2026, 66(3): 1311-1325
CSTR: 32112.14.j.AMS.20250787
DOI: 10.13343/j.cnki.wsxb.20250787

微生物学报
Acta Microbiologica Sinica
http://journals.im.ac.cn/actamicrocn

Research Article 研究报告

我国首例携带 *ermB* 与 *optrA* 基因的青霉素耐药鼠口腔链球菌特征分析

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摘　要：【目的】对一株分离自病死仔猪扁桃体、具有多重耐药表型的链球菌进行生物学表征及基因组分析以揭示其表型和基因型特征。【方法】取病死仔猪扁桃体组织研磨液接种于链球菌增菌培养基进行增菌培养；将增菌液接种至哥伦比亚血琼脂培养基进行链球菌分离培养。运用基质辅助激光解吸电离飞行时间质谱(MALDI-TOF MS)、生化鉴定系统及 16S rRNA 基因测序进行种属鉴定；通过稀释法测定最低抑菌浓度(minimum inhibitory concentrations, MIC)；利用 Illumina 二代测序平台完成全基因组测序；借助 ResFinder 平台分析耐药基因，并通过多序列比对分析青霉素结合蛋白(penicillin-binding proteins)基因的变异情况。【结果】经 16S rRNA 基因系统发育分析，该分离株鉴定为鼠口腔链球菌(*Streptococcus orisratti*)。药敏试验显示，该菌株对青霉素 G、红霉素、克林霉素、甲氧苄啶-磺胺甲噁唑耐药，对利奈唑胺不敏感。基因组分析显示，该菌株携带大环内酯类和林可酰胺类耐药基因 *ermB* 以及恶唑烷酮类耐药基因 *optrA*，且其青霉素结合蛋白编码基因多位点发生突变。【结论】本研究首次报道了一株同时携带 *ermB* 与 *optrA* 基因且对青霉素耐药的鼠口腔链球菌，揭示了猪源链球菌菌株具备获得多重耐药基因的能力，应关注动物源链球菌的耐药性和传播风险。

关键词：鼠口腔链球菌；多重耐药；耐药机制；*ermB*；*optrA*

资助项目：国家疾病预防控制局高层次公共卫生人才培养支持项目；广州国家实验室专项项目(GZNL2024A01025)
This work was supported by the Public Health Talent Support Project of National Disease Control and Prevention Administration and the Major Project of Guangzhou National Laboratory (GZNL2024A01025).
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Received: 2025-10-21; Accepted: 2025-12-23; Published online: 2026-01-09

Characterization of the first penicillin-resistant *Streptococcus orisratti* strain carrying both *ermB* and *optrA* in China

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Abstract: [Objective] To perform biological characterization and genomic analysis for a multidrug-resistant *Streptococcus* strain isolated from the tonsils of a dead piglet, thus elucidating its phenotypic and genotypic characteristics. **[Methods]** The tonsillar tissue homogenate from the dead piglet was inoculated into the *Streptococcus* enrichment medium for enrichment culture. The enriched culture was then streaked onto Columbia blood agar medium for *Streptococcus* isolation. Matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS), a biochemical identification system, and 16S rRNA gene sequencing were employed for strain identification. The minimum inhibitory concentrations (MICs) were determined by the broth microdilution method. Whole-genome sequencing was conducted *via* the Illumina platform. The ResFinder database was used to analyze the resistance genes, and mutations in the genes encoding penicillin-binding proteins (PBPs) were investigated through multiple sequence alignment. **[Results]** The phylogenetic analysis based on 16S rRNA gene sequences identified the isolate as *Streptococcus orisratti*. Antimicrobial susceptibility testing revealed that the strain was resistant to penicillin G, erythromycin, clindamycin, and compound sulfamethoxazole, and it exhibited reduced susceptibility to linezolid. Whole-genome analysis identified the presence of the macrolide and lincoamide resistance gene *ermB* and the oxazolidinone resistance gene *optrA*. Furthermore, multiple mutations were detected in the genes encoding PBPs. **[Conclusion]** This study reports the first isolate of *S. orisratti* harboring both *ermB* and *optrA* and exhibiting resistance to penicillin. It highlights the capacity of porcine-derived streptococcal strains to acquire multidrug resistance genes, underscoring the need for increased vigilance regarding the resistance traits and transmission risks of animal-derived streptococci.

Keywords: *Streptococcus orisratti*; multidrug resistance; resistance mechanisms; *ermB*; *optrA*

鼠口腔链球菌(*Streptococcus orisratti*)是一种革兰氏阳性球菌，隶属于厚壁菌界(Bacillati)芽孢杆菌门(*Bacillota*)乳酸菌纲(*Bacilli*)乳杆菌目(*Lactobacillales*)链球菌科(*Streptococcaceae*)链球菌属(*Streptococcus*)。该菌种于 2000 年由 Zhu 等[1]从实验室 SD 大鼠下臼齿表面分离获得并命名。2005 年，Hill 等[2]基于 16S rRNA 和 *cpn 60* 基因序列的系统发育分析，建议将猪链球菌(*Streptococcus suis*) 32 和 34 血清型也归入该种，但该分类观点迄今尚未得到官方权威认证。目

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前，关于鼠口腔链球菌的研究报道十分有限，NCBI 数据库中仅收录 11 株基因组序列，其中 10 株具备完整背景信息，模式菌株 DSM 15617 分离自鼠口腔，8 株分离自家猪，1 株分离自猫。

甲基转移酶(erythromycin ribosome methylation, Erm)基因家族在 20 世纪 80 年代被首次报道[3-4]，其介导的甲基化修饰可介导对大环内酯类、林可酰胺类及链阳菌素 B 交叉耐药(macrolide-lincosamide streptogramin B resistance, MLSB)[5]，主要包括 *ermA*、*ermB*、*ermC*、*ermF* 等基因亚型[6]。其中，*ermB* 基因编码红霉素核糖体甲基化酶，介导细菌的 23S rRNA 亚基的腺嘌呤 N6 位点甲基化[7]。该基因已在多种链球菌中检出[8]，如无乳链球菌(*Streptococcus agalactiae*)[9-10]、肺炎链球菌(*Streptococcus pneumoniae*)[11]和酿脓链球菌(*Streptococcus pyogenes*)等[12]。在耐药基因的水平转移方面，移动遗传元件(mobile genetic elements, MGEs)，特别是整合和共轭元件(integrative and conjugative elements, ICEs)介导的 *ermB* 基因的获得被认为是大环内酯耐药链球菌菌株快速传播的主要原因[13]。

