综 述

临床常见革兰阴性菌异质性耐药研究进展

张菲阳，周英顺
西南医科大学基础医学院　病原生物学教研室病原生物学技术平台（泸州　646000）

【摘　要】异质性耐药是指遗传背景相同的细菌种群中存在着对抗生素敏感性不同的亚群。异质性耐药是细菌从敏感到耐药进化的中间阶段。目前越来越多研究表明细菌一旦对某种抗菌药物异质性耐药，在抗菌药物作用下，细菌种群中的敏感亚群被杀死，小部分的耐药亚群被选择出来并使整个细菌种群表现出高水平耐药最终导致临床抗感染治疗失败。异质性耐药如果不加遏制将导致细菌完全耐药。研究异质性耐药对于了解临床常见病原菌的耐药性发展过程，指导临床合理应用抗菌药物具有重要的意义。革兰阴性菌是引起医院及社区感染的主要病原菌，为进一步加深临床工作者对此类细菌异质性耐药的认识，本文对临床常见的革兰阴性菌异质性耐药的研究现状进行了综述。

【关键词】革兰阴性菌　耐药　异质性耐药
【中图分类号】R378.2　文献标志码 A　DOI：10.3969/j.issn.2096-3351.2021.05.015

Research advances in heteroresistance of common Gram-negative bacteria in clinical practice

ZHANG Fei-yang, ZHOU Ying-shun
Department of Pathogen Biology, School of Basic Medicine, Public Center of Experimental Technology of Pathogen Biology technology platform, Southwest Medical University, Luzhou, 646000, China

【Abstract】 Heteroresistance refers to the presence of subpopulations with different susceptibilities to a particular antibiotic within the bacterial population with the same genetic background. Heteroresistance is the intermediate stage in the evolution of bacteria from susceptibility to resistance. At present, more and more studies have shown that once a bacterial population develops heteroresistance to an antimicrobial agent, the susceptible subpopulations in this bacterial population will be killed in the presence of the antimicrobial agent and a small part of resistant subpopulations will be screened out, which makes the whole bacterial population develop a high level of antibiotic resistance and eventually leads to the failure of anti-infective therapy in clinical practice. Heteroresistance will lead to resistance without proper control. The research on heteroresistance is of great significance for understanding the development process of antibiotic resistance in common clinical pathogens and guiding the rational clinical application of antimicrobial agents. Gram-negative bacteria are the main pathogens causing hospital and community infections. This article reviews the current status of research on heteroresistance of common Gram-negative bacteria in clinical practice, so as to further improve the awareness of the heteroresistance of such bacteria among clinicians .

【Key words】 Gram-negative bacteria　Resistance　Heteroresistance

---

基金项目：国家自然科学基金（31500114）；四川省科技厅基金（2020YJ0338）
第一作者简介：张菲阳，硕士生。
通信作者：周英顺，副教授，博士。E-mail：yingshunzhou@swmu.edu.cn

革兰阴性菌是引起社区及医院获得性感染的重要病原菌。近年来,由于抗生素长期不合理应用,该类细菌对包括β-内酰胺类、氨基糖苷类、粘菌素在内的多种抗生素均产生耐药性且耐药率逐年增高并出现流行趋势[1]。除了对抗生素耐药外,革兰阴性菌对多种抗生素也出现异质性耐药现象。早在1947年研究人员就在流感嗜血杆菌观察到异质性耐药现象,从那之后越来越多的细菌被报道对多种不同抗生素异质性耐药[2]。异质性耐药广泛存在于多种细菌对不同抗菌药物的反应中,革兰阴性菌中异质性耐药现象主要在铜绿假单胞菌、肺炎克雷伯菌、鲍曼不动杆菌、大肠杆菌等细菌中被发现。自异质性耐药现象被发现以来,异质性耐药机制的研究已经成为临床微生物工作者关注的热点。为了全面了解革兰阴性菌的异质性耐药流行状况并进一步探究异质性耐药现象背后的隐藏机制,现将主要的革兰阴性菌异质性耐药研究进展进行综述。

