基于质谱的蛋白质组学技术在细菌β-内酰胺酶检测中的应用进展

姜皓文[1,2,3], 吴慧鸽[1,2,3], 李聪然[1,2,3], 卢 芸[1,2,3*], 游雪甫[1,2,3*]

(1. 中国医学科学院 北京协和医学院, 医药生物技术研究所, 抗感染新药研发关键技术及应用北京市重点实验室, 北京 100050; 2. 中国医学科学院病原微生物菌 (毒) 种保藏中心药用微生物相关菌 (毒) 种保藏分中心, 北京 100050; 3. 中国医学科学院 北京协和医学院, 医药生物技术研究所, 天然药物活性物质与功能国家重点实验室, 北京 100050)

摘要: 随着细菌耐药性问题的日益严重, β-内酰胺酶作为导致β-内酰胺类抗生素耐药的关键酶, 其快速精准检测对临床治疗和耐药性监测具有重要意义。近年来, 蛋白质组学技术的快速发展为β-内酰胺酶的高通量鉴定提供了新策略。本文综述了基于质谱的蛋白质组学技术在β-内酰胺酶检测中的应用进展, 相较于传统表型检测和基因测序, 蛋白质组学技术能直接捕获酶表达水平信息, 为阐明耐药表型与蛋白质功能的关联提供了多维数据支持。此外, 靶向蛋白质组学的发展进一步提高了检测的灵敏度和特异性, 推动了个性化抗感染治疗的探索。未来, 结合人工智能辅助及微型化高通量检测设备的开发, 蛋白质组学技术有望在细菌耐药性实时监测和精准诊疗中发挥更重要的作用。

关键词: β-内酰胺酶; 质谱; 蛋白质组学; 细菌耐药性; 肽段标志物

中图分类号: R917 **文献标识码**: A **文章编号**: 0513-4870(2026)04-1085-14

Advances in the application of mass spectrometry-based proteomics in the detection of bacterial β-lactamases

JIANG Hao-wen[1,2,3], WU Hui-ge[1,2,3], LI Cong-ran[1,2,3], LU Yun[1,2,3*], YOU Xue-fu[1,2,3*]

(*1. Beijing Key Laboratory of Technology and Application for Anti-Infective New Drugs Research and Development, Institute of Medicinal Biotechnology, Chinese Academy of Medical Sciences & Peking Union Medical College, Beijing 100050, China; 2. Division for Medicinal Microorganism-Related Strains, CAMS Collection Center of Pathogenic Microorganisms, Beijing 100050, China; 3. State Key Laboratory of Bioactive Substance and Function of Natural Medicines, Institute of Medicinal Biotechnology, Chinese Academy of Medical Sciences & Peking Union Medical College, Beijing 100050, China*)

Abstract: With the increasing severity of bacterial resistance issues, β-lactamase, as the key enzyme mediating resistance to β-lactam antibiotics, requires rapid and accurate detection that plays significant importance for clinical treatment and antimicrobial resistance surveillance. In recent years, the rapid development of proteomic technologies has provided novel strategies for high-throughput identification of β-lactamases. This article reviews the application progress of mass spectrometry-based proteomic technologies in β-lactamase detection, compared with traditional phenotypic detection and genetic sequencing methods, proteomic technologies enable direct capture of enzyme expression-level information, offering multidimensional data support for elucidating the association between resistant phenotypes and protein functions. Furthermore, advancements in targeted proteomics have enhanced detection

收稿日期: 2025-11-06; 修回日期: 2025-12-27.

基金项目: 国家自然科学基金资助项目 (82330110, 32141003, 81803593); 中国医学科学院医学科学创新基金 (2021-I2M-1-030, 2021-I2M-1-039); 中央高校基本科研业务费(3332018094); 科技部国家科技资源共享服务平台“国家病原微生物资源库”项目 (No. NPRC-32).

*通讯作者 E-mail: yunluly@imb.pumc.edu.cn;

Tel: 86-10-67010489, E-mail: xuefuyou@imb.pumc.edu.cn

DOI: 10.16438/j.0513-4870.2025-1307

sensitivity and specificity, facilitating the exploration of personalized anti-infective therapies. Looking forward, through the integration of artificial intelligence-assisted analysis and the development of miniaturized high-throughput detection devices, proteomic technologies are expected to play a more prominent role in real-time antimicrobial resistance monitoring and precision medicine.

Key words: *β*-lactamase; mass spectrometry; proteomic; antimicrobial resistance; peptide marker

抗生素的发现和应用, 堪称20世纪医学史上具里程碑意义的突破之一。从青霉素的偶然发现到大规模临床应用, 这类药物不仅成功攻克了诸多致命的细菌感染性疾病, 极大地提升了全球人均寿命, 更奠定了外科手术、肿瘤化疗等现代医疗技术发展的安全性基石[1]。然而, 抗生素的广泛使用导致了日益严重的细菌耐药性问题, 给感染性疾病诊疗带来了严峻挑战。世界卫生组织 (WHO) 预测, 若人类不采取行动遏制微生物耐药性的发展, 到2050年, 抗生素耐药性每年或将导致1 000万人死亡[2]。中国作为一个细菌耐药形势严峻的国家, 近些年来, 在遏制细菌耐药性方面的工作已取得了诸多进展, 如抗生素使用率下降、部分耐药菌检出率下降等, 但目前仍面临耐药菌不断进化、新型耐药机制涌现、流行亚型动态变化等挑战[3]。中国细菌耐药监测网 (CHINET, www.chinets.com) 的数据显示, 我国临床分离菌的耐药形势依然严峻, 尤其是第三代头孢菌素和碳青霉烯类耐药革兰阴性菌的检出率持续处于高位。*β*-内酰胺类抗生素的细菌耐药性问题, 已然成为各界高度关注的焦点。

β-内酰胺类抗生素包括青霉素类、头孢菌素类、碳青霉烯类和单环*β*-内酰胺类抗生素, 其化学结构中都包含一个四元*β*-内酰胺环, 这一环状结构是该类抗生素与青霉素结合蛋白 (penicillin binding proteins, PBPs) 结合并发挥抗菌作用的关键所在[4]。细菌产生一种或多种*β*-内酰胺酶, 是导致此类抗生素产生耐药性的主要原因[5,6]。同时, *β*-内酰胺酶具有底物特异性, 不同*β*-内酰胺酶对特定抗生素的分解效率不同[7]。

目前对*β*-内酰胺酶及相关耐药性的检测方法大致分为如下几种: ① 以药敏试验为代表的表型检测方法; ② 以PCR方法[8]和基因测序为代表的基因型检测方法; ③ 以mRNA表达水平检测为代表的转录过程检测方法[9]; ④ 以免疫印迹 (Western blot)[10]和侧流免疫层析 (lateral flow immunoassay)[11]为代表的基于免疫反应的检测方法; ⑤ 以Carba NP[12]、头孢硝噻吩试验等为代表的基于酶活性的检测方法; ⑥ 基于质谱的蛋白质组学检测方法等。

