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花鲈源杀鱼爱德华菌耐药谱及毒力相关性分析

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摘要: 从广东省珠海市养殖花鲈(*Lateolabrax maculatus*)体内分离得到 96 株爱德华菌属(*Edwardsiella* spp.), 经 *gyrB* 基因鉴定出 87 株杀鱼爱德华菌(*E. piscicida*)。耐药谱分析显示, 杀鱼爱德华菌对利福平(98.85%)、麦迪霉素(96.55%)、红霉素(95.40%)、青霉素(68.96%)、磺胺异恶唑(58.62%)、复方新诺明(28.73%)、阿莫西林(21.83%)、庆大霉素(13.79%)、新霉素(10.34%)、呋喃唑酮(3.45%)、诺氟沙星(2.29%)、氯霉素(2.29%)、多西环素(2.29%)、土霉素(1.15%)、氟苯尼考(1.14%)、恩诺沙星(0%)耐药。杀鱼爱德华菌共有 32 种耐药谱型且均为多重耐药菌株, 多重耐药指数为 0.423。斑马鱼致死率结果发现, 杀鱼爱德华菌是一株中高毒力菌株; 进一步的相关性分析揭示, 杀鱼爱德华菌毒力与庆大霉素抗性呈正相关($P<0.05$), 与恩诺沙星、氟苯尼考($P<0.05$)和土霉素抗性($P<0.01$)呈负相关。综上所述, 花鲈源杀鱼爱德华菌为高毒、多重耐药的菌种, 其毒力与耐药性多呈现负相关, 推测是由于细菌因获得外源 DNA 而产生额外的生物成本所致。

关键词: 花鲈; 杀鱼爱德华菌; 耐药性; 毒力
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花鲈(*Lateolabrax maculatus*), 又称海鲈, 是中国第二大海水养殖鱼类, 在广东珠海养殖面积超过 16 km^2, 产量分别约占广东省的 80%、全国的 50%[1], 是广东省特色水产养殖鱼类之一。随着养殖集约化程度的增加, 病害问题限制了花鲈产业的可持续发展。其中, 杀鱼爱德华菌(*Edwardsiella piscicidas*)是花鲈养殖的主要病原之一, 此菌在世界各地养殖区域爆发流行[2-3], 广泛存在于环境和宿主中, 可感染多种经济鱼类[4-6]。

杀鱼爱德华菌与迟缓爱德华菌(*E. tarda*)有许多相同的表型特征[7], 目前仍存在一些菌株被误归为迟缓爱德华菌的现象。Griffin 等[8-9]通过 *gyrB* 来对爱德华菌进行菌株分类, 将迟缓爱德华菌和杀鱼爱德华菌进行区分。自 1995 年 Yamamoto 等[10]首次用 *gyrB* 基因鉴别恶臭假单胞菌(*Pseudomonas putida*)近缘种后, 越来越多的学者用该基因进行菌种鉴定。*gyrB* 基因作为在分类学中被应用最广泛的管家基因, 其遗传密码子具有优良的兼并性, 在 DNA 序列发生替换时可保证氨基酸序列稳定, 其表达水平明显高于 16S rDNA 基因, 可更正确地区分近缘种。此外, *gyrB* 基因不易在水平间转移, 可作为系统发育分析中的靶基因[11]。

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细菌耐药性的出现和迅速传播使其成为公共卫生安全的焦点[12], 病原菌的耐药机制已经成为临床微生物学中重要且广泛的研究内容[13]。许多研究发现, 病原菌抗生素耐药性机制的主要决定因素是从其他生物水平基因转移获得的, 同时在对病原菌另一大致病因素——毒力研究中同样发现水平基因转移的共同基本特征。毒力和抗生素抗性都可被认为是细菌探索和开拓生存新环境的方法, 这些方法使细菌的基因组表现出高度可塑性, 所以抗生素抗性和毒力在机制研究中同时被称为“量子跃迁的进化”[14]。有研究表明抗生素抗性基因和毒力基因可以处于同个可移动元件(如质粒、转座子、噬菌体、整合子和基因簇)中[15-16], 如迟缓爱德华菌中发现pCK41质粒同时存在耐药基因和毒力基因[17]; 大肠杆菌中发现 pCERC3 质粒同时具有毒力和耐药基因[18]; 多重耐药(MDR)外 排泵能外排抗生素和群体感应信号等物质[19]。进一步研究表明, 毒力和抗生素之间存在相关性联系[20], Ghorbel 等[21]研究发现毒力模式与抗菌药物图谱存在显著相关性; Azzam 等[22]研究表明 MAR 与污水生态系统细菌毒力具有强相关性; Vila 等[23]发现细菌获得抗生素耐药时可导致毒力下降, 对其耐药机制研究表明大部分菌株通过改变自身表达或蛋白结构来增加耐药性[24]。目前对于其相关性的研究大多集中在人类医学和畜牧行业中, 在水产养殖领域研究较少, 所以通过对杀鱼爱德华菌毒力与耐药的相关性研究, 可以为该菌的抗生素使用提供指导。

本研究团队基于 *gyrB* 基因序列信息, 对分离自 2018 年 4—12 月广东省珠海市池塘养殖花鲈的杀鱼爱德华菌菌株耐药谱和毒力特征评价, 为阐明花鲈源杀鱼爱德华菌的流行趋势和耐药规律奠定基础, 并对耐药性和毒力相关性研究提供数据支持。

