线粒体抗氧化剂Visomitin对肠球菌的体外和体内抗菌作用

杨琪，吴苑，吴若兰，佘鹏飞

(中南大学湘雅三医院检验科，长沙 410013)

[摘 要] 目的：随着肠球菌对抗生素的耐药性不断攀升，新型抗菌药物的挖掘已成为研究热点。本研究旨在通过药物重新利用，探究靶向线粒体的抗氧化剂Visomitin对肠球菌的体外和体内抗菌活性。**方法：**肠球菌临床菌株分离自中南大学湘雅三医院住院患者。首先，通过微量肉汤稀释实验、纸片扩散实验和时间-杀菌曲线探究Visomitin对肠球菌的抗菌敏感性及其杀菌效率。通过连续诱导耐药实验检测亚抑菌浓度的Visomitin对肠球菌的耐药诱导作用。通过蛋白酶定量实验检测Visomitin对肠球菌蛋白酶产生的抑制作用。随后，通过结晶紫染色和SYTO9荧光探针检测Visomitin对肠球菌生物膜的抑制和清除作用。最后，通过构建小鼠皮下脓肿模型，检测Visomitin对肠球菌的体内抗菌活性。**结果：**Visomitin对肠球菌具有良好的体外抗菌活性，其最低抑菌浓度(minimal inhibitory concentration，MIC)和最低杀菌浓度分别为4~8 μg/mL和4~16 μg/mL。纸片扩散实验提示Visomitin对肠球菌的生长具有剂量依赖的抑制活性，时间-杀菌曲线进一步显示Visomitin对肠球菌具有浓度和时间依赖的杀菌活性。2×MIC浓度的Visomitin与肠球菌标准菌株ATCC 19434和ATCC 29212分别作用2 h和12 h均可将活菌量降低到最低检测限以下。虽然万古霉素可诱导肠球菌的耐药性增加，但亚抑菌浓度的Visomitin连续传代14次，仍未见明显的耐药诱导作用。亚抑菌浓度的Visomitin还可明显抑制肠球菌蛋白酶的生成，Visomitin在1/8×MIC浓度时可使ATCC 19434蛋白酶产量由100%±2.01%降至96.25%±1.16% (q=3.661，P<0.05)，而在1/2×MIC浓度下则使ATCC 29212蛋白酶产量由100%±2.95%降至41.49%±0.45%(q=35.790，P<0.001)。结晶紫染色提示，1×MIC~4×MIC浓度的Visomitin可显著抑制肠球菌生物膜的形成；当其浓度上升至2×MIC~4×MIC时，能不同程度清除已形成的生物膜。SYTO9荧光探针进一步验证了Visomitin对肠球菌的抗生物膜活性。此外，Visomitin可显著降低小鼠脓肿大小和活菌载量，与仅注射溶媒的对照组相比，Visomitin可使菌载量从[2.90(2.52, 3.18)]×10^7 CFU/abscess降低到[1.56(0.35，4.78)]×10^6 CFU/abscess(U=0.000，P<0.01)。苏木精-伊红染色结果提示，Visomitin能明显减少脓肿组织中的炎症细胞浸润。**结论：**Visomitin对肠球菌具有良好的体外和体内抗菌活性，且不易诱导耐药产生，具备再利用潜力，为其可能成为临床治疗难治性肠球菌感染的新型候选药物提供了重要的临床前证据。

[关键词] 药物重新利用；Visomitin；肠球菌；生物膜；感染模型；体内

In vitro and in vivo antibacterial effects of the mitochondrial antioxidant Visomitin against *Enterococcus*

YANG Qi, WU Yuan, WU Ruolan, SHE Pengfei

(Department of Laboratory Medicine, Third Xiangya Hospital, Central South University, Changsha 410013, China)

收稿日期(Date of reception)：2025-09-10

第一作者(First author)：杨琪，Email: 238311045@csu.edu.cn, ORCID: 0009-0002-6557-6935；吴苑，Email: korchim@126.com, ORCID: 0000-0002-9358-9146

通信作者(Corresponding author)：佘鹏飞，副主任技师，Email: shepengfei_xy@csu.edu.cn, ORCID: 0000-0002-4246-1361

基金项目(Foundation item)：湖南省自然科学基金(2024JJ5514)。This work was supported by the Natural Science Foundation of Hunan Province, China (2024JJ5514).

开放获取(Open access)：本文遵循知识共享许可协议，允许第三方用户按照署名-非商业性使用-禁止演绎4.0(CC BY-NC-ND 4.0)的方式，在任何媒介以任何形式复制、传播本作品(https://creativecommons.org/licenses/by-nc-nd/4.0/)。

©Journal of Central South University (Medical Science). All rights reserved.

ABSTRACT

Objective: With the increase in antibiotic resistance among *Enterococcus* species, the discovery of novel antimicrobial agents has become a major research focus. This study aims to investigate the in vitro and in vivo antibacterial activity of the mitochondria-targeted antioxidant Visomitin against *Enterococcus* through a drug repurposing strategy.

Methods: Clinical *Enterococcus* isolates were obtained from hospitalized patients at The Third Xiangya Hospital of Central South University. Antibacterial susceptibility and bactericidal efficacy of Visomitin against *Enterococcus* were first evaluated using broth microdilution assays, disk diffusion assays, and time-kill curve analyses. Resistance induction by sub-inhibitory concentrations of Visomitin was assessed through serial passage experiments. Protease quantification assays were performed to evaluate the inhibitory effect of Visomitin on protease production in *Enterococcus*. Subsequently, crystal violet staining and SYTO9 fluorescent probe assays were used to determine the inhibitory and eradication effects of Visomitin on *Enterococcus* biofilms. Finally, a mouse subcutaneous abscess model was established to evaluate the in vivo antibacterial activity of Visomitin.

Results: Visomitin exhibited potent antibacterial activity against *Enterococcus* with the MIC and minimal bactericidal concentration of 4 to 8 μg/mL and 4 to 16 μg/mL, respectively. Disk diffusion assay revealed dose-dependent growth inhibition activity against *Enterococcus* by Visomitin, while the concentration- and time-dependent bactericidal effects were observed by time-kill curves. For instance, 2×MIC of Visomitin could reduce the viable bacterial counts of *E. faecium* ATCC 19434 and *E. faecalis* ATCC 29212 to the detection limit after 2 h and 12 h treatment, respectively. Unlike vancomycin, sub-MIC of Visomitin did not induce obvious resistance occurrence even after 14 serial passages. At 1/8×MIC, visomitin reduced the protease production of ATCC 19434 from 100%±2.01% to 96.25%±1.16% (q=3.661, P<0.05). At 1/2×MIC, visomitin decreased the protease production of ATCC 29212 from 100%±2.95% to 41.49%±0.45% (q=35.790, P<0.001). Furthermore, as determined by crystal violet staining, 1×MIC to 4×MIC of Visomitin could inhibit the *Enterococcus* biofilm formation, while it could also partially eradicate the preformed biofilms at the concentrations of 2×MIC to 4×MIC, as confirmed by SYTO9 staining. Furthermore, Visomitin significantly reduced both abscess size and bacterial load in mice. Compared to the vehicle control group, treatment with Visomitin decreased the bacterial load from [2.90 (2.52, 3.18)]×10^7 CFU/abscess to [1.56 (0.35, 4.78)]×10^6 CFU/abscess (U=0.000, P<0.01).

Conclusion: Visomitin demonstrates potent antibacterial activity against *Enterococci* both in vitro and in vivo, with a low risk of inducing resistance and potential for repurposing, providing key preclinical evidence for its development as a novel therapeutic candidate against refractory enterococcal infections.