恶唑烷酮类抗菌药物利奈唑胺于 2000 年被美国食品药品监督管理局(Food and Drug Administration, FDA)批准用于治疗耐甲氧西林金黄色葡萄球菌(methicillin-resistant *Staphylococcus aureus*, MRSA)、多重耐药肺炎链球菌(multidrug-resistant *Streptococcus pneumoniae*, MDR-SP)或耐万古霉素屎肠球菌(vancomycin resistant *Enterococcus faecalis*, VREF)引起的感染，是治疗革兰氏阳性多重耐药菌感染的关键药物之一[14]。2015 年，Wang 等[15]在肠球菌中鉴定出新型可转移恶唑烷酮耐药基因 *optrA*，属 ABC-F 蛋白基因家族，可通过核糖体保护机制介导对多种临床相关抗生素的耐药[16]。在肠球菌中 *optrA* 基因的水平转移主要通过质粒进行[17-18]。除肠球菌外，NCBI 数据库显示 *optrA* 基因也可见于空肠弯曲杆菌(*Campylobacter jejuni*)，在链球菌中比较少见，仅在猪链球菌(*Streptococcus suis*)[19]、副猪链球菌(*Streptococcus parasuis*)[20]和马链球菌(*Streptococcus equi*)[21]等部分链球菌中有少量报道。在猪链球菌中 *optrA* 基因的传播主要通过横向基因转移方式实现[19]。Dai 等[22]证明，猪链球菌中利奈唑胺的耐药性快速增加与携带多种 *optrA* 基因的 MGEs 有关。

链球菌对 β-内酰胺类药物的耐药通常由青霉素结合蛋白(penicillin-binding proteins, PBP)基因突变介导[23]。据 CARSS 监测网报道，我国肺炎链球菌对青霉素的耐药率约为 1%[24]。在肺炎链球菌中对青霉素的敏感性降低，需 PBP2x 和 PBP2b 同时突变[25]。青霉素和头孢菌素的高水平耐药性还需 PBP1a 发生额外的突变[25-26]。van der Linden 等[27]发现肺炎链球菌 PBP2x 基因存在多种链球菌基因序列转移的可能。近年也有研究报道在猪链球菌中出现青霉素耐药株[28-30]。在猪链球菌 PBP 中，氨基酸替代主要发生在 PBP2x 和 PBP2b[28,31]。无乳链球菌和酿脓链球菌中也有分离获得了青霉素不敏感菌株的报道[32-34]。

本研究首次报道一株同时对大环内酯类抗生素及青霉素耐药，并对利奈唑胺不敏感的链球菌通过对其表型特征和基因型特征的分析，阐述其生物学特征和耐药机制，旨在为掌握链球菌的耐药特征和耐药机制提供数据。

1 材料与方法

1.1 材料

1.1.1 主要试剂和仪器

脱纤维羊血，天津市北辰区科技园区三牧畜技术有限公司；哥伦比亚血琼脂基础培养基，维百奥(北京)生物科技有限公司；革兰氏染色液，珠海贝索生物技术有限公司；质谱样本预处理试剂，郑州安图生物工程股份有限公司；API 50 CH 碳水化合物鉴定试剂条、API 酶活性

科研试剂条(ZYM)、链球菌药敏卡片，梅里埃诊断产品(上海)有限公司；链球菌鉴定/药敏板条，碧迪医疗器械(上海)有限公司；Wizard® Genomic DNA Purification Kit，Promega 公司。

全自动平板分装仪，Systec GmbH 公司；光学显微镜，Echo 公司；基质辅助激光解吸电离飞行时间质谱，郑州安图生物工程股份有限公司；临界点干燥仪，Tousimis 公司；喷金仪、扫描电镜，日立高新技术公司；全自动微生物鉴定药敏分析仪，梅里埃诊断产品(上海)有限公司；全自动微生物鉴定药敏分析仪，碧迪医疗器械(上海)有限公司。

1.1.2 培养基

哥伦比亚血琼脂培养基：哥伦比亚血琼脂基础培养基 39.0 g/L，121 ℃灭菌 20 min，冷却至 48 ℃，按体积分数 5% 加入脱纤维绵羊血，充分混匀后制备琼脂平板。

改良胰蛋白胨大豆肉汤培养基(modified tryptone soybean broth, mTSB)：胰蛋白胨大豆肉汤培养基(tryptone soybean broth, TSB) 30.0 g/L，121 ℃灭菌 20 min，冷却至室温，每升 TSB 中添加 10.0 mL 多黏菌素 B 水溶液(1.0 g/L)及 10.0 mL 萘啶酮酸的 0.05 mol/L 氢氧化钠溶液(1.0 g/L)。

1.2 菌株分离培养

2024 年 11 月，对中国广西百色某养猪场死亡的仔猪进行表面消毒，无菌操作采集扁桃体样品，研磨后接种于 mTSB 液体培养基中，37 ℃、5% CO_2 条件下培养 16−18 h。使用 10 μL 一次性接种环进行三区划线，接种于含 5% 绵羊血的哥伦比亚血琼脂平板，37 ℃、5% CO_2 培养 24 h。挑取单菌落，在新鲜血平板上进行三区划线分离，经传代纯化后获得纯培养菌株。

1.3 菌株鉴定

采用基质辅助激光解吸电离飞行时间质谱和全自动微生物鉴定药敏分析仪进行初步细菌种属鉴定。使用临界点干燥仪对菌株进行干燥处理，之后使用喷金仪对干燥后的菌株进行喷金，最后使用扫描电镜拍摄菌株。使用 API 50 CH 和 API 酶活性科研试剂条检测菌株代谢特征。同时，使用 Wizard® Genomic DNA Purification Kit 提取菌株基因组 DNA，参考文献[35]设计 16S rRNA 基因引物，参考文献[1]完成 16S rRNA 基因扩增并测序。从 NCBI 数据库下载 32 种链球菌标准菌株 16S rRNA 基因序列，利用 SILVA 在线工具(https://www.arb-silva.de/)进行序列比对[36]，采用 MEGA 12.0.11 软件构建系统发育树(邻接法，bootstrap=1 000)[37]，进行种属鉴定。使用 EZBioCloud 在线网站(http://www.ezbiocloud.net/tools/an)计算分离菌株与模式株的平均核苷酸一致性(average nucleotide identity, ANI)[38]，对分离菌株与模式株进行 DNA (基因组)在线杂交(https://tygs.dsmz.de/)以验证种属鉴定的准确性[39]。使用 CD-HIT 软件提取核心基因组[40]，使用 FastTree 软件绘制系统发育树[41]，使用 iTOL 在线网站进行可视化[42]。

