微生物学报
Acta Microbiologica Sinica
2023, 63(11): 4101–4117
http://journals.im.ac.cn/actamicrocn
DOI: 10.13343/j.cnki.wsxb.20230191

Review 综 述

新型可移动 RND 家族外排泵 TMexCD-TOprJ 的研究进展

孙敬都[1]，贾程皓[1,2]，唐标[3]，赵国屏[4,5,6]，乐敏[1,2*]

1 浙江大学动物科学学院，浙江 杭州 310030
2 浙江大学海南研究院，海南 三亚 572025
3 浙江省农业科学院农产品质量安全与营养研究所，浙江 杭州 310021
4 中国科学院分子植物科学卓越创新中心 合成生物学重点实验室，上海 200031
5 中国科学院上海营养与健康研究所 生物医学大数据中心，上海 200031
6 中国科学院大学国科大杭州高等研究院 生命与健康科学学院，浙江 杭州 310024

孙敬都, 贾程皓, 唐标, 赵国屏, 乐敏. 新型可移动 RND 家族外排泵 TMexCD-TOprJ 的研究进展[J]. 微生物学报, 2023, 63(11): 4101-4117.
SUN Jingdu, JIA Chenghao, TANG Biao, ZHAO Guoping, YUE Min. Research progress in a novel mobile RND efflux pump TMexCD-TOprJ[J]. Acta Microbiologica Sinica, 2023, 63(11): 4101-4117.

摘　要：抗生素被认为是现代医学的基石之一，但包括抗生素在内抗菌药物的滥用也加速了可抵抗多种抗菌药物“超级细菌”的出现。耐药基因是导致细菌产生耐药性的关键因素，可通过质粒、转座子(transposon, Tn)、插入序列(insertion sequence, IS)等可移动元件(mobile genetic elements, MGEs)进行水平转移，严重威胁公共卫生安全。近年来，面对碳青霉烯类药物和多黏菌素耐药性的暴发，替加环素被视为人类面临多重耐药细菌感染的最后一道防线。近期发现了一种主要存在于质粒上的新型可移动外排泵基因簇 *tmexCD-toprJ*，可编码耐药结节细胞分化家族(resistance-nodulation-cell division, RND)外排泵，排出菌体内包括替加环素在内的多种抗生素，大幅提升了细菌的耐药性。*tmexCD-toprJ* 基因簇可以随质粒等可移动元件进行水平转移，已经传播至人、动物和环境中，给公共卫生健康造成了严重威胁。然而，目前人们对于其具体结构和功能作用机制等研究仍不透彻。本文系统总结 *tmexCD-toprJ* 耐药基因的分布特征、传播机制及外排泵结构等研究现状，并基于同一健康(One Health)理念提出了阻遏其扩散的措施，为减缓 *tmexCD-toprJ* 传播提供科学依据。

资助项目：浙江省“领雁”研发攻关计划(2023C03045)；杭州市重点研发计划(2022ZDSJ0135)
This work was supported by the “Leading Goose” Research and Development Project of Zhejiang Province (2023C03045) and the Key Research and Development Project of Hangzhou (2022ZDSJ0135).
*Corresponding author. E-mail: myue@zju.edu.cn
Received: 2023-03-20; Accepted: 2023-05-24

关键词：替加环素；耐药结节细胞分化家族外排泵；*tmexCD-toprJ*；耐药基因；水平传播

Research progress in a novel mobile RND efflux pump TMexCD-TOprJ

SUN Jingdu[1], JIA Chenghao[1,2], TANG Biao[3], ZHAO Guoping[4,5,6], YUE Min[1,2*]

1 College of Animal Sciences, Zhejiang University, Hangzhou 310030, Zhejiang, China
2 Hainan Institute of Zhejiang University, Sanya 572025, Hainan, China
3 Institute of Agricultural Product Quality, Safety and Nutrition, Zhejiang Academy of Agricultural Sciences, Hangzhou 310021, Zhejiang, China
4 Key Laboratory of Synthetic Biology, Center for Excellence in Molecular Plant Sciences, Chinese Academy of Sciences, Shanghai 200031, China
5 Biomedical Big Data Center, Shanghai Institute of Nutrition and Health, Chinese Academy of Sciences, Shanghai 200031, China
6 College of Life and Health Sciences, Hangzhou Institute for Advanced Study, University of Chinese Academy of Sciences, Hangzhou 310024, Zhejiang, China

Abstract: Antibiotics are considered one of the cornerstones of modern medicine. However, the abuse of antimicrobial agents, including antibiotics, has accelerated the emergence of super bacteria that can resist multiple antimicrobial agents. Resistance genes are the key factors leading to bacterial resistance and can be horizontally transmitted by mobile genetic elements (MGEs), such as plasmids, transposons, and insertion sequences, posing a serious threat to public health. In recent years, facing the outbreak of carbapenem-resistant and polymyxin-resistant bacteria, tigecycline has been regarded as the "last line of defense" against multiple drug-resistant bacterial infections in humans. Recently, a novel mobile efflux pump gene cluster *tmexCD-toprJ*, mainly existing in plasmids, was found. This gene cluster encodes resistance-nodulation-cell division (RND) efflux pumps, which can expel a variety of antibiotics, including tigecycline, out of bacteria, greatly enhancing the resistance of bacteria. The *tmexCD-toprJ* gene cluster has been horizontally transferred to humans, animals, and the environment by mobile elements such as plasmids, posing a serious threat to public health. However, research on the specific structure and functional mechanism of TMexCD-TOprJ remains to be carried out. This article systematically summarizes the distribution characteristics, transmission mechanisms, and efflux pump structures of *tmexCD-toprJ* and proposes measures to block its spread from the concept of One Health, offering a scientific basis for slowing down the dissemination of *tmexCD-toprJ*.

Keywords: tigecycline; RND efflux pump; *tmexCD-toprJ*; antimicrobial resistance gene; horizontal transfer

抗生素等抗菌药物在临床上通常被用于预防和治疗细菌感染，并在手术和癌症治疗等现代医学领域中广泛使用，被誉为现代医学的基石。Gerhard Domagk 曾于 1935 年合成了第一种商品化的抗菌药物 prontosil 用以对抗链球菌感染[1]。但随着抗生素等抗菌药物的大量使用，越来越多

✉ actamicro@im.ac.cn, ☎ 010-64807516

的细菌出现了多重耐药性(multi-drug resistance, MDR)。这类细菌可以通过突变细菌靶蛋白、编码药物失活酶、改变细胞膜渗透性和外排药物等机制单独或协同抵抗药物作用，并在人、动物和环境中广泛传播[2-5]。近年来碳青霉烯类耐药肠杆菌的出现使临床死亡率急剧上升，有报道指出，临床上碳青霉烯类耐药肺炎克雷伯菌(carbapenem-resistant *Klebsiella pneumoniae*, CRKP)感染患者的合并死亡率可达到 42.14%[6]。此外，随着多黏菌素耐药基因 *mcr* 的广泛传播，多黏菌素的临床作用也被大大削弱[7-8]。在此背景下，替加环素被认为是治疗碳青霉烯及多黏菌素耐药细菌等致命细菌感染的最后一道防线[9]。

替加环素是辉瑞公司开发的第三代四环素类药物，具有广泛的底物谱，能够与 30S 核糖体亚基可逆性结合阻止肽链延长，从而达到抗菌效果。但随着高水平替加环素耐药的 *tet*(X)和 *tmexCD-toprJ* 基因的广泛传播，替加环素也可能面临严重的危机[10]。本文聚焦于新型可移动外排泵基因 *tmexCD-toprJ* 的传播机制及研究现状等，为减缓 *tmexCD-toprJ* 耐药基因的传播提供参考。

1 耐药结节细胞分化家族(resistance-nodulation-cell division, RND)外排泵的作用机制

将药物通过外排泵从体内排出是细菌中常见的耐药机制。外排泵是一类位于细胞膜上，并能将抗菌药物、重金属、去垢剂等多种物质从细胞内泵出至细胞外的蛋白，可分为耐药结节细胞分化家族(resistance-nodulation-cell division, RND)、主要协助转运蛋白超家族(major facilitator superfamily, MFS)、ATP 结合盒家族(ATP-binding cassette, ABC)等[11-13]。RND 外排泵多存在于革兰氏阴性细菌中，主要由负责底物识别和主动转运的质子动力驱动的内膜转运蛋白(inner membrane protein, IMP)、负责连接转运蛋白与通道蛋白使结构稳定的膜间质融合蛋白(membrane fusion protein, MFP)和为底物提供穿越外膜孔道的外膜蛋白(outer membrane protein, OMP)共同构成一个完整的跨越细胞内膜、周质空间和外膜的通道(图 1)，是最常见的多重药物外排转运蛋白类型之一，并能协助形成生物

