淋病奈瑟菌耐药机制研究进展

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中国医学科学院医学与健康创新工程协同创新团队项目(批准号: 2016-I2M-3-021)、重要传染病病原系统生物学研究(批准号: 2018PT51009)和重要传染病病原组学研究项目(批准号: 2019PT310006)资助

摘要 淋病是由淋病奈瑟菌(淋球菌)感染引起的一种性传播疾病, 其流行和蔓延已成为全球公共卫生事业巨大的负担。抗菌药物治疗是目前淋病防治的主要方式, 但随着抗菌药物在临床上的广泛应用, 淋球菌的耐药性问题日益严重。深入探究淋球菌的耐药机制, 对完善其耐药监测体系和调整临床用药方案均有重要意义。本文在回顾近几年淋球菌耐药机制相关研究的基础上, 就临床上几种常见抗菌药物耐药机制的最新研究进展进行了综述, 旨在为我国淋病防治策略的制定提供依据。

关键词 淋病, 淋病奈瑟菌, 抗菌药物, 耐药机制

淋病奈瑟菌(*Neisseria gonorrhoeae*, Ng), 又称淋球菌, 是引起淋病及相关疾病的病原菌。目前, 淋病发病率居世界性传播疾病第二位, 世界卫生组织(World Health Organization, WHO)报告显示, 全球每年新增病例约8700万左右^[1]。淋球菌除感染人体泌尿生殖系统引发化脓性感染外, 还可侵犯眼睛、咽部和直肠等多个部位^[2,3], 同时可增加感染艾滋病的风险^[4]。由于目前尚无针对淋球菌的有效疫苗上市, 抗菌药物治疗仍是淋病防治的主要手段^[5], 但抗菌药物在临床上的广泛应用使耐药菌株大量出现并广泛传播。近年来, 已有多种分子检测技术应用于淋球菌的耐药检测, 包括基于核酸质谱技术的淋球菌常见耐药位点的检测^[6]、基于高分辨率熔解分析技术的淋球菌常见耐药位点的检测^[7,8]、基于多重PCR技术和纳米孔测序技

术的淋球菌耐药基因的检测等^[9]。耐药机制研究是基于分子检测技术的全方面耐药监测策略的理论基石, 深入研究耐药机制, 在分子水平上不断拉近耐药机制与耐药分布之间的联系, 有利于人们及时调整监测策略和更新临床用药方案。本文综述了淋球菌耐药机制方面的最新研究进展, 以期完善淋球菌的耐药监测和检测技术体系, 为临床治疗方案的制订和新药研发提供助力。

1 淋球菌耐药演化史

抗菌药物的应用史, 同时也伴随着淋球菌的耐药史, 导致淋病治疗的一线抗菌药物也在不断发生变化(图1)。

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图 1 抗菌药物应用于淋病治疗的历史^[10-20]和淋球菌耐药史^[11,29,30,34-37,60,68]

Figure 1 A timeline showing the use of antibacterial agents in the treatment of gonorrhea^[10-20] and the resistance of *N. gonorrhoeae* against them^[11,29,30,34-37,60,68]

20世纪30年代,磺胺类药物首次被引入临床用于淋球菌抗菌治疗,但在几年时间内,淋球菌就产生了对该药物的普遍耐药性^[10,11]。1943年青霉素开始应用于临床,在随后的几十年中,多地分离到了由染色体和质粒介导耐药性的菌株,尤其是介导高水平青霉素耐药性质粒的广泛传播,使人们不得不放弃使用青霉素作为一线淋病治疗药物^[12-14]。作为青霉素替代药物的四环素类药物自1962年应用以来,在之后约20年的时间内,淋球菌中就出现了质粒介导的高水平耐药株^[15,16]。对于其他应用于淋病治疗的抗菌药物,如氨基糖苷类和喹诺酮类药物,在淋球菌中也先后通过纯培养得到了高水平的耐药株^[17-19],目前,WHO建议采用头孢曲松加阿奇霉素的双重疗法用于淋病治