1.4 药物敏感性试验

以肺炎链球菌 ATCC 49619 为质控菌株，使用碧迪医疗器械(上海)有限公司的商业链球菌药敏板条和梅里埃公司的链球菌药敏卡片，通过肉汤微量稀释法测定抗菌药物青霉素 G、美罗培南、红霉素、克林霉素、四环素、头孢噻肟、莫西沙星、氯霉素、替考拉宁、甲氧苄啶-磺胺甲噁唑、利奈唑胺等的最低抑菌浓度(minimum inhibitory concentration, MIC)，同时自配药敏板，使用琼脂稀释法对青霉素 G 的药物敏感性进行复核。参照 CLSI M100 Ed35[43]文件对结果进行判读。

1.5 核酸提取及全基因组分析

使用 Wizard® Genomic DNA Purification Kit 提取菌株基因组 DNA。采用 Illumina NovaSeq PE 150 平台(Illumina 公司，https://www.illumina.com)进行全基因组测序。通过 Rast 网站(https://rast.nmpdr.org/rast.cgi)对全基因组序列进行功能注释[44]。使用 ICEfinder (https://tool2mml.sjtu.

edu.cn/ICEberg3/ICEfinder.php)分析菌株中的可移动作用遗传元件[45]。采用 ResFinder 4.7.2 (http://genepi.food.dtu.dk/resfinder) 鉴定耐药基因[46]，使用 Easyfig_2.2.5 绘制耐药基因侧翼序列线性图[47]。

从 NCBI 数据库下载全部鼠口腔链球菌基因组序列，经质量评估后筛选出 10 株用于后续分析。同时，下载与鼠口腔链球菌高度同源且 PBP 结构明确的猪链球菌 PBP1a、PBP1b、PBP2a、PBP2b、PBP2x 及 PBP3 氨基酸序列作为参考序列，进行多序列比对及突变位点分析。

2 结果与分析

2.1 分离培养与鉴定

分离得到优势菌株 A128，其为革兰氏阳性球菌，37 ℃、5% CO_2 条件下培养 24 h 菌落呈圆形、光滑、湿润、边缘整齐，具体培养与形态特征详见图 1。质谱鉴定结果为豕链球菌(*Streptococcus porcinus*)，全自动微生物鉴定分析仪鉴定为唾液链球菌(*Streptococcus salivarius*)。16S rRNA 基因测序获得 1 337 bp 序列。通过与 32 种链球菌模式菌株 16S rRNA 基因比较构建系统发育树，16S rRNA 基因系统发育树及全基因组核心基因系统发育分析显示该菌株与鼠口腔链球菌亲缘关系最近(图 2、图 3)。BLAST 比对显示，其与模式菌株 DSM 15617 的 16S rRNA 基因一致性为 97.54% (1 308/1 341)，存在 7 个位点的缺失，但与分离自澳大利亚犬类动物的鼠口腔链球菌 9-70MP 的 16S rRNA 基因一致性为 98.80%，序列覆盖度为 100.00%。菌株 A128 代谢特征与模式菌株 DSM 15617 基本一致(表 1)。菌株 A128 全基因组序列存储于国家微生物科学数据中心 (http://nmdc.cn)，编号为 NMDC60215640。A128 菌株与模式株平均核苷酸一致性为 97.82%，ANI 值大于 96.00% 可判定为同一个细菌种，dDDH 结果为 83.1%，DDH 值大于 70.0% 可判定为同一个细菌种。综合评估上述结果，认定该菌株 A128 为鼠口腔链球菌。

2.2 药敏实验结果

药敏结果参照 CLSI M100 Ed35 中草绿色链球菌群(*Streptococcus viridans* group)的折点进行判读(利奈唑胺及甲氧苄啶-磺胺甲噁唑参考

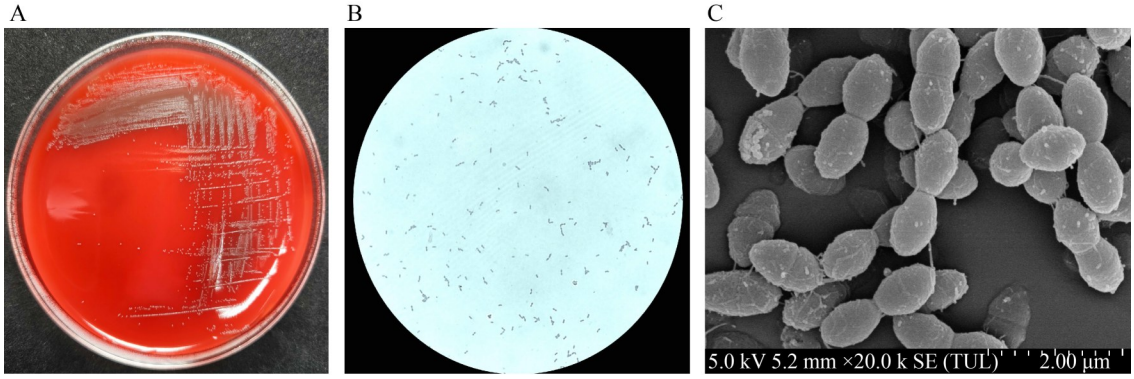

图1 A128菌株培养与形态特征。A：含5%绵羊血的哥伦比亚血琼脂平板37 ℃、5% CO_2培养24 h的菌落形态；B：A128菌株在100倍光学显微镜下的革兰氏染色形态；C：A128菌株在20 000倍扫描电镜下的图片。

Figure 1 Culture and morphological characteristics of strain A128. A: Colonial morphology of the bacteria after 24 hours of incubation at 37 °C under 5% CO_2 on Columbia blood agar plates containing 5% sheep blood; B: Gram-staining morphology of strain A128 observed under a light microscope at 100×; C: Scanning electron microscope (SEM) image of strain A128 at 20 000×.