KEY WORDS drug repurposing; Visomitin; *Enterococcus*; biofilm; infection model; in vivo

肠球菌(*Enterococcus*)是一种临床上常见的革兰氏阳性球菌，可引起各种类型医疗相关感染[1]，包括感染性心内膜炎和脑膜炎等[2]，同时也是尿路感染[1-3]和血流感染的致病菌[4]。高耐药性肠球菌的感染率在全球范围内逐年增加[5]。其中，万古霉素(vancomycin，VAN)耐药的肠球菌检出率不断上升，

©Journal of Central South University (Medical Science). All rights reserved.

尤其在屎肠球菌(*Enterococcus faecium*，EFM)中更为常见[6]。肠球菌在非洲的感染率已达到26.8%[7]。肠球菌对抗生素的耐药性不断攀升而且越来越难以治疗[8]，使得医院获得性感染变成重大威胁[5]。

肠球菌可附着于医疗器械或人体组织表面而形成生物膜。肠球菌的生物膜成分包括菌体、细胞外基质、分泌因子(例如分泌抗原A、溶细胞素和明胶酶)及细胞表面因子等[9]。生物膜与单个状态下的浮游细菌相比，对抗菌药物表现出更强的耐药性，从而常导致感染慢性化。其中，细胞外基质的特定成分可以改变生物膜的结构，增强细菌之间的相互交流，并极大地降低抗生素在生物膜中的渗透能力，导致细菌对抗生素产生耐药性[10]。此外，蛋白酶是肠球菌产生的最重要的毒力因子之一，已被证实能破坏保护黏膜的免疫球蛋白和上皮细胞之间的紧密连接，从而导致组织侵袭和损伤[11]。

Visomitin作为靶向线粒体的抗氧化剂，常用于治疗干眼症[12]。近年来，研究者发现Visomitin对其他疾病或功能失调也具有潜在的治疗效果：1)Visomitin可减轻癌症恶病质诱导的雄性小鼠肌肉损失，同时改善雌性小鼠的肌肉收缩功能[13]；2)Visomitin可通过中和线粒体活性氧，关闭细胞因子风暴[14]；3)Visomitin能恢复角膜上皮细胞功能[15]。同时，Visomitin也被发现对枯草芽孢杆菌和结核分枝杆菌等细菌表现出一定的抗菌活性[16-18]。然而，尚未见Visomitin对肠球菌抗菌活性的系统性研究。

本研究通过微量肉汤稀释实验和时间-杀菌曲线等检测了Visomitin对肠球菌的体外抗菌活性，并探究了其对肠球菌生物膜的抑制、清除作用及对蛋白酶的抑制效果，还通过小鼠皮下脓肿模型探究了Visomitin的体内抗菌作用。本研究旨在为Visomitin在临床中治疗肠球菌相关难治性感染提供新的实验依据。

1 材料与方法

1.1 伦理声明

本研究中的动物实验已获得中南大学湘雅三医院伦理委员会批准(审批号：CSU-2024-0280)。

1.2 主要试剂

Visomitin和结晶紫染液均购自美国MedChemExpress公司。二甲基亚砜(dimethyl sulfoxide，DMSO)购自上海碧云天生物技术有限公司。SYTO9染液购自美国赛默飞公司。Mueller-Hinton(MH)肉汤和脑心浸液肉汤培养基(brain-heart infusion broth，BHI)均购自北京索莱宝科技有限公司。羊血琼脂平板购自郑州安图生物科技有限公司。

1.3 主要仪器

恒温培养箱购自美国赛默飞公司。恒温摇床购自上海申能博彩生物科技有限公司。II级生物安全柜购自济南鑫贝西生物技术有限公司。VITEK 2 Compact全自动微生物鉴定系统和麦氏比浊仪购自法国BioMérieux公司。iMark酶标仪购自美国Bio-Rad公司。基质辅助激光解吸电离飞行时间质谱仪购自德国布鲁克公司。

1.4 菌株来源及保存

EFM标准菌株ATCC 19434和粪肠球菌(*Enterococcus faecalis*，EFA)标准菌株ATCC 29212均购自美国模式培养物集存库，EFM临床菌株EFM 1~12及EFA临床菌株EFA 1~11均分离自中南大学湘雅三医院的住院患者，并通过VITEK 2 Compact全自动微生物鉴定系统及基质辅助激光解吸电离飞行时间质谱仪进行双重鉴定。所有菌株接种于甘油磁珠管后置于−80 ℃进行冻存。

1.5 微量肉汤稀释实验

采用MH肉汤将处于对数期的细菌稀释至1×10^6 CFU/mL后，再将50 μL含倍比稀释的Visomitin的MH肉汤和等体积的细菌悬液转移到96孔板中并混匀。将菌种放置于湿盒，在37 ℃下培养16~18 h。最小抑菌浓度(minimal inhibitory concentration，MIC)被定义为肉眼下完全抑制细菌生长(即培养液澄清)的最低药物浓度。在96孔板中每孔取5 μL培养液点样于羊血琼脂平板并培养24 h，在羊血琼脂平板上没有生长菌落的最低药物浓度被定义为最低杀菌浓度(minimum bactericidal concentration，MBC)[19-20]。

1.6 纸片扩散实验

肠球菌ATCC 19434和ATCC 29212在BHI肉汤中培养至对数期，用生理盐水调至0.5麦氏浊度(McFarland，McF)，再用棉签均匀地涂布于MH琼脂板上。待菌液吸收后，将直径为5 mm的无菌圆形纸片贴至平板表面，将不同剂量的Visomitin药液滴加于纸片上，同时滴加DMSO作为阴性对照，商品化VAN纸片作为阳性对照。在37 ℃下培养18 h后测量抑菌圈的直径，并以抑菌圈面积进行统计学分析[21]。

1.7 时间-杀菌曲线

将BHI肉汤过夜培养的肠球菌ATCC 19434和ATCC 29212悬液用新鲜的BHI肉汤1∶200稀释，终体

©Journal of Central South University (Medical Science). All rights reserved.

积为15 mL，并加入不同浓度(1/4×MIC~2×MIC)的Visomitin及DMSO(对照组)，在37 ℃下以200 r/min摇菌培养。在不同时间点(0、2、4、8、12和24 h)取样，并用生理盐水连续稀释后吸取5 μL在羊血琼脂平板上过夜培养，计数菌落数[17]。

1.8 连续诱导耐药实验

根据上述微量肉汤稀释实验确定Visomitin及VAN对肠球菌ATCC 19434和ATCC 29212的MIC值。培养24 h后，取1/2×MIC孔中的培养液按照1∶1 000稀释至终体积为3 mL的MH肉汤中，以重新测定次日的MIC，实验连续进行14 d，记录每日MIC值[22]。

1.9 蛋白酶定量实验

将肠球菌ATCC 19434和ATCC 29212的过夜培养菌悬液在含有1/8×MIC~1/2×MIC的Visomitin及DMSO的胰蛋白胨大豆肉汤中孵育24 h，以10 000 r/min离心10 min，取上清。将总体积为500 μL的培养上清液与1 mL 1.25%(wt/vol)脱脂牛奶在37 ℃下孵育30 min。拍照记录并测量600 nm处的吸光度值[23]。

1.10 结晶紫染色法定量检测生物膜

将肠球菌ATCC 19434和ATCC 29212用BHI肉汤在37 ℃下以200 r/min条件下培养12 h，并用BHI肉汤1∶200稀释备用。对于生物膜抑制实验，用新鲜BHI肉汤将Visomitin稀释至终浓度为1~16 μg/mL，同时以DMSO作为对照，与等体积备用菌液加入96孔板中并混匀。将96孔板置于37 ℃湿盒中孵育24 h。随后，弃去培养上清液，用无菌生理盐水洗涤2~3次后，每孔加入100 μL含有0.25%(wt/vol)的结晶紫溶液，孵育15 min，弃去结晶紫溶液，用生理盐水洗涤2~3次，加入200 μL 95%乙醇溶解结晶紫，检测570 nm处的吸光度值[24]。对于生物膜清除实验，在96孔板每孔加入备用菌液孵育24 h后，弃去上清并用生理盐水洗涤2~3次，再加入含有2~16 μg/mL Visomitin或DMSO的BHI肉汤，在37 ℃下于湿盒孵育24 h。染色步骤同上。