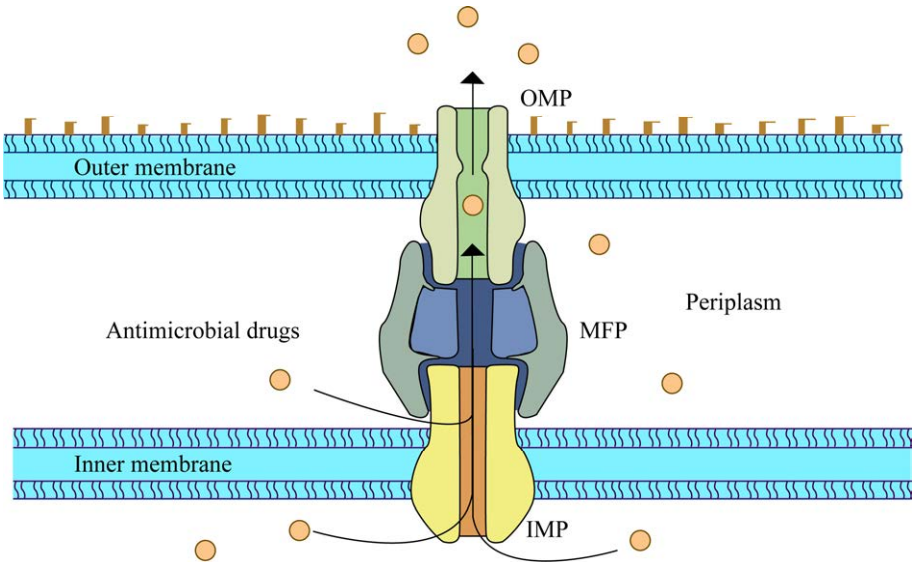

图 1 RND 外排泵模式图

Figure 1 Schematic diagram of RND efflux pump mode.

膜[14-15]。不同于 Tet(A)等单组份外排泵仅能将药物排至周质空间，RND 外排泵可以将药物直接外排至外界环境中，并与外膜协同作用减少药物再次进入细菌体内的机会，从而更有效地降低体内药物浓度，增强细菌的耐药能力[4]。

2 *tmexCD-toprJ* 基因的发现及分布

mexCD-oprJ 是存在于铜绿假单胞菌染色体上的外排泵基因，能编码 RND 家族外排泵 MexCD-OprJ，提升细菌对多种抗菌药物的耐药性。最近，LV 等[16]于肺炎克雷伯菌的质粒中发现了与 *mexCD-oprJ* 基因具有较高同源性的基因簇，二者的氨基酸序列同一性超过了 60%。由于该基因簇由质粒携带并具有可转移性，因此被命名为 *tmexCD1-toprJ1* (t 代表可转移性)。其编码的外排泵蛋白 TMexCD1-TOprJ1 与 RND 家族蛋白 MexCD-OprJ、MexAB-OprM 及 AcrAB-TolC 有高度相似性；外膜蛋白 TMexD1 拥有 RND 家族蛋白共有的 12 个跨膜片段，预示 TMexCD1-TOprJ1 是 RND 外排泵家族的一员。几乎同时，有报道称在山东的养鸡场内分离出了 *tmexCD1-toprJ1* 阳性的肺炎克雷伯菌，提示 *tmexCD1-toprJ1* 或许早已广泛传播[17]。*tmexCD-toprJ* 在不同种类的细菌分布具有差异性，目前收录于 NCBI 数据库中的 *tmexCD1-toprJ1* 阳性菌株以假单胞菌和气单胞菌为主，肠杆菌科细菌中以克雷伯氏菌检出最多[18]，但在沙门菌中罕见[19-21]。

随后，tmexCD1-*toprJ1* 的同源基因 *tmexCD2-toprJ2*、*tmexCD3-toprJ3*、*tmexCD4-toprJ4* 等被陆续报道。此外，在 NCBI 数据库中还检索到了大量的 *tmexCD-toprJ* 基因，最早可追溯至 1997 年的印度。其中超过 70%的 *tmexCD-toprJ* 基因由质粒携带，仅有少部分位于细菌染色体上，目前已经广泛分布于人、动物及环境中。研究表明，*tmexCD-toprJ* 基因可能起源于假单胞菌属，在位点特异性整合酶等作用下从染色体上转移，并通过可移动元件的介导在不同细菌间传播；耐药性方面，*tmexCD-toprJ* 能使细菌对替加环素的 MIC 值上升 4 至 32 倍，还表现出对环丙沙星、氯霉素、强力霉素和头孢噻肟等多种药物的耐药性，并可与多黏菌素耐药基因 *mcr* 等多种耐药基因共存和共转移，大大增加了“超级细菌”出现的风险[22]。目前，*tmexCD-toprJ* 基因在中国等亚洲国家检出较多(表 1，表 2，表 3)。总之，这

表 1 *tmexCD-toprJ* 基因在世界范围内的检出情况

Table 1 Detection of *tmexCD-toprJ* gene in the worldwide

Continent	Country/Region	Isolated year	Source	Quantity
Asia	China	2010−2022	Human, animal and environment	156
	Vietnam	2015, 2021	Human and environment	13
	India	1997, 2012	Human and environment	2
	Japan	2020	Environment	2
	Lebanon	2018	Human	2
	Hong Kong, China	2017	Human	1
	Thailand	2013	Human	1
Africa	Ghana	2017	Environment	2
	Kenya	UN	UN	UN
Europe	Sweden	2001	Human	1
	Poland	2014	Human	1
	Britain	UN	UN	UN
North America	USA	2020	Human	2
	Mexico	UN	UN	UN

Date as of March 1, 2023, based on NCBI database with positive strains in unlabeled isolated areas; UN presents the data unknown.

表 2 *tmexCD-toprJ* 基因在国内的检出情况

Table 2 Detection of *tmexCD-toprJ* gene in China

Administrative region	Source	Quantity	Total
Jiangsu	Animal	25	37
	Environment	7	
	Human	4	
	UN	1	
Henan	Human	16	19
	Animal	3	
Zhejiang	Human	11	17
	Animal	2	
	Environment	2	
	UN	2	
Beijing	Human	6	12
	Environment	3	
	UN	3	
Shandong	Animal	8	11
	Human	3	
Guangdong	Environment	3	9
	Human	3	
	Animal	2	
	Vegetable	1	
Sichuan	Environment	4	7
	Human	3	
Hunan	Animal	3	8
	Human	3	
	UN	2	
Hainan	Human	2	3
	Environment	1	
Liaoning	Animal	2	2
Fujian	Human	2	2
Hubei	Human	2	2
Chongqing	Human	2	2
Yunnan	Human	2	2
Anhui	Animal	1	1
Xizang	Animal	1	1
Hong Kong	Human	1	1
UN	Animal	9	20
	Human	9	
	Environment	1	
	UN	1	
China (total)	Human	69	156
	Animal	56	
	Environment	21	
	Vegetable	1	
	UN	9	

Date as of March 1, 2023, based on NCBI database with positive strains in unlabeled isolated areas; UN presents the data unknown.

类可移动外排泵基因的广泛分布及其外排泵的底物广泛性，对人畜健康以及食品安全都构成了巨大的潜在风险，值得人们警惕。

3 *tmexCD-toprJ* 的水平转移机制

耐药细菌是目前全世界公共卫生健康面临的最大挑战之一，细菌耐药基因的获得和传播与可移动遗传元件有着密切的联系。可移动遗传元件包括质粒、插入序列、转座子、整合性接合元件(integrative and conjugative elements, ICEs)等，是能介导耐药基因在细菌之间传播的基因组片段[47]。*tmexCD-toprJ* 基因可以由多种可移动遗传元件介导，在细菌种内和种间水平转移[48]。

3.1 质粒

质粒被认为是最重要的移动元件，与许多耐药基因的水平转移有关，在 *tmexCD-toprJ* 的传播中也扮演着重要角色[49]。分析表明，杂合型质粒可能是 *tmexCD1-toprJ1* 阳性肺炎克雷伯菌中 *tmexCD1-toprJ1* 基因的主要载体，特别是 IncFIB(Mar)/IncHI1B 杂合型质粒[27,29-31]。该质粒具有有限的宿主范围和特定的插入位点，主导基因簇在肺炎克雷伯菌中的水平传播，因此肠杆菌科其他属中很少检测到 *tmexCD1-topJ1*[31]。此外，IncHI5[24]、IncR[31]、IncFIA[17]、IncFIB[17,23]和 IncC[35]等质粒也是 *tmexCD1-toprJ1* 的重要载体。与 IncFIB(Mar)/IncHI1B 质粒相比，IncR、IncC 等质粒具有更广泛的宿主范围，增加了耐药基因转移的风险。*tmexCD2-toprJ2* 与 IncFII、IncHI1B 和 IncQ 等多种质粒密切相关，这也可能是 *tmexCD2-toprJ2* 分布更广泛的原因之一。携带 *tmexCD3-toprJ3*、*tmexCD4-toprJ4* 等基因的质粒也大多有着广泛的宿主菌范围和可转移特性[22,45]。总之，*tmexCD-toprJ* 基因在质粒上的广泛分布大大增加了其在不同细菌之间传播的风险，需要人们特别关注。

表 3　*tmexCD-toprJ* 基因的种类、分布、来源及共存的耐药基因

Table 3　Types, distribution, sources and co-existing drug resistance genes of *tmexCD-toprJ* genes