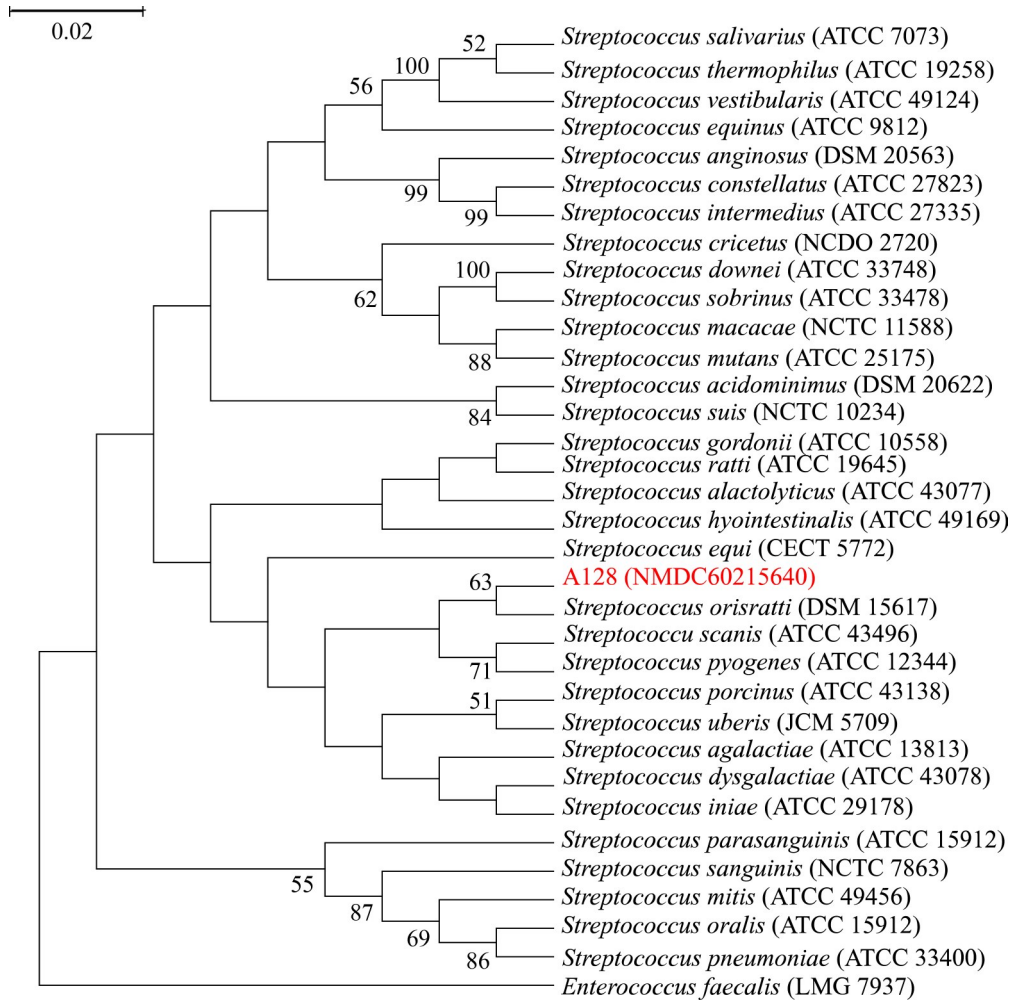

图2　基于16S rRNA基因序列构建的A128菌株系统发育树(邻接法)。引入粪肠球菌(*Enterococcus faecalis*) LMG 7937 16S rRNA作为外群，采用MEGA 12.0.11软件中的邻接法(neighbor-joining method)构建系统发育树，并通过1 000次重复抽样计算自展值(bootstrap values)；菌种拉丁名后为菌株编号。

Figure 2　Neighbor-joining phylogenetic tree based on 16S rRNA gene sequences of A128 strain. The 16S rRNA gene sequence of *Enterococcus faecalis* LMG 7937 was used as the outgroup. Phylogenetic trees were constructed using the neighbor-joining method in MEGA version 12.0.11 software, and bootstrap values were calculated with 1 000 replicates. The strain numbers are presented in order after the strain name.

EUCAST 标准)。如表 2 所示，菌株 A128 对青霉素 G、红霉素、克林霉素和甲氧苄啶-磺胺甲噁唑耐药；对利奈唑胺不敏感；对氨苄西林、头孢吡肟、头孢噻肟、头孢曲松、左氧氟沙星、氯霉素、万古霉素、四环素敏感。

2.3　基因组测序结果

菌株 A128 基因组全长为 2 371 027 bp，G+C 含量为 38.45%。共预测到 2 406 个蛋白质编码基因，其中包括 618 个假设蛋白和 1 788 个功能蛋白。功能蛋白中包含 54 个移动元件相关蛋白及 16 个噬菌体蛋白。此外，鉴定出 59 个 tRNA 基因和 5 个 rRNA 基因。