1　材料与方法

1.1　菌株分离及鉴定

采样时间为 2018 年 4—12 月, 样品采自广东省珠海市养殖患病的花鲈内脏器官, 具体细菌分离方法参照鱼病调查手册[25], 分离菌株进行革兰氏染色, 通过 16S rDNA 通用引物和 *gyrB* 基因引物进行分子鉴定(表 1), 对鉴定为杀鱼爱德华菌的菌株进行后续实验。

表 1　基因引物序列
Tab. 1　Gene primer sequence

基因 gene	引物 primer	序列(5′-3′) sequence	目的片段/bp target fragment	退火温度/℃ annealing temperature
16S rDNA	8F	AGAGTTTGATCCTGGCTCAG	1500	55
	1492R	GGTTACCTTGTTACGACTT		
gyrB	F	GAAGTCATCATGACCGTTCTGCA	1100	56
	R	AGCAGGGTACGGATGTGCGAGCC		

1.2　杀鱼爱德华菌药敏试验及谱型分析

将鉴定获得的 87 株杀鱼爱德华菌制备菌悬液, 菌液浓度为 1×10^8 CFU/mL, 采用纸片扩散法(K-B 法)进行药敏试验, 记录药敏纸片抑菌圈的直径。药敏纸片为 16 种常见的抗生素(均购于杭州微生物试剂有限公司): 阿莫西林(amoxicillin, AMO, 20 μg)、麦迪霉素(midecamycin, MD, 30 μg)、利福平(rifampin, RFP, 5 μg)、青霉素(penicillin, P, 10 μg)、复方新诺明(norepinephrine, SXT, 1.25 μg)、诺氟沙星(norfloxacin, NOR, 10 μg)、红霉素(erythromycin, E, 15 μg)、氟苯尼考(florfenicol, FFC, 30 μg)、氯霉素(chloramphenicol, C, 30 μg)、呋喃唑酮(furazolidone, FUR, 100 μg)、新霉素(neomycin, N, 10 μg)、庆大霉素(gentamicin, GM, 10 μg)、磺胺异恶唑(sulfamethoxazole, FS, 300 μg)、土霉素(oxytetracycline, OT, 30 μg)、多西环素(doxycycline, DO, 30 μg)、恩诺沙星(enrofloxacin, ENR, 30 μg)。参照美国临床和实验室标准协会抗生素敏感试验标准分析菌株耐药谱(表 2)[26]。将杀鱼爱德华菌对每一种抗生素的耐药情况以 *S*、*I*、*R* 记录,

表 2 药物敏感实验判定标准
Tab. 2 Criteria for antibiotics sensitivity test

药名 medicine	判定标准 judging standard			药名 medicine	判定标准 judging standard		
	R	*I*	*S*		*R*	*I*	*S*
磺胺异恶唑 sulfamethoxazole, FS	≤10	11-15	≥16	红霉素 erythromycin, E	≤13	14-22	≥23
利福平 rifampin, RFP	≤16	17-19	≥20	青霉素 penicillin, P	≤12	13-16	≥17
麦迪霉素 midecamycin, MD	≤13	14-17	≥18	多西环素 doxycycline, DO	≤12	13-15	≥16
呋喃唑酮 furazolidone, FUR	≤9	10-14	≥15	氟苯尼考 florfenicol, FFC	≤12	13-17	≥18
阿莫西林 amoxicillin, AMO	≤13	12-18	≥19	新霉素 neomycin, N	≤12	13-16	≥17
庆大霉素 gentamicin, GM	≤12	13-14	≥15	氯霉素 chloramphenicol, C	≤12	13-17	≥18
恩诺沙星 enrofloxacin, ENR	≤16	17-22	≥23	诺氟沙星 norfloxacin, NOR	≤12	13-16	≥17
复方新诺明 norepinephrine, SXT	≤10	11-15	≥16	土霉素 oxytetracycline, OT	≤14	15-18	≥19

则每株受试菌株对所有抗生素形成唯一的耐药谱, 对所有耐药谱进行归类并命名。计算耐药率及多重抗生素耐药指数(MARI), MARI=a/bc 其中 a 代表某来源细菌的总抗菌药物耐药值, b 代表检测的抗菌药物种类数目, c 代表某来源细菌菌株[27], 分析比较耐药谱型所包括的抗生素及菌株量差异, 用 Origin 软件进行作图分析。

1.3 杀鱼爱德华菌对斑马鱼的毒力试验

选择体长 4 cm 左右且健康的 AB 品系斑马鱼, (25±1) ℃恒温暂养一周。将培养好的 87 株杀鱼爱德华菌用生理盐水稀释至浓度约为 3×10^5 CFU/mL, 腹腔注射剂量 20 μl/尾, 每株菌注射 30 尾鱼, 分 3 个平行组, 记录注射后 2 周内的死亡率。

1.4 数据处理

将杀鱼爱德华菌对 16 种抗生素的耐药情况进行赋值(*R*=4, *I*=2, *S*=0), 对每种抗生素耐药情况(*R*=4, *I*=2, *S*=0)与斑马鱼死亡率结果结合, 利用 IBM SPSS 22 进行 Spearman 相关性分析, 得到其相关性结果。