1.11 SYTO9荧光染色观察生物膜

挑取羊血琼脂平板上的单个肠球菌菌落于BHI肉汤中过夜培养，并用BHI肉汤1∶200稀释。对于生物膜抑制实验，将稀释菌液和Visomitin(终浓度为1×MIC)混匀培养于含有无菌玻片的6孔板中，同时设置含有DMSO的对照组，置于37 ℃湿盒培养24 h。弃去培养液，用无菌生理盐水洗涤2~3次，加入1 mL含有终浓度为10 μmol/L的SYTO9染液，于37 ℃避光孵育15 min后，用无菌生理盐水漂洗2~3次以去除多余染液。在488 nm激发波长和503 nm发射波长下通过LSM800型激光共聚焦显微镜(德国卡尔蔡司公司)观察生物膜结构。对于生物膜清除实验，先将稀释菌液培养于6孔板24 h后，弃去上清，并用无菌生理盐水漂洗2~3次后，再将含Visomitin(终浓度为4×MIC)的BHI肉汤加入孔板孵育24 h[25]，其余染色步骤和生物膜抑制实验相同。

1.12 皮下脓肿模型

对于皮下脓肿模型，使用7周龄、体重约25 g的无特定病原体ICR雌性小鼠进行实验。将肠球菌ATCC 29212接种于BHI肉汤中过夜培养，洗涤2~3次后，重悬于生理盐水中备用。通过腹腔注射50 mg/kg戊巴比妥钠麻醉小鼠，并用电动剃须刀去除背部毛发。随后，将100 μL含有1 McF(活菌计数约为1.23×10^7 CFU)的菌悬液皮下注射到小鼠的背部。将小鼠随机分为2组，每组6只。感染后1 h，将皮下注射20 mg/kg的Visomitin的小鼠作为实验组，同时将注射0.5% DMSO的小鼠作为对照组。感染24 h后，对小鼠实施安乐死，无菌切除感染部位脓肿。用生理盐水研磨，连续稀释10倍后，取5 μL稀释液在羊血琼脂平板上过夜孵育，计算脓肿组织中的活菌数量[26]。同时，各组取完整脓肿组织，用5 mL 4%中性多聚甲醛固定溶液固定后，进行苏木精-伊红(hematoxylin and eosin，HE)染色[25]。

1.13 统计学处理

所有数据均采用GraphPad Prism 9.0软件进行分析。符合正态分布的计量资料以均数±标准差表示，2组间的比较采用Student *t*检验，多组数据比较则采用单因素方差分析(One-way ANOVA)及Dunn's多重比较检验。非正态分布的计量资料以中位数(第1分位数，第3分位数)表示，采用Mann-Whitney *U*检验进行比较。$P<0.05$为差异具有统计学意义。

2 结 果

2.1 Visomitin对肠球菌具有良好的杀菌活性

微量肉汤稀释实验显示，Visomitin对EFA和EFM(包括VAN耐药的临床菌株)的MIC为4~8 μg/mL，MBC为4~16 μg/mL。纸片扩散实验(图1A)结果显示，DMSO纸片周围未见抑菌圈的形成，而含有Visomitin的纸片周围出现明显的浓度依赖性抑菌圈，即随着Visomitin的含量增加，其抑菌圈直径明显扩大。抑菌圈面积定量分析(图1B)进一步提示，与DMSO对照组相比，Visomitin能显著抑制肠球菌的增殖，其抑菌圈面积随Visomitin的含量增加而显著

©Journal of Central South University (Medical Science). All rights reserved.

扩大。时间-杀菌曲线(图1C)显示，Visomitin对肠球菌还具有良好的杀菌活性。2×MIC浓度的Visomitin与ATCC 19434和ATCC 29212分别作用2 h和12 h即可将其活菌量分别从(5.48±0.15)Log_{10}CFU/mL和(4.91±0.41)Log_{10}CFU/mL降低至最低检测限。

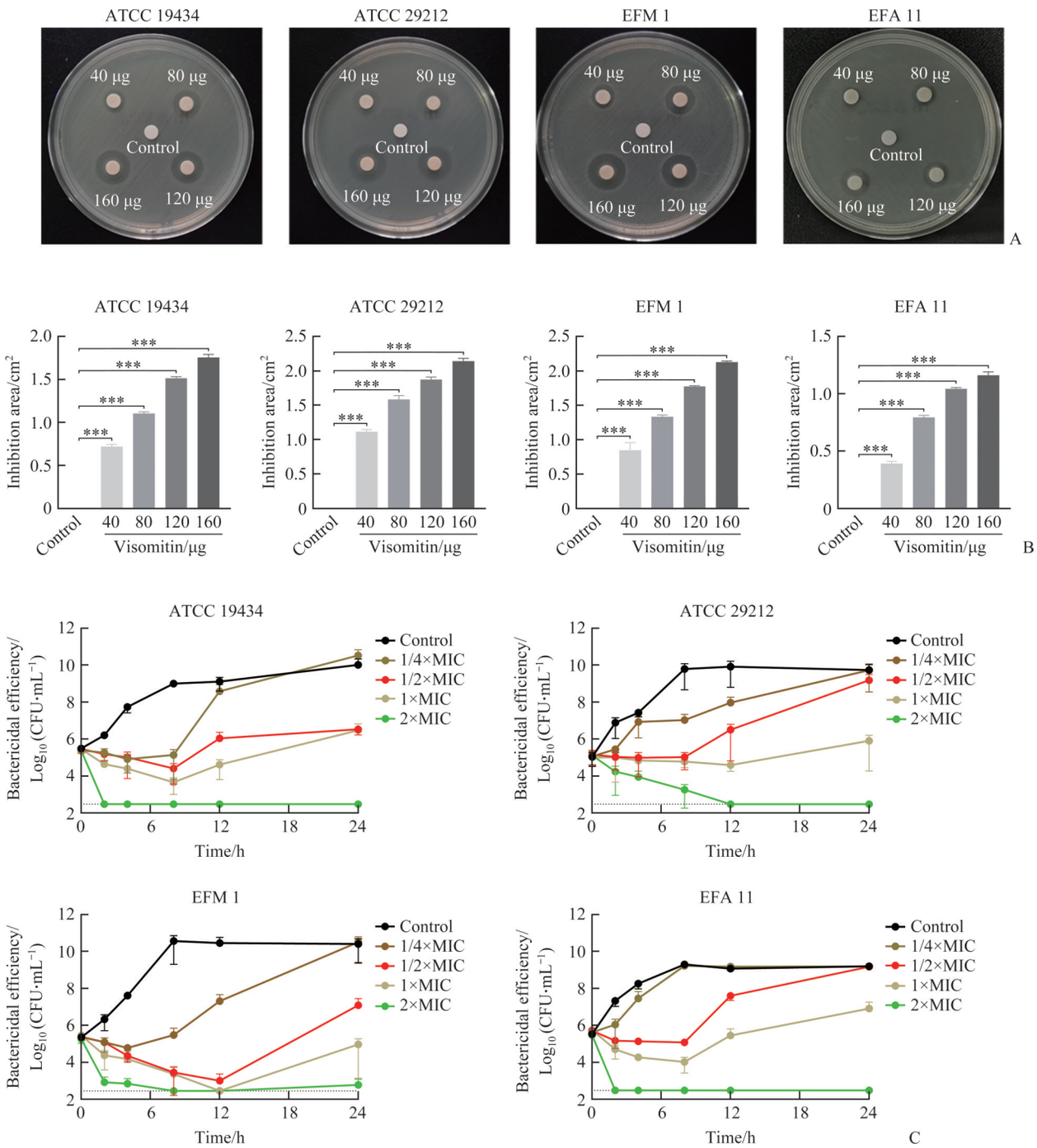


图1　Visomitin的浓度依赖性抗菌活性

Figure 1　Concentration-dependent antimicrobial activity of Visomitin

A: Antibacterial activity of Visomitin against *Enterococcus* was assessed by the disk diffusion assay. B: Quantitative analysis of the inhibition zone areas from the disk diffusion assay. C: Bactericidal efficiency of Visomitin was evaluated using time-kill curves. Data are analyzed using one-way ANOVA and Dunn's multiple comparison test, expressed as mean±standard deviation. ATCC 19434 and EFM 1 were EFM; ATCC 29212 and EFA 11 were EFA. ***P<0.001. ATCC: American Type Culture Collection; EFM: *Enterococcus faecium*; EFA: *Enterococcus faecalis*; MIC: Minimal inhibitory concentration.

