Cas9 cleavage[J]. ACS Sens, 2020, 5(4): 1082-1091. DOI: 10.1021/acssensors.0c00034.

[31] Ai J W, Zhou X, Xu T, *et al*. CRISPR-based rapid and ultra-sensitive diagnostic test for *Mycobacterium tuberculosis*[J]. Emerg Microbes Infect, 2019, 8(1): 1361-1369. DOI: 10.1080/22221751.2019.1664939.

[32] Huang L, Yuan N, Guo W, *et al*. An electrochemical biosensor for the highly sensitive detection of Staphylococcus aureus based on SRCA-CRISPR/Cas12a[J]. Talanta, 2023, 252: 123821. DOI: 10.1016/j.talanta.2022.123821.

[33] Wu H, Cao X, Meng Y, *et al*. DropCRISPR: a LAMP-Cas12a based digital method for ultrasensitive detection of nucleic acid[J]. Biosens and Bioelectron, 2022, 211: 114377. DOI: 10.1016/j.bios.2022.114377.

[34] Yang J W, Kim H, Hyeon L S, *et al*. Development of a recombinase polymerase amplification-coupled CRISPR/Cas12a platform for rapid detection of antimicrobial-resistant genes in carbapenem-resistant *enterobacterales*[J]. Biosensors (Basel), 2024, 14(11): 536. DOI: 10.3390/bios14110536.

[35] Tan M, Yi X, Liao C, *et al*. Establishment of a platform based on dual RPA combined with CRISPR/Cas12a for the detection of *Klebsiella pneumoniae* and its KPC resistance gene[J]. Front Bioeng Biotechnol, 2024, 12: 1447963. DOI: 10.3389/fbioe.2024.1447963.

[36] Curti L A, Pereyra-Bonnet F, Repizo G D, *et al*. CRISPR-based platform for carbapenemases and emerging viruses detection using Cas12a (Cpf1) effector nuclease[J]. Emerg Microbes Infect, 2020, 9(1): 1140-1148. DOI: 10.1080/22221751.2020.1763857.

[37] Cao X, Chang Y, Tao C, *et al*. Cas12a/Guide RNA-based platforms for

rapidly and accurately identifying *Staphylococcus aureus* and methicillin-resistant *S. aureus*[J/OL]. Microbiol Spectr, 2023, 11(2): e04870-22. https://journals.asm.org/doi/10.1128/spectrum.04870-22. DOI: 10.1128/spectrum.04870-22.

[38] Suea-Ngam A, Howes P D, deMello A J. An amplification-free ultrasensitive electrochemical CRISPR/Cas biosensor for drug-resistant bacteria detection[J]. Chem Sci, 2021, 12(38): 12733-12743. DOI: 10.1039/D1SC02197D.

[39] Yan K, Wang X, Han Y, *et al*. Simultaneous detection of *helicobacter pylori* and clarithromycin resistance mutations using RAA-CRISPR/Cas13a assay[J]. Infect Drug Resist, 2024, 17: 3001-3010. DOI: 10.2147/IDR.S462963.

[40] Li Y, Shi Z, Hu A, *et al*. Rapid one-tube RPA-CRISPR/Cas12 detection platform for methicillin-resistant *Staphylococcus aureus*[J]. Diagnostics (basel), 2022, 12(4): 829. DOI: 10.3390/diagnostics12040829.

[41] Jiang S, Li H, Zhang L, *et al*. Generic Diagramming Platform (GDP): a comprehensive database of high-quality biomedical graphics[J]. Nucleic Acids Res, 2025, 53(D1): D1670-D1676. DOI: 10.1093/nar/gkae973.

[42] Li S Y, Cheng Q X, Wang J M, *et al*. CRISPR-Cas12a-assisted nucleic acid detection[J]. Cell Discov, 2018, 4(1): 20. DOI: 10.1038/s41421-018-0028-z.

[43] Mao Y, Shisler J L, Nguyen T H. Enhanced detection for antibiotic resistance genes in wastewater samples using a CRISPR-enriched metagenomic method[J]. Water Res, 2025, 274: 123056. DOI: 10.1016/j.watres.2024.123056.

[44] Pardee K, Green A A, Takahashi M K, *et al*. Rapid, low-cost detection of zika virus using programmable biomolecular components[J]. Cell, 2016, 165(5): 1255-1266. DOI: 10.1016/j.cell.2016.04.059.

[45] Mukama O, Wu J, Li Z, *et al*. An ultrasensitive and specific point-of-care CRISPR/Cas12 based lateral flow biosensor for the rapid detection of nucleic acids[J]. Biosens Bioelectron, 2020, 159: 112143. DOI: 10.1016/j.bios.2020.112143.

[46] Peng R, Chen X, Xu F, *et al*. Pneumatic nano-sieve for CRISPR-based detection of drug-resistant bacteria[J]. Nanoscale Horiz, 2023, 8(12): 1677-1685. DOI: 10.1039/D3NH00365E.

[47] Zhang X, Huang Z, Zhang Y, *et al*. Mitigating antibiotic resistance: the utilization of CRISPR technology in detection[J]. Biosensors (Basel), 2024, 14(12): 633. DOI: 10.3390/bios14120633.