Gene	Country	Region	Source	Bacteria	Type of plasmid	Co-carried drug resistance genes and references
tmexCD1-toprJ1	China	Shandong	Chicken	*Klebsiella pneumoniae*	IncFIA, IncFII, IncFIB, etc.	bla_{NDM-1}, bla_{NDM-5}, *mcr-8*, etc.[17]
			Flies in pig farms	*Klebsiella pneumoniae*	IncFIB	*ampC/R*, *sul1*, *rmtB*, *msr(E)*, bla_{NDM-1}, *mcr-8*, etc.[23]
		Sichuan	Hospital wastewater	*Raoultella planticola*	IncHI5	*aac(6′)-Ib3*, *aadA5*, *ant(2″)-Ia*, *armA*, *rmtC*, bla_{KPC-2}, bla_{NDM-1}, $bla_{CTX-M-14}$, bla_{PLA2a}, bla_{PER-1}, etc. [24]
			Human	*Klebsiella quasipneumoniae*	IncU/IncFIB (Mar)/IncHI1B	bla_{IMP-4}, *tet*(A), bla_{IMP-4}, *strB*, *strA*, *aac(3)-lld*, bla_{TEM-1B}, *aac(6′)-lb-cr*, bla_{OXA-1}, *catB*, *sul1*, bla_{PER-1}, etc.[25]
		Zhejiang	Migrant bird	*Pseudomonas putida*	UN	*tet*(C), *arr-3*, *aadA1*, *sul1*, *qnrVC1*, *dfrB1*, bla_{VIM-2}, *floR*, *strA*, *strB*, etc.[26]
			Chicken	*Klebsiella pneumoniae*	IncFIB-HI1B	*mcr-10*, *mcr-8*, *msr(E)*, *aph(3′)-Ib*, *aadA1*, *aadA2b*, *armA*, *aph(6)-Id*, *aph(3′)-Ia*, *qnrB4*, *sul1*, *sul3*, bla_{DHA-1}, *mph(E)*, *qacE*, etc.[27]
			Hospital wastewater	*Klebsiella michiganensis*, *Klebsiella oxytoca*, *Citrobacter youngae*, *Raoultella ornithinolytica*, etc.	UN	bla_{NDM-1}, bla_{KPC-2}, bla_{IMP-1}, *mcr-3*, etc.[28]
			Chicken farm	*Klebsiella pneumoniae*	IncFIB-HI1B	*msr(E)*, *aph(3′)-Ib*, *aadA1*, *armA*, *aph(3′)-Ia*, *qnrB4*, *sul1*, *cmlA1*, etc.[27]
		Yunnan	Human	*Klebsiella pneumoniae*	IncHI1B/FIB	*aadA1*, *aadA2*, *cmlA1*, *sul3*, *aac(3)-Iva*, *strA*, *strB*, *msr(E)*, *armA*, etc.[29]
		Jiangsu	Chicken	*Klebsiella pneumoniae*	IncFIB/IncFII, IncFIA/IncFIB, etc.	$bla_{CTX-M-27}$, $bla_{SHV-11/12}$, bla_{TEM-1B}, *floR*, *sul1/3*, *oqxAB*, *fosA*, *rmtB*, *aac(6′)Ib*, *aadA*, *ant(2″)- Ia*, *aph(3″)-Ib*, *aph(6)-Id*, *mcr-8*, etc.[30]
			Retail pork, slaughterhouse	*Klebsiella pneumoniae*, *Proteus mirabilis*	IncR, IncFIB(Mar)/Inc HI1B, etc.	*fosA*, *sul2*, *armA*, *qnrS1*, *mph(A)*, etc.[31]
		Henan	Human sputum and blood	*Klebsiella pneumoniae*	IncHI5	*sul1*, bla_{NDM-1}, *mph(A)*, *trpF*, *aac(3)*, etc.[32]
		Hong Kong	Human blood	*Klebsiella pneumoniae*	IncFIB/IncHI1B	*strAB*, *armA*, *aph(3′)-Ia*, *qnrB4*, *sul1*, *mphA*, *mphE*, *msrE*, bla_{DHA-1}, etc.[33]
		Liaoning, Shanxi, Hunan, Guizhou	Farm manure	UN	UN	UN[34]

(待续)

(续表 3)

Gene	Country	Region	Source	Bacteria	Type of plasmid	Co-carried drug resistance genes and references
		Guangdong	Food market sewage	*Citrobacter portucalensis*	IncC	*bla*$_{TEM-1b}$, *rmtB*, *qepA1*, *tet*(A), *strAB*, *sul2*, etc.[35]
			Food market sewage	*Klebsiella pneumoniae*	IncHI1B-IncFIB	*oqxAB*, *fosA*,*aac(3)-IV*, *aadA1*, *aadA2b*, *strA*, *strB*, *aph(4)-Ia*, etc.[36]
				Klebsiella quasipneumoniae		*aac(3)-IV*, *aadA1*, *aph(4)-Ia*, *strAB*, *armA*, *mph(E)*, *msr(E)*, *cmlA1*, *sul1*, *sul3*, *dfrA1*, etc.[36]
		UN	Human blood and urine	*Klebsiella pneumoniae*, *Klebsiella quasipneumoniae*	IncFIA-IncFII, etc.	UN[37]
		Anhui	Chicken feces	*Klebsiella pneumoniae*	IncFIA	*bla*$_{NDM-1}$, *mcr-1.1*, *mcr-8.1*, *armA*, *tet*(A), *floR*, etc.[16]
		Jiangxi	Human	*Klebsiella pneumoniae*	UN	*bla* $_{SHV-119}$, *bla* $_{DHA-1}$, *qnrB6*, *qnrB4*, *aac(6′)-Ib-cr*, *oqxA*, *oqxB*, *mph(E)*, *ARR-3*, *sul1*, *sul2*, *sul3*, *tet*(A), *dfrA27*, etc.[38]
		Beijing	Human	*Klebsiella pneumoniae*	IncHI1B, IncFIB	*bla*$_{NDM-1}$, etc.[39]
				Enterobacter cloacae	IncU	
	Vietnam	Hanoi	Human	*Klebsiella pneumoniae*	IncHI1B/FIB	/[40]
tmexCD2-toprJ2	China	Shandong	Human sputum	*Klebsiella variicola*	IncHI1B	*aac(6′)-Ib*, *aadA16*, *qnrS1*, *mphE*, *msrE*, *catB3*, *catII.2*, *arr-3*, *sul1*, *dfrA27*, etc.[41]
		Fujian	Human urine	*Klebsiella quasipneumoniae*		*sul1*, *aac(3)-Iid*, *aadA16*, *qnrS1*, *mphA*, *dfrA27*, *bla*$_{TEM-1D}$, *arr-3*, etc.[41]
			Human blood	*Klebsiella michiganensis*		*bla*$_{MBL}$, *bla*$_{NDM-1}$, etc.[41]
		Jiangxi	Human sputum	*Raoultella ornithinolytica*	IncFIBK	*bla*$_{NDM-1}$, *bla*$_{KPC-2}$, *rmtC*, *oqxAB*, *fosA*, etc.[42]
		Henan, Shandong	Human	*Klebsiella oxytoca*	lncU	*bla*$_{NDM-1}$, *mcr-6602*, etc.[39]
		Sichuan	Human	*Klebsiella pneumoniae*	IncHI1B, IncFIB	*bla*$_{NDM-1}$, etc.[39]
			Human	*Klebsiella variicola*	IncFIB(Mar)/IncHI1B	*bla*$_{IMP-4}$, *bla*$_{NDM-1}$, *qnrS1*, *strA*, *bla*$_{SFO-1}$, *aac(6′)-lld*, *bla*$_{IMP-4}$, etc.[25]
		Zhejiang	Animal feces, hospital wastewater, clinical patients, etc.	*Aeromonas caviae*, *Aeromonas hydrophilia*, *Aeromonas salmonicida*, *Aeromonas veronii*	UN	*bla*$_{KPC-2}$, *bla*$_{NDM-1}$, *bla*$_{VIM-1}$, *bla*$_{KPC-2}$, *mcr-3*, etc.[43]

(待续)

(续表 3)

