·综　　述·

鲍曼不动杆菌的外膜囊泡和外排泵在耐药中的作用*

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摘　要：多重耐药鲍曼不动杆菌的院内感染日益增多，严重影响临床住院患者尤其是重症患者治疗的总体效果。多重耐药鲍曼不动杆菌感染目前已成为临床抗感染治疗的难题，其耐药机制复杂多样，该文拟就细菌外膜囊泡，及其与外排泵的关系做简要综述。

关键词：鲍曼不动杆菌；　耐药；　外排泵；　微囊泡
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The role of Acinetobacter baumannii outer membrane vesicles and efflux pumps in drug resistance*

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Abstract: The nosocomial infection of multidrug-resistant Acinetobacter baumannii is increasing day by day, which seriously affects the overall therapeutic efficacy of hospitalized patients, especially for severe patients. Multi-drug resistant Acinetobacter baumannii infection has become a difficult problem in clinical anti-infection treatment, and its resistance mechanism is complex and diverse. This paper reviews the bacterial outer membrane vesicles and their relationship with efflux pumps.

Key words: Acinetobacter baumannii;　drug resistance;　efflux pump;　micro vesicles

在我国，鲍曼不动杆菌大多分离于重症住院患者的送检标本，其耐药性呈逐年上升的趋势，并呈现地区差异[1]。多重耐药鲍曼不动杆菌的医院内传播与流行已成为普遍关注的公共卫生问题，由此带来治疗成本与经济负担的增加，促使人们寻找更好地应对策略[2]。鉴于抗菌药物研发的相对缓慢[3]，对鲍曼不动杆菌耐药机制的研究成为热点。

1　鲍曼不动杆菌耐药机制的研究现状

临床报道的多重耐药鲍曼不动杆菌可以通过多种机制对各类抗菌药物产生耐药，主要包括产生 β-内酰胺酶，外膜通透性下降，外膜蛋白的表达及其基因结构的变化，拓扑异构酶基因突变，产生氨基糖苷类钝化酶，耐药质粒、整合子或转座子等可移动基因元件的转移，细菌生物膜的形成和外排泵的表达等。其中，β-内酰胺酶的产生是鲍曼不动杆菌重要且常见的一种耐药机制[4-5]。β-内酰胺酶分为 A、B、C、D，其中 A、C、D 三类同为青霉素结合蛋白演变而来的丝氨酸 β-内酰胺酶，B 类为金属 β-内酰胺酶[6]；D 类又有窄谱（OXA-1、OXA-10）、超广谱（OXA-13、OXA-17）和水解亚胺培南的 D 型 β-内酰胺酶之分（OXA-23、OXA-24/40、OXA-48、OXA-51、OXA-58）[7-8]，国内报道最多的为 OXA-23[9]。此外，构成外膜蛋白耐药机制的青霉素结合蛋白 3（PBP3），其 S390 突变导致苏氨酸侧链产生空间位阻，限制了底物与 S336 的相互作用，从而降低 PBP3 对氨苄青霉素和舒巴坦的反应性；S336 附近的 S395 侧链疏水苯丙氨酸位点突变，也可降低众多 β-内酰胺的反应性。鲍曼不动杆菌还可通过使其合成脂质的基因失活，导致胞膜脂质缺失而对多黏菌素耐受，如出现插入序列 ISAba11 抑制 lpxA 与 lpxC 产生的耐药现象[10-17]。

近年来研究证实，鲍曼不动杆菌表达的外排泵，是对包括内酰胺、氯霉素、大环内酯、四环素、氟喹诺酮和氨基糖苷类等在内的多种抗菌药物耐药的主要因素[11-14]。外排泵系统还能通过 EmrAB 的作用，降低鲍曼不动杆菌对于多黏菌素的敏感性。外排泵表达于鲍曼不动杆菌的细胞壁或细胞膜，而鲍曼不动杆菌微囊泡可能有传递耐药基因如碳青霉烯酶 OXA-24 使周围鲍曼不动杆菌获得耐药性的作用[18-19]。因此，

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除了对外排泵在鲍曼不动杆菌耐药中的作用及机制进行深入探讨外，开展细菌微囊泡与外排泵关系的研究，也将有助于对鲍曼不动杆菌院内感染的防控和指导抗菌药物的应用。