[48] Song Y, Hu Q, Han Y, *et al*. Detection assay of polymyxin resistance coding *mcr-1* gene based on CRISPR/Cas13a system[J]. Front Cell Infect Microbiol, 2025, 15: 1553681. DOI: 10.3389/fcimb.2025.1553681.

[49] Tram T T B, Ha V T N, Trieu L P T, *et al*. FLASH-TB: an application of next-generation CRISPR to detect drug resistant tuberculosis from direct sputum[J/OL]. J Clin Microbiol, 2023, 61(4): e01634-22. https://journals.asm.org/doi/10.1128/jcm.01634-22. DOI: 10.1128/jcm.01634-22.

[50] Thakku S G, Ackerman C M, Myhrvold C, *et al*. Multiplexed detection of bacterial nucleic acids using Cas13 in droplet microarrays[J/OL]. PNAS Nexus, 2022, 1(1): pgac021. https://academic.oup.com/pnasnexus/article/1/1/pgac021/6569121?login=false. DOI: 10.1093/pnasnexus/pgac021.

[51] Sun Y, Yu L, Liu C, *et al*. One-tube SARS-CoV-2 detection platform based on RT-RPA and CRISPR/Cas12a[J]. J Transl Med, 2021, 19(1): 74. DOI: 10.1186/s12967-021-02741-5.

[52] Jiang Y, Hu M, Liu A A, *et al*. Detection of SARS-CoV-2 by CRISPR/Cas12a-enhanced colorimetry[J]. ACS sens, 2021, 6(3): 1086-1093. DOI: 10.1021/acssensors.0c02365.

[53] Hang X M, Wang H Y, Liu P F, *et al*. Cas12a-assisted RTF-EXPAR for accurate, rapid and simple detection of SARS-CoV-2 RNA[J]. Biosens Bioelectron, 2022, 216: 114683. DOI: 10.1016/j.bios.2022.114683.

[54] Mao K, Zhang H, Ran F, *et al*. Portable biosensor combining CRISPR/Cas12a and loop-mediated isothermal amplification for antibiotic resistance gene *ermB* in wastewater[J]. J Hazard Mater, 2024, 462: 132793. DOI: 10.1016/j.jhazmat.2023.132793.

[55] Anderson E M, Haupt A, Schiel J A, *et al*. Systematic analysis of CRISPR-Cas9 mismatch tolerance reveals low levels of off-target activity[J]. J Biotechnol, 2015, 211: 56-65. DOI: 10.1016/j.jbiotec.2015.06.427.

[56] Kim M G, Go M J, Kang S H, *et al*. Revolutionizing CRISPR technology with artificial intelligence[J]. Exp Mol Med, 2025, 57(7): 1419-1431. DOI: 10.1038/s12276-025-01462-9.

[57] Dixit S, Kumar A, Srinivasan K, *et al*. Advancing genome editing with artificial intelligence: opportunities, challenges, and future directions[J]. Front Bioenga Biotechnol, 2023, 11: 1335901. DOI: 10.3389/fbioe.2023.1335901.

[58] Li Y, Liu Y, Tang X, *et al*. CRISPR/Cas-powered amplification-free detection of nucleic acids: current state of the art, challenges, and futuristic perspectives[J]. ACS sens, 2023, 8(12): 4420-4441. DOI: 10.1021/acssensors.3c01463.

[59] Chen Y, Qian S, Yu X, *et al*. Microfluidics: the propellant of CRISPR-based nucleic acid detection[J]. Trends Biotechnol, 2023, 41(4): 557-574. DOI: 10.1016/j.tibtech.2022.07.015.

[60] Li L, Shen G, Wu M, *et al*. CRISPR-Cas-mediated diagnostics[J]. Trends Biotechnol, 2022, 40(11): 1326-1345. DOI: 10.1016/j.tibtech.2022.04.006.

[61] Song D, Xu W, Zhuo Y, *et al*. Orthogonal CRISPR/Cas system facilitated dual-color fluorescence fiber-embedded optofluidic nano-biochip for parallel amplification-free on-site detection of bacterium and virus[J]. Microchim Acta, 2025, 192(7): 417. DOI: 10.1007/s00604-025-07289-5.

[62] Sen A, Masetty M, Weerakoon S, *et al*. Paper-based loop-mediated isothermal amplification and CRISPR integrated platform for on-site nucleic acid testing of pathogens[J]. Biosens and Bioelectron, 2024, 257: 116292. DOI: 10.1016/j.bios.2024.116292.

[63] Kellner M J, Koob J G, Gootenberg J S, *et al*. SHERLOCK: nucleic acid detection with CRISPR nucleases[J]. Nat Protoc, 2019, 14(10): 2986-3012. DOI: 10.1038/s41596-019-0210-2.

(2025 – 10 – 16收稿, 2026 – 03 – 04修回)

编辑 姜 恬

开放获取 本文使用遵循知识共享署名—非商业性使用4.0国际许可协议(CC BY-NC 4.0),详细信息请访问https://creativecommons.org/licenses/by-nc/4.0/。

OPEN ACCESS This article is licensed for use under Creative Commons Attribution-NonCommercial 4.0 International license (CC BY-NC 4.0). For more information, visit https://creativecommons.org/licenses/by-nc/4.0/.

© 2026《四川大学学报(医学版)》编辑部

Editorial Office of *Journal of Sichuan University (Medical Sciences)*