2.4　耐药基因分析

ResFinder 4.7.2 耐药基因库比对分析显示，

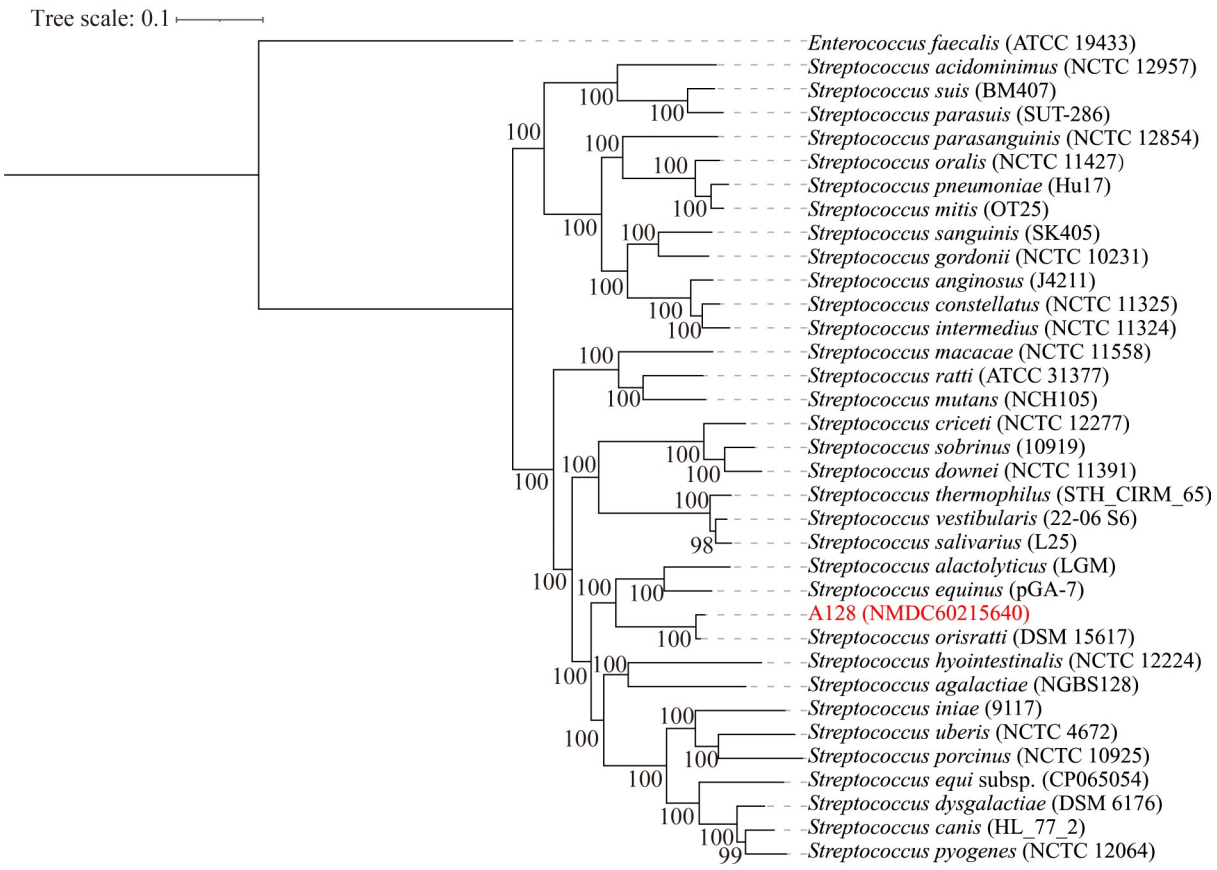

图3 基于534个核心基因组序列构建的A128菌株系统发育树(最大似然法)。使用FastTree软件并采取最大似然法(maximum-likelihood method)构建系统发育树，并通过1 000次重复抽样计算自展值(bootstrap values)；菌种拉丁名后为菌株编号。

Figure 3 Phylogenomic tree of strain A128 was inferred from an alignment of 534 core genome sequences using the maximum-likelihood algorithm implemented in FastTree. Branch support was evaluated with 1 000 bootstrap pseudo-replicates. The strain identifier is appended to the Latin name of each species.

菌株A128携带4个耐药基因，分别为介导MLSB耐药的*lsaE*、*lnuB*和*ermB*基因，以及恶唑烷酮耐药基因*optrA*。

2.4.1 *ermB*基因侧翼序列分析

A128菌株携带的*ermB*基因全长737 bp，与参考序列(NC_014959.1)相比存在3个位点的核苷酸突变(c.75T>C、c.515A>G、c.517G>A)，导致2个氨基酸替换(I75T、N222D)。无乳链球菌(CP021867.1、MW602970.1、OQ054582.1)和猪链球菌(MZ960465.1、CP032064.1)存在与A128菌株携带*ermB*基因突变位置一致的基因序列。如图4所示，携带*ermB*基因的contig长度为73 092 bp，其上、下游区域与猪链球菌1081基因组中的同源区高度相似，且同源区均含有噬菌体位点特异性酶等移动元件，非同源区域富含细菌代谢相关基因。

2.4.2 *optrA*基因侧翼序列分析

A128携带的*optrA*基因全长为1 967 bp。与参考序列(NG_048023)高度同源，存在3处点突变(c.219G>A、c.626T>G、c.1541A>C)，导致3个氨基酸位点突变(G24D、Y160D、T465D)。本研究将获得的*optrA*基因与NCBI数据库中的

表1　A128菌株与模式菌株DSM 15617代谢特征

Table 1　Metabolic characteristics of strain A128 and the type strain DSM 15617

Characteristic	DSM 15617	A128
Hydrolysis of		
Arginine*	−	−
Aesculin*	+	+
Starch*	+	+
Activity of		
Pyrrolidonylarylamidase*	−	−
α-galactosidase*	−	−
β-glucuronidase*	−	−
β-galactosidase*	−	−
Alkaline phosphatase*	−	−
Leucine arylamidase*	+	+
Growth in/at		
6.5% NaCl	−	−
pH 9.6	+	+
45 ℃	+	+
Fermentation of		
Ribose*	−	−
L-arabinose*	−	−
Mannitol*	−	−
Sorbitol*	−	−
Lactose*	+	+
Trehalose*	+	+
Inulin*	+	−
Raffinose*	+	+
Glycogen*	+	+
Amygdalin*	+	+

*：数据来源于商品化试剂盒(API ZYM与API 50 CH)。

*: Data obtained from commercially available kits (API ZYM and API 50 CH).

序列进行 BLAST 比对。结果显示，未找到一致性为 100% 的序列。同源性最高的序列为粪肠球菌的 *optrA* 基因(MN731744.1)，该基因存在 1 个不同的点突变(c.219A>G)，携带此 *optrA* 基因的粪肠球菌对利奈唑胺耐药[48]。

如图 5 所示，A128 携带的 *optrA* 基因上游检测到整合酶基因，其所在区域与猪链球菌 3372 的一个携带整合酶的质粒序列高度同源；下游携带 *optrA* 基因的区域与粪肠球菌 661 质粒 p661-b 表现出高度同源性。

2.5　青霉素结合蛋白序列多态性分析

本研究基于从 NCBI 数据库中获取的 10 株鼠口腔链球菌全基因组序列，对其青霉素结合蛋白 PBP1a、PBP1b、PBP2a 和 PBP2x 的氨基酸序列进行了比较分析。结果显示，这些蛋白均存在多个氨基酸位点变异，其中 PBP2a 的变异程度最高，系统发育分析表明其序列多态性显著高于其他 3 种 PBP 蛋白。相反，PBP1b 最为保守，所观察到的氨基酸替换位点数量最少。