疗^[20],然而,世界范围内头孢曲松和阿奇霉素双重耐药株的出现^[21-23],使淋病治疗在不久的将来可能面临着无药可用的困境。

2 淋球菌耐药机制

过去几十年中,虽然淋球菌的耐药问题日益严重,但其耐药机制的研究也取得了巨大进展^[24]。淋球菌耐药机制主要包括改变青霉素结合蛋白(penicillin-binding proteins, PBPs)、DNA促旋酶及拓扑异构酶等药物靶点蛋白的结构,降低药物亲和性; MtrCDE, MacAB, NorM^[25]和FarAB^[26]等主动外排系统高表达,增加药物排出量;细胞膜孔蛋白PorB低表达,减少药物摄入量;合成 β -内酰胺酶等抗生素灭活酶,使抗菌药物失活^[27]等,表1总结了淋球菌对常见抗菌药物的耐药机制。

2.1 磺胺类

磺胺类药物通过竞争细菌的二氢喋呤合酶(dihydropteroate synthase, DHPS)来抑制细菌叶酸合成^[28]。其耐药机制主要包括:合成过量对氨基苯甲酸,稀释抗菌药物剂量^[29];编码DHPS的*folP*基因突变,使药物亲和性降低^[11,30]; MtrF外排泵高表达,药物排出增加等^[31,32]。

2.2 青霉素类

青霉素类属于 β -内酰胺类抗菌药物,含有 β -内酰胺环^[33]。青霉素类药物通过竞争细菌转肽酶,阻碍细菌细胞壁中黏肽形成,破坏细胞壁结构,达到杀菌效果。淋球菌对青霉素类药物的耐药性可由质粒和染色体介导。

质粒介导的耐药性主要来源于质粒上携带的*bla*_{TEM}基因,该基因编码可水解 β -内酰胺环的 β -内酰胺酶,可随质粒在细菌间传递,造成细菌的广泛耐药性^[34-37]。此外,*bla*_{TEM}基因的单核苷酸突变可造成TEM型 β -内酰胺酶的多态性,如TEM-135型与TEM-1型 β -内酰胺酶的差异只表现在M182T氨基酸改变(T539C),TEM-220型与TEM-135型 β -内酰胺酶相比,增加了一个额外的A185T氨基酸突变(G547A)^[35]。而 β -内酰胺酶不同突变体间也具有较大差异,例如M182T突变提高了酶的稳定性,使其具有更强的酶活性,增加了TEM-135型和TEM-220型 β -内酰胺酶发展为可水解头孢菌

表 1 淋球菌对常见抗菌药物的耐药机制

Table 1 The resistance mechanisms of *N. gonorrhoeae* against common antibacterial agents