Gene	Country	Region	Source	Bacteria	Type of plasmid	Co-carried drug resistance genes and references
			Hospital wastewater	*Klebsiella oxytoca*, *Citrobacter youngae*, *Raoultella ornithinolytica*, *Aeromonas*, etc.	UN	bla_{NDM-1}, bla_{KPC-2}, bla_{IMP-1}, *mcr-3*, etc.[28]
tmexCD3-toprJ3	China	Jiangsu	Pig feces	*Proteus mirabilis*	UN	*catA1*, *floR*, *sul2*, *aph(4)-Ia*, *aac(3)-IVa*, *aph(3')-Ia*, *strA*, *strB*, *aac(3)-IId*, *sul1*, *arr-3*, *catB3*, $bla_{CTX-M-65}$, *fosA*, *aadA5*, *dfrA17*, *aadA1*, etc.[44]
		Zhejiang	Animal feces, hospital wastewater, clinical patients, etc.	*Aeromonas caviae*, *Aeromonas hydrophilia*, *Aeromonas salmonicida*, *Aeromonas veronii*	UN	bla_{KPC-2}, bla_{NDM-1}, bla_{VIM-1}, bla_{KPC-2}, *mcr-3*, etc.[43]
			Hospital wastewater	*Klebsiella michiganensis*, *Klebsiella oxytoca*, *Raoultella ornithinolytica*, *Aeromonas*, etc.	UN	bla_{NDM-1}, bla_{KPC-2}, bla_{IMP-1}, *mcr-3*, etc.[28]
	Vietnam	Hanoi	Urban sewerage	*Klebsiella aerogenes*	IncC-IncX3	bla_{NDM-4}, *tet*(X), *sul2*, etc.[45]
tmexCD3-toprJ1b	China	Anhui, Shandong, etc.	Chicken feces	*Proteus cibarius*, *Pseudomonas aeruginosa*, *Proteus mirabilis*	UN	*tet*(X391), *arr-3*, *aac(6')-Ib-cr5*, bla_{DHA-1}, *lnu(F)*, *sul1*, etc.[46]
tmexCD4-toprJ4	China	Guangdong	Chicken	*Klebsiella quasipneumoniae*	UN	*oqxAB*, *fosA*, *aac(3)-IV*, *aph(4)-Ia*, *aph(3')-Ia*, *aadA2*, *aph(3')-Ia*, *tet*(A), *floR*, etc.[22]
			Farm product market environment	*Enterobacter roggenkampii*	UN	bla_{MIR-6}, *qnrS1*, *tet*(A), *floR*, etc.[22]

Date as of March 1, 2023, based on NCBI database; UN and "/" presents the data unknown.

3.2 插入序列

以 IS*26* 为代表的插入序列能促进 *tmexCD-toprJ* 基因的水平传播。肺炎克雷伯菌 GD111RT 的 pHN111RT-1 和拟肺炎克雷伯 HN111WT 的 pHN111WT-1 质粒上的 *tmexCD1-toprJ1* 可能是通过形成的环状中间体上的 IS*26* 和原始质粒上的 IS*26* 的作用而被共整合到新质粒之中[36]。在肺炎克雷伯菌和拟肺炎

克雷伯菌中也发现了 IS*903B* 和 IS*Kpn47* 元件，这两个插入序列均可能介导 *tmexCD1-toprJ1* 的水平转移[37]。FU 等[34]通过实时定量荧光 PCR 等方法证实 *tmexCD1-toprJ1* 的丰度与 IS*26* 呈强正相关，进一步证实了 IS*26* 促进了 *tmexCD1-toprJ1* 的转移。IS*L3* 和 IS*1182* 等插入序列也可能在 *tmexCD3-toprJ3* 的水平传播中起到推动作用[45]。

3.3 转座子

转座子包含转座酶或重组酶，可以通过剪切、插入等机制携带耐药基因在基因组内移动，从而实现耐药基因的传播[50]，在 *tmexCD-toprJ* 基因的水平传播中起到了重要的作用。其中，转座子 Tn*5393* 是 *tmexCD1-toprJ1* 基因移动的关键移动元件之一。在质粒 pHNAH8I 中，*tmexCD1-toprJ1* 基因与 2 个编码位点特异性整合酶(*int*)的基因和 2 个功能未知的基因(*hp1* 和 *hp2*)插入转座子 Tn*5393* 的转座酶基因 *tnpA* 中，共同形成了 15 695 bp 的插入片段[16]；在肺炎克雷伯菌和恶臭假单胞菌的质粒 pKA3-2、pZXPA-20-602k[17,26,33]等质粒中也发现了相似的插入结构，表明转座子 Tn*5393* 可能促进 *tmexCD1-toprJ1* 基因在不同细菌间的传播。此外，转座子 Tn*1721*、Tn*6347* 等也可能促进了 *tmexCD1-toprJ1* 的传播[24,35]。

3.4 整合性接合元件

整合性接合元件(ICEs)是一种存在于细菌染色体上并且具有切除、接合和转移等能力的遗传移动元件，对耐药基因的传播起到重要作用。SXT/ R391 ICE是目前研究最多的整合性接合元件家族，在肠杆菌科细菌中分布广泛。在奇异变形杆菌 RGF134-1 中，*tmexCD3-toprJ3* 被插入到 SXT/R391 ICE 可变区III的 *umuC* 基因中[44]；在卡氏变形杆菌的 SXT/R391 ICE 中也发现了 *tmexCD3-toprJ1b*，并且与 *tet*(X6)基因共存。相同的结构也在肺炎克雷伯菌、假单胞菌属、气单胞菌属等细菌中发现，表明 *tmexCD3-toprJ1b* 基因可能通过 SXT/R391 ICE 在细菌种间进行转移[46]。值得注意的是，*tmexCD-toprJ* 插入 *umuC* 基因的现象并不少见。在变栖克雷伯菌中，*tmexCD2-toprJ2* 同样被插入 *umuC* 基因中[25]，相似的现象也越来越多地被报道，包括 *tmexCD1-toprJ1*、*tmexCD2-toprJ2* 等，表明 *umuC* 基因可能是 *tmexCD-toprJ* 的特异性整合热点[32,41]。另外一个功能相似的细菌聚合酶基因，其热点突变同样也驱动细菌耐药性产生[51]。

此外，含有 *tmexCD-toprJ* 的保守移动单元 *int-int-hp-hp-tnfxB-tmexCD-toprJ* 及其类似的移动单元存在于许多细菌中，可能是 *tmexCD-toprJ* 的水平转移的关键结构。这意味着这些 *tmexCD-toprJ* 基因可能源于共同的祖先，随着可移动元件被转移到不同种类的细菌中[25]；假定的整合酶 XerD 介导的位点特异性整合系统等也可能介导 *tmexCD-toprJ* 转移，但其生物学功能还需要进一步研究[32,39]。

总之，*tmexCD-toprJ* 基因的水平转移与质粒、插入元件、ICE、转座子及整合酶等因素密切相关。尽管质粒介导的 *tmexCD-toprJ* 的水平转移更为普遍，但 *tmexCD-toprJ* 基因在其他移动元件(如 ICE)中的出现和传播无疑加剧了当前细菌耐药现状。其中，*umuC* 基因作为 SXT/R391 ICE 家族可变区III的整合位点显示出捕获并转移 *tmexCD-toprJ* 基因的能力，这应当引起人们的重视。SXT/R391 家族 ICE 元件本身携带有大量耐药基因，加上其捕获与转移 *tmexCD-toprJ* 基因的能力，可能会大幅增强细菌的耐药性。可以预见，这种“超级细菌”的持续传播将给公共卫生健康带来巨大隐患，加强监测并研究 SXT/R391 ICE 等移动元件在 *tmexCD-toprJ* 等基因在传播和进化中的作用迫在眉睫[44]。值得注意的是，各种可移动遗传元

件并不一定单独存在，在进化过程中可移动遗传元件也可能发生分裂或被其他元件插入，最终共同发挥作用，促进 *tmexCD-toprJ* 基因在细菌之间的扩散。

4 RND家族及TMexCD-TOprJ结构研究进展

4.1 RND家族外排泵结构

常用的蛋白结构解析技术包括 X 射线晶体衍射技术、冷冻电镜技术、多维核磁共振技术等，RND 外排泵蛋白结构的解析大多通过 X 射线晶体衍射技术和冷冻电子显微镜技术进行。例如多噬伯克霍尔德氏菌的 HpnN[52]、铜绿假单胞菌的 OprN 与 OprJ[53]等膜蛋白都通过X射线晶体衍射技术进行了解析。冷冻电镜(cryogenic electron microscopy, Cyro-EM)技术能够追踪蛋白的多种构象变化，对于蛋白的浓度要求更低，且适用于大型的蛋白复合物结构解析，因此在膜蛋白结构研究中应用日渐广泛。目前已经使用该方法解析出大肠杆菌的 AcrAB-TolC 以及铜绿假单胞菌的 MexAB-OprM 等结构[54-55]。

为了解决去垢剂对蛋白天然构象的影响，脂质体、纳米盘、脂立方相等人造脂质双分子层模拟物等膜稳定技术近年来逐渐兴起[56]。随着 AlphaFold、RoseTTAFold 等人工智能技术的发展，绝大多数蛋白均可以通过人工智能技术快速预测，并且具有很高的精确度，极大推动了结构生物学的发展。

4.2 TMexCD-TOprJ结构预测情况

截至目前，TMexCD-TOprJ 高分辨率蛋白结构仍未被解析。虽然 RND 外排泵大都形成三聚体，但仍有单体及二聚体结构被报道。例如与霍类物质转移至外膜密切相关的 HpnN 蛋白，其晶体结构表明 HpnN 是二聚体，整体呈现独特的蝴蝶状，而非传统的三聚体复合物形态[52,57]。尽管 RND 外排泵在结构上具有相似性，但其个体的底物运输机制及亚基装配等均存在一定的差异，而这些差异也反映在蛋白的功能上，同时也意味着外排泵抑制剂的设计需要针对每个单独的蛋白结构来进行[58]。根据 AlphaFold 对 TMexCD2-TOprJ2 等蛋白的预测，该外排泵内膜蛋白、膜融合蛋白和外膜蛋白的组装与 RND 家族外排泵代表 AcrAB-TolC 高度相似(图 2)。但其真实性与准确性仍需要通过实验验证。