2.5.1　PBP1a 结构分析

PBP1a 蛋白全长 733 个氨基酸，共发现 20 个高变位点。变异频率最高(100%)的氨基酸位点包括 L409A/V、I286V 和 E704Q/H。另有 7 个高频发生替代的主要位点，分别为 V39I、K248E、V345I、T374A、I411F、V498I 和 A572P，这些位点在 80% (8/10)的菌株中均发生突变。此外，发现 6 个菌株特异性突变：SUG2382 (Y38F)、SUG2381 (K96R)、SRR11489755 (A589T)、SGI. 063 (A648T，A717T)、SUG1074 (V718I)。

2.5.2　PBP1b 结构分析

PBP1b 蛋白由 766 个氨基酸组成，共包含 17 个高变区位点，是本研究中唯一未发现 100% 氨基酸替代的蛋白，表明其序列在进化上高度保守。主要高频变异位点为 T566A、S567G 和 I572V (90%，9/10)；其次为 Q332K、R371K 和 A372T (80%，8/10)。此外，鉴定出 5 个仅发生于单个菌株的低频氨基酸替代，分别位于以下菌株：A128 中的 T308N、SRR11489755 中的 A286T、SGI.245 中的 A381S、SRS10476997 中的 T394S 与 A616T。这些变异位点分散于不同菌株中，表明其可能属于随机突变事件。

2.5.3　PBP2a 结构分析

PBP2a 蛋白全长 760 个氨基酸，共鉴定出 53 个高变位点。其中，L20F 与 I24F 为发生频率最高的突变位点，在所有菌株中均出现

表2 A128菌株对18种抗菌药物的MIC值及药敏结果判读

Table 2 Antimicrobial susceptibility profile of A128 strain against 18 drugs

药物种类 Types of drugs	抗生素 Antibiotic	MIC (μg/mL)	药敏判读结果 Drug susceptibility	耐药判读折点 Antibiotic resistance		
				S	I	R
Penicillins	Penicillin G	4	R	≤0.12	0.25−2	≥4
	Ampicillin	≤0.25	S	≤0.25	0.5−4	≥8
	Amoxicillin	≤0.25				
Cephalosporins	Cefepime	1	S	≤1	2	≥4
	Cefotaxime	1	S	≤1	2	≥4
	Ceftriaxone	0.5	S	≤1	2	≥4
Carbapenem	Meropenem	0.125	S	≤0.5		
Fluoroquinolone	Levofloxacin	1	S	≤2	4	≥8
	Moxifloxacin	0.125				
Macrolide	Erythromycin	>8	R	≤0.25	0.5	≥1
Lincosamides	Clindamycin	>1	R	≤0.25	0.5	≥1
Amphenicols	Chloramphenicol	4	S	≤4	8	≥16
Glycopeptides	Vancomycin	0.5	S	≤1		
	Teicoplanin	≤0.12				
Tetracyclines	Tetracycline	2	S	≤2	4	≥8
	Tigecycline	≤0.06				
Sulfonamide	TMP-SMX*	>2/38	R			>2/38
Oxazolidinones	Linezolid	4	NS	≤2		

MIC：最低抑菌浓度。S：敏感；I：中介；R：耐药；NS：非敏感。TMP-SMX：甲氧苄啶-磺胺甲噁唑；*：草绿色链球菌EUCAST标准。

MIC: Minimum inhibitory concentration. S: Susceptible; I: Intermediate; R: Resistant; NS: Non-susceptible. TMP-SMX: Trimethoprim sulfamethoxazole; *: *Streptococcus viridans* group EUCAST.

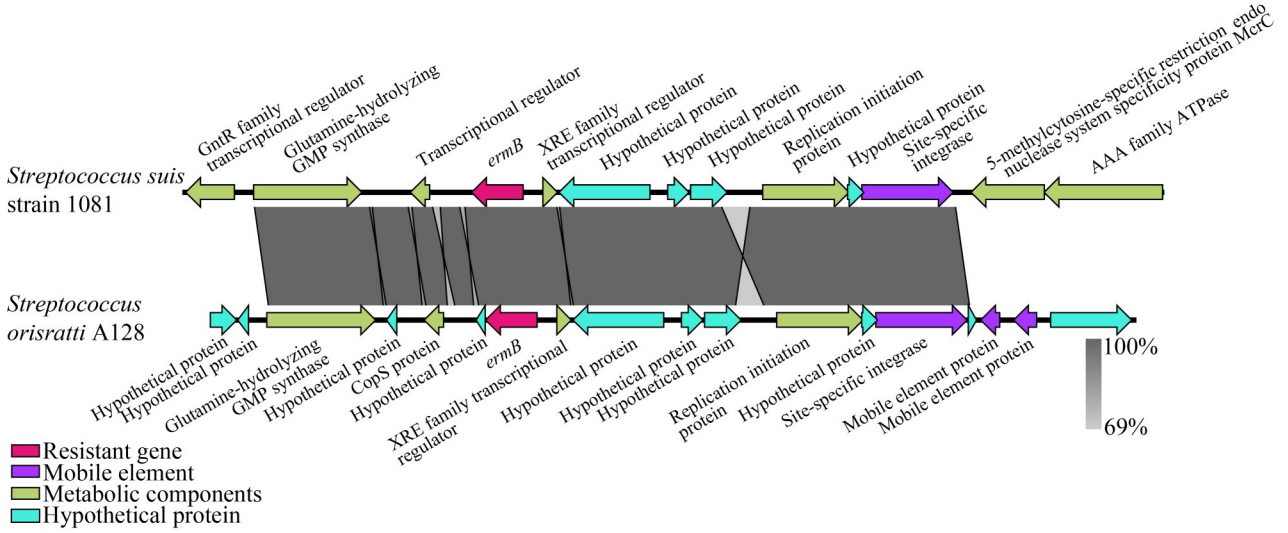

图4 A128菌株*ermB*基因侧翼序列与猪链球菌1081中*ermB*基因簇的线性比较。紫色：整合酶基因；红色：耐药基因；绿色：代谢相关基因；蓝色：假想蛋白基因。

Figure 4 Linear comparison between *ermB* gene of A128 strain and homologous region of *Streptococcus suis* 1081 genome. Integrase genes are in purple; The resistance genes are in red; The metabolism related genes are in green; The hypothetical proteins are in blue.