1 常见革兰阴性菌异质性耐药

1.1 粘菌素异质性耐药

目前发现存在粘菌素异质性耐药现象的肠杆菌科细菌主要是肺炎克雷伯菌和阴沟肠杆菌[3-4]。此外也有研究报道在伤寒沙门菌、大肠杆菌中也存在粘菌素异质性耐药现象[5]。研究发现粘菌素异质性耐药的肺炎克雷伯菌中异质性耐药机制包括以下方面:(1)粘菌素耐药亚群出现小菌落变异且生物膜形成能力增强[6]。(2)粘菌素耐药亚群中粘菌素耐药相关基因*mgrB*、双组份调节系统基因*phoP*[7]、*phoQ*、*lpxM*、*yciM*[8]、*PmrAB*[9-10]等出现突变。此外研究人员发现在阴沟肠杆菌中粘菌素异质性耐药机制与细菌对宿主抗菌溶解酶交叉耐药相关[11]。其他研究也证明了阴沟肠杆菌可通过L-Ara4N掺入脂质A,对脂质A进行修饰并进一步导致粘菌素异质性耐药[3]。最近的一项研究也发现阴沟肠杆菌中一个新鉴定出的基因*ecr*编码的小分子跨膜蛋白可以调节粘菌素异质性耐药[12]。尽管粘菌素异质性耐药的临床意义目前仍然存在很大争议,近年来越来越多的动物实验证明细菌种群中存在的少数耐药亚群可导致抗生素治疗失败,且可能与疾病的治疗失败有关[13-14]。

1.2 碳青霉烯异质性耐药

碳青霉烯异质性耐药的肠杆菌科细菌主要见于肺炎克雷伯菌和大肠杆菌。此外也有研究报道碳青霉烯异质性耐药现象也存在于阴沟肠杆菌[15]、产气肠杆菌中[16],但研究人员未对异质性耐药机制进行深入探究。目前国内外已经报道了产VIM酶、KPC酶及OXA酶的碳青霉烯异质性耐药的肺炎克雷伯菌[17-18]。此外研究人员已经证明了碳青霉烯异质性耐药机制与耐药亚群中bla_{VIM}和bla_{KPC}基因表达水平增加及编码OmpK36外膜蛋白基因突变或缺失相关[18-19]。与肺炎克雷伯菌不同,对碳青霉烯异质性耐药的大肠杆菌的研究主要局限于流行情况及危险因素等方面。如超广谱β-内酰胺酶(Extended-spectrum β-lactamase,ESBLs)的产生被认为是厄他培南及亚胺培南异质性耐药的共同独立危险因素[20]。这提示我们ESBLs的产生可能是碳青霉烯异质性耐药的另一重要机制。

1.3 β-内酰胺类抗生素异质性耐药

β-内酰胺类抗生素异质性耐药主要在大肠杆菌中被报道。2014年,研究人员发现向大肠杆菌敏感菌株中导入pUA66-bla CTX-M-14重组质粒能够构建头孢曲松异质性耐药模型。研究人员还发现随着抗生素浓度增加细菌种群中生长缓慢、头孢菌素水解酶表达水平增加的亚群的比例逐渐增高。这说明异质性耐药是细菌在抗生素作用下降低生长速率的结果而不是抗生素选择的结果[21]。近年来研究人员也发现了哌拉西林-他唑巴坦异质性耐药的大肠杆菌[22]。然而研究人员并未对其异质性耐药机制进行深入探究。此外,有文献报道了头孢吡肟异质性耐药大肠杆菌的流行病学特点及危险因素[23]。这篇报道显示了头孢吡肟异质性耐药大肠杆菌的独立危险因素包括:头孢菌素用药史、产ESBLs、肠外营养。

1.4 四环素类异质性耐药

四环素类异质性耐药的肠杆菌科细菌包括肺炎克雷伯菌、阴沟肠杆菌、伤寒沙门菌。目前发现的四环素类异质性耐药的肠杆菌科细菌并不多见,相应的研究也比较少。2017年,Chen Yi等[24]发现AcrAB-Tolc和OqxAB外排泵过度表达导致伤寒沙门菌对替加环素异质性耐药。近年来,Hang Liu等[25]发现外排泵基因*acrAB*、*oqxAB*及转录调控因子soxS、ramA的表达水平增加导致阴沟肠杆菌对替加环素异质性耐药。此外研究人员也发现OqxAB和MacAB外排泵的过表达以及转录调节因子RamA可能参与了肺炎克雷伯菌对eravacycline(一种新型的