早在1929年体外抗菌药敏试验方法就被首次引入, 并沿用至今[13]。表型检测方法虽操作简单且能直接反映临床菌耐药表型, 但难以直接提供细菌携带与表达的*β*-内酰胺酶的详细信息。例如, 由于OXA-48-like在体外对碳青霉烯类药物产生低水平耐药性, 因此携带有OXA-48-like酶的细菌, 对美罗培南最低抑菌浓度 (minimum inhibitory concentration, MIC) 值常低于耐药性判定折点, 在表型检测中易出现耐药性低估或误判[14]。微生物鉴定分析仪 (如bioMérieux Vitek® 2)在检测效率上有很大提升, 但检测范围有限。基因检测因其高灵敏度和特异性, 可直接识别耐药基因的存在, 在耐药流行病学监测和分子溯源研究中具有重要价值。然而, 该方法无法确认所检测到的基因是否被功能性表达, 因此其结果不一定对应实际的耐药表型, 在临床应用中应结合表型试验综合判断。基于转录过程或免疫学的检测方法具有特异性强的优势, 但因操作复杂、成本高、通量低, 限制了其在临床常规检测中的大规模应用。针对特定碳青霉烯酶 (如KPC、NDM、OXA-48-like等) 的侧流免疫层析法已在快速筛查中得到应用[15], 但其检测范围与检测通量, 仍是限制其临床广泛普及的关键因素。酶活性方法兼具快速直观的优势, 但在检测低水平产酶菌株时, 其灵敏度可能偏低, 且无法明确酶的具体类型。其中, 通过分析抗生素水解产物来判定耐药表型的质谱检测方法[16-20], 也属于酶活性方法的一种, 常被用于反映*β*-内酰胺酶的表达情况。基于质谱的蛋白质组学方法, 直接检测*β*-内酰胺酶或其肽段, 可以更准确地解释细菌的耐药表型特征。本文就基于质谱的蛋白质组学技术直接检测*β*-内酰胺酶的方法 (图1)、应用现状及进展进行综述, 为后续开展相关研究提供参考。

1 *β*-内酰胺酶的分类及与其相关的细菌耐药表型

β-内酰胺酶种类繁多, 截至2025年11月份, *β*-内酰胺酶数据库 (BLDB, www.bldb.eu) 已记录超过12 000种*β*-内酰胺酶[21]。目前*β*-内酰胺酶最主要的分类方法有两种, 一是Ambler分类法, 根据氨基酸序列将其分为A、B、C和D四类, 其中A、C和D类以丝氨酸作为活性位点, 所以也称之为丝氨酸*β*-内酰胺酶, B类以金属Zn^{2+}作为活性位点, 又叫金属*β*-内酰胺酶[22]。二是Bush-Jacoby功能分类法, 将酶以各自的底物谱、抑制剂及生化特性为标准, 与临床分离株耐药表型相

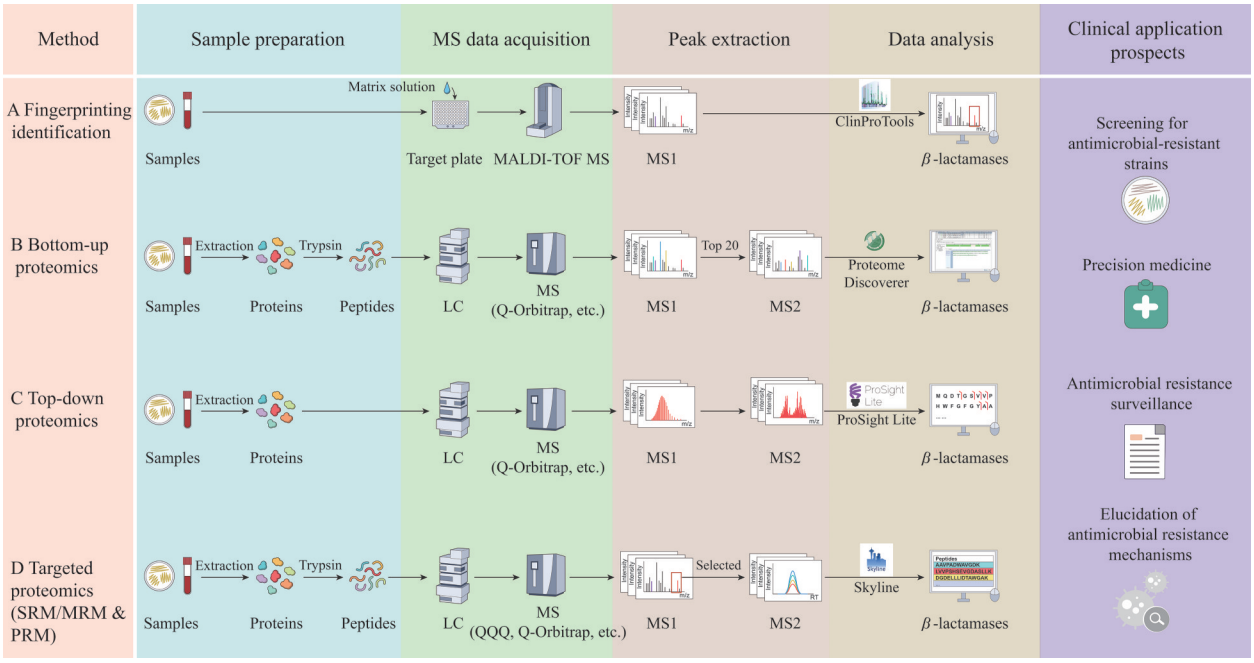


Figure 1 The workflow of detecting β-lactamase using proteomics technology. The "Data analysis" column presents representative analytical software. SRM: Selected reaction monitoring; MRM: Multiple reaction monitoring; PRM: Parallel reaction monitoring; MALDI-TOF MS: Matrix-assisted laser desorption ionization time-of-flight mass spectrometry; LC: Liquid chromatography; MS: Mass spectrometry; QQQ: Triple quadrupole

结合, 分为包含多个功能亚群的4个大组[23,24]。

2024年5月17日, WHO发布的细菌类重点病原体名单 (bacterial priority pathogens list, BPPL), 将耐碳青霉烯类药物的鲍曼不动杆菌及耐碳青霉烯类和第三代头孢菌素的肠杆菌列入关键优先级病原体。碳青霉烯类抗生素被认为是治疗细菌感染的最后一道防线, 用于治疗多药耐药的革兰阴性菌感染, 然而, 近二十年来, 可水解多种抗生素的碳青霉烯酶的不断涌现, 引起了极大关注[25]。最常见的A类碳青霉烯酶为KPC型, 主要分布于肺炎克雷伯菌[26]。D类碳青霉烯酶以OXA酶为主。其中, OXA-23、OXA-58等常见于不动杆菌属; 而OXA-48常见于肠杆菌目, 亦可见于不动杆菌属[19,27]。B类碳青霉烯酶全球流行类型包括IMP、VIM和NDM等。除氨曲南外, 它们可水解几乎所有β-内酰胺类抗生素[28]。NDM、KPC及OXA-48是目前碳青霉烯耐药肠杆菌目 (carbapenem-resistant Enterobacteriaceae, CRE) 菌株中最常见的三大碳青霉烯酶类型[29]。

第三代头孢菌素耐药的主要原因是超广谱β-内酰胺酶 (extended spectrum beta-lactamases, ESBLs) 的产生和AmpC的过表达。ESBLs通常由质粒介导, 可水解青霉素类、第一至第三代头孢菌素类及单环β-内酰胺类抗生素 (如氨曲南), 对碳青霉烯类和头霉素类水解作用较差, 可被克拉维酸和他唑巴坦等β-内酰胺酶抑制剂抑制[7,30]。ESBLs广泛存在于革兰阴性肠杆菌科细菌, 如大肠埃希菌、肺炎克雷伯菌等常见临床分离株中, 常见类型包括A类丝氨酸酶中的SHV、TEM和CTX-M等[7,24]。AmpC属于Ambler C类酶, 通常存在于细菌染色体上, 在某些β-内酰胺类抗生素存在的情况下可被诱导表达, 部分也可以通过质粒来高水平表达和传播, 例如CMY-2、DHA-1等[31]。此类酶可水解大多数第一至第三代头孢菌素, 菌株仅高水平表达AmpC通常不影响其对碳青霉烯类抗生素的敏感性[31], 但也有报道显示携带编码AmpC质粒且孔蛋白缺失的肺炎克雷伯菌对碳青霉烯类药物耐药[32]。

