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Research Article 研究报告

浙江某市屠宰场猪链球菌血清型、耐药及致病特征

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摘　要：【目的】猪链球菌(*Streptococcus suis*)是猪的重要病原菌，同时也是人畜共患病原。猪的扁桃体是猪链球菌主要定殖部位之一，是易感猪和人的重要传染源。因此，对屠宰场健康猪进行猪链球菌流行病学调查，具有重要的公共卫生学意义。【方法】本研究自 2020 年至 2021 年，从浙江某市屠宰场采集健康猪扁桃体样品，分离鉴定猪链球菌，采用血清型特异性 PCR 法分型，通过耐药基因检测、药敏试验、斑马鱼毒力实验分析其耐药及致病特征。【结果】131 份健康猪扁桃体样品猪链球菌阳性率为 62.59% (82/131)，共分离猪链球菌 68 株，其中 16 型分离率最高，占比 16.18% (11/68)，其次为 31 型(11.76%，8/68)、9 型(7.35%，5/68)、3 型(7.35%，5/68)等。含 2 种及以上血清型的扁桃体样品占 15.85% (13/82)。药敏试验表明，分离株主要对林可酰胺类(100%，68/68)、大环内酯类(98.53%，67/68)、四环素类(100%，68/68)抗生素耐药，所有菌株均属于多药耐药。值得关注的是，有 18 株菌对青霉素耐药、3 株菌对头孢噻肟耐药、2 株菌对利福平耐药、11 株菌对利奈唑胺耐药。大环内酯类/林可酰胺类耐药基因、四环素类耐药基因检出率均为 82.35% (56/68)，这是猪链球菌对这些抗生素耐药的主要原因。按不同批次分离的菌株，选择 25 株代表株进行斑马鱼毒力实验，结果显示：5 株菌对斑马鱼致病力强，攻毒剂量

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为 3×10^6 CFU/尾时死亡率为 80%–100%。【结论】该地区健康猪不仅猪链球菌携带率高，而且存在多药耐药和致病性强的菌株，这为了解该地区猪链球菌病的发病规律和制定相关的防控策略提供参考。

关键词：猪链球菌；浙江；屠宰场；健康猪；耐药；致病

Serotypes, antimicrobial resistance, and pathogenic characteristics of *Streptococcus suis* isolated from a slaughterhouse in an area of Zhejiang Province

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Abstract: [Objective] *Streptococcus suis* is a major swine pathogen and a zoonotic agent. Swine tonsil is a natural habitat of *S. suis*, and the tonsillar *S. suis* from healthy pigs is considered as an important source of infection for susceptible pigs and humans. Thus, the epidemiological investigation of *S. suis* from healthy pigs in slaughterhouses is of great significance for public health. **[Methods]** We collected healthy pigs' tonsils from a slaughterhouse in Zhejiang in 2020–2021, isolated and identified *S. suis*, and serotyped the strains by the serotype-specific PCR assay. Through the resistance gene detection, antimicrobial susceptibility testing, and zebrafish infection experiment, we examined the antimicrobial resistance and pathogenic characteristics of these isolates. **[Results]** The positive rate of *S. suis* in 131 tonsil samples of healthy pigs was 62.59% (82/131), and we isolated a total of 68 strains. The strains were dominated by serotype 16 (16.18%, 11/68), followed by serotype 31 (11.76%, 8/68), serotype 9 (7.35%, 5/68), and serotype 3 (7.35%, 5/68). In addition, tonsil samples containing 2 or more serotypes of *S. suis* accounted for 15.85% (13/82). The antimicrobial susceptibility testing showed that the isolates were mainly resistant to lincosamides (100%, 68/68), macrolides (98.53%, 67/68), and tetracyclines (100%, 68/68), and all isolates were multidrug-resistant. It is worth noting that 18 isolates were resistant to penicillin, 3 resistant to cefotaxime, 2 resistant to rifampicin, and 11 resistant to linezolid. The detection rate of both macrolides/lincosamides resistance genes and tetracycline resistance genes was 82.35% (56/68), which was the main reason why these isolates were resistant to the above antimicrobials. We selected 25 representative strains for the zebrafish infection experiment, and the results showed that 5 strains were highly virulent with mortality of 80%–100% when challenged with the dose of 3×10^6 CFU/fish. **[Conclusion]** Healthy pigs in this area had a high detection rate of *S. suis*. All isolates were multidrug-resistant, and some were highly virulent. These results contribute to understanding the incidence of *S. suis* and formulating relevant prevention and control strategies in this area.