氟四环素类抗生素）的耐药和异质性耐药[26]。上述研究提示我们四环素类异质性耐药的机制可能与耐药亚群中外排泵相关基因表达水平增加或活性增强有关。

1.5 磷霉素异质性耐药

目前关于磷霉素异质性耐药的肠杆菌科细菌的报道主要见于大肠杆菌、肺炎克雷伯菌及阴沟肠杆菌。2017年，Ballestero-Téllez M等[27]发现提高细菌的初始接种量能够增加磷霉素异质性耐药的大肠杆菌及肺炎克雷伯菌的检出效率。随后Abbott Iain J等[28]发现肺炎克雷伯菌及大肠杆菌对磷霉素的反应存在差异，异质性耐药亚群的存在导致磷霉素不能完全杀灭大肠杆菌。研究人员也在阴沟肠杆菌中发现了磷霉素异质性耐药现象[29]。他们在磷霉素暴露前检测到了高水平耐药亚群并认为纸片扩散实验能更好的预测异质性耐药。然而这些报道并未深入探究异质性耐药机制。随后，Campos Ana Carolina C等[30]调查了巴西某医院磷霉素异质性耐药大肠杆菌的流行情况。发现磷霉素异质性耐药大肠杆菌流行率较高。机制研究发现耐药亚群中转运调控基因突变导致磷霉素摄取系统缺陷及磷霉素耐药基因*murA*过度表达是引起异质性耐药的原因。在近期一项研究中，Lucas AE等[31]发现磷霉素异质性耐药的大肠杆菌中耐药亚群的*uhpT*基因（编码磷霉素转运蛋白介导磷霉素转运入细菌体内）缺失导致大肠杆菌对磷霉素异质性耐药，见图1。

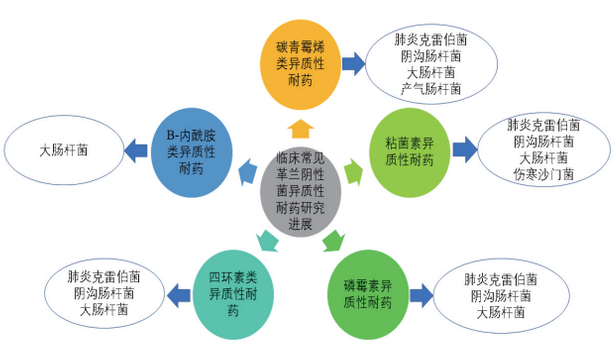


图1 常见革兰阴性菌的异质性耐药情况

2 结语及展望

异质性耐药现象广泛存在于多种细菌对多种抗生素的反应中。异质性耐药机制十分复杂，不同种属细菌对同种抗生素异质性耐药机制各不相同，即使是同种细菌对同种抗生素异质性耐药机制也不尽相同。目前仍然需要进一步研究异质性耐药机制。异质性耐药的检测方法在不同实验室中也存在着巨大差异，缺乏统一评估异质性耐药的标准。目前常用的异质性耐药表型筛查实验包括K-B纸片扩散实验、E-test等。但这些方法耗时较长、重复性较差且对异质性耐药菌株的检测结果往往不可靠。不能及时、准确的向临床提供用药指导。菌谱分析（Population analysis profile，PAP）实验是评估异质性耐药的金标准，但因其耗时、繁琐，很少应用于临床或仅用于确定特定的临床病例。若不能及时检测出异质性耐药菌株，在抗菌药物的选择压力下，异质性耐药将最终进展至完全耐药，导致临床抗感染治疗失败。

目前还没有关于治疗异质性耐药菌株感染的规范用药方案。除了提高异质性耐药菌株的检出率、加强对异质性耐药菌株性的认识外临床还应该规范合理应用抗生素。同时还应防止异质性耐药菌株的传播。总而言之，异质性耐药已经对临床抗感染治疗造成了巨大的威胁。临床工作者应当加强对异质性耐药的认识，深入探究异质性耐药机制。

参 考 文 献

[1] Uc-Cachón AH, Gracida-Osorno C, Luna-Chi IG, *et al*. High prevalence of antimicrobial resistance among gram-negative isolated bacilli in intensive care units at a tertiary-care hospital in Yucatán Mexico[J]. Medicina (Kaunas), 2019, 55(9): 588-601.