5 针对 *tmexCD-toprJ* 扩散的防治策略

One Health 理念又称同一健康理念、全健康理念，它从人类、动物与环境健康的交界面出发，强调人、动物和环境健康的整体性及可持续发展。One Health 理念认为，一个生态系统的耐药性很容易传播至另一个生态系统，进而导致耐药性快速且广泛地传播[59-60]。例如，与动物密切相关的饲养员、兽医很容易将来自于动物的耐药基因传播至人类或者环境中，这也说明了“同一个世界，同一个健康”的重要性。因此，应当从人类、动物与环境健康的整体出发，加强源头控制、阻断传播并加强相关药物的研发，抑制 *temxCD-toprJ* 等耐药基因的扩散。

5.1 加强耐药基因源头控制

从 One Health 理念整体出发，应该多管齐下，从源头控制耐药基因的扩散，加强人、动物和环境多方面的共同治理[10,59-60]。在动物健康方面，应加强兽药监管，减少四环素等抗菌药物的滥用，使用替抗方案保障动物生产，并积极发挥兽医在公共卫生体系中的作用；在人类健康方面，应减少临床抗菌药物的使用，继续推进新药的研发，对已分离的菌株进行 *tmexCD-toprJ* 基

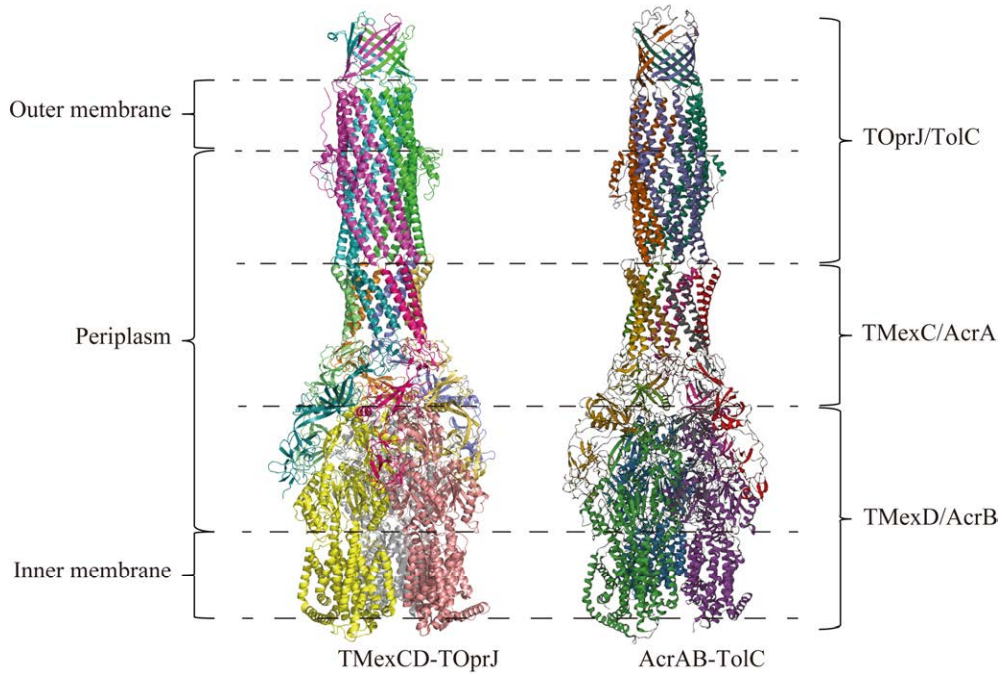

图 2　TMexCD-TOprJ 的预测结构与 RND 家族蛋白 AcrAB-TolC 三级结构比对

Figure 2　Alignment of the predicted structure of TMexCD-TOprJ with the tertiary structure of the RND family protein AcrAB-TolC. TMexCD-TOprJ GeneBank: QHW08915.1, QHW08916.1, QHW08917.1; Use AlphaFold-Multimer to predict and PyMOL to export; AcrAB-TolC PDB ID: 5V5S.

因回顾性筛查，有必要利用全基因组测序开展风险识别，并全局系统研究耐药基因的流行分布，做好监测预警工作[61-62]。对养殖人员、医护人员等高风险人群应重点检测，防止耐药基因大范围传播；在环境健康方面，应该加强对医院、畜禽批发市场、屠宰场等 *tmexCD-toprJ* 基因高检出率场所废弃物的妥善处理，对已经污染的环境进行净化，并减少耐药细菌再进入环境的机会，降低耐药基因扩散的可能性。此外，相关部门应各司其职并加强合作，建立共同治理机制，阻断耐药基因的传播。国际也应加强合作，建立起跨部门的协调机制，加强对 *tmexCD-toprJ* 等耐药基因的监测，从人类、动物和环境健康 3 方面共同发力，阻止耐药基因的进一步传播(图 3)。

5.2　阻断“畜禽粪便及污水”等耐药基因传播

我国作为畜禽养殖大国，每年产生大量的畜禽粪便、养殖污水等。据报道，我国每年产生的畜禽粪便总量将近 40 亿 t，其中有大量未经处理被用作农业肥料或排放到水体中[63]。环境因素与耐药形成密切相关，粪污中残留有大量的耐药细菌与抗菌药物等，显著影响环境中耐药基因的丰度[64-65]。数据显示，有大量的 *tmexCD-toprJ* 阳性细菌来自于动物粪便以及污水等环境中(表 1，表 2，表 3)，动物粪便及污水可能成为 *tmexCD-toprJ* 的巨大储存库，耐药基因可以通过水源、食品链、人畜接触等各种途径传到人或其他动物并进一步扩散。因此，应该以粪污为源头，对粪便及污水进行及时治理，防止耐药细菌的二次传播。一方面，要坚持养殖科学规划、科学选址，考虑环境承载能力，并严格按照标准进行粪便排放，从源头控制好粪污的排放；另一方面，应加快畜禽粪污资源化利用，加速动物粪便的无

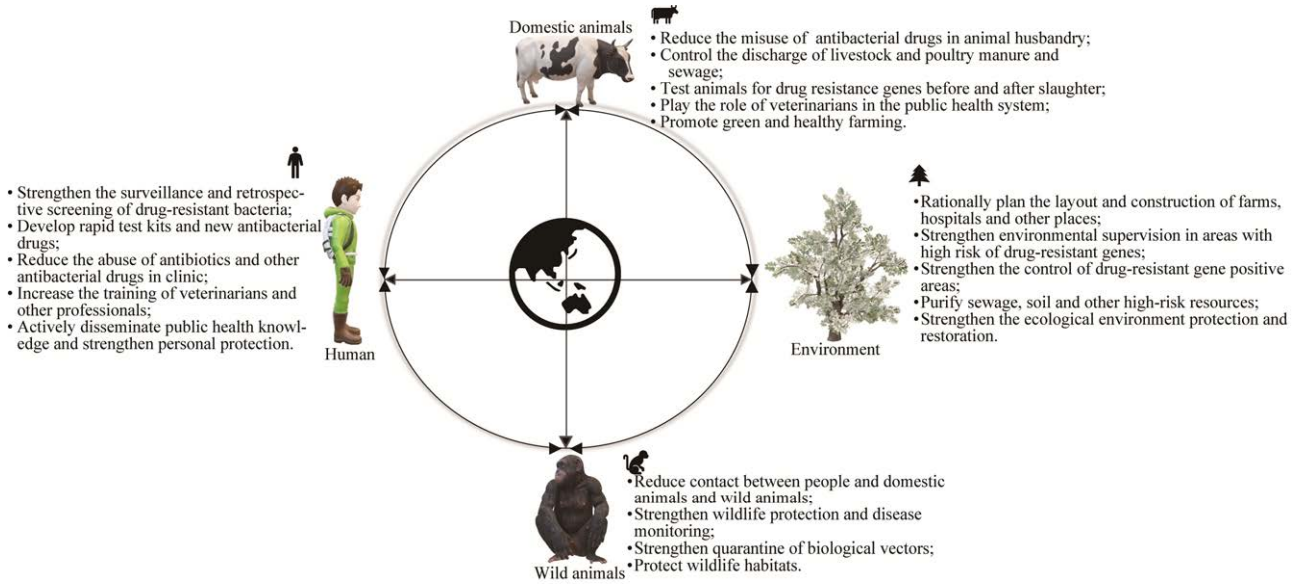

图 3　从 One Health 理念角度抑制 *tmexCD-toprJ* 等耐药基因的扩散

Figure 3　Inhibiting the spread of antibiotic resistance genes such as *tmexCD-toprJ* from the perspective of One Health.