抗菌药物	耐药机制	参考文献
磺胺类	1. 合成过量对氨基苯甲酸, 稀释抗菌药物剂量. 2. 编码DHPS的 <i>folP</i> 基因突变, 使药物亲和性降低. 3. MtrF外排泵高表达, 药物排出增加.	[11,29~32]
青霉素类	1. 质粒上携带的 <i>bla</i> _{TEM} 基因(<i>bla</i> _{TEM-1} , <i>bla</i> _{TEM-135} 和 <i>bla</i> _{TEM-220}), 可编码水解β-内酰胺环的β-内酰胺酶. 2. <i>ponA</i> 基因突变, 导致编码的PBP1发生L421P氨基酸改变, 显著降低药物酰化率. 3. <i>penA</i> 基因突变, 导致编码的PBP2在345位点插入精氨酸, 蛋白结构发生改变, 使药物亲和性降低. 4. <i>mtrR</i> 基因启动子区插入T/TT、缺失A, 使MtrCDE外排泵高表达, 药物排出增加. 5. <i>porB</i> 基因突变, 导致G120K/D/N/T, A121/D/N/G氨基酸替换, 使药物摄入减少.	[34~46]
四环素类	1. 质粒上携带的 <i>tetM</i> 基因编码细菌核糖体保护蛋白, 阻断四环素类药物与细菌核糖体结合, 是造成高水平耐药性的主要原因. 2. <i>rpsJ</i> 基因突变, 导致V57M/L氨基酸置换, 破坏药物与细菌核糖体的结合. 3. <i>mtrR</i> 基因突变(见青霉素类). 4. <i>porB</i> 基因突变(见青霉素类).	[45,46,48]
氨基糖苷类 (壮观霉素)	1. <i>rpsE</i> 基因突变, 导致的V27缺失和K28E氨基酸改变可使MIC达到2048 mg/L, 此外, V25缺失、T24P和K26E等突变降低了药物亲和性. 2. 16S rRNA螺旋34中的C1192G, C1066U, G1193A, C1063U, U1189C氨基酸改变以及首个在螺旋34之外区域发现的U1183C突变.	[50~52]
喹诺酮类	1. <i>gyrA</i> 基因点突变, 导致DNA促旋酶发生S91F和D95G/N/A氨基酸置换. 2. <i>parC</i> 基因点突变, 导致拓扑异构酶IV发生E91G/K氨基酸置换. 3. <i>mtrR</i> , <i>norM</i> 基因突变造成药物外排增加. 4. <i>porB</i> 基因突变(见青霉素类).	[56~59]
大环内酯类 (阿奇霉素)	1. 23S rRNA 发生A2059G突变, 导致阿奇霉素高水平耐药性. 2. 23S rRNA 发生的C2611T突变, 与阿奇霉素低水平的耐药性相关. 3. 编码MtrCDE外排泵的马赛克样 <i>mtr</i> 序列, 如马赛克样 <i>mtrD</i> 基因, 造成的S821A及K823E氨基酸改变.	[29,61~64]
头孢菌素类	1. <i>penA</i> 基因突变, 导致的T483S, G545S, V316P, I312M, A311V, A501R, A501P等氨基酸变化导致耐药性增加, 最新发现的 <i>penA-121.001</i> 型可导致PBP2发生75个氨基酸变化, 包括从未在马赛克 <i>penA</i> 中报道过的A516G. 2. <i>mtrR</i> 基因突变(见青霉素类). 3. <i>porB</i> 基因突变(见青霉素类). 4. RNA聚合酶全酶突变, 如 <i>rpoB</i> 基因突变导致的R201H, C470T, P157L, G473T及G158V氨基酸变化, <i>rpoD</i> 基因突变导致的E98K及92~95位氨基酸缺失.	[38,65~69]

素的广谱β-内酰胺酶的可能性^[38]; 当*bla*_{TEM-1}基因从起始密码子ATG的G开始缺失GAGTAT 6个碱基时, 生成的24 kD截短型β-内酰胺酶的活性比正常32 kD TEM-1型β-内酰胺酶的活性更弱, 可使淋球菌获得中等程度的耐药性^[39]. 总之, TEM型β-内酰胺酶的多态性是淋球菌表现出不同程度耐药性的重要原因, 也是延缓淋球菌耐药性问题出现的重要突破口.

染色体介导的青霉素类药物的耐药性主要是由染色体上基因位点的突变引起的. 例如, *ponA*基因点突变, 导致编码的PBP1发生L421P氨基酸改变, 显著降低青霉素类药物的酰化率^[40]; *penA*基因点突变, 导致编码的PBP2在345位点插入精氨酸, 其蛋白结构发生

改变, 对药物亲和性降低^[41~43]; *mtrR*基因启动子区域插入T/TT、缺失A, 导致MtrCDE外排泵高表达^[44,45], 使药物排出增加; *porB*基因突变, 导致编码的孔蛋白PorB1b发生G120K/D/N/T, A121/D/N/G等氨基酸替换, 使药物摄入减少^[46]等. 值得注意的是, 染色体基因突变介导的耐药性具有累积效应, 即突变基因数量越多, 其导致的耐药性可能越强.