[2] Anderson SE, Sherman EX, Weiss DS, *et al*. Aminoglycoside heteroresistance in Acinetobacter baumannii AB5075[J]. mSphere, 2018, 3(4): 271-289.

[3] Kang KN, Klein DR, Kazi MI, *et al*. Colistin heteroresistance in Enterobacter cloacae is regulated by PhoPQ-dependent 4-amino-4-deoxy-l-arabinose addition to lipid A[J]. Mol Microbiol, 2019, 111(6): 1604-1616.

[4] Uechi K, Tada T, Shimada K, *et al*. Emergence of a carbapenem-resistant and colistin-heteroresistant Enterobacter cloacae clinical isolate in Japan[J]. J Infect Chemother, 2019, 25(4): 285-288.

[5] Hjort K, Nicoloff H, Andersson DI. Unstable tandem gene amplification generates heteroresistance (variation in resistance within a population) to colistin in Salmonella enterica[J]. Mol Microbiol, 2016, 102(2): 274-289.

[6] Silva A, Sousa AM, Alves D, *et al*. Heteroresistance to colistin in Klebsiella pneumoniae is triggered by small col-

ony variants sub-populations within biofilms[J]. Pathog Dis, 2016, 74(5): 36-57.

[7] Jayol A, Nordmann P, Brink A, *et al*. Heteroresistance to colistin in Klebsiella pneumoniae associated with alterations in the PhoPQ regulatory system[J]. Antimicrob Agents Chemother, 2015, 59(5): 2780-2784.

[8] Halaby T, Kucukkose E, Janssen AB, *et al*. Genomic characterization of colistin heteroresistance in Klebsiella pneumoniae during a nosocomial outbreak[J]. Antimicrob Agents Chemother, 2016, 60(11): 6837-6843.

[9] Cheong HS, Kim SY, Wi YM, *et al*. Colistin heteroresistance in Klebsiella pneumoniae isolates and diverse mutations of PmrAB and PhoPQ in resistant subpopulations [J]. J Clin Med, 2019, 8(9): 1444-1453.

[10] Lin J, Xu CQ, Fang RC, *et al*. Resistance and heteroresistance to colistin in Pseudomonas aeruginosa isolates from Wenzhou, China[J]. Antimicrob Agents Chemother, 2019, 63(10): 556-575.

[11] Napier BA, Band V, Burd EM, *et al*. Colistin heteroresistance in Enterobacter cloacae is associated with cross-resistance to the host antimicrobial lysozyme[J]. Antimicrob Agents Chemother, 2014, 58(9): 5594-5597.

[12] Huang L, Feng Y, Zong ZY. Heterogeneous resistance to colistin in Enterobacter cloacae complex due to a new small transmembrane protein[J]. J Antimicrob Chemother, 2019, 74(9): 2551-2558.

[13] Band VI, Satola SW, Burd EM, *et al*. Carbapenem-Resistant Klebsiella pneumoniae exhibiting clinically undetected colistin heteroresistance leads to treatment failure in a murine model of infection[J]. MBio, 2018, 9(2): 2448-2465.

[14] Andersson DI, Nicoloff H, Hjort K. Mechanisms and clinical relevance of bacterial heteroresistance[J]. Nat Rev Microbiol, 2019, 17(8): 479-496.

[15] da Silva AEB, Martins AF, Nodari CS, *et al*. Carbapenem-heteroresistance among isolates of the Enterobacter cloacae complex: is it a real concern?[J]. Eur J Clin Microbiol Infect Dis, 2018, 37(1): 185-186.

[16] Gordon NC, Wareham DW. Failure of the MicroScan WalkAway system to detect heteroresistance to carbapenems in a patient with Enterobacter aerogenes bacteremia [J]. J Clin Microbiol, 2009, 47(9): 3024-3025.

[17] Tato M, Morosini M, García L, *et al*. Carbapenem heteroresistance in VIM-1-producing Klebsiella pneumoniae isolates belonging to the same clone: consequences for routine susceptibility testing[J]. J Clin Microbiol, 2010, 48(11): 4089-4093.

[18]López-Camacho E, Paño-Pardo JR, Sotillo A, *et al*. Meropenem heteroresistance in clinical isolates of OXA-48-producing Klebsiella pneumoniae[J]. Diagn Microbiol Infect Dis, 2019, 93(2): 162-166.