害化处理。例如对粪便进行堆肥处理，利用高温加速抗生素和耐药基因的消除，并转换为农家肥进行资源的循环利用[66]，或通过纳米材料、酶、电催化降解等方式处理医院等的污水以去除抗生素与耐药基因[67]。值得注意的是，粪污资源化利用不仅可以降低 *tmexCD-toprJ* 等耐药基因的传播风险，还能改善环境，并将粪污变废为宝、节约资源，因此也是国家当前鼓励发展的重要措施之一，值得进一步落实与推广。

5.3　研发 TMexCD-TOprJ 外排泵特异性抑制剂

在加强对 *tmexCD-toprJ* 等耐药基因的防控的同时，也应积极开发新药物，更好地应对临床耐药细菌感染性治疗。目前研究发现质子动力(proton-motive force, PMF)是产生 ATP 的驱动力，也是细菌的物质运输和能量合成不可或缺的条件。而非甾体消炎药芐达明能够通过破坏细菌质子动力抑制 TMexCD-TOprJ 外排泵的功能，从而增加替加环素在细菌体内的积累，对革兰氏阴性菌有强大的杀菌作用；抗糖尿病药物二甲双胍也能通过耗散电势可以破坏细菌质子动力，进而削弱外排泵 TMexCD1-TOprJ1 的活性，从而协同增强替加环素的抗菌能力。这 2 种药物都能够增强替加环素的临床作用[68-69]。除了破坏质子动力之外，还可以从模仿底物竞争、降低外排泵对抗生素的亲和力、下调外排泵蛋白表达、提高抗菌药物渗透率等方法降低 TMexCD-TOprJ 外排泵蛋白活性[70-75]。然而，现有的外排泵抑制剂大多具有显著的毒性，如何开发出专一性强、毒性低、安全性高的临床药物还需进一步探索。

6　总结与展望

细菌耐药性的传播已经是人类无法回避的问题。随着以替加环素耐药细菌为代表的多重耐药细菌的扩散，或许即将面临无药可用的窘境。*tmexCD-toprJ* 等耐药基因通过质粒等可移动元件可以在不同种属之间水平转移，大大加速了替加环素等耐药性的传播，使人类面临着巨大的危

机。目前人们对 TMexCD-TOprJ 的研究大多限于其基因的来源进化及传播机制，但对于耐药基因的监测、如何控制耐药基因扩散及研制针对性的外排泵抑制剂等方面仍处于起步阶段。只有加强对 *tmexCD-toprJ* 基因的监测，全局系统研究耐药基因的流行分布，采取积极措施阻止其传播，继续对 TMexCD-TOprJ 蛋白进行研究，并进一步加强对抗菌药物的管理和环境污染的治理，团结协作，才能有效应对这一全球公共卫生危机。

参考文献

[1] ROSSOLINI GM, ARENA F, PECILE P, POLLINI S. Update on the antibiotic resistance crisis[J]. Current Opinion in Pharmacology, 2014, 18: 56-60.

[2] FERRI M, RANUCCI E, ROMAGNOLI P, GIACCONE V. Antimicrobial resistance: a global emerging threat to public health systems[J]. Critical Reviews in Food Science and Nutrition, 2017, 57(13): 2857-2876.

[3] PIDDOCK LJV. Clinically relevant chromosomally encoded multidrug resistance efflux pumps in bacteria[J]. Clinical Microbiology Reviews, 2006, 19(2): 382-402.

[4] LI XZ, PLÉSIAT P, NIKAIDO H. The challenge of efflux-mediated antibiotic resistance in Gram-negative bacteria[J]. Clinical Microbiology Reviews, 2015, 28(2): 337-418.

[5] VERGALLI J, ATZORI A, PAJOVIC J, DUMONT E, MALLOCI G, MASI M, VARGIU AV, WINTERHALTER M, RÉFRÉGIERS M, RUGGERONE P, PAGÈS JM. The challenge of intracellular antibiotic accumulation, a function of fluoroquinolone influx versus bacterial efflux[J]. Communications Biology, 2020, 3: 198.

[6] XU LF, SUN XX, MA XL. Systematic review and meta-analysis of mortality of patients infected with carbapenem-resistant *Klebsiella pneumoniae*[J]. Annals of Clinical Microbiology and Antimicrobials, 2017, 16(1): 18.

[7] EL-SAYED AHMED MAE, ZHONG LL, SHEN C, YANG YQ, DOI Y, TIAN GB. Colistin and its role in the era of antibiotic resistance: an extended review (2000−2019)[J]. Emerging Microbes & Infections, 2020, 9(1): 868-885.

[8] HUSSEIN NH, AL-KADMY IMS, TAHA BM, HUSSEIN JD. Mobilized colistin resistance (*mcr*) genes from 1 to 10: a comprehensive review[J]. Molecular Biology Reports, 2021, 48(3): 2897-2907.

[9] POURNARAS S, KOUMAKI V, SPANAKIS N, GENNIMATA V, TSAKRIS A. Current perspectives on tigecycline resistance in *Enterobacteriaceae*: susceptibility testing issues and mechanisms of resistance[J]. International Journal of Antimicrobial Agents, 2016, 48(1): 11-18.

[10] ANYANWU MU, NWOBI OC, OKPALA COR, EZEONU IM. Mobile tigecycline resistance: an emerging health catastrophe requiring urgent one health global intervention[J]. Frontiers in Microbiology, 2022, 13: 808744.

[11] KORNELSEN V, KUMAR A. Update on multidrug resistance efflux pumps in *Acinetobacter* spp.[J]. Antimicrobial Agents and Chemotherapy, 2021, 65(7): e0051421.

[12] GLAVIER M, PUVANENDRAN D, SALVADOR D, DECOSSAS M, PHAN G, GARNIER C, FREZZA E, CECE Q, SCHOEHN G, PICARD M, TAVEAU JC, DAURY L, BROUTIN I, LAMBERT O. Antibiotic export by MexB multidrug efflux transporter is allosterically controlled by a MexA-OprM chaperone-like complex[J]. Nature Communications, 2020, 11: 4948.

[13] BOLLA JR, HOWES AC, FIORENTINO F, ROBINSON CV. Assembly and regulation of the chlorhexidine-specific efflux pump AceI[J]. Proceedings of the National Academy of Sciences of the United States of America, 2020, 117(29): 17011-17018.

[14] ALAV I, SUTTON JM, RAHMAN KM. Role of bacterial efflux pumps in biofilm formation[J]. Journal of Antimicrobial Chemotherapy, 2018, 73(8): 2003-2020.

[15] GUNDOLF T, KALB R, ROSSMANITH P, MESTER P. Bacterial resistance toward antimicrobial ionic liquids mediated by multidrug efflux pumps[J]. Frontiers in Microbiology, 2022, 13: 883931.

[16] LV LC, WAN M, WANG CZ, GAO X, YANG QW, PARTRIDGE SR, WANG Y, ZONG ZY, DOI Y, SHEN JZ, JIA PY, SONG QH, ZHANG QH, YANG J,

HUANG XH, WANG MG, LIU JH. Emergence of a plasmid-encoded resistance-nodulation-division efflux pump conferring resistance to multiple drugs, including tigecycline, in *Klebsiella pneumoniae*[J]. mBio, 2020, 11(2): e02930-19.

[17] SUN SJ, GAO H, LIU YD, JIN LY, WANG RB, WANG XJ, WANG Q, YIN YY, ZHANG YW, WANG H. Co-existence of a novel plasmid-mediated efflux pump with colistin resistance gene *mcr* in one plasmid confers transferable multidrug resistance in *Klebsiella pneumoniae*[J]. Emerging Microbes & Infections, 2020, 9(1): 1102-1113.

[18] YANG XM, DONG N, CHAN EW, ZHANG R, CHEN S. Carbapenem resistance-encoding and virulence-encoding conjugative plasmids in *Klebsiella pneumoniae*[J]. Trends in Microbiology, 2021, 29(1): 65-83.

[19] PAN H, JIA CH, PAUDYAL N, LI F, MAO JY, LIU X, DONG CH, ZHOU K, LIAO XY, GONG JS, FANG WH, LI XL, KEHRENBERG C, YUE M. Comprehensive Assessment of Subtyping Methods for Improved Surveillance of Foodborne *Salmonella*[J]. Microbiology Spectrum, 2022, 10(5): e0247922.

[20] TANG B, SIDDIQUE A, JIA CH, ED-DRA A, WU J, LIN H, YUE M. Genome-based Risk Assessment for Foodborne *Salmonella enterica* from Food Animals in China: A One Health Perspective[J]. International Journal of Food Microbiology, 2023, 390: 110120.

[21] LI Y, TENG L, XU XB, LI XM, PENG XQ, ZHOU X, DU JX, TANG YT, JIANG ZJ, WANG ZN, JIA CH, MULLER A, KEHRENBERG C, WANG HQ, WU BB, WEILL FX, YUE M. A non-typhoidal *Salmonella* serovar domestication accompanying enhanced niche adaptation[J]. EMBO Molecular Medicine, 2022, 14(11): e16366.

[22] GAO X, WANG CZ, LV LC, HE XT, CAI ZP, HE WY, LI T, LIU JH. Emergence of a novel plasmid-mediated tigecycline resistance gene cluster, *tmexCD4-toprJ4*, in *Klebsiella quasipneumoniae* and *Enterobacter roggenkampii*[J]. Microbiology Spectrum, 2022, 10(4): e0109422.