如上所述, 细菌β-内酰胺酶的产生给临床用药和治疗带来了极大压力, 能够快速和准确地鉴定如此多且复杂的β-内酰胺酶可以极大地提高临床诊断效率, 助力合理用药和减缓耐药性的发生。

2 质谱技术与蛋白质组学的发展

质谱技术自其诞生以来, 经过了一个多世纪的发展, 逐步演变为现代蛋白质组学的核心工具。早期质谱主要用于分析气态分子和原子, 然而, 在分析复杂的生物大分子时, 其应用受到了明显限制。这一瓶颈的突破始于二十世纪中后期, 快速原子轰击 (fast atom bombardment, FAB)[33]和化学电离 (chemical ionization, CI)[34]技术的相继问世, 显著拓展了质谱的分析范围。20世纪80年代, 基质辅助激光解吸电离 (matrix-assisted laser desorption ionization, MALDI) 和电喷雾

电离 (electrospray ionization, ESI) 两项革命性电离技术的诞生, 真正攻克了生物大分子 (尤其是蛋白质) 高效、温和离子化的技术难题, 为蛋白质组学这门学科的兴起奠定了核心技术基础[35-37]。在此后的二十年里, 相关技术体系不断完善, 基于质谱的蛋白质鉴定与定量检测的标准方法学逐步建立。伴随着基因组学研究的发展和质谱分析技术与生物信息学之间的有机结合, 高通量蛋白质鉴定效率产生了质的飞跃。值得关注的是, Orbitrap™和Astral™等高分辨率质量分析器的迭代创新, 为解析复杂生物样本中的低丰度蛋白质及蛋白翻译后修饰提供了全新解决方案。当前, 基于高精度质谱的蛋白质组学技术已具备单次实验定量解析人类细胞系中逾10 000种蛋白质的能力[38]。特别在转化医学领域, 靶向蛋白质组学技术通过选择反应监测/多重反应监测 (selected reaction monitoring/multiple reaction monitoring, SRM/MRM) 和平行反应监测 (parallel reaction monitoring, PRM) 技术, 能够对特定蛋白的肽段标志物进行定量, 在临床生物标志物的发现与验证领域展现出独特优势[39]。这种技术演进推动了微生物表征的标准化, 并为精准医学时代的疾病机制研究和诊疗优化提供了强大支撑。

3 基于质谱的非靶向蛋白质组学在*β*-内酰胺酶检测中的应用

3.1 基于一级质谱指纹图谱的鉴定技术 基质辅助激光解吸电离−飞行时间质谱 (MALDI-TOF MS) 技术是一种适合在100～100 000 Da内快速评估分子质量的分析方法, 可用于蛋白质的分子量测定、鉴定和高通量筛选等[40]。此技术已广泛应用于微生物鉴定、蛋白质分析、疾病诊断等多个领域[16,20]。在体外诊断领域, 美国食品药品监督管理局 (FDA) 目前已批准两种商用系统—布鲁克 (Bruker Daltonics Inc.) 的MALDI Biotyper CA系统和生物梅里埃 (bioMérieux Inc.) 的Vitek® MS系统[41]。基于蛋白质谱图与数据库指纹图谱匹配的原理, 这些系统可实现对微生物的快速鉴定。此外, 该技术也适用于分析菌株间的亲缘或同源性关系。

在细菌耐药性检测方面, 该技术主要通过比较耐药与敏感菌株 (或数据库) 的蛋白质或酶解肽段的MALDI-TOF MS指纹谱图, 识别差异峰 (或特征峰) 来推测细菌耐药性。Camara等[42]使用MALDI-TOF MS分析了野生型与氨苄西林耐药的大肠埃希菌的蛋白提取物, 发现可能与*β*-内酰胺酶相关的*m*/*z* 29 000左右处的特征峰, 并通过凝胶电泳和肽指纹图谱分析加以证实。Espinosa等[43]建立了一种基于MALDI-TOF MS的快速检测策略, 研究发现所有产CMY-2大肠埃希菌中均存在约*m*/*z* 39 800的特征峰, 该技术方法可实现从阳性血培养样本中检测对应耐药酶。在此基础上, 研究者[44]进一步从菌落和阳性血培养样本中发现约*m*/*z* 28 544的特征峰与KPC-2相关, 为临床碳青霉烯酶的快速筛查提供了高效可行的新途径。2020年的一项研究运用机器学习方法, 通过比较多药耐药大肠埃希菌在有无抗生素压力培养下的蛋白提取物, 利用MALDI-TOF MS检测到了多种抗生素耐药性相关的特征峰[45]。Yu等[46]将机器学习和结果评估方法应用于MALDI-TOF MS数据, 建立了快速预测肺炎克雷伯菌对碳青霉烯类药物和黏菌素耐药性的模型, 发现*m*/*z* 4 520～4 529内的峰是区分KPC与OXA、NDM、IMP、VIM的关键潜在生物标志峰。

正如上文所述, 图谱鉴定的方法操作简便、检测高效, 但其进一步发展仍受到若干固有局限的制约: ① 可检测性和可重复性差: 该方法高度依赖于敏感与耐药菌株间存在的特异性差异峰, 导致其在实际应用中的可检测性和结果重复性均面临挑战, 亟需通过后续研究进行深入验证; ② 低丰度蛋白易漏检: 该技术的鉴定效果在很大程度上依赖于特征峰信息匹配质量, 当前数据库的准确性和覆盖广泛性需进一步标准化; ③ 共存蛋白干扰: 在复杂样本中, 仅依赖一级质谱峰信号进行判断, 耐药酶的特征峰易受共存蛋白质信号的干扰或掩盖, 从而影响鉴定可靠性且无法区分近缘突变体; ④ 定量能力弱。因此, 为了进一步提高鉴定的准确性和可靠性, 二级质谱和基于肽段的自下而上的蛋白质组 (bottom-up proteomics) 策略逐渐被引入到*β*-内酰胺酶的检测中。通过二级质谱对母离子进行碎片化分析, 获得更丰富的结构信息, 提升了鉴定的精确性。例如, Russell等[47]对携带*β*-内酰胺酶质粒的大肠埃希菌进行全蛋白及酶解肽段的MALDI-TOF MS PSD碎裂分析, 通过图谱变化成功判断质粒插入与表达情况。Papagiannitsis等[48]使用MALDI-TOF MS直接检测临床肠杆菌科细菌, 发现约*m*/*z* 39 850处存在特征峰, 经胶内酶解和MALDI-TOF/TOF MS进一步证实为一种*C. freundii*-like *β*-内酰胺酶, 且仅在产CMY的菌株中发现。

3.2 自下而上蛋白质组学非靶向策略 自下而上蛋白质组学又称鸟枪法蛋白质组学 (shotgun proteomics), 是全蛋白质谱分析的常用方法。它通过酶解蛋白质为肽段后进行串联质谱分析, 实现蛋白质的定性和定量。该方法高效便捷, 可在短时间内完成微生物全蛋白组分析[49]。该方法中, *β*-内酰胺酶经酶解消化后, 所产生的肽段经一级质谱全扫描检测, 随后筛选丰度较高的母离子进行碎裂处理, 再通过二级质谱分析获得肽段碎片离子信息, 将该信息与*β*-内酰胺酶数据库进行搜