©Journal of Central South University (Medical Science). All rights reserved.

2.2 Visomitin难以诱导肠球菌耐药的形成

连续诱导耐药实验提示，亚抑菌浓度VAN连续诱导14 d后，可使EFM ATCC 19434的MIC上升8倍，而Visomitin处理组的MIC仅上升2倍(图2A)。此外，VAN和Visomitin对EFA ATCC 29212均无明显的耐药诱导作用，传代14 d后，其MIC值与初代细菌相比均无明显变化(图2B)。VAN对肠球菌的MIC详见表1。

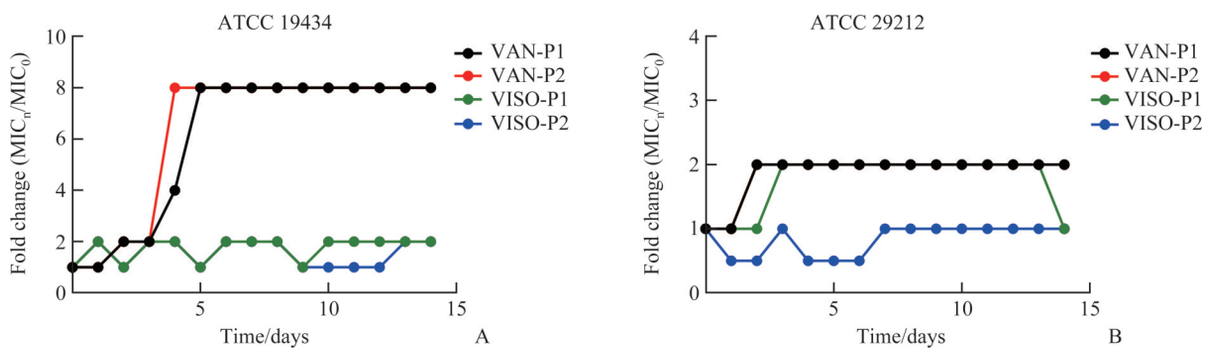


图2 Visomitin的耐药诱导活性低

Figure 2 Low resistance inducing activity by Visomitin

A: EFM ATCC 19434. B: EFA ATCC 29212. P1: Replicate experiment 1; P2: Replicate experiment 2; VAN: Vancomycin; VISO: Visomitin.

表1 Visomitin对肠球菌的抗菌敏感性实验

Table 1 Antimicrobial susceptibility of Visomitin against *Enterococcus*

Strain	Type	VISO/(μg·mL^{-1})		VAN/(μg·mL^{-1})		Strain	Type	VISO/(μg·mL^{-1})		VAN/(μg·mL^{-1})	
		MIC	MBC	MIC	MBC			MIC	MBC	MIC	MBC
EFM						EFA					
19434	ATCC	4	4	1	16	29212	ATCC	4	8	2	>32
EFM 1	Clinical isolate	4	4	>256	>256	EFA 1	Clinical isolate	4	8	2	16
EFM 2	Clinical isolate	4	8	1	16	EFA 2	Clinical isolate	4	8	1	16
EFM 3	Clinical isolate	4	4	0.5	16	EFA 3	Clinical isolate	4	8	2	16
EFM 4	Clinical isolate	4	8	>256	>256	EFA 4	Clinical isolate	8	16	2	16
EFM 5	Clinical isolate	4	8	2	16	EFA 5	Clinical isolate	4	8	1	8
EFM 6	Clinical isolate	4	4	0.5	16	EFA 6	Clinical isolate	4	8	2	16
EFM 7	Clinical isolate	4	4	1	16	EFA 7	Clinical isolate	4	8	1	16
EFM 8	Clinical isolate	4	4	1	1	EFA 8	Clinical isolate	4	8	4	>32
EFM 10	Clinical isolate	4	4	>256	>256	EFA 9	Clinical isolate	8	16	2	16
EFM 11	Clinical isolate	4	8	>256	>256	EFA 10	Clinical isolate	8	8	1	16
EFM 12	Clinical isolate	4	8	>256	>256	EFA 11	Clinical isolate	4	8	0.5	4

EFM: *Enterococcus faecium*; EFA: *Enterococcus faecalis*; ATCC: American Type Culture Collection; MIC: Minimal inhibitory concentration; MBC: Minimum bactericidal concentration; VAN: Vancomycin; VISO: Visomitin.

2.3 Visomitin抑制肠球菌蛋白酶的生成

亚抑菌浓度的Visomitin可明显抑制肠球菌蛋白酶的生成，从而降低其对脱脂牛奶的分解能力(图3A)。定量分析(图3B)结果显示，与仅添加DMSO的

©Journal of Central South University (Medical Science). All rights reserved.

对照组相比，1/8×MIC浓度的Visomitin即可显著抑制EFM ATCC 19434和EFM 1的蛋白酶生成，使其蛋白酶的总量分别从100%±2.01%和100%±1.64%降低至96.25%±1.16%(q=3.661，P<0.05)和83.55%±0.46%(q=19.640，P<0.001)。同时，1/2×MIC浓度的Visomitin也可显著抑制EFA ATCC 29212和EFA 11的蛋白酶生成，使其蛋白酶的总量分别从100%±2.95%和100%±2.12%降低至41.49%±0.45%(q=35.790，P<0.001)和84.80%±2.88%(q=53.730，P<0.001)。