[19] Adams-Sapper S, Nolen S, Donzelli GF, *et al*. Rapid induction of high-level carbapenem resistance in heteroresistant KPC-producing Klebsiella pneumoniae[J]. Antimicrob Agents Chemother, 2015, 59(6): 3281-3289.

[20] Sun JD, Huang SF, Yang SS, *et al*. Impact of carbapenem heteroresistance among clinical isolates of invasive Escherichia coli in Chongqing, southwestern China[J]. Clin Microbiol Infect, 2015, 21(5): 469.e1-10.

[21] Wang XR, Kang Y, Luo CX, *et al*. Heteroresistance at the single-cell level: adapting to antibiotic stress through a population-based strategy and growth-controlled interphenotypic coordination[J]. MBio, 2014, 5(1): 942-955.

[22] Rodríguez-Villodres Á, Ortiz de la Rosa JM, Álvarez-Marín R, *et al*. Heteroresistance to piperacillin-tazobactam in clinical isolates of Escherichia coli sequence type 131[J]. Antimicrob Agents Chemother, 2018, 62 (1): 1923-1940.

[23] Ma W, Sun J, Yang S, *et al*. Epidemiological and clinical features for cefepime heteroresistant Escherichia coli infections in southwest China[J]. Eur J Clin Microbiol Infect Dis, 2016, 35(4): 571-578.

[24] Chen Y, Hu DX, Zhang QJ, *et al*. Efflux pump overexpression contributes to tigecycline heteroresistance in salmonella enterica serovar typhimurium[J]. Front Cell Infect Microbiol, 2017, 7(37):153-161.

[25] Liu H, Jia XJ, Zou H, *et al*. Detection and characterization of tigecycline heteroresistance in E. cloacae: clinical and microbiological findings[J]. Emerg Microbes Infect, 2019, 8(1): 564-574.

[26] Zheng JX, Lin ZW, Sun X, *et al*. Overexpression of OqxAB and MacAB efflux pumps contributes to eravacycline resistance and heteroresistance in clinical isolates of Klebsiella pneumoniae[J]. Emerg Microbes Infect, 2018, 7(1): 139-150.

[27] Ballestero-Téllez M, Docobo-Pérez F, Rodríguez-Martínez JM, *et al*. Role of inoculum and mutant frequency on fosfomycin MIC discrepancies by agar dilution and broth microdilution methods in Enterobacteriaceae[J]. Clin Microbiol Infect, 2017, 23(5): 325-331.

[28] Abbott IJ, Dekker J, van Gorp E, *et al*. Impact of bacterial species and baseline resistance on fosfomycin efficacy

in urinary tract infections[J]. J Antimicrob Chemother, 2020, 75(4): 988-996.

[29] Abbott IJ, van Gorp E, Wijma RA, *et al*. Oral fosfomycin efficacy with variable urinary exposures following single and multiple doses against Enterobacterales: the importance of heteroresistance for growth outcome[J]. Antimicrob Agents Chemother, 2020, 64(3): 1982-2001.

[30] Campos ACDC, Andrade NL, Couto N, *et al*. Characterization of fosfomycin heteroresistance among multidrug-resistant Escherichia coli isolates from hospitalized patients in Rio de Janeiro, Brazil[J]. J Glob Antimicrob Resist, 2020(22): 584-593.

[31] Lucas AE, Ito R, Mustapha MM, *et al*. Frequency and mechanisms of spontaneous fosfomycin nonsusceptibility observed upon disk diffusion testing of Escherichia coli[J]. J Clin Microbiol, 2018, 56(1): 1368-1385.

(2021-06-17 收稿)



专 家 简 介

周英顺，西南医科大学副教授，博士，硕士研究生导师，四川省第十二批学术和技术带头人后备人选，中华医学会微生物与免疫学分会第十届委员会青年委员会委员，中国医药教育协会微生态与健康教育专业委员会委员，西南医科大学青年科技人才特别培育计划，《西南医科大学学报》编委。近年来从事病原微生物学的科研、教学工作。科研的主要研究方向为病原微生物感染与耐药机制研究。主持国家自然科学基金青年基金，四川省科技厅应用基础重点项目等，近年来以第一作者或者通讯作者发表SCI论文25篇。