索比对, 进而获取β-内酰胺酶相关信息。例如, Chang等[50]基于鸟枪法技术检测鲍曼不动杆菌的酶解肽段, 并构建了专属肽段鉴定数据库, 进而开发出该菌快速耐药性鉴定方法。Fleurbaaij等[51]采用毛细管电泳−电喷雾电离−串联质谱同步鉴定OXA-48、KPC等β-内酰胺酶。该团队后续[52]借助鸟枪法在血培养样本中检测CTX-M, 在170份样本中表现出95%的灵敏度与100%的特异性, 进一步证实自下而上蛋白质组学方法在β-内酰胺酶检测中的可行性。Hart等[53]通过LC-MALDI MS/MS, 在大肠埃希菌周质中检测到多种β-内酰胺酶的肽段标志物。Foudraine等[54]对187株大肠埃希菌和肺炎克雷伯菌进行了质谱分析, 证实鸟枪法蛋白质组学能表征β-内酰胺酶等多种耐药相关蛋白。此外, Hillyer等[55]结合凝胶分离与LC-MS/MS技术, 鉴定了鲍曼不动杆菌在不同抗生素压力下的β-内酰胺酶表达谱, 既揭示了耐药性发展机制, 也为解决多药耐药性问题提供了新视角。

上述研究共同证实了自下而上蛋白质组学在检测与表征β-内酰胺酶中的有效性。然而, 该方法的局限性在于: ① 蛋白质整体信息丢失。由于需将完整的β-内酰胺酶酶解成肽段分析, 从而无法直接获得完整蛋白质的精确分子量、复杂的翻译后修饰组合以及不同蛋白质异构体信息等; ② 肽段检测遗漏。低丰度的耐药酶可能被高丰度的蛋白质掩盖; 在复杂样本中, 检测的深度和通量易受色谱分离条件, 如上样量、洗脱梯度等因素制约, 可能导致某些肽段无法有效被鉴定, 序列覆盖度不完整, 从而可能遗漏重要的变异体或修饰位点信息; ③ 亚型区分有限。数据分析高度依赖于能否可靠检测到酶亚型特异性的肽段, 因此对β-内酰胺酶亚型的区分能力有限; ④ 数据分析制约。数据分析的准确性高度依赖于数据库的完整性与更新程度, 并受到不同分析软件、参数设置以及数据库本身可能缺失特定β-内酰胺酶信息的共同制约。鉴于此, 着眼于提升低丰度靶标检出能力与分辨率的迫切需求, 靶向蛋白质组学策略 (例如SRM、PRM) 已成为重要解决方案, 有望显著推动β-内酰胺酶检测的灵敏度、特异性和定量准确性方面的技术进展。

3.3 自上而下蛋白质组学策略 自上而下蛋白质组学 (top-down proteomics) 是通过MS技术直接对完整蛋白质进行分析的策略, 相较于仅依赖于一级质谱检测策略, 此方法通常使用串联质谱, 旨在实现蛋白质全局和综合性分析, 从而发现新的蛋白质形态、完成深入的序列和翻译后修饰 (post-translational modifications, PTMs) 表征等[56]。

目前, 该技术通常被应用于β-内酰胺酶耐药相关生物标志物峰的鉴定, 例如, Maus等[57]开发了离线LC结合MALDI-TOF-MS/MS的技术方案, 对10株来自欧洲椋鸟肠道的β-内酰胺耐药/敏感大肠杆菌进行分析, 筛选出与β-内酰胺耐药强相关的*m*/*z* 9 355蛋白标志物, 经自上而下MS/MS推断此峰对应一种DNA结合HU蛋白, 由IncA/C质粒上*bla*CMY-2基因转录而来。Yoon等[58]使用LC-MS/MS确认重组株中KPC-2完整蛋白分子质量为28 718.13 Da, 并基于此建立MALDI-TOF MS检测KPC的方法, 实现对产KPC肠杆菌的快速鉴定。McGee等[59]建立了一种基于纳升液相和高分辨率质谱的完整蛋白质检测策略, 可直接在未经纯化的细菌裂解液中直接检测肺炎克雷伯菌产生的KPC, 是该方法应用于β-内酰胺酶的突破性进展。该研究采用自下而上与自上而下联合分析策略, 可区分不同KPC变体 (KPC-2, 3, 4, 5)。随后, 他们[60]进一步拓展该研究框架, 可在细菌裂解液中直接检测共表达的OXA-48-like和CTX-M-15完整蛋白。

尽管自上而下蛋白质组学能够在单次分析中直接解析完整蛋白的完整序列与翻译后修饰信息, 但其主要应用局限在于: ① 灵敏度和通量低。本技术一次只能分析单个蛋白或者有限种类蛋白, 限制了检测通量和覆盖范围; ② 数据分析困难。在数据解卷积及算法标准化等方面仍存在瓶颈; ③ 蛋白质电离过程局限。与肽段相比, 完整蛋白质在ESI过程中不仅电离效率偏低, 造成检测灵敏度受限, 还会产生更宽的电荷态分布和更为复杂的碎片离子信息, 显著增加了谱图解析复杂度[61]。在β-内酰胺酶检测应用中, 目前该方法仅适用于有限的几种而非所有的β-内酰胺酶种类。

4 基于质谱的靶向蛋白质组学在β-内酰胺酶检测中的应用

4.1 基于质谱的靶向蛋白质组学简介 基于质谱的靶向蛋白质组学 (主要包括SRM/MRM和PRM技术) 利用串联质谱的高效分离检测能力, 通过精准选择监测特定肽段序列, 实现对复杂生物样本中目标蛋白的高灵敏度、高特异性与定量分析[62-65]。

SRM/MRM技术在靶向蛋白质组学中较早出现且应用广泛, 它通常借助QQQ等仪器实现, 精准选择目标母子离子对进行检测和定量分析[66]。随着高分辨质谱技术的不断发展, PRM技术也崭露头角并不断发展完善。定量性能方面, 其与SRM/MRM技术相比, 具备相似的线性范围、动态范围、定量精度及可重复性。此外, 由于PRM技术可选择特定母离子并监测其全部子离子碎片信息, 不仅更易构建数据采集方法, 还表现出更高的特异性[67,68]。通过SureQuant等技术优化和升级, 其检测通量和灵敏度进一步提高[69]。

4.2 靶向蛋白质组学技术在*β*-内酰胺酶检测中的应用 靶向蛋白组学作为一种针对性强、定量精确的蛋白质分析技术, 在生物医学研究、临床诊断以及系统生物学等领域得到了广泛应用[65]。随着靶向技术的不断发展, 在以*β*-内酰胺酶相关特异性肽段作为生物标志物的检测方法展现出巨大潜力。

早期研究确立了SRM/MRM在检测*β*-内酰胺酶方面的可靠性。这些研究通常采用多种技术手段筛选出候选标志性肽段, 然后基于标志性肽段的母离子和离子碎片开发方法进行验证。例如, Charretier等[70]针对铜绿假单胞菌的抗生素耐药机制检测开发SRM方法, 发现该方法在外排泵、OprD及AmpC *β*-内酰胺酶等机制的分类与耐药预测上与药敏试结果高度一致, 并优于RT-qPCR方法。随后, 多项研究成功将SRM/MRM应用于各类*β*-内酰胺酶的检测, 如Wang等[71,72]在不到90 min内检测3条KPC标志性肽段或3条NDM标志性肽段; Strich等[73]在不到90 min内检测的2条OXA-48标志性肽段; Lovison等[74]在具有多耐药背景的肠杆菌目细菌中检测4条KPC标志性肽段, 以上研究结果均在临床分离株样本中展现100%的特异性和高灵敏度。Cecchini等[75]整合基因组学和无标记SRM技术, 在39株鲍曼不动杆菌中成功定量检测与抗生素耐药性相关的16种蛋白质, 包含*β*-内酰胺酶和外排泵等, 从而解析多重耐药表型的分子机制。