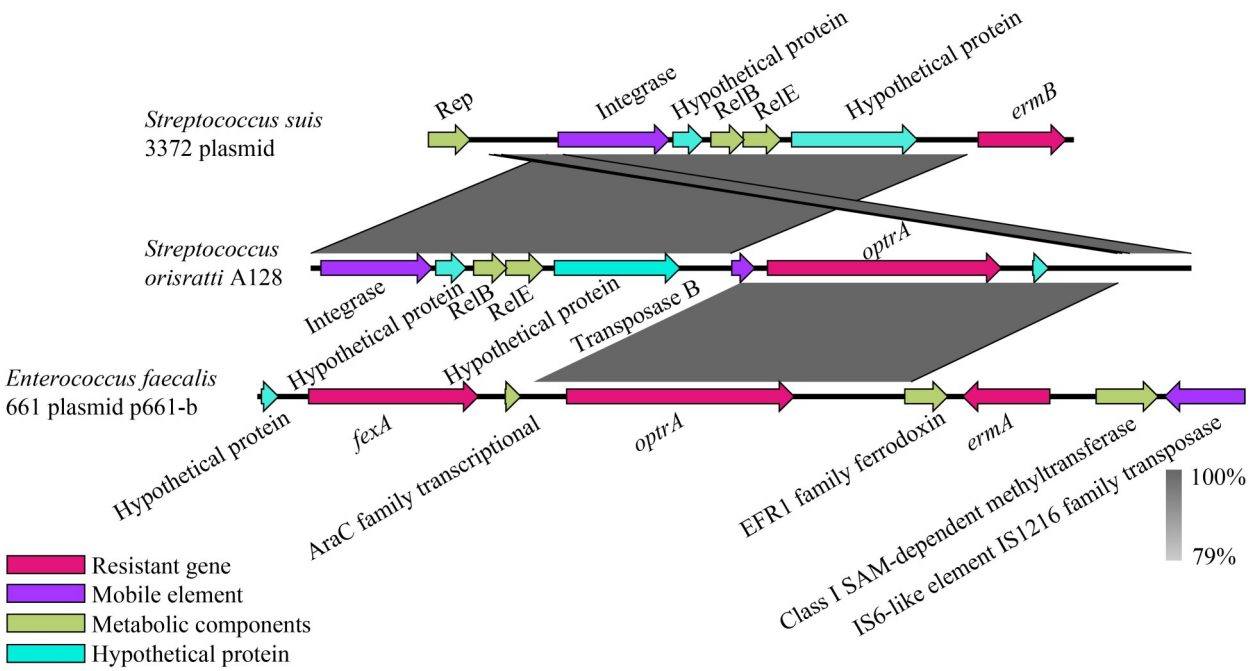

图5　A128菌株*optrA*侧翼序列与猪链球菌3372和粪肠球菌661基因组同源区的线性比较。紫色：整合酶基因；红色：耐药基因；绿色：代谢相关基因；蓝色：假想蛋白基因。

Figure 5　Linear comparison between *optrA* gene of A128 strain and homologous region of *Streptococcus suis* 3372 with *Enterococcus faecalis* 661 genome. Integrase genes are in purple; The resistance genes are in red; The metabolism related genes are in green; The hypothetical proteins are in blue.

(100%，10/10)，其次为 V28I 和 D724N (90%，9/10)。此外，共发现 21 个具备唯一性的氨基酸替代位点，这些变异集中分布于 5 个菌株：其中 A128 菌株中存在 7 个位点突变(M202V、W205G、S324A、I372N、P574S、D638N、A706T)；SGI.245 菌株中有 5 个突变位点；SUG1074 菌株中有 4 个；SRR11489755 菌株中有 3 个；SGI.063 菌株中有 2 个。

2.5.4　PBP2X 结构分析

PBP2X 蛋白全长为 755 个氨基酸，包含 24 个高变区位点。其中，F469L 为发生频率最高的突变位点，出现在所有菌株中(100%，10/10)；其次为 G8V、T626I 和 A630T，频率为 90% (9/10)。此外，发现 6 个菌株特异性突变：SUG2382 (Y38F)、SUG2381 (K96R)、SRR11489755 (A589T)、SGI.063 (A648T，A717T)、SUG1074 (V718I)。

基于 PBP 氨基酸序列构建的系统发育树显示，不同 PBP 蛋白的进化关系存在差异，提示其可能经历独立的进化历程(图 6)。

3　讨论与结论

本研究中，质谱法鉴定结果(豕链球菌)、全自动微生物鉴定分析仪结果(唾液链球菌)与 16S rRNA 鉴定结果(鼠口腔链球菌)存在差异，提示商品化的质谱法和生化鉴定法在链球菌种属鉴定方面存在局限性[49-50]。对于非常见链球菌菌株，结合 16S rRNA 基因测序乃至全基因组测序进行确认是确保鉴定准确性的必要策略。

本研究首次在鼠口腔链球菌中同时检出 *ermB* 和 *optrA* 基因，*ermB* 基因的存在介导了菌株对红霉素和克林霉素的高水平耐药，该基因在酿脓链球菌、无乳链球菌、肺炎链球菌等链球菌各属菌株中普遍存在，已成为介导链球菌