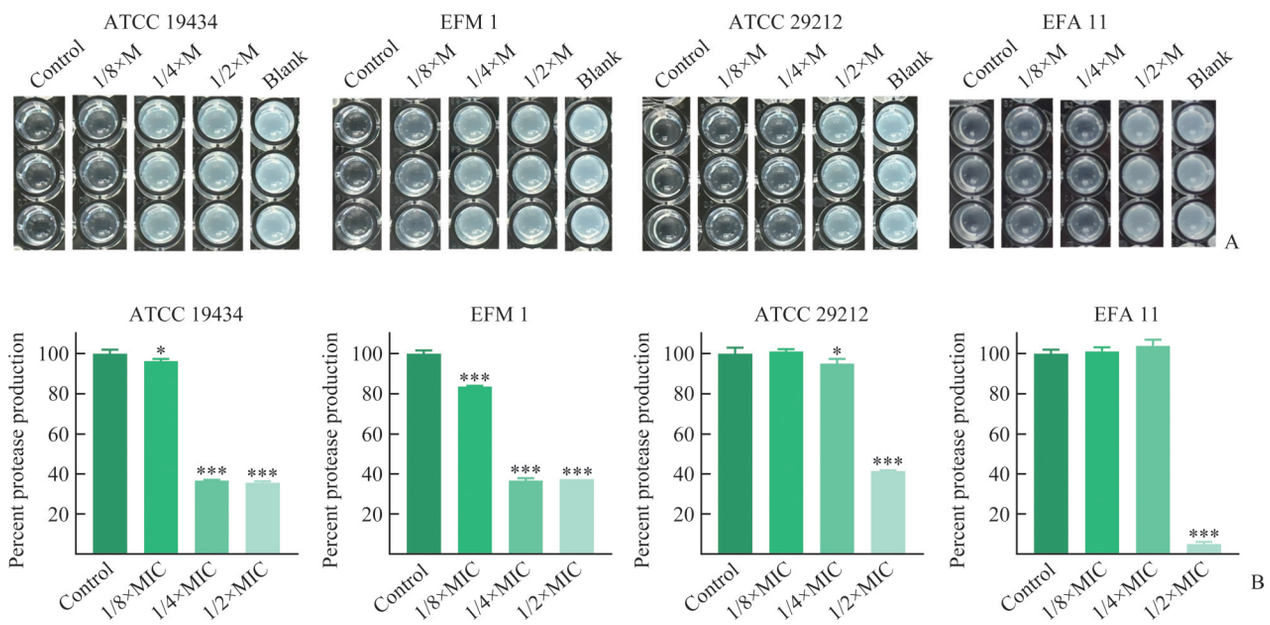


图3　Visomitin抑制肠球菌的蛋白酶生成

Figure 3　Visomitin inhibits the protease production by *Enterococcus*

A: Representative images showing the inhibition of protease production in *Enterococcus* by Visomitin. B: Quantitative analysis of total protease levels. Data are analyzed using one-way ANOVA and Dunn's multiple comparison test, expressed as mean±standard deviation. *P<0.05, ***P<0.001 vs the Control group. M: MIC.

2.4　Visomitin对肠球菌具有良好的抗生物膜活性

结晶紫染色提示，1×MIC~4×MIC浓度的Visomitin可显著抑制肠球菌生物膜的形成。与仅加DMSO的对照组相比，1×MIC Visomitin可使ATCC 19434和ATCC 29212的生物膜形成量($A_{570\ nm}$)分别从2.35±0.11和2.36±0.18降低至0.34±0.04(q=19.320，P<0.001)和0.74±0.48(q=8.509，P<0.001；图4A)。同时，除ATCC 29212外，2×MIC~4×MIC的Visomitin还能显著清除肠球菌已形成的生物膜，2×MIC Visomitin可使ATCC 19434已形成的生物膜从1.68±0.07减少至0.48±0.22(q=11.010，P<0.001；图4B)。SYTO9荧光染色进一步提示，与对照组相比，1×MIC Visomitin可明显抑制肠球菌生物膜的形成(图4C)并降低其荧光强度(图5A)，而4×MIC Visomitin能明显清除已形成的生物膜(图4D)，使生物膜的结构破坏，荧光强度显著降低(图5B)。

2.5　Visomitin具有良好的体内抗菌活性

通过构建EFA ATCC 29212皮下脓肿模型检测Visomitin的体内抗菌活性。由图6A可知，未用药的对照组可见明显的皮下脓肿形成，而Visomitin处理组未见脓肿。活菌计数结果(图6B和6C)显示，与仅注射溶剂的对照组相比，Visomitin可显著降低脓肿组织中的细菌载量，使细菌量从[2.90(2.52, 3.18)]×10^7 CFU/abscess降低到[1.56(0.35, 4.78)]×10^6 CFU/abscess(U=0.000，P<0.01)。脓肿组织的HE染色(图6D)提示，对照组可见明显炎症细胞浸润，而Visomitin处理组的皮下组织趋于正常，未见明显炎症细胞浸润。

©Journal of Central South University (Medical Science). All rights reserved.

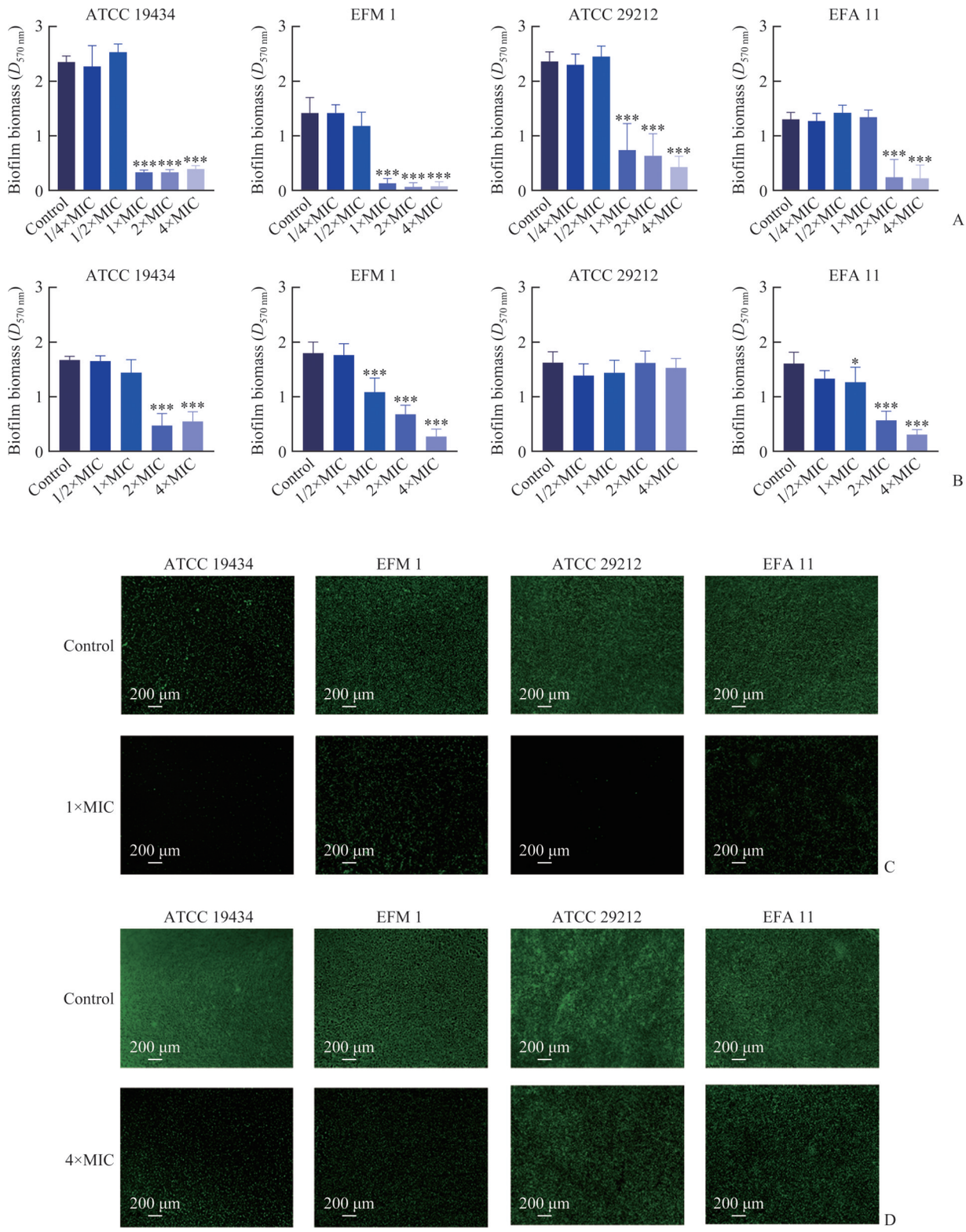


图4 Visomitin的抗生物膜活性

Figure 4 Antibiofilm effects of Visomitin

A and B: Quantitative assessment of the inhibitory effect (A) and the eradicating effect (B) of Visomitin on *Enterococcus* biofilm formation by crystal violet staining. C: CLSM observation using SYTO9 fluorescent staining, showing biofilm inhibition by 1×MIC Visomitin. D: CLSM observation using SYTO9 fluorescent staining, showing biofilm eradication by 4×MIC Visomitin. Data are analyzed using one-way ANOVA and Dunn's multiple comparison test, expressed as mean±standard deviation. **P*<0.05; ****P*<0.001 vs the Control group. CLSM: Confocal laser scanning microscopy

©Journal of Central South University (Medical Science). All rights reserved.