2.3 四环素类

四环素类药物的抑菌机制是通过结合到细菌核糖体30S亚基上, 抑制氨酰基tRNA与核糖体结合, 阻止肽链增长从而影响细菌蛋白质合成^[47]. 淋球菌对四环素

类药物的耐药性也可由质粒和染色体介导。

淋球菌质粒上携带的*tetM*基因编码细菌核糖体保护蛋白,可阻断四环素类药物与细菌核糖体结合,是造成淋球菌高水平耐药性的主要原因^[46,48]。而淋球菌对四环素类药物低水平的耐药性主要由染色体所介导,例如,染色体上编码细菌30S核糖体亚基蛋白S10的*rpsJ*基因突变,导致V57M/L氨基酸置换,破坏了药物与细菌核糖体的结合^[46]; *mtrR*基因突变,使药物外排增加^[45]; *porB*基因突变,使药物摄入减少^[46]等。由于质粒和染色体介导的耐药性水平的不同,人们可以通过淋球菌的耐药性水平粗略判断其耐药性是否由质粒介导,从而对质粒的传播趋势做出判断。值得注意的是,染色体上突变基因的累积也可能造成淋球菌的高水平耐药性。

2.4 氨基糖苷类

壮观霉素是氨基糖苷类药物中常用于淋病治疗的抗菌药物,可通过与细菌30S核糖体亚基的16S rRNA相互作用,抑制细菌蛋白质合成^[49]。淋球菌主要通过阻断抗菌药物与靶标的结合获得对壮观霉素类药物的耐药性,例如,*rpsE*基因80~82位碱基TTA缺失,导致细菌30S核糖体亚基蛋白S5发生V27缺失和K28E氨基酸置换,可使壮观霉素药物最小抑菌浓度(minimum inhibitory concentration, MIC)达到2048 mg/L^[50];此外,核糖体蛋白S5发生的其他可导致淋球菌耐药性水平增加的突变还包括V25缺失、T24P和K26E等突变^[51]。此外,壮观霉素与16S rRNA结合区域的螺旋34发生的C1192G, C1066U, G1193A, C1063U, U1189C氨基酸改变和最新研究发现的首个发生在螺旋34区域之外的U1183C氨基酸改变都与壮观霉素的耐药性增加相关^[52]。然而,目前对于螺旋34区域之外发现的氨基酸突变导致耐药性增加的相关机制尚不清晰,需要进一步的实验探究。

2.5 喹诺酮类

喹诺酮类药物抑菌作用的产生主要是通过抑制细菌DNA促旋酶和拓扑异构酶IV活性,阻断细菌DNA复制^[53]。细菌*gyrA*和*gyrB*基因分别编码细菌DNA促旋酶的A亚基和B亚基^[54], *parC*和*parE*基因分别编码细菌拓扑异构酶IV的C亚基和E亚基^[53]。淋球菌对喹诺酮类药物的耐药性主要是由*gyrA*和*parC*基因突变引起的,

*gyrA*和*parC*基因中热点突变区域又称为喹诺酮抗性决定区(quinolone resistance determining region, QRDR)^[55]。研究发现, *gyrA*基因点突变导致的S91F和D95G/N/A氨基酸置换^[56]及*parC*基因点突变导致的E91G/K氨基酸置换^[57],是造成喹诺酮类药物高水平耐药的重要原因^[56,57]。此外, *mtrR*, *norM*基因突变造成的药物外排增加^[58], *porB*基因突变导致的药物摄入减少等,也加速了淋球菌对喹诺酮类药物耐药性的发展^[59]。