随着高分辨率质谱仪的普及, PRM技术因其更高的特异性和更强的多重检测能力得到广泛应用。它能够同时监测多个目标蛋白的所有碎片离子, 减少了方法开发的复杂性, 并适合大规模靶标筛查。为解决临床中碳青霉烯酶检测方法的局限, Foudraine等[76]采用PRM技术同时检测KPC、OXA-48-like、NDM和VIM的肽段标志物, 在83株菌株中实现100%特异性。随后, 在针对阳性血液样本的检测, 他们[77]开发实现了27种包含常见的*β*-内酰胺酶、氨基糖苷类和氟喹诺酮耐药机制的快速检测, 从血液样本到结果耗时约3 h, 且结果与全基因测序高度一致, 为血液样本PRM方法开发提供支持。有意思的是, 研究者[78]通过对耐药诱导过程中部分耐药机制相关蛋白表达的监测, 成功揭示了大肠埃希菌在美罗培南作用下通过减少孔蛋白表达和增加CMY-2型*β*-内酰胺酶表达而产生耐药性的机制, 进一步拓展了靶向蛋白组在耐药机制解析中的应用。作者前期研究[79]建立了一种基于PRM技术的检测方法, 可直接对单菌落来源的蛋白质进行鉴定, 成功覆盖了碳青霉烯酶和ESBLs在内的8类*β*-内酰胺酶。该方法对大部分目标肽段的检测均显示出较高的灵敏度和特异性。鉴于特异性肽段的检测方法的灵敏度和特异性在很大程度上取决于这些特异性肽段对不同亚型的覆盖范围, 以及这些肽段与临床菌株中实际存在的亚型的匹配程度。因此, 进一步提出基于肽段组合的阳性判别方法, 结果显示OXA-1、CMY-2和CTX-M-9 group的检测灵敏度得到了显著提升, 为临床耐药菌的快速识别和合理用药指导提供了更有力的支持[79]。随着新技术手段发展, 作者[80]建立了基于TEM和SHV的5个高亚型覆盖率特异性肽段的SureQuant检测方法, 并发现了较大比例菌株存在*bla*TEM和*bla*SHV的低表达与不表达情况, 这提示靶向蛋白质组学技术在表型预测方面具有巨大的优势。表1[70-80]总结了目前已报道的用于靶向检测各类*β*-内酰胺酶的特异性肽段及其应用性能。

Table 1 Peptide markers of targeted proteomics in the detection of *β*-lactamase. [a]Methodological data are presented herein; refer to the literature for detailed peptide results. CI: Confidence intervals; NA: Not applicable

Category	Subtypes included	Peptide	Method	Sample	Sample size	Sensitivity/%, CI/%	Specificity/%, CI/%	Ref.
Ambler A								
TEM	Uncovered subtypes: 60, 139, 178, 210	VGYIELDLNSGK	PRM	Strains	188	91, 83-96	74, 64-83	[79]
	Uncovered subtypes: 60, 147, 167, 176, 183 and 4 unannotated subtypes	SALPAGWFIADK	PRM	Blood culture	241	98, 93-100[a]	96, 90-98[a]	[77]
	NA	VDAGQEQLGR	SRM	Strains	39	NA	NA	[75]
	NA	VLLC[CAM]GAVLSR	SRM	Strains	39	NA	NA	[75]
	Uncovered subtypes: 101, 104, 151, 164 and 6 unannotated subtypes	QIAEIGASLIK	PRM	Blood culture	241	98, 93-100[a]	96, 90-98[a]	[77]
	1, 4-6, 9, 10, 12, 15, 17, 19, 20, 26, 28-37, 39, 40, 43, 45, 47-49, 52-55, 57, 63, 68, 70, 71, 76-88, 90-99, 102-112, 115, 116, 120, 122-128, 131-133, 135-138, 142-154, 156-159, 163, 166-169, 171, 176, 181-191, 193-198, 201, 205-208, 210-215, 217, 219, 220, 224-226, 228-240, 242-252	DAEDQLGAR	SureQuant	Strains	102	NA	NA	[80]

Continued

Category	Subtypes included	Peptide	Method	Sample	Sample size	Sensitivity/%, CI/%	Specificity/%, CI/%	Ref.
	1-4, 15, 17, 19-22, 30-37, 40, 45, 47-49, 52, 54, 55, 57, 67, 68, 70-72, 76, 77, 79-82, 84, 88, 90, 92-99, 101-106, 108, 110-113, 116, 120, 122-124, 126-128, 135, 139, 141, 142, 145, 146, 148, 150, 153, 156, 157, 159, 160, 162-164, 166-168, 171, 176, 181, 183, 188, 189, 191, 196-198, 201, 206-217, 219, 220, 225-231, 233-235, 237, 239, 240, 243, 244, 246-249	WEPELNEAIPNDER	SureQuant	Strains	102	NA	NA	[80]
SHV	Uncovered subtypes: 75, 103, 137, 150 and 9 unannotated subtypes	GIVALLGPNNK	PRM	Blood culture	241	89, 79-95[a]	97, 92-99[a]	[77]
	Uncovered subtypes: 15, 135, 156 and 4 unannotated subtypes	VDAGDEQLER	PRM	Blood culture	241	89, 79-95[a]	97, 92-99[a]	[77]
	1, 1b-b, 2a, 2-9, 11-16, 18, 19, 24-32, 34-38, 40-46, 48-52, 55, 57, 59, 60-67, 69-71, 74-82, 85, 86, 89, 92-111, 115, 116, 119-121, 122b, 128, 129, 132-135, 137, 141-165, 168, 171-173, 175, 178-180, 182, 183, 185-191, 193-207, 209-230, 232-237, 239-244	SVLPAGWFIADK	SureQuant	Strains	102	NA	NA	[80]
	1, 1b-b, 2a, 2-5, 7-9, 11-16, 18, 24-36, 38, 40-46, 48-52, 55-57, 59-67, 69-82, 85, 86, 89, 92-111, 115, 116, 119-121, 122b, 132-135, 137, 141-147, 149-154, 156-162, 164, 165, 168, 171-173, 175, 178-180, 182, 183, 185-189, 191, 193-195, 197-210, 212-218, 220-225, 227-243	NQQIAGIGAALIEHWQR	SureQuant	Strains	102	NA	NA	[80]
	1, 1b-b, 2a, 2-9, 11-16, 18, 24-38, 40-46, 48-50, 52, 55, 56, 59-67, 69-78, 80-82, 84-86, 89, 92-110, 115, 116, 119-121, 128, 129, 132-135, 137, 141, 142, 144-165, 168, 171-173, 175, 178-180, 182, 183, 185-187, 189-191, 193, 194, 196-234, 238-242, 244, 245	WETELNEALPGDAR	SureQuant	Strains	102	NA	NA	[80]
CTX-M	1, 3, 10, 12, 15, *etc*. (CTX-M-1 group)	APLILVTYFTQPQPK	PRM	Strains	188	98, 89-100	99, 95-100	[79]
	1, 2, 5, 12, 15, *etc*. (CTX-M-1 group partial)	SDLVNYNPIAEK	PRM	Strains	188	76, 83-86	95, 89-98	[79]
	Uncovered subtypes: 68, 177 and 3 unannotated subtypes (CTX-M-1 group)	QLGDETFR	PRM	Blood culture	241	100, 94-100[a]	100, 97-100[a]	[77]
	Uncovered subtypes: 4 anonymous types (CTX-M-1 group)	AQLVTWMK	PRM	Blood culture	241	100, 94-100[a]	100, 97-100[a]	[77]
	9, 13, 14, 17, 21, 19, 24, 65, 81, *etc*. (CTX-M-9 group)	QLTLGHALGETQR	PRM	Strains	188	39, 27-51	100, 96-100	[79]
	9, 13, 14, 16, 21, 17, 19, 81, *etc*. (CTX-M-9 group)	LIAQLGGPGGVTAFAR	PRM	Strains	188	81, 70-89	100, 96-100	[79]
	Uncovered subtypes: 4, 6, 7, 19, 23, 35, 42, 52, 54, 58, 62, 74, 87, 93, 99, 117, 126, 144, 147, 151, 155, 157, 168, 204, 219, 212, 221	TEPTLNTAIPGDPR	PRM	Strains	188	98, 93-100	97, 89-100	[79]
GES	NA	AAEIGVAIVDPQGEIVAGHR	SRM	Strains	39	NA	NA	[75]
	NA	DWVVGEK	SRM	Strains	39	NA	NA	[75]
	NA	DYAVAVYTTAPK	SRM	Strains	39	NA	NA	[75]
	NA	LSYGPDMIVEWSPATER	SRM	Strains	39	NA	NA	[75]
	NA	NDIGFFK	SRM	Strains	39	NA	NA	[75]
PER	NA	EQIESIVIGK	SRM	Strains	39	NA	NA	[75]
	NA	FPMQSVFK	SRM	Strains	39	NA	NA	[75]
	NA	GAAEILK	SRM	Strains	39	NA	NA	[75]
	NA	GLLPAGTVVAHK	SRM	Strains	39	NA	NA	[75]