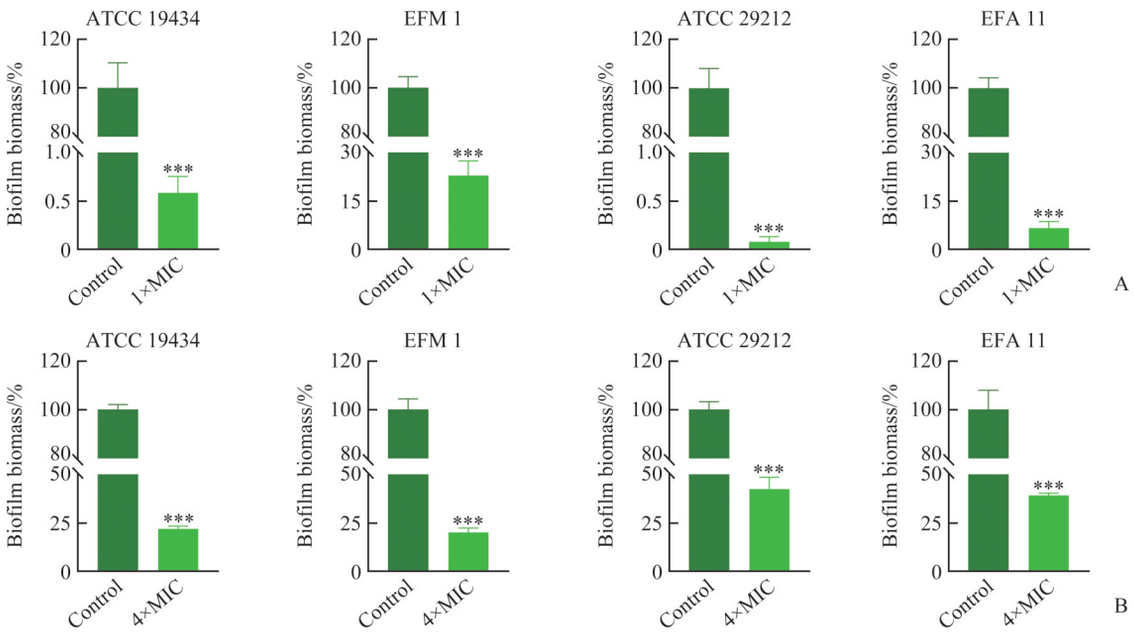


图5　Visomitin的抗生物膜活性(基于荧光强度)

Figure 5　Antibiofilm effects of Visomitin (based on fluorescence intensity)

A: Fluorescence-based quantitative analysis of biofilm inhibition. B: Fluorescence-based quantitative analysis of biofilm eradication. Data are analyzed using independent sample *t*-test, expressed as mean±standard deviation. ****P*<0.001 vs the Control group.

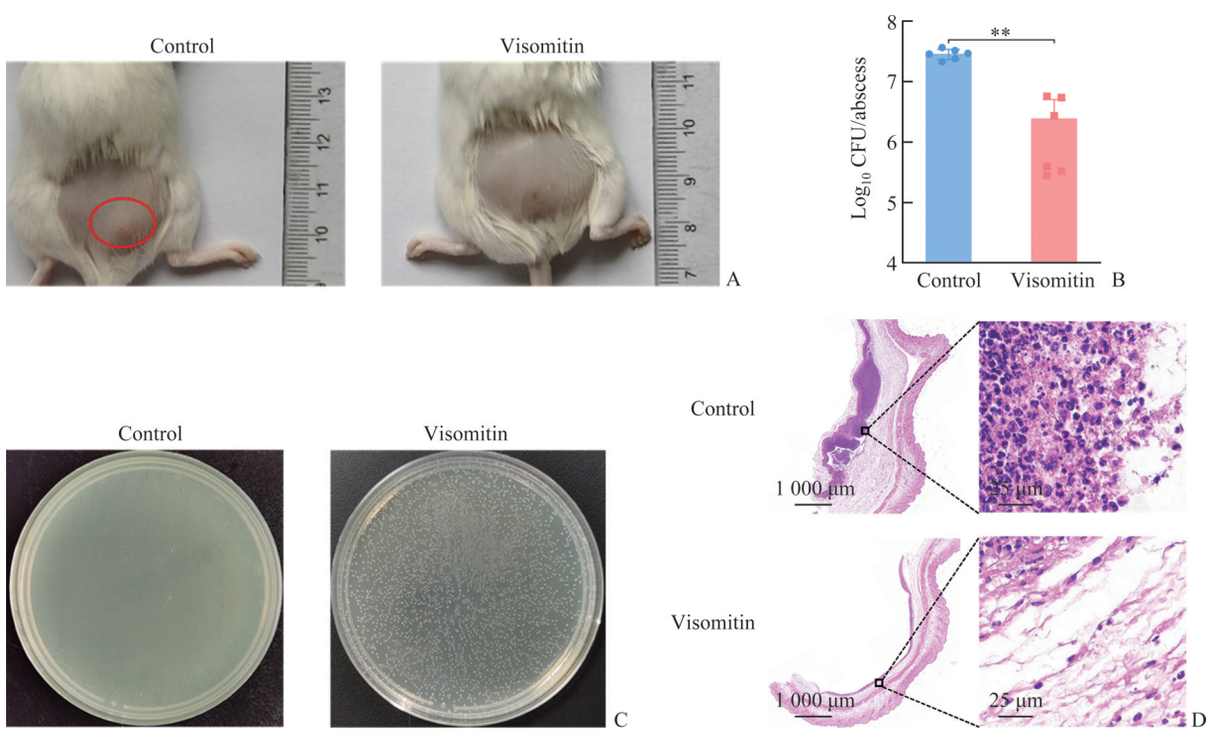


图6　Visomitin在EFA ATCC 29212皮下脓肿模型中的体内抗菌活性

Figure 6　In vivo antimicrobial effects of Visomitin against EFA ATCC 29212 in abscess model

A: Representative images of subcutaneous abscesses before and after treatment. B: Visomitin significantly reduces bacterial load in abscess tissues. Data are compared using the Mann-Whitney *U* test and presented as median (25th percentile, 75th percentile). C: A visualization of bacterial quantity in undiluted abscess homogenates. D: HE staining showing inflammatory cell infiltration in abscess tissues. ***P*<0.01. HE: Hematoxylin and eosin.

©Journal of Central South University (Medical Science). All rights reserved.

3 讨 论

本研究发现Visomitin对肠球菌具有良好的抗菌活性，并且难以诱导肠球菌耐药。在亚抑菌浓度下，Visomitin显著抑制肠球菌毒力因子蛋白酶的合成。同时，Visomitin不仅能抑制肠球菌生物膜的形成，还能对已形成的生物膜表现出清除效果。在体内实验中，Visomitin也表现出良好的抗菌活性。

本研究重点研究了Visomitin对肠球菌的抗菌效果。微量肉汤稀释实验显示，Visomitin对肠球菌的MIC和MBC分别为4~8 μg/mL和4~16 μg/mL；纸片扩散法和时间-杀菌曲线也证明了Visomitin对肠球菌具有良好的抗菌活性。Nazarov等[16]指出Visomitin在微摩尔浓度下对革兰氏阳性菌(枯草芽孢杆菌、金黄色葡萄球菌和分枝杆菌)和革兰氏阴性菌(大肠杆菌、磷酸光杆菌和球形红杆菌)具有抗菌活性。研究者[16, 27]发现，革兰氏阴性菌的多重耐药泵AcrAB-TolC会使其对Visomitin耐药，但革兰氏阳性菌由于不存在这些外排泵，对Visomitin更为敏感。既往研究[28]已报道Visomitin可通过降低细菌的膜电位而发挥抗菌活性。Dong等[18]发现Visomitin可通过靶向50S核糖体亚基和破坏蛋白质合成发挥抗结核分枝杆菌的作用。这些研究提示Visomitin对肠球菌的抗菌作用也可能存在类似机制。此外，本研究中的亚抑菌浓度连续诱导耐药实验结果显示，Visomitin持续14 d诱导肠球菌后，其MIC值只上涨1倍或无变化，表现为极低频率的耐药性。既往研究[17]中也有类似结果：Visomitin不会引起金黄色葡萄球菌的耐药。