2.6 大环内酯类

阿奇霉素属于大环内酯类抗菌药物,通过结合到细菌50S核糖体亚基的23S rRNA上,阻止肽酰基tRNA移位,抑制细菌蛋白质的合成^[60],达到抑菌效果。23S rRNA发生A2059G突变,可降低细菌对药物的亲和性,导致淋球菌产生对阿奇霉素高水平的耐药性^[61,62],而C2611T突变则与阿奇霉素低水平的耐药性相关^[29]。此外,马赛克样*mtr*序列造成的外排泵表达异常也是阿奇霉素耐药性增加的重要原因。阿奇霉素耐药株CDC2中包含耐药性突变基因——*mtrR*(D79N, S183N, M197I),与阿奇霉素敏感株FA19相比,携带核苷酸突变的*mtrCDE-mtrR*启动子区域及马赛克样*mtrD*序列,其CDC2的MIC增加了8倍;进一步实验发现,将CDC2的*mtrR*和*mtrCDE-mtrR*启动子耐药基因导入FA19序列时,其MIC只增加了4倍,当在此基础上导入马赛克样*mtrD*序列时,其MIC值增加了8倍,而马赛克样*mtrD*基因主要导致S821A及K823E氨基酸改变,并且显示出强烈的连锁不平衡和上位性效应^[63,64]。重要的是, MtrCDE外排泵表达异常,可使淋球菌获得对多种抗菌药物的耐药性,所以应该加强对MtrCDE外排泵耐药机制的相关研究,尤其是马赛克样*mtr*序列对外排泵表达的影响。

2.7 头孢菌素类

头孢菌素类药物作用于细菌的PBPs,干扰细菌细胞壁合成^[29]。头孢菌素类药物也属于β-内酰胺类抗菌药物,虽然目前为止,淋球菌中尚未发现能够水解头孢菌素的广谱β-内酰胺酶(extended-spectrum beta-lactamases, ESBLs),但质粒编码的TEM-135型和TEM-220型β-内酰胺酶,具有发展成为ESBLs的可能^[38]。另外,本课题组^[65]在湖南发现的五株耐药株中,有三株携带*bla*_{TEM-135}基因,其头孢曲松MIC明显高于其余

两株。

有研究发现, 淋球菌PBPs活性位点附近的氨基酸改变将导致蛋白质结构刚性增加, 无法产生药物酰化过程中所需的构象变化, 从而降低药物亲和性, 造成药物耐药性, 例如, *penA*基因编码的PBP2中T498附近的T483S, G545S氨基酸改变, S310附近的V316P, I312M和A311V氨基酸改变^[66], 紧邻T500的501位氨基酸改变, 尤其是A501R, A501P突变都与头孢菌素MIC值升高相关^[67]。此外, 本课题组最新发现的*penA*-121.001型可导致PBP2发生75个氨基酸变化, 包括从未在马赛克*penA*基因中报道过的A516G突变^[68], 毫无疑问的是, 头孢菌素耐药株在传播过程中正在不断进化以提高生物适应性甚至获得新的耐药表型^[65]。此外, 其他传统的耐药机制还包括*mtrR*基因突变、*porB*基因突变等。除了传统耐药机制外, 新的研究发现, RNA聚合酶全酶突变也会导致淋球菌对头孢曲松低敏, 例如编码RNA聚合酶 β 亚基的*rpoB*基因突变导致的R201H, C470T, P157L, G473T及G158V氨基酸变化, 编码持家蛋白 σ 因子的*rpoD*基因突变导致的E98K及92~95位氨基酸缺失都与药物MIC升高相关^[69], 这一发现为淋球菌耐药机制提供了新的解释, 更提示了耐药机制的多样性, 以及持续监测新的耐药突变的重要性。

3 新抗菌药物研发进展

在淋球菌耐药现状日趋严重的背景下, 新抗菌药物的研发已成为亟需深入开展的工作。2017年, WHO将淋球菌列为12种需要优先研究和开发抗菌药物的细菌之一^[70]。虽然目前尚未研发出高效抗菌药物, 但已经在新抗菌药物研发领域取得了不错的进展, 有三种较有应用潜力的淋病抗菌药物已经进入到临床试验阶段——索利霉素(Solithromycin)、吉泊达星(Gepotidacin)和唑氟达星(Zoliflodacin), 这为新抗菌药物的问世带来了希望。