[23] WANG XM, WANG Y, JIANG XT, GONG XW, WANG Y, SHEN ZQ. Co-transfer of *mcr-8* with $bla_{\text{NDM-1}}$ or *tmexCD1-toprJ1* by plasmid hybridisation[J]. International Journal of Antimicrobial Agents, 2022, 60(2): 106619.

[24] LI Y, QIU YC, GAO Y, CHEN WB, LI CW, DAI XY, ZHANG LH. Genetic and virulence characteristics of a *Raoultella planticola* isolate resistant to carbapenem and tigecycline[J]. Scientific Reports, 2022, 12: 3858.

[25] XIAO T, PENG K, CHEN Q, HOU XQ, HUANG WF, LV H, YANG XR, LEI GP, LI RC. Coexistence of *tmexCD-toprJ*, $bla_{\text{NDM-1}}$, and $bla_{\text{IMP-4}}$ in one plasmid carried by clinical *Klebsiella* spp.[J]. Microbiology Spectrum, 2022, 10(3): e0054922.

[26] LI RC, PENG K, XIAO X, LIU Y, PENG DX, WANG ZQ. Emergence of a multidrug resistance efflux pump with carbapenem resistance gene $bla_{\text{VIM-2}}$ in a *Pseudomonas putida* megaplasmid of migratory bird origin[J]. The Journal of Antimicrobial Chemotherapy, 2021, 76(6): 1455-1458.

[27] XU LN, WAN F, FU H, TANG B, RUAN Z, XIAO YH, LUO QX. Emergence of colistin resistance gene *mcr–10* in *Enterobacterales* isolates recovered from fecal samples of chickens, slaughterhouse workers, and a nearby resident[J]. Microbiology Spectrum, 2022, 10(2): e0041822.

[28] WU YC, DONG N, CAI C, ZHANG R, CHEN S. Hospital wastewater as a reservoir for the tigecycline resistance gene cluster *tmexCD-toprJ*[J]. The Lancet Microbe, 2023, 4(3): e134.

[29] LIU CC, WU YC, FANG YF, SANG Z, HUANG L, DONG N, ZENG Y, LU JY, ZHANG R, CHEN GX. Emergence of an ST1326 (CG258) multi-drug resistant *Klebsiella pneumoniae* co-harboring *mcr-8.2*, ESBL genes, and the resistance-nodulation-division efflux pump gene cluster *tmexCD1-toprJ1* in China[J]. Frontiers in Microbiology, 2022, 13: 800993.

[30] WANG XM, SUN NY, LIU X, LI F, SUN JJ, HUANG JH, LI RC, WANG LP. Small clone dissemination of *tmexCD1-toprJ1*-carrying *Klebsiella pneumoniae* isolates in a chicken farm[J]. Journal of Global Antimicrobial Resistance, 2022, 29: 105-112.

[31] PENG K, WANG Q, YIN Y, LI Y, LIU Y, WANG MZ, QIN SS, WANG ZQ, LI RC. Plasmids shape the current prevalence of *tmexCD1-toprJ1* among *Klebsiella pneumoniae* in food production chains[J]. mSystems, 2021, 6(5): e70221.

[32] QIN SS, PENG JK, DENG RP, PENG K, YAN TT, CHEN FF, LI RC. Identification of two plasmids coharboring carbapenemase genes and *tmexCD1-toprJ1* in clinical *Klebsiella pneumoniae* ST2667[J]. Antimicrobial Agents and Chemotherapy, 2021, 65(6): e00625-21.

[33] YANG XM, YE LW, CHAN EWC, ZHANG R, CHEN S. Characterization of an IncFIB/IncHI1B plasmid encoding efflux pump TMexCD1-TOprJ1 in a clinical tigecycline- and carbapenem-resistant *Klebsiella pneumoniae* strain[J]. Antimicrobial Agents and Chemotherapy, 2021, 65(4): e02340-20.

[34] FU YL, CHEN YQ, LIU DJ, YANG DW, LIU ZH, WANG YY, WANG JY, WANG XY, XU XY, LI X, HE JJ, JIANG JY, ZHAI WS, HUANG LL, HE T, XIA X, CAI C, WANG Y, JIANG HY. Abundance of tigecycline resistance genes and association with antibiotic residues in Chinese livestock farms[J]. Journal of Hazardous Materials, 2021, 409: 124921.

[35] LIU YY, GAO X, HE XT, LV LC, JIAO YX, YU RY, LIU JH. Emergence of the tigecycline resistance gene cluster *tmexCD1-toprJ1* in an IncC plasmid and *Citrobacter portucalensis*[J]. Journal of Antimicrobial Chemotherapy, 2022, 77(7): 2030-2033.

[36] WAN M, GAO X, LV LC, CAI ZP, LIU JH. IS*26* mediates the acquisition of tigecycline resistance gene cluster *tmexCD1-toprJ1* by IncHI1B-FIB plasmids in *Klebsiella pneumoniae* and *Klebsiella quasipneumoniae* from food market sewage[J]. Antimicrobial Agents and Chemotherapy, 2021, 65(3): e02178-20.

[37] HE RW, YANG YQ, WU YP, ZHONG LL, YANG YX, CHEN GP, QIN MY, LIANG XX, EL-GAWAD EL-SAYED AHMED MA, LIN MM, YAN B, XIA Y, DAI M, CHEN HT, TIAN GB. Characterization of a plasmid-encoded resistance-nodulation-division efflux pump in *Klebsiella pneumoniae* and *Klebsiella quasipneumoniae* from patients in China[J]. Antimicrobial Agents and Chemotherapy, 2021, 65(2): e02075-20.

[38] LIU YL, LIN YQ, WANG ZW, HU NY, LIU Q, ZHOU WK, LI XZ, HU LH, GUO J, HUANG XT, ZENG LB. Molecular mechanisms of colistin resistance in *Klebsiella pneumoniae* in a tertiary care teaching hospital[J]. Frontiers in Cellular and Infection Microbiology, 2021, 11: 673503.

[39] SUN SJ, WANG Q, JIN LY, GUO YF, YIN YY, WANG RB, BI L, ZHANG RF, HAN YG, WANG H. Identification of multiple transfer units and novel subtypes of *tmexCD-toprJ* gene clusters in clinical carbapenem-resistant *Enterobacter cloacae* and *Klebsiella oxytoca*[J]. Journal of Antimicrobial Chemotherapy, 2022, 77(3): 625-632.

[40] HIRABAYASHI A, HA VTT, van NGUYEN A, NGUYEN ST, SHIBAYAMA K, SUZUKI M. Emergence of a plasmid-borne tigecycline resistance in *Klebsiella pneumoniae* in Vietnam[J]. Journal of Medical Microbiology, 2021, 70(3): https://doi.org/10.1099/jmm.0.001320.

[41] WANG YJ, ZHU B, LIU M, DONG XT, MA JP, LI XF, CHENG F, GUO JZ, LU SM, WAN FR, HAO YY, MA WS, HAO MJ, CHEN L. Characterization of IncHI1B plasmids encoding efflux pump TmexCD2-ToprJ2 in carbapenem-resistant *Klebsiella variicola*, *Klebsiella quasipneumoniae*, and *Klebsiella michiganensis* strains[J]. Frontiers in Microbiology, 2021, 12: 759208.

[42] WANG CZ, GAO X, YANG QW, LV LC, WAN M, YANG J, CAI ZP, LIU JH. A novel transferable resistance-nodulation-division pump gene cluster, *tmexCD2-toprJ2*, confers tigecycline resistance in *Raoultella ornithinolytica*[J]. Antimicrobial Agents and Chemotherapy, 2021, 65(4): e02229-20.

[43] WU YC, DONG N, CAI C, ZENG Y, LU JY, LIU CC, WANG HY, ZHANG YY, HUANG L, ZHAI WS, SHAO DY, LI RC, LIU DJ, CHEN S, ZHANG R. *Aeromonas* spp. from hospital sewage act as a reservoir of genes resistant to last-line antibiotics[J]. Drug Resistance Updates, 2023, 67: 100925.

[44] WANG Q, PENG K, LIU Y, XIAO X, WANG ZQ, LI RC. Characterization of TMexCD3-TOprJ3, an RND-type efflux system conferring resistance to tigecycline in *Proteus mirabilis*, and its associated integrative conjugative element[J]. Antimicrobial Agents and Chemotherapy, 2021, 65(7): e271220.

[45] HIRABAYASHI A, DAO TD, TAKEMURA T, HASEBE F, TRANG LT, THANH NH, TRAN HH, SHIBAYAMA K, KASUGA I, SUZUKI M. A transferable IncC-IncX3 hybrid plasmid cocarrying bla_{NDM-4}, *tet*(X), and *tmexCD3-toprJ3* confers resistance to carbapenem and tigecycline[J]. mSphere, 2021, 6(4): e0059221.