Continued

Category	Subtypes included	Peptide	Method	Sample	Sample size	Sensitivity/%, CI/%	Specificity/%, CI/%	Ref.
	NA	LDLNQTVIVNR	SRM	Strains	39	NA	NA	[75]
	NA	TAATNDLGIILLPDGR	SRM	Strains	39	NA	NA	[75]
	NA	TQLSETSQALLWK	SRM	Strains	39	NA	NA	[75]
	NA	VLQNTWAPIMK	SRM	Strains	39	NA	NA	[75]
	NA	WMVETTTGPER	SRM	Strains	39	NA	NA	[75]
VEB	NA	IEITPQDLLPK	SRM	Strains	39	NA	NA	[75]
	NA	IISDIAK	SRM	Strains	39	NA	NA	[75]
	NA	LLIDTYNNK	SRM	Strains	39	NA	NA	[75]
KPC	2-15, 25	APIVLAVYTR	SRM	Strains	100	100[a]	100[a]	[71]
	NA	APIVLAVYTR	MRM	Strains	111	96.07[a]	100[a]	[74]
	Uncovered subtypes: none	APIVLAVYTR	PRM	Blood culture	241	100, 52-100[a]	99, 97-100[a]	[77]
	2-15, 25	LTLGSALAAPQR	SRM	Strains	100	100[a]	100[a]	[71]
	NA	LTLGSALAAPQR	MRM	Strains	111	96.07[a]	100[a]	[74]
	NA	LALEGLGVNGQ	MRM	Strains	111	96.07[a]	100[a]	[74]
	2-15, 25	GFLAAAVLAR	SRM	Strains	100	100[a]	100[a]	[71]
	NA	GFLAAAVLAR	MRM	Strains	111	96.07[a]	100[a]	[74]
	Uncovered subtypes: none	GFLAAAVLAR	PRM	Blood culture	241	100, 52-100[a]	99, 97-100[a]	[77]
	1-19, 21, 22, 24-26, 28 and 1 unannotated subtype	SIGDTTFR	PRM	Strains	83	100, 76-100	100, 93-100	[76]
	1-19, 21, 22, 24-26, 28 and 1 unannotated subtype	FPLCSSFK	PRM	Strains	83	100, 76-100	100, 93-100	[76]
	1-95	AAVPADWAVGDK	PRM	Strains	188	100, 91-100	100, 97-100	[79]
	1-95 except for 13, 45, 59	SQQQAGLLDTPIR	PRM	Strains	188	98, 88-100	100, 97-100	[79]
Ambler B								
VIM	1-12, 14-20, 23-46, 49-51 and 11 unannotated subtypes	VGGVDVLR	PRM	Strains	83	100, 72-100	100, 94-100	[76]
	1-12, 14-20, 23-46, 49-51 and 11 unannotated subtypes	VGGVDVLR	PRM	Blood culture	241	100, 46-100[a]	100, 98-100[a]	[77]
	1-6, 8-12, 14-20, 23-46, 49-51 and 11 unannotated subtypes	NTAALLAEIEK	PRM	Strains	83	92, 62-100	100, 94-100	[76]
	1-6, 8-12, 14-20, 23-46, 49-51 and 11 unannotated subtypes	NTAALLAEIEK	PRM	Blood culture	241	100, 46-100[a]	100, 98-100[a]	[77]
	1, 4, 5, *etc*.	LAEAEGNEIPTHSLEGLSSSGDAVR	PRM	Strains	188	100, 20-100	100, 97-100	[79]
	Uncovered subtypes: 7, 13, 47, 61, 69	DGDELLLIDTAWGAK	PRM	Strains	188	100, 20-100	100, 97-100	[79]
IMP	1, 5, 7, 10, 28, 30, 34, 40, 42, 43, 52, 55, 60, 61, 66, 70, 73, 76, 77, 79, 81, 85, 88 (IMP-1)	LVVPSHSEVGDASLLK	PRM	Strains	188	100, 60-100	100, 97-100	[79]
	1, 3, 6, 10, 25, 30, 34, 40, 42, 52, 55, 60, 61, 66, 70, 76-80, 88 (IMP-1)	VQATNSFSGVNYWLVK	PRM	Strains	188	100, 60-100	100, 97-100	[79]
	4, 26, 38, 59, 89 (IMP-4)	NSFGGVNYWLVK	PRM	Strains	188	58, 37-77	100, 97-100	[79]
	1, 3, 4, 5, 6, 7, 15, 25, 28, 29, 34, 38, 51, 52, 60, 61, 62, 59, 70, 79, 81, 82, 85	LDEGVYVHTSFEEVNGWGVVPK	PRM	Strains	188	92, 73-99	85, 79-90	[79]
NDM	1, 2, 3, 4, 5, 7, 8, 9, 10, 11, 13, 14	SLGNLGDADTEHYAASAR	MRM	Strains	100	87.5, 67.6-97.3[a]	100, 95.3-100[a]	[72]
	1-15	ASMIVMSHSAPDSR	MRM	Strains	100	87.5, 67.6-97.3[a]	100, 95.3-100[a]	[72]
	1-15	AFGAAFPK	MRM	Strains	100	87.5, 67.6-97.3[a]	100, 95.3-100[a]	[72]
	1-31	AFGAAFPK	PRM	Strains	188	100, 80-100	100, 97-100	[79]
	Uncovered subtypes: none	AFGAAFPK	PRM	Blood culture	241	100, 75-100[a]	100, 98-100[a]	[77]
	1-16 and 1 unannotated subtype	FGDLVFR	PRM	Strains	83	100, 76-100	100, 93-100	[76]
	1-16 and 1 unannotated subtype	FGDLVFR	PRM	Blood culture	241	100, 75-100[a]	100, 98-100[a]	[77]
	1	FGDLVFR	SRM	Strains	39	NA	NA	[75]
	1-16 and 1 unannotated subtype	QEINLPVALAVVTHAHQDK	PRM	Strains	83	25, 8-53	100, 93-100	[76]