2　鲍曼不动杆菌外膜囊泡

鲍曼不动杆菌在不同的生长时期可以产生形态及组成不同的外膜囊泡或微囊泡，在对鲍曼不动杆菌进行冷电镜扫描观察时发现，培养早期的细菌，其远端可见小于 30 nm 的囊泡状凸起，及 200～500 nm 大小、表面光滑且无胞膜蛋白的细菌外膜囊泡（OMVs）；而在平稳期，形成较多含胞质及胞膜蛋白的内-外膜囊泡（IOMVs）[20]。这可能与利用动态光散射技术所观察到的 10～150 nm 大小的外膜囊泡部分相一致，它包含胞质、包膜及胞质间隙的各种蛋白成分，而这些蛋白成分与鲍曼不动杆菌生物膜的形成、群体感应、氧化应激耐受性和细胞毒性等功能有关[21]。有报道称，IOMVs 只占细菌所产生的总囊泡量的 0.23%～1.20%，但其所包含的 DNA、胞质及内膜蛋白与细菌耐药基因横向转移、细胞蛋白的转移等相关，正吸引人们的关注和形成新的研究领域[22]。研究表明，鲍曼不动杆菌可以通过 Omvs 传递耐药基因，近年来的报道又证实 OMVs 横向传递 β-内酰胺酶基因的作用[19,23]。诸多研究结果已经显示，OMVs 参与细菌耐药性形成的形式并非单一，且有一定的致病性和免疫性。例如产 β 内酰胺酶的细菌可以通过释放含酶的 Omvs 直接降解周围的相关抗菌药物，使敏感菌免受抗菌药物的影响；OMVs 还能参与针对细胞膜的抗菌药物的耐药[24-29]；鲍曼不动杆菌囊泡可以传递 omp33-36 导致受感染细胞的凋亡与自噬，并向细胞传递具有细胞毒性的 OmpA，构成鲍曼不动杆菌的致病性[25-26]；鉴于细菌囊泡含有细菌胞质及外膜蛋白等成分，用作细菌疫苗也引起了人们的研究兴趣。有报道表明，通过干预细菌 TolA 基因产生的富含 OmpA 与 Lps 的囊泡具有保护小鼠免受感染的作用。然而，值得注意的是，鲍曼不动杆菌的细胞壁外层含有荚膜多糖，这将阻碍抗体与 OmpA 的结合，构成 Omvs 用作疫苗激发保护性体液免疫的障碍[27-28]。鲍曼不动杆菌 OMVs 参与的耐药机制、OMVs 的致病性与免疫性仍待进一步深入研究。

3　鲍曼不动杆菌外排泵

目前，公认的鲍曼不动杆菌外排泵包括 RND、MATE、MFS、SMR 和 ABC 超家族[30]，此外还有新发现的 pace 家族[31]。在 RND 家族中，adeABC 系统通过质子动力依赖的外排机制参与氨基糖苷类药物的抵抗，也影响氟喹诺酮类、四环素类、氯霉素、红霉素和替加环素的抗菌效果[32-33]。此系统受 adeR-adeS 双组分系统调节，针对这一靶点可以设计出新的药物，从而避免鲍曼不动杆菌 adeABC 过表达导致的耐药性。adeIJK、adeFGH 等其他 RND 家族成员，也参与鲍曼不动杆菌对多种抗菌药的耐药[34-36]，有报道称在低剂量抗菌药物作用下，adeFGH 可外排自诱导分子，具有在经久不愈的创面上形成生物膜的潜能[37]。SMR 家族的 AbeS，则可通过氢-药逆向转运体排出菌体内的药物，形成对卡那霉素、红霉素和氯霉素的耐药性[38-39]；而在 MFS 家族，磷霉素可诱导 AbaF 表达，高表达的 AbaF 可降低鲍曼不动杆菌对磷霉素的敏感性和促进生物膜的形成[40]，MFS 家族外排泵还有 AmvA、CraA、TetA、ClmA 和导致对米诺环素耐药的 TetB[41-45]。

近期发现的一种小分子外排泵抑制剂 IITR08027，其可通过对外排泵 MATE 家族成员 AbeM 的抵抗，从而逆转对氟喹诺酮类药物的敏感性[46-47]；然而，在对氨基糖苷类药物外排过程中，adeJ 等发挥主要作用，而 AbeM 仅为次要作用，提示 AbeM 的药物外排作用可能存在着底物特异性[48]。此外，ABC 家族相关蛋白的表达与舒巴坦的抗菌药效相关；而近年来新报道的 Pace 家族外排泵 AceI，则与细菌形成对消毒剂洗必泰的抗性相关。研究表明，在洗必泰的作用下，刺激 AceR 对 AceI 表达的调控，增强对洗必泰的抗性[49-50]。鲍曼不动杆菌固有或获得性的外排泵家族种类繁多，对不同种类抗菌药物外排效果也存有差异，探讨不同外排泵表达的调控机制和药物外排过程，将为控制细菌耐药性的发生及临床耐药菌感染的治疗提供新的思路。

4　鲍曼不动杆菌外膜囊泡与外排泵的关系

已有的研究表明，一些抗菌药物如庆大霉素、多黏菌素 B、多黏菌素 E、头孢他啶和亚胺培南等，可以增加鲍曼不动杆菌外膜囊泡的产生[51]；而另一些抗菌药物如亚胺培南等，则能诱导外排泵过表达从而增强其对抗菌药物的耐药性[52]。此外，在对绿脓假单胞菌及其他革兰阴性杆菌的研究发现，其外膜囊泡含有多药耐药外排泵 Mex、Mtr、TolC 和外膜蛋白 OmpA、OmpC、OmpF。由于鲍曼不动杆菌外排泵和外膜囊泡均能因抗菌药物诱导增加，那么在特定抗菌药物的诱导下，多重耐药鲍曼不动杆菌产生的外膜囊泡上各外排泵蛋白总量及活性，理应发生相应改变。通过检测和分析细菌外膜囊泡外排泵的表达种类与水平，对评估和监测临床鲍曼不动杆菌的耐药性及流行趋势将有促进作用，也有助于对鲍曼不动杆菌耐药机制的进一步认识和指导抗菌药物的临床应用。

5　结　　语

多重耐药鲍曼不动杆菌院内感染对住院患者构成了严重威胁，其耐药机制的复杂性、耐药范围的广泛性、院内感染的普遍性，正吸引越来越多研究者们的关注。初步研究业已显示，鲍曼不动杆菌外膜囊泡在致病性和耐药性形成的过程中发挥重要作用，但其确切机制及与外排泵等其他耐药机制有何关系，尚不十分清楚；能否通过外膜囊泡的检测，达到全面了解细菌耐药性之目的，也有待进一步的试验追溯。

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