2 结果与分析

2.1 细菌分离鉴定

对优势菌进行革兰氏染色, 发现有 96 株菌株在显微镜下呈现红色短杆状。通过 16S rDNA 通用引物和 *gyrB* 基因引物进行扩增, 测序后分析基因序列的同源性, 发现杀鱼爱德华菌占 87 株, 迟缓爱德华菌仅占 9 株。大部分采样鱼呈现鱼眼部肿大、眼球缺失、头骨处皮肤溃烂、腹水以及内脏器官肿大等症状。

2.2 杀鱼爱德华菌耐药谱

87 株杀鱼爱德华菌对 16 种抗生素的耐药存在差异(图 1), 其中对麦迪霉素、红霉素和利福平的耐药率超过了 90%及以上, 对青霉素、磺胺异恶唑耐药率在 80%~50%, 对庆大霉素、阿莫西林和复方新诺明耐药率均低于 50%~10%, 对多西环素、氟苯尼考、恩诺沙星、氯霉素、诺氟沙星和土霉素的耐药率低于 10%。杀鱼爱德华菌的耐药谱型共 32 种, 分为 E1~E32 (表 3), 谱型丰富度为 36.78%。E1~E3 型谱型对 3 种抗生素耐药, 包含 14 菌株, 占总菌数的 16.09%; E4~E10 谱型对 4 种抗生素耐药, 包含 20 菌株, 占总菌数的 22.98%; E11~E17 谱型对 5 种抗生素耐药, 包含 20 菌株, 占总菌数的 22.98%; E18~E23 谱型对 6 种抗生素耐药, 包含 17 菌株, 占总菌数的 19.10%; E24~E29 谱型对 7 种抗生素耐药, 包含 12 菌株, 占总菌数的 13.80%; E30、E31 谱型对 8 种抗生素耐药,

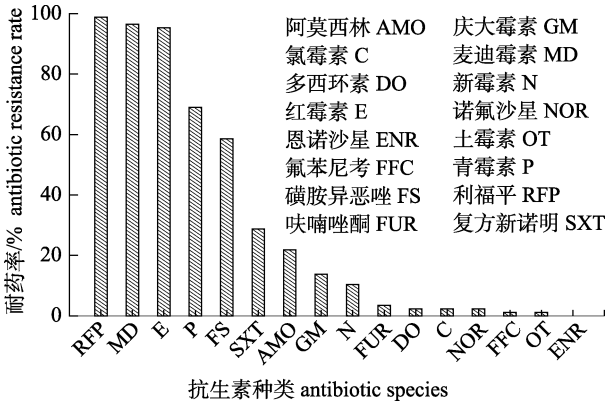

图 1 87 株杀鱼爱德华菌菌株对 16 种抗生素的耐药性
Fig. 1 Antibiotic resistance of 87 *Edwardsiella piscicida* strains to 16 antibiotics