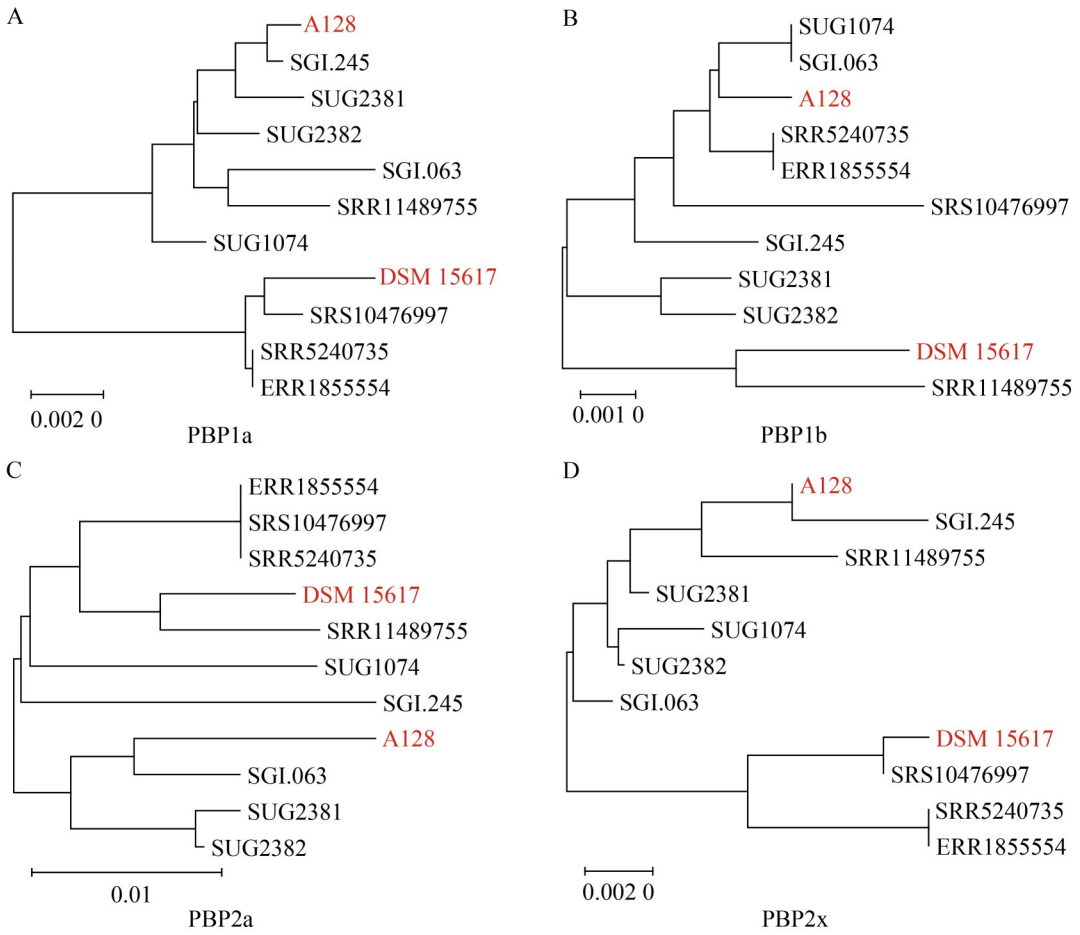

图6 使用NJ法绘制的鼠口腔链球菌青霉素结合蛋白的氨基酸系统发育树。A：PBP1a的氨基酸系统发育树；B：PBP1b的氨基酸系统发育树；C：PBP2a的氨基酸系统发育树；D：PBP2x的氨基酸系统发育树。

Figure 6 Amino acid evolutionary tree of penicillin binding protein in *Streptococcus orisratti* using NJ method. A: The amino acid evolutionary tree of *S. orisratti* PBP1a; B: The amino acid evolutionary tree of *S. orisratti* PBP1b; C: The amino acid evolutionary tree of *S. orisratti* PBP2a; D: The amino acid evolutionary tree of *S. orisratti* PBP2x.

菌株对大环内酯类和克林霉素类耐药的最主要机制[51-52]。

利奈唑胺是针对革兰阳性菌感染治疗的高级别药物，在链球菌中较少发现耐药情况。该菌株携带 *optrA* 基因，导致其对利奈唑胺的敏感性降低。基因环境分析显示，*ermB* 和 *optrA* 基因侧翼均存在可移动遗传元件(如整合酶)，且 *optrA* 基因所在的 contig 与粪肠球菌 661 中质粒序列高度同源，推测 *optrA* 基因可能源自粪肠球菌 661 质粒基因。该发现为理解多重耐药基因在链球菌种间及属间的传播机制提供了重要线索。

青霉素耐药是链球菌耐药领域的重要问题。目前，青霉素作为链球菌感染治疗的一线用药[51]，已在肺炎链球菌[53]、猪链球菌[28]等链球菌中出现耐药株。在酿脓链球菌[34]和口腔链球菌(*Streptococcus oralis*)[54]中也观察到敏感性下降。据我们所知，本研究为首次报道鼠口腔链球菌青霉素耐药株。

有研究表明，PBP 多个活性位点的氨基酸

替换是链球菌对 β-内酰胺类药物敏感性降低的主要机制[29]。在肺炎链球菌中，PBP2b 和 PBP2x 同时存在是肺炎链球菌存活所必需的[55-56]。肺炎链球菌对青霉素的耐药与 PBP2b 特定突变相关[57-58]。然而，本研究未在鼠口腔链球菌中鉴定出 PBP2b，仅发现 PBP2x。在链球菌中，有研究证明头孢噻肟可诱导肺炎链球菌 PBP2x 突变[59]，头孢类药物也可引起无乳链球菌 PBP2x 突变[60-61]，而 PBP2x 突变与酿脓链球菌和肺炎链球菌对头孢噻肟耐药相关[60,62]。由于菌株数量有限，目前鼠口腔链球菌尚无药物敏感、耐药折点以及流行病学界值折点。根据草绿色链球菌折点判断 A128 对头孢噻肟敏感，但其 MIC 值为 1 μg/mL，远超肺炎链球菌的流行病学界值折点(ECV 为 0.060 μg/mL)、酿脓链球菌(ECV 为 0.030 μg/mL)和无乳链球菌(ECV 为 0.125 μg/mL)，提示 A128 菌株中 PBP2x 的氨基酸替换可能是其对头孢噻肟敏感性下降的分子机制。

综上所述，本研究从病死仔猪扁桃体中分离出一株同时携带 *ermB* 和 *optrA* 基因且青霉素耐药的鼠口腔链球菌，揭示了猪源链球菌菌株具备获得多重耐药基因的能力，应关注动物源链球菌的耐药特征和传播风险。

作者贡献声明

邰浩文：数据处理分析及论文撰写修改；张嘉铭：论文修改及实验指导；郑翰：实验指导；刘雨清：生化实验准备；张静宜：试剂耗材准备；付慧强：实验协助；张艺凡：技术支持；李娟：获取基金，实验指导和论文修改。

作者利益冲突公开声明

作者声明不存在任何可能会影响本文所报告工作的已知经济利益或个人关系。

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