Continued

Category	Subtypes included	Peptide	Method	Sample	Sample size	Sensitivity/%, CI/%	Specificity/%, CI/%	Ref.
Ambler C								
CMY	2, 4, 5, 6, 7, *etc*. (CMY-2 family)	ADIANNHPVTQQTLFELGSVSK	PRM	Strains	188	27, 12-50	100, 97-100	[79]
	2, 4, 5, 6, 7, *etc*. (CMY-2 family)	TLQQGIALAQSR	PRM	Strains	188	82, 59-94	100, 97-100	[79]
	36, 55, 60, 145 (CMY-2 like)	TFNGVLGGDAIAR	PRM	Blood culture	241	100, 66-100[a]	99, 96-100[a]	[77, 78]
	40, 45, 49, 144, 157 (CMY-2 like)	TGSTGGFGSYVAFVPEK	PRM	Blood culture	241	100, 66-100[a]	99, 96-100[a]	[77, 78]
cAmpC	14, 20, 75, 112, 113, 121, 157-159, 169, 212, 225, 247-249, 254, 272, 281, 292, 325, 333, 374, 384-395	SSSDLLR	PRM	Blood culture	241	7, 4-12[a]	100, 95-100[a]	[77, 78]
	14, 36, 75, 112, 113, 121, 169, 223, 237, 247, 248, 249, 281, 291, 297, 333, 374, 384-395	QPVTQQTLFELGSVSK	PRM	Blood culture	241	7, 4-12[a]	100, 95-100[a]	[77, 78]
	NA	AVNSSTIFELGSVSK	SRM	Strains	39	NA	NA	[75]
	NA	HSYVNVPK	SRM	Strains	39	NA	NA	[75]
	NA	ISFDDTPGK	SRM	Strains	39	NA	NA	[75]
	NA	LFTATAGGYAK	SRM	Strains	39	NA	NA	[75]
	NA	LVDQNFKPLLEK	SRM	Strains	39	NA	NA	[75]
	NA	TGSTNGFGTYVVFIPK	SRM	Strains	39	NA	NA	[75]
	NA	DLGLVILANR	SRM	Strains	44	NA	NA	[70]
	NA	FVDANLHPER	SRM	Strains	44	NA	NA	[70]
	NA	LPAPQALEGQR	SRM	Strains	44	NA	NA	[70]
	NA	SLGQPFER	SRM	Strains	44	NA	NA	[70]
	NA	TGSTNGFGAYVAFVPGR	SRM	Strains	44	NA	NA	[70]
	NA	TSAADLLR	SRM	Strains	44	NA	NA	[70]
	NA	VGPGPLDAEGYGVK	SRM	Strains	44	NA	NA	[70]
Ambler D								
OXA-1-like	1, 4, 31, 47, 224, 320, 392, 534, 675	NNGLTEAWLESSLK	PRM	Strains	188	78, 52-93	100, 97-100	[79]
	1, 4, 31, 47, 224, 320, 392, 534, 675	IINHNLPVK	PRM	Strains	188	78, 52-93	100, 97-100	[79]
	Uncovered subtypes: none	TLQNGWFEGFIISK	PRM	Blood culture	241	100, 90-100[a]	100, 98-100[a]	[77]
	Uncovered subtypes: 1 unannotated subtype	TGAGFTANR	PRM	Blood culture	241	100, 90-100[a]	100, 98-100[a]	[77]
OXA-48-like	48, 54, 162, 163, 181, 199, 204, 232, 244, 245, 247, 370	ANQAFLPASTFK	MRM	Strains	98	100[a]	100[a]	[73]
	48, 162, 163, 181, 199, 204, 232, 244, 245, 247, 370 and 3 unannotated subtypes	ANQAFLPASTFK	PRM	Strains	83	100, 81-100	100, 93-100	[76]
	48, 162, 163, 181, 199, 204, 232, 244, 245, 247, 370 and 3 unannotated subtypes	ANQAFLPASTFK	PRM	Blood culture	241	100, 60-100[a]	100, 97-100[a]	[77]
	48, 54, 162, 163, 181, 199, 204, 232, 244, 247, 370	YSVVPVYQEFAR	MRM	Strains	98	100[a]	100[a]	[73]
	48, 162, 163, 181, 204, 232, 244, 245, 247, 370	SWNAHFTEHK	PRM	Strains	83	100, 81-100	100, 93-100	[76]
	Uncovered subtypes: 199 and 3 unannotated subtypes	SQGVVVLWNENK	PRM	Blood culture	241	100, 60-100[a]	100, 97-100[a]	[77]
OXA	23	ANTEYVPASTFK	SRM	Strains	39	NA	NA	[75]
	23	DMTLGEAMK	SRM	Strains	39	NA	NA	[75]
	23	INLYGNALSR	SRM	Strains	39	NA	NA	[75]
	23	LSAVPVYQELAR	SRM	Strains	39	NA	NA	[75]
	23	MLNALIGLENQK	SRM	Strains	39	NA	NA	[75]
	23	NMLLLEESNGYK	SRM	Strains	39	NA	NA	[75]
	23	NTSGVLVIQTDK	SRM	Strains	39	NA	NA	[75]
	23	TDINEIFK	SRM	Strains	39	NA	NA	[75]
	24	ATTNEIFK	SRM	Strains	39	NA	NA	[75]

Continued

Category	Subtypes included	Peptide	Method	Sample	Sample size	Sensitivity/%, CI/%	Specificity/%, CI/%	Ref.
24		EGMSGSIR	SRM	Strains	39	NA	NA	[75]
24		IPFSLNLEMK	SRM	Strains	39	NA	NA	[75]
24		ITPVQEVNFADDLAHNR	SRM	Strains	39	NA	NA	[75]
24		MLNALIGLENHK	SRM	Strains	39	NA	NA	[75]
24		NLSTYGNALAR	SRM	Strains	39	NA	NA	[75]
24		TGLELMQK	SRM	Strains	39	NA	NA	[75]
51		ASTEYVPASTFK	SRM	Strains	39	NA	NA	[75]
51		ATTTEVFK	SRM	Strains	39	NA	NA	[75]
51		ITPQQEAQFAYK	SRM	Strains	39	NA	NA	[75]
51		VQDEVQSMLFIEEK	SRM	Strains	39	NA	NA	[75]
58		AGDDIALR	SRM	Strains	39	NA	NA	[75]
58		IANALIGLENHK	SRM	Strains	39	NA	NA	[75]
58		TAYIPASTFK	SRM	Strains	39	NA	NA	[75]

4.3 靶向蛋白质组学技术的应用挑战 靶向蛋白组学技术凭借其在耐药酶检测中的优异表现展现出巨大潜力, 然而其在实际应用过程中仍有待优化。首先, 样品前处理效率与通量: 样品处理过程及液相洗脱时间是主要限速因素。例如, 检测菌落样品仍需较长的培养周期; 对于细菌浓度低的血液样本, 通常需待血培养报阳后才能检测。若能直接从血液样本中检测β-内酰胺酶, 将其能够实时与抗生素血药浓度关联, 不仅为药效学评估和药物研究提供关键数据支持, 更能极大提升临床应用的便捷性和效率, 有力推动精准医疗发展, 但目前尚缺乏直接从患者血液中快速、灵敏检测标志性肽段的方法。现有样品前处理步骤繁琐、成本高、通量低。例如传统的蛋白酶解过程, 需16～24 h, 不过随着处理技术的不断优化, 多个研究已证实快速酶解方法的可行性, 借助微波或温度 (55 ℃) 辅助酶解, 可将样品酶切时间缩短至15 min, 提高检测效率[71,75,79]。此外, 液相洗脱时间及梯度是肽段在质谱分析前有效分离的关键, 尽管已有研究将液相洗脱时间缩短至7 min[73], 但在高通量检测中, 过短的洗脱时间可能会导致灵敏度下降。因此, 开发兼具快速、高效的前处理及洗脱方法是靶向蛋白组学走向临床检测的关键所在。