表 3　87 株杀鱼爱德华菌的耐药谱型

Tab. 3　Antibiotic resistance spectrum of 87 strains of *Edwardsiella piscicida*

编号 code	耐药谱 antibiotic resistance spectrum																谱型 spectrum
	利福平 RFP	红霉素 E	麦迪霉素 MD	青霉素 P	磺胺异恶唑 FS	复方新诺明 SXT	呋喃唑酮 FUR	新霉素 N	阿莫西林 AMO	庆大霉素 GM	多西环素 DO	氟苯尼考 FFC	恩诺沙星 ENR	氯霉素 C	诺氟沙星 NOR	土霉素 OT	
18QW10	*R*	*R*	*R*	*S*	*S*	*S*	*S*	*I*	*S*	*I*	*S*	*S*	*S*	*S*	*S*	*S*	E1
18QW45	*R*	*R*	*R*	*S*	*S*	*I*	*S*	*I*	*S*	*S*	*S*	*S*	*I*	*S*	*S*	*I*	E1
18QW46	*R*	*R*	*R*	*S*	*S*	*I*	*I*	*I*	*S*	*S*	*S*	*S*	*I*	*S*	*S*	*S*	E1
18QW49	*R*	*R*	*S*	*I*	*R*	*S*	*S*	*I*	*I*	*S*	*S*	*S*	*S*	*S*	*S*	*S*	E2
18QW62	*R*	*R*	*R*	*S*	*S*	*S*	*S*	*I*	*S*	*S*	*S*	*S*	*S*	*S*	*S*	*S*	E1
18QW70	*R*	*R*	*R*	*I*	*S*	*S*	*S*	*I*	*S*	*S*	*S*	*S*	*S*	*S*	*S*	*S*	E1
18BJ134	*R*	*R*	*R*	*S*	*I*	*S*	*S*	*I*	*S*	*S*	*S*	*S*	*S*	*S*	*S*	*S*	E1
18BJ141	*R*	*R*	*R*	*I*	*S*	*S*	*S*	*I*	*I*	*S*	*S*	*S*	*S*	*S*	*S*	*S*	E1
18QW83	*R*	*R*	*R*	*I*	*S*	*S*	*S*	*I*	*I*	*I*	*S*	*S*	*S*	*S*	*S*	*S*	E1
18QW84	*R*	*R*	*R*	*I*	*S*	*S*	*S*	*S*	*I*	*I*	*S*	*S*	*S*	*S*	*S*	*S*	E1
18BJ225	*R*	*R*	*S*	*R*	*S*	*S*	*I*	*I*	*I*	*S*	*S*	*S*	*S*	*S*	*S*	*S*	E3
18BJ315	*R*	*R*	*R*	*S*	*S*	*S*	*S*	*I*	*I*	*S*	*S*	*S*	*S*	*S*	*S*	*S*	E1
18QW226	*R*	*I*	*R*	*R*	*S*	*S*	*S*	*I*	*S*	*S*	*S*	*S*	*S*	*S*	*S*	*S*	E4
18QW231	*R*	*R*	*R*	*I*	*S*	*S*	*S*	*I*	*I*	*S*	*S*	*S*	*S*	*S*	*S*	*S*	E1
18QW248	*R*	*R*	*R*	*S*	*S*	*S*	*S*	*I*	*S*	*S*	*S*	*S*	*S*	*S*	*S*	*S*	E1
18QW60	*R*	*R*	*R*	*I*	*R*	*S*	*S*	*I*	*S*	*S*	*S*	*S*	*S*	*S*	*S*	*S*	E6
18QW61	*R*	*R*	*R*	*S*	*R*	*S*	*S*	*I*	*I*	*I*	*S*	*S*	*S*	*S*	*S*	*S*	E6
18BJ135	*R*	*R*	*R*	*R*	*S*	*S*	*S*	*I*	*I*	*S*	*I*	*S*	*S*	*S*	*S*	*S*	E5
18BJ136	*R*	*R*	*R*	*R*	*S*	*S*	*S*	*I*	*S*	*S*	*S*	*S*	*S*	*S*	*S*	*S*	E5
18BJ139	*R*	*R*	*R*	*R*	*S*	*S*	*S*	*I*	*I*	*S*	*S*	*S*	*S*	*S*	*S*	*S*	E5
18BJ140	*R*	*R*	*R*	*R*	*S*	*S*	*S*	*I*	*I*	*I*	*S*	*S*	*S*	*S*	*S*	*S*	E5
18BJ151	*R*	*R*	*R*	*R*	*S*	*S*	*S*	*I*	*I*	*I*	*S*	*S*	*S*	*S*	*S*	*S*	E5
18QW76	*R*	*R*	*R*	*R*	*S*	*S*	*S*	*I*	*I*	*S*	*S*	*S*	*S*	*S*	*S*	*S*	E5
18QW77	*R*	*R*	*R*	*I*	*I*	*S*	*I*	*I*	*I*	*R*	*S*	*S*	*S*	*I*	*S*	*S*	E7
18BJ206	*R*	*R*	*R*	*I*	*S*	*S*	*S*	*I*	*R*	*I*	*S*	*S*	*S*	*S*	*I*	*S*	E8
18QW109	*R*	*R*	*R*	*I*	*R*	*S*	*S*	*I*	*S*	*S*	*S*	*S*	*S*	*S*	*I*	*S*	E6
18QW126	*S*	*S*	*S*	*S*	*R*	*R*	*R*	*S*	*S*	*S*	*S*	*S*	*S*	*S*	*R*	*S*	E9
18BJ269	*R*	*R*	*R*	*R*	*S*	*S*	*S*	*I*	*S*	*S*	*S*	*S*	*S*	*S*	*S*	*S*	E5

(待续　to be continued)

(续表 3 Tab. 3 continued)