3.1 索利霉素

索利霉素属于大环内酯类抗菌药物, 能够与核糖体50S亚基的23S rRNA更紧密地结合, 提高对耐大环内酯类药物淋球菌的有效性^[71]。该药进行的2期临床试验结果显示, 59名无并发症淋病患者(感染部位包括生殖道、咽部、直肠)在接受单剂量口服索利霉素1 g

或1.2 g治疗后, 经淋球菌培养发现全部转为阴性^[72]。之后进行的3期临床试验比较了单剂量口服索利霉素1 g与肌注头孢曲松0.5 g加口服阿奇霉素1 g对无并发症淋病患者的疗效, 结果显示, 单剂量口服索利霉素1 g不能替代双重疗法作为淋病治疗的一线药物, 失败的原因可能与药物作用时间及服用剂量有关^[73], 还需要进行更深入的试验研究。

3.2 吉泊达星

吉泊达星是一种新型的II型拓扑异构酶抑制剂类药物, 具有双靶向作用机制, 可选择性地与DNA促旋酶和拓扑异构酶IV相互作用, 抑制细菌DNA复制^[74]。已经完成的2期临床试验显示, 69名无并发症淋病患者(感染部位包括生殖道、咽部、直肠)在接受单剂量口服吉泊达星1.5 g或3 g治疗后, 淋球菌的细菌根除率至少为95%^[75]。虽然目前吉泊达星对淋病治疗有较好的效果, 但对于其疗效、抗菌效果和安全性还需要3期临床试验进行研究。

3.3 唑氟达星

唑氟达星属于螺喹啉三酮II型拓扑异构酶抑制剂类药物, 同样具有双靶向作用机制, 通过抑制细菌DNA合成达到抑菌效果^[76]。该药物的2期临床试验结果显示, 在对泌尿生殖道淋病患者的治疗中, 单剂量口服唑氟达星2 g或3 g和单剂量注射头孢曲松0.5 g, 其泌尿生殖道淋球菌感染治愈率分别为96%、96%和100%, 咽部感染治愈率分别为50%、82%和100%, 直肠感染治愈率均为100%^[77], 该药物显示了对生殖道和直肠部位淋球菌感染的良好治愈效果, 但是对咽部感染的疗效, 还需要进一步探究。

4 总结和展望

随着淋球菌耐药性的不断增加及耐药菌株的广泛传播, 淋病的防治已成为一个全球性的公共健康问题。淋球菌可通过多种机制产生耐药性, 需要不断完善耐药监测机制, 以应对复杂耐药机制给耐药性检测带来的困难。在治疗上, 目前的抗菌药物可能面临着失效的困境, 但新抗菌药物的研发为调整治疗策略带来了希望, 同时, 临床治疗方面也进行了调整治疗策略、单次药物剂量等研究, 为延缓淋球菌的耐药性提供了

有意义的探索^[78]。此外, 未来的淋病防治不应只局限在“治”, “防”也同样重要, 对大众普及预防淋病传播的

科学知识, 规范合理地使用抗菌药物, 加强疫苗研发也是淋病防治的重要方面。

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Advances in research on the resistance mechanism of *Neisseria gonorrhoeae*

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Gonorrhea is a sexually transmitted disease caused by *Neisseria gonorrhoeae* infection. Its prevalence and spread have become a huge burden on global public health. Antimicrobial therapy is currently the main method for the prevention and treatment of gonorrhea. However, with the widespread use of antibacterial agents in clinical practice, the resistance of *N. gonorrhoeae* has become an increasingly serious problem. Exploration of the resistance mechanism of *N. gonorrhoeae* is essential for improving its resistance monitoring system and adjusting the antimicrobial treatment regimens. We reviewed the recent advances in research on the resistance mechanism of *N. gonorrhoeae*, aiming to provide reference for the prevention and treatment of gonorrhea in China.

gonorrhea, *Neisseria gonorrhoeae*, antibacterial agents, mechanism of resistance

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