肠球菌可借助生物膜实现定植，不仅易诱发难治性根尖周炎，还可引发泌尿系统感染、伤口感染、心内膜炎等多种感染性疾病[29]。同时，具备生物膜形成能力的肠球菌占比居高不下[30]，此类菌株对多数抗生素均表现出耐药性，进一步加大了肠球菌感染的临床治疗难度[31]。因此，除对浮游菌的抗菌活性外，对抗生物膜的能力也是抗生素研究的重点。本研究通过结晶紫染色发现，Visomitin在1×MIC浓度时就可显著抑制肠球菌生物膜的形成，而当浓度上升到2×MIC时能显著清除肠球菌已形成的生物膜。SYTO9荧光染色进一步提示了Visomitin对肠球菌生物膜的抑制和清除作用，使生物膜的结构破坏，荧光强度显著降低。值得注意的是，在结晶紫染色中，Visomitin处理EFA ATCC 29212已形成的生物膜后与对照组无明显差异，但SYTO9染色显示4×MIC的Visomitin能明显降低已形成生物膜的绿色荧光强度，这可能提示Visomitin不足以清除ATCC 29212已形成生物膜的基质结构，但对生物膜内细菌的生理状态造成了影响，如细菌DNA降解或构象变化。此外，蛋白酶作为肠球菌主要毒力因子之一，可促进细菌的侵袭和组织的损伤[11]。已有研究[32]发现肠球菌蛋白酶的产生可促进其眼内炎症的发生、发展。本研究发现，Visomitin在亚抑菌浓度下，即可显著抑制肠球菌蛋白酶的产生。因此，Visomitin可抑制肠球菌在人体附着和侵入宿主组织方面的能力。

皮下脓肿实质上是高细菌密度相关的感染，抗生素常难以治疗[33]，从而给医疗机构带来了沉重的负担[34]。全球性抗菌药物耐药性监测项目(SENTRY Antimicrobial Surveillance Program)[35]报告表明，肠球菌在皮肤与软组织感染中仅次于金黄色葡萄球菌和铜绿假单胞菌。因此，评估抗生素对肠球菌皮肤脓肿感染的有效性具有重要的临床意义。脓肿模型已被广泛用于研究各种抗菌药物的体内抗菌活性[36]，如Pletze等[26]通过该模型研究了合成肽与抗生素联合使用效果。本研究通过小鼠皮下脓肿模型评估了Visomitin的体内抗菌活性，结果表明，Visomitin对肠球菌形成的脓肿具有良好的抗菌活性。Visomitin作为一种老药新用的药物，其毒理学特征已较为明确，具有较低的毒性和良好的人体安全性，有望为临床治疗肠球菌相关感染提供新的策略。

本研究还存在一定的局限性，例如由于VAN耐药的EFA临床菌株难以收集，缺乏Visomitin对该类肠球菌抗菌能力的研究。虽然Visomitin对肠球菌的抗菌和抗生物膜作用已被证实，但其具体分子靶点(如线粒体氧化应激通路、蛋白酶抑制的具体机制)尚未明确。

Visomitin对肠球菌具有良好的体外和体内活性，且不易诱导细菌产生耐药，具备再利用潜力。本研究为Visomitin成为临床治疗难治性肠球菌感染的新型候选药物提供了重要的临床前证据。

作者贡献声明：杨琪 研究实施，数据统计与分析，论文撰写；吴苑 研究指导，数据整理；吴若兰 研究设计；佘鹏飞 研究设计与指导，论文修改。所有作者阅读并同意最终的文本。

利益冲突声明：作者声称无任何利益冲突。

参考文献

[1] Mwikuma G, Kainga H, Kallu SA, et al. Determination of the prevalence and antimicrobial resistance of *Enterococcus faecalis* and *Enterococcus faecium* associated with poultry in four districts in Zambia[J]. Antibiotics (Basel), 2023, 12(4): 657. https://doi.org/10.3390/antibiotics12040657.

©Journal of Central South University (Medical Science). All rights reserved.

[2] Yang S, Meng X, Zhen Y, et al. Strategies and mechanisms targeting *Enterococcus faecalis* biofilms associated with endodontic infections: a comprehensive review[J]. Front Cell Infect Microbiol, 2024, 14: 1433313. https://doi.org/10.3389/fcimb.2024.1433313.

[3] Archambaud C, Nunez N, da Silva RAG, et al. *Enterococcus faecalis*: an overlooked cell invader[J/OL]. Microbiol Mol Biol Rev, 2024, 88(3): e0006924[2025-09-02]. https://doi.org/10.1128/mmbr.00069-24.

[4] Scharloo F, Cogliati Dezza F, López-Hernández I, et al. Clinical characteristics, predisposing factors and outcomes for *Enterococcus faecalis* versus *Enterococcus faecium* bloodstream infections: a prospective multicentre cohort study [J]. Eur J Clin Microbiol Infect Dis, 2024, 43(10): 2011-2022. https://doi.org/10.1007/s10096-024-04917-5.

[5] Guan L, Beig M, Wang L, et al. Global status of antimicrobial resistance in clinical *Enterococcus faecalis* isolates: systematic review and meta-analysis[J]. Ann Clin Microbiol Antimicrob, 2024, 23(1): 80. https://doi.org/10.1186/s12941-024-00728-w.

[6] Rosselli Del Turco E, Bartoletti M, Dahl A, et al. How do I manage a patient with enterococcal bacteraemia? [J]. Clin Microbiol Infect, 2021, 27(3): 364-371. https://doi.org/10.1016/j.cmi.2020.10.029.

[7] Alemayehu T, Hailemariam M. Prevalence of vancomycin-resistant *Enterococcus* in Africa in one health approach: a systematic review and meta-analysis[J]. Sci Rep, 2020, 10(1): 20542. https://doi.org/10.1038/s41598-020-77696-6.

[8] Radadiya N N, Shah K, Mehta S. Study of Antimicrobial Resistance among *Enterococcus* Species in Tertiary Care Teaching Hospital [J]. Saudi Journal of Pathology and Microbiology, 2023, 8(6): 122-125. https://doi.org/10.36348/sjpm.2023.v08i06.002.

[9] Schiopu P, Toc DA, Colosi IA, et al. An overview of the factors involved in biofilm production by the *Enterococcus* genus[J]. Int J Mol Sci, 2023, 24(14): 11577. https://doi.org/10.3390/ijms241411577.

[10] Sadanandan B, Yogendraiah KM. Enterococcus faecalis Biofilm: A Clinical and Environmental Hazard[Z]. The 4th International Electronic Conference on Antibiotics, 2025. https://doi.org/10.3390/msf2025035005.

[11] Heras B, Scanlon MJ, Martin JL. Targeting virulence not viability in the search for future antibacterials[J]. Br J Clin Pharmacol, 2015, 79(2): 208-215. https://doi.org/10.1111/bcp.12356.

[12] Brzheskiy VV, Efimova EL, Vorontsova TN, et al. Results of a multicenter, randomized, double-masked, placebo-controlled clinical study of the efficacy and safety of visomitin eye drops in patients with dry eye syndrome[J]. Adv Ther, 2015, 32(12): 1263-1279. https://doi.org/10.1007/s12325-015-0273-6.