编号 code	耐药谱 antibiotic resistance spectrum																谱型 spectrum
	利福平 RFP	红霉素 E	麦迪霉素 MD	青霉素 P	磺胺异恶唑 FS	复方新诺明 SXT	呋喃唑酮 FUR	新霉素 N	阿莫西林 AMO	庆大霉素 GM	多西环素 DO	氟苯尼考 FFC	恩诺沙星 ENR	氯霉素 C	诺氟沙星 NOR	土霉素 OT	
18BJ270	*R*	*R*	*R*	*S*	*S*	*S*	*I*	*S*	*S*	*I*	*R*	*S*	*S*	*S*	*S*	*S*	E10
18QW168	*R*	*R*	*R*	*I*	*R*	*S*	*I*	*I*	*S*	*I*	*S*	*S*	*I*	*S*	*S*	*S*	E6
18QW189	*R*	*R*	*R*	*I*	*R*	*S*	*S*	*I*	*I*	*S*	*S*	*S*	*S*	*I*	*S*	*S*	E6
18QW92	*R*	*R*	*R*	*I*	*S*	*S*	*S*	*I*	*I*	*R*	*S*	*S*	*S*	*S*	*S*	*S*	E7
18QW209	*R*	*R*	*R*	*R*	*S*	*S*	*S*	*I*	*S*	*I*	*S*	*S*	*S*	*S*	*S*	*S*	E5
18QW210	*R*	*R*	*R*	*R*	*S*	*S*	*S*	*I*	*I*	*I*	*S*	*S*	*S*	*S*	*S*	*S*	E5
18QW50	*R*	*R*	*R*	*S*	*R*	*R*	*S*	*I*	*S*	*S*	*S*	*S*	*S*	*S*	*S*	*S*	E13
18QW53	*R*	*R*	*R*	*I*	*R*	*R*	*S*	*I*	*I*	*I*	*S*	*S*	*S*	*S*	*S*	*S*	E13
18QW56	*R*	*R*	*R*	*I*	*R*	*S*	*S*	*I*	*R*	*I*	*S*	*S*	*S*	*S*	*S*	*S*	E14
18BJ154	*R*	*R*	*R*	*R*	*S*	*S*	*S*	*I*	*I*	*S*	*R*	*S*	*S*	*S*	*S*	*S*	E15
18BJ155	*R*	*R*	*R*	*R*	*S*	*S*	*I*	*I*	*R*	*I*	*I*	*S*	*S*	*S*	*S*	*S*	E12
18BJ137	*R*	*R*	*R*	*R*	*R*	*S*	*I*	*I*	*I*	*S*	*S*	*S*	*S*	*S*	*S*	*S*	E11
18QW85	*R*	*R*	*R*	*R*	*S*	*S*	*S*	*I*	*R*	*I*	*S*	*S*	*S*	*S*	*S*	*S*	E12
18QW119	*R*	*R*	*R*	*R*	*R*	*S*	*S*	*S*	*S*	*S*	*S*	*S*	*S*	*S*	*S*	*S*	E11
18BJ249	*R*	*R*	*R*	*R*	*R*	*S*	*S*	*I*	*I*	*I*	*S*	*S*	*S*	*I*	*I*	*S*	E11
18QW161	*R*	*R*	*R*	*R*	*S*	*S*	*S*	*I*	*I*	*R*	*S*	*S*	*S*	*S*	*S*	*S*	E16
18QW162	*R*	*R*	*R*	*R*	*R*	*S*	*S*	*I*	*I*	*S*	*S*	*S*	*S*	*S*	*S*	*S*	E11
18QW163	*R*	*R*	*R*	*R*	*R*	*S*	*S*	*I*	*I*	*I*	*S*	*S*	*S*	*S*	*S*	*S*	E11
18QW164	*R*	*R*	*R*	*R*	*R*	*S*	*S*	*I*	*I*	*S*	*S*	*S*	*S*	*S*	*S*	*S*	E11
18QW165	*R*	*R*	*R*	*R*	*R*	*S*	*S*	*I*	*I*	*S*	*S*	*S*	*S*	*S*	*S*	*S*	E11
18QW167	*R*	*R*	*R*	*R*	*R*	*S*	*S*	*S*	*S*	*I*	*S*	*S*	*S*	*S*	*S*	*S*	E11
18QW169	*R*	*R*	*R*	*R*	*R*	*S*	*S*	*I*	*I*	*I*	*S*	*S*	*S*	*S*	*S*	*S*	E11
18QW228	*R*	*I*	*R*	*R*	*R*	*S*	*S*	*R*	*S*	*I*	*S*	*S*	*S*	*S*	*S*	*S*	E17
18BJ345	*R*	*R*	*R*	*R*	*S*	*S*	*S*	*I*	*R*	*S*	*S*	*S*	*S*	*S*	*S*	*S*	E12
18QW236	*R*	*R*	*R*	*R*	*R*	*S*	*S*	*I*	*I*	*I*	*S*	*S*	*S*	*S*	*S*	*S*	E11
18BJ316	*R*	*R*	*R*	*R*	*R*	*S*	*S*	*I*	*I*	*S*	*S*	*S*	*S*	*S*	*S*	*S*	E11
18QW52	*R*	*R*	*R*	*R*	*R*	*R*	*S*	*I*	*I*	*I*	*S*	*S*	*S*	*S*	*S*	*S*	E18
18QW55	*R*	*R*	*R*	*S*	*R*	*R*	*S*	*I*	*I*	*R*	*S*	*S*	*S*	*S*	*S*	*S*	E18
18BJ138	*R*	*R*	*R*	*R*	*S*	*S*	*S*	*R*	*I*	*R*	*S*	*S*	*S*	*S*	*S*	*S*	E22

(待续 to be continued)

(续表 3 Tab. 3 continued)