[46] WANG CZ, GAO X, LV LC, CAI ZP, YANG J, LIU JH. Novel tigecycline resistance gene cluster *tnfxB3-tmexCD3-toprJ1b* in *Proteus* spp. and *Pseudomonas aeruginosa*, co-existing with Tet(X6) on an SXT/R391 integrative and conjugative element[J]. Journal of Antimicrobial Chemotherapy, 2021, 76(12): 3159-3167.

[47] FROST LS, LEPLAE R, SUMMERS AO, TOUSSAINT A. Mobile genetic elements: the agents of open source evolution[J]. Nature Reviews Microbiology, 2005, 3(9): 722-732.

[48] LIU C, DU PC, YANG P, ZHENG JJ, YI J, LU M, SHEN N. Emergence and inter- and intrahost evolution of pandrug-resistant *Klebsiella pneumoniae* coharboring *tmexCD1-toprJ1*, bla_{NDM-1}, and bla_{KPC-2}[J]. Microbiology Spectrum, 2023, 11(2): e0278622.

[49] CARROLL AC, WONG A. Plasmid persistence: costs, benefits, and the plasmid paradox[J]. Canadian Journal of Microbiology, 2018, 64(5): 293-304.

[50] ZHANG YX, LI ZY, XU X, PENG X. Transposon mutagenesis in oral *streptococcus*[J]. Journal of Oral Microbiology, 2022, 14(1): 2104951.

[51] 吴小磊, 贾程皓, 王剑峰, 王雷杰, 乐敏. DNA 聚合酶 IV：抗细菌的潜在药靶[J]. 微生物学通报, 2022, 49(12): 5342-5358.
WU XL, JIA CH, WANG JF, WANG LJ, YUE M. DNA polymerase IV - a potential drug target against bacteria[J]. Microbiology China, 2022, 49(12): 5342-5358 (in Chinese).

[52] KUMAR N, SU CC, CHOU TH, RADHAKRISHNAN A, DELMAR JA, RAJASHANKAR KR, YU EW. Crystal structures of the *Burkholderia multivorans* hopanoid transporter HpnN[J]. Proceedings of the National Academy of Sciences of the United States of America, 2017, 114(25): 6557-6562.

[53] YONEHARA R, YAMASHITA E, NAKAGAWA A. Crystal structures of OprN and OprJ, outer membrane factors of multidrug tripartite efflux pumps of *Pseudomonas aeruginosa*[J]. Proteins: Structure, Function, and Bioinformatics, 2016, 84(6): 759-769.

[54] WANG Z, FAN GZ, HRYC CF, BLAZA JN, SERYSHEVA II, SCHMID MF, CHIU W, LUISI BF, DU DJ. An allosteric transport mechanism for the AcrAB-TolC multidrug efflux pump[J]. eLife, 2017, 6: 24905.

[55] TSUTSUMI K, YONEHARA R, ISHIZAKA-IKEDA E, MIYAZAKI N, MAEDA S, IWASAKI K, NAKAGAWA A, YAMASHITA E. Structures of the wild-type MexAB-OprM tripartite pump reveal its complex formation and drug efflux mechanism[J]. Nature Communications, 2019, 10: 1520.

[56] 方家豪, 曹雨虹, 何宇臻, 洪战英, 柴逸峰. 膜蛋白稳定技术及其在药物筛选中的应用进展[J]. 药学学报, 2021, 56(9): 2325-2334.
FANG JH, CAO YH, HE YZ, HONG ZY, CHAI YF. Advancements in stabilization technologies for membrane protein and its application in drug screening[J]. Acta Pharmaceutica Sinica, 2021, 56(9): 2325-2334 (in Chinese).

[57] DOUGHTY DM, COLEMAN ML, HUNTER RC, SESSIONS AL, SUMMONS RE, NEWMAN DK. The RND-family transporter, HpnN, is required for hopanoid localization to the outer membrane of *Rhodopseudomonas palustris* TIE-1[J]. Proceedings of the National Academy of Sciences of the United States of America, 2011, 108(45): E1045-E1051.

[58] KLENOTIC PA, MOSENG MA, MORGAN CE, YU EW. Structural and functional diversity of resistance-nodulation-cell division transporters[J]. Chemical Reviews, 2021, 121(9): 5378-5416.

[59] MCEWEN SA, COLLIGNON PJ. Antimicrobial resistance: a one health perspective[J]. Microbiology Spectrum, 2018, 6(2): http://10.1128/microbiolspec.ARBA-0009-2017.

[60] SINCLAIR JR. Importance of a One Health approach in advancing global health security and the sustainable development goals[J]. Revue Scientifique et Technique De L'OIE, 2019, 38(1): 145-154.

[61] PENG X, ED-DRA A, YUE M. Whole genome sequencing for the risk assessment of probiotic lactic acid bacteria[J]. Critical Reviews in Food Science and Nutrition, 2022, 13: 1-19.

[62] ELBEDIWI M, LI Y, PAUDYAL N, PAN H, LI XL, XIE SH, RAJKOVIC A, FENG YJ, FANG WH, RANKIN SC, YUE M. Global burden of colistin-resistant bacteria: mobilized colistin resistance genes study (1980−2018)[J]. Microorganisms, 2019, 7(10): 461.

[63] 武淑霞, 刘宏斌, 黄宏坤, 雷秋良, 王洪媛, 翟丽梅, 刘申, 张英, 胡钰. 我国畜禽养殖粪污产生量及其资源化分析[J]. 中国工程科学, 2018, 20(5): 103-111.
WU SX, LIU HB, HUANG HK, LEI QL, WANG HY, ZHAI LM, LIU S, ZHANG Y, HU Y. Analysis on the amount and utilization of manure in livestock and poultry breeding in China[J]. Engineering Science, 2018, 20(5): 103-111 (in Chinese).

[64] LI Y, ED-DRA A, TANG B, KANG XM, MÜLLER A, KEHRENBERG C, JIA CH, PAN H, YANG H, YUE M. Higher tolerance of predominant *Salmonella* serovars circulating in the antibiotic-free feed farms to environmental stresses[J]. Journal of Hazardous Materials, 2022, 438: 129476.

[65] KARKMAN A, PÄRNÄNEN K, LARSSON DGJ. Fecal pollution can explain antibiotic resistance gene abundances in anthropogenically impacted environments[J]. Nature Communications, 2019, 10: 80.

[66] EZZARIAI A, HAFIDI M, KHADRA A, AEMIG Q, EL FELS L, BARRET M, MERLINA G, PATUREAU D, PINELLI E. Human and veterinary antibiotics during composting of sludge or manure: global perspectives on persistence, degradation, and resistance genes[J]. Journal of Hazardous Materials, 2018, 359: 465-481.

[67] 刘海洋. 污水中典型抗生素、耐药菌及耐药基因的分布及其电催化降解研究[D]. 长春: 东北师范大学博士学位论文, 2020.
LIU HY. Distribution and electrocatalytic degradation of typical antibiotics, drug-resistant bacteria and drug-resistant genes in sewage[D]. Changchun: Doctoral Dissertation of Northeast Normal University, 2020 (in Chinese).

[68] GLASSER NR, KERN SE, NEWMAN DK. Phenazine redox cycling enhances anaerobic survival in *Pseudomonas aeruginosa*by facilitating generation of ATP and a proton-motive force[J]. Molecular Microbiology, 2014, 92(2): 399-412.

[69] XIAO X, HUAN QM, HUANG YH, LIU Y, LI RC, XU XL, WANG ZQ. Metformin reverses *tmexCD1-toprJ1*- and Tet(A)-mediated high-level tigecycline resistance in *K. pneumoniae*[J]. Antibiotics, 2022, 11(2): 162.

[70] SEYEDHOSSEINI GHAHEH H, DAMAVANDI MS, SADEGHI P, MASSAH AR, ASL TH, SALARI-JAZI A, HEJAZI SH. Targeting and ultrabroad insight into molecular basis of resistance-nodulation-cell division efflux pumps[J]. Scientific Reports, 2022, 12: 16130.

[71] DAVIN-REGLI A, PAGES JM, FERRAND A. Clinical status of efflux resistance mechanisms in Gram-negative bacteria[J]. Antibiotics, 2021, 10(9): 1117.

[72] VERMA P, TIWARI V. Targeting outer membrane protein component AdeC for the discovery of efflux pump inhibitor against AdeABC efflux pump of multidrug resistant *Acinetobacter baumannii*[J]. Cell Biochemistry and Biophysics, 2018, 76(3): 391-400.

[73] AYHAN DH, TAMER YT, AKBAR M, BAILEY SM, WONG M, DALY SM, GREENBERG DE, TOPRAK E. Sequence-specific targeting of bacterial resistance genes increases antibiotic efficacy[J]. PLoS Biology, 2016, 14(9): e1002552.

[74] NAGANO K, NIKAIDO H. Kinetic behavior of the major multidrug efflux pump AcrB of *Escherichia coli*[J]. Proceedings of the National Academy of Sciences of the United States of America, 2009, 106(14): 5854-5858.

[75] NIKAIDO H, PAGÈS JM. Broad-specificity efflux pumps and their role in multidrug resistance of Gram-negative bacteria[J]. FEMS Microbiology Reviews, 2012, 36(2): 340-363.