[13] Tsitkanou S, Morena da Silva F, Cabrera AR, et al. Mitochondrial antioxidant SkQ1 attenuates C26 cancer-induced muscle wasting in males and improves muscle contractility in female tumor-bearing mice[J]. Am J Physiol Cell Physiol, 2024, 327(5): C1308-C1322. https://doi.org/10.1152/ajpcell.00497.2024.

[14] Skulachev VP, Vyssokikh MY, Chernyak BV, et al. Mitochondrion-targeted antioxidant SkQ1 prevents rapid animal death caused by highly diverse shocks[J]. Sci Rep, 2023, 13(1): 4326. https://doi.org/10.1038/s41598-023-31281-9.

[15] Somayajulu M, Wright R, Muhammed F, et al. PM_{10} dysregulates epithelial barrier function in human corneal epithelial cells that is restored by antioxidant SKQ1[J]. Toxicol Appl Pharmacol, 2024, 492: 117122. https://doi.org/10.1016/j.taap.2024.117122.

[16] Nazarov PA, Osterman IA, Tokarchuk AV, et al. Mitochondria-targeted antioxidants as highly effective antibiotics[J]. Sci Rep, 2017, 7(1): 1394. https://doi.org/10.1038/s41598-017-00802-8.

[17] Wu R, Wu Y, Wu P, et al. Bactericidal and anti-quorum sensing activity of repurposing drug Visomitin against *Staphylococcus aureus*[J]. Virulence, 2024, 15(1): 2415952. https://doi.org/10.1080/21505594.2024.2415952.

[18] Dong W, Wang G, Bai Y, et al. Repurposing an antioxidant to kill *Mycobacterium tuberculosis* by targeting the 50S subunit of the ribosome[J]. Biomolecules, 2023, 13(12): 1793. https://doi.org/10.3390/biom13121793.

[19] She P, Liu Y, Xu L, et al. L007-0069 kills *Staphylococcus aureus* in high resistant phenotypes[J]. Cell Mol Life Sci, 2022, 79(11): 552. https://doi.org/10.1007/s00018-022-04588-5.

[20] Yasir M, Dutta D, Kumar N, et al. Interaction of the surface bound antimicrobial peptides melimine and Mel4 with *Staphylococcus aureus*[J]. Biofouling, 2020, 36(9): 1019-1030. https://doi.org/10.1080/08927014.2020.1843638.

[21] Haq SU, Wang L, Guo W, et al. Enhancing activity of β-lactam and fluoroquinolones antibiotics by artemisinin and its derivatives against MDR *Escherichia coli*[J]. Front Vet Sci, 2022, 9: 1048531. https://doi.org/10.3389/fvets.2022.1048531.

[22] Wu Y, Wu P, Wu R, et al. Simeprevir restores the anti-*Staphylococcus* activity of polymyxins[J]. AMB Express, 2023, 13(1): 122. https://doi.org/10.1186/s13568-023-01634-8.

[23] Ashraf D, Shaaban M I, Hassan R, et al. Polidocanol inhibits *Enterococcus faecalis* virulence factors by targeting fsr quorum sensing system[J]. BMC Microbiol, 2024, 24(1): 411. https://doi.org/10.1186/s12866-024-03548-2.

[24] Zhang N, Li W, Du X, et al. Identification and functional characterization of a novel Acinetobacter pittii bacteriophage-encoded depolymerase [J]. Front Cell Infect Microbiol, 2025, 15: 1608526. https://doi.org/10.3389/fcimb.2025.1608526.

[25] Zhao Y, Guo Q, Dai X, et al. A biomimetic non-antibiotic approach to eradicate drug-resistant infections[J/OL]. Adv Mater, 2019, 31(7): e1806024[2025-09-02]. https://doi.org/10.1002/adma.201806024.

[26] Pletzer D, Mansour SC, Hancock REW. Synergy between conventional antibiotics and anti-biofilm peptides in a murine, sub-cutaneous abscess model caused by recalcitrant ESKAPE pathogens[J/OL]. PLoS Pathog, 2018, 14(6): e1007084[2025-09-02]. https://doi.org/10.1371/journal.ppat.1007084.

©Journal of Central South University (Medical Science). All rights reserved.

[27] Nazarov PA, Kotova EA, Skulachev VP, et al. Genetic variability of the AcrAB-TolC multidrug efflux pump underlies SkQ1 resistance in gram-negative bacteria[J]. Acta Naturae, 2019, 11(4): 93-98. https://doi.org/10.32607/20758251-2019-11-4-93-98.

[28] Nazarov PA, Majorov KB, Apt AS, et al. Penetration of triphenylphosphonium derivatives through the cell envelope of bacteria of *Mycobacteriales* order[J]. Pharmaceuticals (Basel), 2023, 16(5): 688. https://doi.org/10.3390/ph16050688.

[29] 徐智博, 姜鑫淼, 甄雨琦, 等. 氯己定对粪肠球菌耐药性和致病性的影响及其机制[J]. 吉林大学学报(医学版), 2024, 50(3): 676-681. https://doi.org/10.13481/j.1671-587X.20240311.
XU Zhibo, JIANG Xinmiao, ZHEN Yuqi, et al. Effect of chlorhexidine on drug resistance and pathogenicity of *Enterococcus faecalis* and its mechanism[J]. Journal of Jilin University. Medicine Edition, 2024, 50(3): 676-681. https://doi.org/10.13481/j.1671-587X.20240311.

[30] 曹阳, 赵建平. 肠球菌生物被膜形成能力与耐药性的相关性[J]. 临床与病理杂志, 2025, 45(7): 841-846. https://doi.org/10.11817/j.issn.2095-6959.2025.240954.
CAO Yang, ZHAO Jianping. Correlation between biofilm formation and antibiotic resistance in *Enterococcus*[J]. Journal of Clinical and Pathological Research, 2025, 45(7): 841-846. https://doi.org/10.11817/j.issn.2095-6959.2025.240954.

[31] Khalil MA, Alorabi JA, Al-Otaibi LM, et al. Antibiotic resistance and biofilm formation in *Enterococcus* spp. isolated from urinary tract infections [J]. Pathogens, 2022, 12(1): 34. https://doi.org/10.3390/pathogens12010034.

[32] Engelbert M, Mylonakis E, Ausubel FM, et al. Contribution of gelatinase, serine protease, and fsr to the pathogenesis of *Enterococcus faecalis* endophthalmitis[J]. Infect Immun, 2004, 72(6): 3628-3633. https://doi.org/10.1128/IAI.72.6.3628-3633.2004.

[33] Udekwu KI, Parrish N, Ankomah P, et al. Functional relationship between bacterial cell density and the efficacy of antibiotics[J]. J Antimicrob Chemother, 2009, 63(4): 745-757. https://doi.org/10.1093/jac/dkn554.

[34] Giordano P, Weber K, Gesin G, et al. Skin and skin structure infections: treatment with newer generation fluoroquinolones [J]. Ther Clin Risk Manag, 2007, 3(2): 309-317. https://doi.org/10.2147/tcrm.2007.3.2.309.

[35] Rennie RP, Jones RN, Mutnick AH, et al. Occurrence and antimicrobial susceptibility patterns of pathogens isolated from skin and soft tissue infections: report from the SENTRY Antimicrobial Surveillance Program (United States and Canada, 2000) [J]. Diagn Microbiol Infect Dis, 2003, 45(4): 287-293. https://doi.org/10.1016/s0732-8893(02)00543-6.

[36] Medina E. Murine model of cutaneous infection with *Streptococcus pyogenes*[J]. Methods Mol Biol, 2010, 602: 395-403. https://doi.org/10.1007/978-1-60761-058-8_21.

（责任编辑 田朴）

©Journal of Central South University (Medical Science). All rights reserved.