编号 code	耐药谱 antibiotic resistance spectrum																谱型 spectrum
	利福平 RFP	红霉素 E	麦迪霉素 MD	青霉素 P	磺胺异恶唑 FS	复方新诺明 SXT	呋喃唑酮 FUR	新霉素 N	阿莫西林 AMO	庆大霉素 GM	多西环素 DO	氟苯尼考 FFC	恩诺沙星 ENR	氯霉素 C	诺氟沙星 NOR	土霉素 OT	
18QW79	*R*	*R*	*R*	*R*	*R*	*S*	*I*	*I*	*R*	*S*	*S*	*S*	*S*	*S*	*I*	*S*	E20
18QW80	*R*	*R*	*R*	*R*	*R*	*S*	*I*	*I*	*I*	*R*	*S*	*S*	*S*	*S*	*S*	*S*	E21
18BJ207	*R*	*R*	*R*	*R*	*R*	*R*	*S*	*I*	*S*	*I*	*S*	*S*	*S*	*S*	*S*	*S*	E18
18BJ208	*R*	*R*	*R*	*R*	*R*	*R*	*S*	*S*	*I*	*I*	*S*	*S*	*S*	*S*	*S*	*S*	E18
18QW160	*R*	*R*	*R*	*R*	*R*	*R*	*I*	*I*	*I*	*I*	*S*	*S*	*S*	*S*	*S*	*S*	E18
18QW170	*R*	*R*	*R*	*R*	*R*	*S*	*I*	*I*	*R*	*S*	*S*	*S*	*S*	*S*	*S*	*S*	E20
18QW171	*R*	*R*	*R*	*R*	*R*	*S*	*I*	*I*	*I*	*R*	*S*	*S*	*S*	*S*	*S*	*S*	E21
18QW203	*R*	*R*	*R*	*R*	*S*	*S*	*S*	*I*	*R*	*R*	*S*	*S*	*S*	*S*	*S*	*S*	E23
18QW253	*R*	*R*	*R*	*R*	*R*	*R*	*S*	*I*	*I*	*S*	*S*	*S*	*S*	*S*	*S*	*S*	E18
18QW245	*R*	*R*	*R*	*R*	*R*	*R*	*S*	*I*	*I*	*I*	*S*	*S*	*S*	*S*	*S*	*S*	E18
18BJ317	*R*	*R*	*R*	*R*	*R*	*R*	*S*	*I*	*I*	*S*	*S*	*S*	*S*	*S*	*S*	*S*	E18
18QW237	*R*	*R*	*R*	*R*	*R*	*S*	*S*	*R*	*I*	*S*	*S*	*S*	*S*	*S*	*S*	*S*	E19
18QW234	*R*	*R*	*R*	*R*	*R*	*S*	*S*	*R*	*S*	*S*	*S*	*S*	*S*	*S*	*S*	*S*	E19
18QW232	*R*	*R*	*R*	*R*	*R*	*S*	*S*	*R*	*I*	*I*	*S*	*S*	*S*	*S*	*S*	*S*	E19
18BJ224	*R*	*R*	*R*	*R*	*S*	*S*	*R*	*I*	*R*	*S*	*S*	*I*	*S*	*R*	*S*	*I*	E26
18QW211	*R*	*R*	*R*	*R*	*R*	*R*	*S*	*I*	*S*	*I*	*I*	*S*	*S*	*S*	*R*	*S*	E27
18QW227	*R*	*R*	*R*	*R*	*R*	*R*	*S*	*R*	*S*	*S*	*S*	*S*	*S*	*S*	*S*	*S*	E25
18QW230	*R*	*I*	*R*	*R*	*R*	*R*	*S*	*R*	*S*	*R*	*S*	*S*	*S*	*S*	*S*	*S*	E28
18QW191	*R*	*R*	*R*	*R*	*R*	*R*	*S*	*R*	*I*	*S*	*S*	*S*	*S*	*S*	*S*	*S*	E25
18BJ346	*R*	*R*	*R*	*R*	*R*	*R*	*S*	*I*	*R*	*S*	*S*	*S*	*S*	*S*	*S*	*S*	E24
18BJ344	*R*	*R*	*R*	*R*	*R*	*R*	*S*	*I*	*I*	*R*	*S*	*S*	*S*	*S*	*S*	*S*	E29
18QW254	*R*	*R*	*R*	*R*	*R*	*R*	*S*	*I*	*R*	*S*	*S*	*S*	*S*	*S*	*S*	*S*	E24
18QW246	*R*	*R*	*R*	*R*	*R*	*R*	*S*	*I*	*R*	*S*	*S*	*S*	*S*	*S*	*S*	*S*	E24
18BJ323	*R*	*R*	*R*	*R*	*R*	*R*	*S*	*I*	*R*	*S*	*S*	*S*	*S*	*S*	*S*	*S*	E24
18BJ319	*R*	*R*	*R*	*R*	*R*	*R*	*S*	*I*	*R*	*S*	*S*	*S*	*S*	*S*	*S*	*S*	E24
18QW195	*R*	*R*	*R*	*R*	*R*	*R*	*S*	*I*	*R*	*S*	*S*	*S*	*S*	*S*	*S*	*S*	E24
18QW241	*R*	*R*	*R*	*R*	*R*	*R*	*S*	*I*	*R*	*R*	*S*	*S*	*S*	*S*	*S*	*S*	E30
18BJ338	*R*	*R*	*R*	*R*	*R*	*R*	*S*	*I*	*R*	*R*	*S*	*S*	*S*	*S*	*S*	*S*	E30
18BJ321	*R*	*R*	*R*	*R*	*R*	*R*	*S*	*R*	*R*	*S*	*S*	*S*	*S*	*S*	*S*	*S*	E31
18QW67	*R*	*R*	*R*	*R*	*S*	*S*	*R*	*I*	*R*	*S*	*S*	*R*	*S*	*R*	*S*	*R*	E32

注: QW 代表采样地点为珠海市乾务镇, BJ 代表采样地点为珠海市白蕉镇.

Note: QW means sampling site is Qianwu Town, Zhuhai, Guangdong province, China. BJ means sampling site is Baijiao Town, Zhuhai, Guangdong province, China.

包含 3 菌株, 占总菌数的 3.45%; E32 对 9 种抗生素耐药, 包含 1 菌株, 占总菌数的 1.60%。87 株菌 MARI 指数为 0.423, 抗 4~6 种抗生素的菌株居多, 占到总菌数的 64.36%。

2.3 杀鱼爱德华菌毒力

杀鱼爱德华菌中对斑马鱼致死率大于 90%有 20 株菌, 占总杀鱼爱德华菌株数的 22.99%, 对斑马鱼致死率在 80%~90%的有 17 株菌, 占 19.54%, 对斑马鱼致死率小于 30%有 11 株菌, 占 12.64% (表 4)。

2.4 杀鱼爱德华菌毒力与耐药相关性分析

通过 Spearman 相关性分析, 可知斑马鱼死亡率与庆大霉素耐药性呈正相关(P<0.05), 相关系数 0.255, 与恩诺沙星、氟苯尼考(P<0.05)和土霉素(P<0.01)耐药性呈负相关, 相关系数分别为 0.237、0.245、0.297 (表 5)。

表 4 87 株杀鱼爱德华菌对斑马鱼致死率

Tab. 4 Mortality of *Danio rerio* to 87 *Edwardsiella piscicida* strains

死亡率区间 the range of mortality	死亡率/% mortality				
	⩾90	90–80	80–30	30–10	⩽10
菌株数 the number of strains	20	17	39	5	6
占比/% percentage	22.99	19.54	44.82	5.74	6.9