其次, 标志性肽段筛选与分析挑战: β-内酰胺酶亚型众多, 部分亚型的特异性肽段之间仅差1到2个氨基酸, 其高度相似的质谱图和b/y碎片离子对高通量分离鉴定构成巨大挑战。目前研究中已获得部分具有高特异性的肽段标志物, 但家族覆盖并不完整。不同特异性肽段对相应β-内酰胺酶的覆盖率不一致且覆盖亚型缺乏规律性, 采用肽段组合作为阳性判断标准可能提高灵敏度[79]。同时, 肽段潜在的翻译后修饰以及质谱操作中可能引入的污染物等也是研究者们必须考虑的因素。而部分亚型覆盖率高的肽段因容易被修饰或较差的质谱行为并不适合成为标志性肽段而被排除。因此, 筛选适用于质谱检测的标志性肽段组、对其进行合理分类和分组、优化色谱保留时间并严格验证方法的重现性和稳定性, 是该方法临床应用面临的主要挑战。此外, 各研究中对PRM结果的阳性判定尚未形成统一标准。例如, 有研究[74]以至少检出2个特征肽段作为判据, 而另有研究[76]则采用rdotp值。判读方式的差异, 导致数据分析环节的标准化仍是该领域面临的一个关键挑战, 直接影响研究结果的可比性与可重复性。

第三, 定量能力与表型相关性验证: 靶向蛋白组不仅可以定性检测耐药酶的存在, 还可通过比较内源性肽段与同位素标记肽段的比值, 实现对耐药酶表达水平的相对定量分析。然而, 耐药酶的表达水平和耐药表型之间的定量相关性仍需通过更深入的实验研究加以确证。

5 结论与展望

快速、准确地检测细菌β-内酰胺酶, 对于及时判断细菌耐药性、指导临床合理用药至关重要。这不仅有助于有效降低患者的发病率、死亡率、缩短住院时间并减少医疗费用, 更能减少抗生素压力下细菌的耐药突变, 从而延缓耐药进程。此外, 监测耐药细菌的β-内酰胺酶表达情况, 可为耐药机制的研究提供重要线索, 揭示细菌对抗生素压力的适应性变化, 同时为新型抗菌药物的研发和现有药物的评价奠定科学基础。本文着重探讨了基于质谱的蛋白组学方法在检测各类β-内酰胺酶中的检测性能、实验流程、时效性、实际应用场景及应用挑战 (表2)[43,44,46,48,50,54,59,60,71-74,76-79]。鉴于现有单一方法的局限性, 联合应用多种检测方法, 完整覆盖“基因−蛋白−表型”链路, 是检测β-内酰胺酶的最优策略。特别值得注意的是, 靶向蛋白组学技术凭借其在耐药酶检测中的优异表现展现出巨大潜力, 但其临床应用目前仍受制于仪器成本和操作复杂性等现实因素。因此, 期待未来研究能着力于进一步优化检测手

Table 2 The comparison of proteomics technology in the detection of β-lactamase

Comparison dimension	Fingerprinting identification	Bottom-up proteomics	Targeted proteomics (SRM/MRM)	Targeted proteomics (PRM)	Top-down proteomics
Analyte	Protein or peptide solution.	Total digested peptides.	Predefined precursor-product ion pairs of targeted peptides.	Predefined precursor ions of targeted peptides.	Intact proteins.
Instrument requirements	MALDI-TOF MS (e.g., Bruker Microflex[43,44,46,48]).	High-resolution LC-MS/MS (e.g., Q-Orbitrap[54]).	LC-MS/MS (e.g., QQQ[72,73]).	High-resolution LC-MS/MS (e.g., Q-Orbitrap[76-79]).	High-resolution MS (e.g., Q-Orbitrap[59, 60]).
Spectrum features	Characteristic peaks of β-lactamase.	Unbiased full MS^1 and MS^2 spectra.	Spectra of selected precursor-product ion pairs.	Spectra of all product ions (full MS^2 scans).	Spectra of proteins and product ions.
Output format	Peak lists.	Protein lists with peptide coverage (quantifiable in comparative proteomics).	Qualitative and quantitative peptide lists.	Qualitative and quantitative peptide lists.	Protein sequences and modifications.
Sensitivity	Low.	Medium-high.	Very high.	High.	Low-medium.
Specificity	Low.	Medium.	High.	High-very high.	Very high.
Throughput	Very high.	Medium.	High.	High.	Low.
β-Lactamase types per run	Limited (< 10 types).	Dozens.	Dozens to hundreds.	Dozens to hundreds.	Limited (< 10 types).
Subtype discrimination	Possible inability to discriminate.	Medium-high (specific peptide identification dependent).	Medium-high (dependent on whether the selected transitions cover the variant site).	High (all product ions analyzed; capable of discriminating most peptide variants; specific peptide dependent).	Very high (full-length protein sequences).
Turnaround time	Very fast (≈ 30 min[44]).	Medium (5-6 h[50]).	Fast (< 90 min[71-74]).	Fast (≈ 1 h[79]).	Slow.
Data analysis	Simple.	Medium.	Medium.	Medium.	Complex.
Advantages	Fast, cost-effective, easy to operate.	Unbiased screening; database-supported identification of β-lactamases and resistance-related proteins (e.g., efflux pumps).	High sensitivity; accurate and reproducible quantification.	High sensitivity; accurate and reproducible quantification; capable of discriminating most peptide variants.	Digestion-free direct identification of full-length β-lactamases; intact protein structural information including post-translational modifications; excellent subtype discrimination.
Limitations	Low detectability and reproducibility; risk of missing low-abundance β-lactamases; limited subtype discrimination; poor quantification.	Prone to missing low-abundance enzymes; limited subtype discrimination.	Targeting only predefined transitions; challenges in the selection of precursor-product ion pairs.	High-resolution MS requirements; targeting predefined peptides only.	High-resolution MS requirements; low throughput; complex data analysis; poor protein ionization efficiency.
Typical applications	Rapid β-lactamase screening; preliminary large-scale epidemiological surveys.	Preliminary research on resistance mechanisms; detection of multiple β-lactamase in complex samples (e.g., mixed infections).	Rapid qualitative/quantification of β-lactamase peptides; large-cohort validation; biomarker verification.	Precise quantification of β-lactamase peptides; large-cohort validation; biomarker verification.	β-Lactamase structure -function studies; analysis of protein cleavage/degradation mechanisms.

段、降低仪器成本。此外, 人工智能在蛋白质组学研究中的作用愈发凸显, 不仅有望加速自动化研究平台的搭建, 更能推动相关技术方法的迭代优化。随着技术瓶颈的持续突破, 蛋白质组学技术在临床诊断与抗生素耐药性研究领域, 必将展现出更为广阔的应用前景, 为临床耐药性的精准防控与科学治理提供坚实支撑。

作者贡献: 姜皓文负责文献收集整理、阅读、文章构思、撰写和审阅; 吴慧鸽、李聪然参与文章审阅; 卢芸参与文章构思, 并负责内容讨论、审阅和校对; 游雪甫参与文章审阅和校对。

利益冲突: 所有作者声明本综述内容无任何利益冲突。

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