表 5 杀鱼爱德华菌毒力与耐药相关性分析

Tab. 5 Analysis of the relationship between virulence and antibiotics resistance of *Edwardsiella piscicida*

抗生素种类 antibiotics	耐药与毒力 Spearman 相关性分析 correlation of virulence and antibiotic resistance with Spearman analysis		抗生素种类 antibiotics	耐药与毒力 Spearman 相关性分析 correlation of virulence and antibiotic resistance with Spearman analysis	
	系数 coefficient	显著性 significance		系数 coefficient	显著性 significance
利福平 RFP	0.11	0.312	阿莫西林 AMO	–0.25	0.815
红霉素 E	0.071	0.515	庆大霉素 GM	0.255*	0.017
麦迪霉素 MD	0.009	0.936	多西环素 DO	0.153	0.156
青霉素 P	–0.66	0.546	氟苯尼考 FFC	–0.237*	0.027
磺胺异恶唑 FS	0.128	0.238	恩诺沙星 ENR	–0.245*	0.022
复方新诺明 SXT	0.126	0.243	氯霉素 C	–0.081	0.457
呋喃唑酮 FUR	–0.198	0.08	诺氟沙星 NOR	0.098	0.369
新霉素 N	0.076	0.486	土霉素 OT	–0.297**	0.005

注: *P<0.05, **P<0.01.

Note: *P<0.05, **P<0.01.

3 讨论

3.1 杀鱼爱德华菌耐药性分析

爱德华菌属起初分为迟缓爱德华菌, 鲶爱德华菌(*E. ictaluri*)和保科爱德华菌(*E. hoshinae*) 3 个种, 后有学者发现迟缓爱德华菌又可再分为迟缓爱德华菌、杀鱼爱德华菌和鳗鲡爱德华菌(*E. anguillarum*)[28-29]。近些年来, 杀鱼爱德华菌因从迟缓爱德华菌中分出而成为一种新的鱼类病原菌, 可引起鱼体的败血症[30]、表皮腐烂瘀斑、深部皮肤溃疡暴露、骨头坏死[4]以及多个器官出现肉芽肿病变[5-6]等病症。造成上述症状感染和产生致病性主要是由毒力基因和毒力调节系统造成的[31], 包括溶血素[32]、软骨素酶、三型分泌系统[33]、六型分泌系统等。而本研究分离出杀鱼爱德华菌的花鲈鱼体不完全呈现上述典型的病理症状, 可能是该病原所致的不同临床症状与宿主或菌株间毒力基因(系统)存在差异有关。

在水产养殖中, 细菌对抗生素的耐药性主要来自抗生素的选择性压力[34], 药物的频繁和过量使用, 会导致细菌出现自适应突变, 即出现多重耐药甚至“超级细菌”[35]。以前研究发现, 酰胺醇类抗生素[36]、四环素类[37-38]和喹诺酮类[39-40]抗生素在中国海域检出率较高。而本实验结果显示, 杀鱼爱德华菌对多西环素、氟苯尼考、恩诺沙星、氯霉素、诺氟沙星和土霉素的耐药性较低。显然

从鱼体中分离的菌株部分耐药性与水体的抗生素检测结果不同, 可能与近年来相关部门提倡科学用药和禁止养殖中使用氯霉素、诺氟沙星和土霉素等抗生素在降低药物耐药率方面发挥一定作用, 在杀鱼爱德华菌上得到了体现。本研究中耐药谱显示杀鱼爱德华菌对红霉素、麦迪霉素和利福平有较高的耐药性。而 Xie 等[41]和 Xu 等[42]报道红霉素和麦迪霉素在珠三角水域和养殖区域的检出率较高, 同样 Ma 等[43]发现环境中普遍存在利福平耐药, 即从鱼体中分离的菌株耐药性与水体的抗生素检测结果相同, 与之前结果矛盾, 推测与杀鱼爱德华菌对大环内酯类和利福霉素类抗生素耐药性的持续保持有关, 有待后续进一步研究。

多重抗生素耐药(MARI)是分析指定菌群耐药性的极佳工具, 当菌株暴露于常用抗菌药物, MARI>0.2, 而少用或不用抗菌药物时, MARI<0.2[27]。多重抗生素耐药结果显示, 杀鱼爱德华菌 MARI 指数为 0.423, 说明该菌在珠海养殖区处于抗生素高危暴露状态, 由于本实验暂未对其耐药机制进行研究, 故珠海花鲈源杀鱼爱德华菌高 MARI 指数形成原因暂不明确。

3.2　杀鱼爱德华菌毒力与耐药相关性分析

斑马鱼(*Danio rerio*)是一种研究病原体感染和宿主免疫反应的模式动物[44], 已建立了斑马鱼的气单胞菌[45]、链球菌[46]、沙门氏菌[47]和爱德华菌感染模型[48]。本研究揭示, 分离的 87 株杀鱼爱德华菌菌株对斑马鱼毒力存在一定的差异, 证实杀鱼爱德华菌含有不同毒力类型的菌株[49]。细菌毒力因子主要存在于染色体基因簇中, 或存在于质粒和噬菌体等遗传辅助元件中, 其扩散同细菌耐药基因的扩散存在相关的机制。有学者发现毒力与耐药基因在一定程度上可以相互传播和转化[50-51]。并且在编码细菌素[52]、铁载体[53]、细胞毒素[54]和黏附因子[55]的基因中都存在抗生素抗性。研究发现 ESP_{fm}(肠球菌表面蛋白)与氨苄耐药呈正相关[56]; 细菌生物膜与耐药性呈正相关[57]; 细胞膜蛋白失活导致毒力与耐药均下降[58]。所以研究表明细菌抗生素耐药和毒力存在遗传连锁和表达共轭。这与本实验发现的杀鱼爱德华菌毒力与庆大霉素抗性呈正相关(P<0.05)的结果相似。

当然, 也存在相反的表型, 如耐青霉素的肺炎链球菌菌株致病性可能低于敏感菌株[59]; 多重耐药导致毒力下降[60]; 对利福平耐药导致毒力下降[61]; 从氟喹诺酮耐药菌株中发现毒力基因表达下降[62-63]。对此, 许多学者提出了“生物适应性成本”的概念, 即抗性突变引起细菌的适合度下降[64], 所以当可移动元件接合到细菌中, 可能发生异常的细菌基因调控即生物适应性成本增加。当病原菌抗生素耐药性增加时, 由于新的遗传决定因子可能会使病原菌产生生物适应性成本, 这可能会导致细菌适应度下降, 从而使细菌毒性降低。所以, 当病原菌抗生素耐药和毒力之间由于生物适应性成本的增加出现负相关情况, 并且大多数情况表明主要的相关性为负相关[65-67], 这与本研究中杀鱼爱德华菌毒力与恩诺沙星、氟苯尼考(P<0.05)和土霉素抗性(P<0.01)呈负相关的结果一致。但长远来看, 细菌由于获得性耐药导致调控异常(毒力减弱)会慢慢通过细菌的补偿突变进行回复, 甚至在某些个别的情况中会出现不会改变细菌适应度的突变[68-69]。虽然目前在研究中分析大部分抗生素与毒力呈现负相关, 但由于细菌的补偿突变, 终究会形成高毒力, 高耐药的菌株[70-72]。本研究第一次在杀鱼爱德华菌上提出毒力与耐药之间的相关性概念, 并发现杀鱼爱德华菌耐药与毒力之间大多呈现负相关, 对杀鱼爱德华菌耐药和毒力相关性的分子机制尚有待进一步深入研究。

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Analysis of the relationship between antibiotic resistance and virulence of *Edwardsiella piscicida* strains isolated from *Lateolabrax maculatus*

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Abstract: The emergence and spread of antibiotic resistant bacteria brings risks to disease prevention and control efforts. Although the spread of antibiotic resistance has been a hot topic in recent research, information on antibiotic resistance in aquaculture is still lacking. A bacterial epidemic of *Lateolabrax maculatus* was investigated in fish farms at Zhuhai, Guangdong province, China from April to December 2018, and a total of 96 strains of *Edwardsiella* spp. were obtained. Among them, 87 strains were identified as *E. piscicida* by the *gyrB* gene. These 87 *E. piscicida* strains were tested for resistance to 16 antibiotics and for their virulence in zebrafish using the KB method and challenge tests, respectively. The relationship of antibiotic resistance and virulence was assessed using Spearman correlation analysis. The results were as follows: (1) The percentage of *E. piscicida* strains resistant to each antibiotic was: Rifampin (98.85%), Madinomycin (96.55%), Erythromycin (95.40%), Penicillin (68.96%), Sulfamethoxazole (58.62%), Norepinephrine (28.73%), Amoxicillin (21.83%), Gentamicin (13.79%), Neomycin (10.34%), Furazolidone (3.45%), Norfloxacin (2.29%), Chloramphenicol (2.29%), Doxycycline (2.29%), Oxytetracycline (1.15%), Florfenicol (1.14%), and Enrofloxacin (0%). The antibiotic resistance spectrum revealed 32 total antibiotic resistant types existed in 87 strains. The Multiple Antibiotic Resistance Index (MARI) was 0.423, indicating that the strains were isolated in an environment with high exposure to antibiotics. (2) The virulence challenge tests showed that the lethality rate of 37 *E. piscicida* strains was > 80% in zebrafish, accounting for 42.52% of the total bacterial strains tested; the lethality rate of 39 *E. piscicida* strains was 30%-80% in zebrafish, accounting for 44.83% of the total bacterial strains tested; and the lethality rate of 11 *E. piscicida* strains to zebrafish was < 30%, accounting for 12.65% of the total bacterial strains tested. This demonstrates that *E. piscicida* is a high virulence strain. (3) Spearman correlation analysis showed that the virulence of *E. piscicida* was positively correlated with Gentamicin resistance ($P<0.05$), and negatively correlated with Enrofloxacin, Florfenicol ($P<0.05$), and Oxytetracycline resistance ($P<0.01$). Bacterial virulence may be related to antibiotic resistance and this relationship can be either negative or positive. We conclude that *E. piscicida* strains derived from *Lateolabrax maculatus* are highly toxic and multidrug resistant, and their virulence is negatively correlated with antibiotic resistance. This is presumably produced by the additional biological expenditure of bacteria to the acquisition of foreign DNA. Additional studies on this interaction mechanism may provide new data for the evolution of microorganisms and new ideas for bacterial disease control.

Key words: *Lateolabrax maculatus*; *Edwardsiella piscicida*; antibiotic resistance; virulence

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