Keywords: *Streptococcus suis*; Zhejiang; slaughterhouse; healthy pigs; antimicrobial resistance; pathogenicity

猪链球菌是猪的重要致病菌，可导致猪的关节炎、脑膜炎和败血症等，给养猪业带来重大的经济损失。同时，该菌可通过伤口等途径感染人，是重要的人畜共患病原[1]。人感染猪链球菌的病例遍及各国，其中亚洲国家居多，包括中国、越南、泰国等。该菌主要定殖于猪的扁桃体等部位，表观健康猪携带猪链球菌的阳性率较高，是易感猪和人的重要传染源[2]。根据荚膜多糖抗原的差异，该菌可分为 29 种传统血清型(1–19、21、23–25、27–31、1/2)[3]和 Chz 血清型[4]。此外，根据荚膜基因簇的差异，近年来发现了 27 种新的荚膜基因簇菌株(NCL 1–26[5–7]和 NCL 21′[8])。迄今，感染人的血清型已达 10 种，分别是血清型 2、4、5、7、9、14、16、21、24、31[9–10]。

猪链球菌的耐药现象较为普遍，主要由于在养殖中，抗生素作为药物或饲料添加剂被大量使用。在药物的选择性压力下，猪链球菌可通过突变或获得外源性耐药基因产生耐药性，且该菌还可作为耐药基因的储库，促进耐药基因在链球菌属间传播[11]。本研究自 2020–2021 年从浙江某市屠宰场采集健康猪扁桃体样品，分离鉴定猪链球菌并进行血清型分型，检测分离株耐药基因并进行药敏试验，选择代表菌株进行斑马鱼毒力实验，分析它们的耐药及毒力特征。研究结果为了解该地区猪链球菌病的发病规律和制定相关的防控策略提供参考。

1 材料与方法

1.1 菌株

本研究的猪链球菌菌株于 2020–2021 年分离自浙江某市屠宰场健康猪扁桃体。最小浓度抑菌试验(MIC)所使用的质控菌株金黄色葡萄球菌 ATCC 29213、斑马鱼毒力实验的强毒对照猪链球菌血清 2 型菌株 SC070731 和弱毒对照血清 9 型菌株 SH040917[9]，均由本实验室保存。

1.2 主要试剂和仪器

THB、MH 培养基购于青岛海博生物公司；无菌脱纤维绵羊血、琼脂粉、新生牛血清、无菌 96 孔板、无菌注射器购于南京鼎国生物公司；抗生素购自上海麦克林生化公司；2×Rapid *Taq* Master Mix、DL2000 DNA Marker、FastPure DNA Extraction Mini Kit 购于南京诺唯赞生物公司；FastDigest *Bst*NⅠ限制性核酸内切酶、PCR 扩增仪购自 Thermo Fisher 公司；离心机购自日立公司；匀浆仪购自 MP 公司。

1.3 培养基及抗生素配制

THB、MH 培养基按产品说明书配置；猪链球菌选择性培养基配制：THB 培养基中加入终浓度为 1.2 mg/L 结晶紫、1.2 mg/L 庆大霉素和 30 mg/L 萘啶酮酸[12]。

药敏试验所用抗生素母液参照美国临床和实验室标准协会(M100-Ed31)推荐方法配制，母液浓度为 2 560 μg/mL，用 0.22 μm 滤器过滤，置于–20 °C 保存。

1.4 样品采集及处理

屠宰场中随机采集健康猪扁桃体样品，低温冷藏运送至实验室。使用无菌 PBS 冲洗扁桃体表面，冲洗干净后使用酒精棉擦拭表面，待酒精挥发后，将组织置于酒精灯火焰上方快速灼烧。从扁桃体内部剪下小块组织(约 0.1 g)置于匀浆管中，加入 PBS 配平后，置于匀浆仪上研磨充分。

1.5 猪链球菌分离鉴定

匀浆后将 200 μL 匀浆液转接至 5 mL 猪链球菌选择性液体培养基中，置于 37 °C CO_2 培养箱中静置培养 8 h。取增殖培养后的菌液，以猪链球菌种特异性基因——谷氨酸脱氢酶基因 *gdh* 和 DNA 修复酶基因 *recN* 为目的片段设计的引物进行 PCR 反应，两者均为阳性则鉴定为猪

链球菌阳性样品，引物信息详见表 1。无菌接种环蘸取阳性菌液，划线至猪链球菌选择性血平板，置于 37 °C 培养箱中培养 8 h。培养后的平板可见多种不同的菌落形态，挑取猪链球菌疑似菌落(呈圆形、边缘整齐、灰白色、针尖大小、含 α 溶血环的菌落)于 THB 液体培养基中，每份平板挑 8 个单菌落，置于 37 °C 摇床 180 r/min 培养至对数期。将菌液以基因 *gdh* 和 *recN* 鉴定引物进行 PCR 反应，两者均为阳性则鉴定为猪链球菌，将菌株冻存至–80 °C 冰箱保存。

1.6 猪链球菌血清型分型

将冻存的猪链球菌菌株划线至 THB 血平板复苏，挑取单菌落至 THB 液体培养基中，置于 37 °C 摇床 180 r/min 培养至菌液浑浊。依次以 29 种传统血清型(1–19、21、23–25、27–31、1/2)、26 种新荚膜基因簇菌株(NCL 1–26)、Chz 血清型鉴定引物进行 PCR 反应，阳性则鉴定为相应血清型，引物信息见表 1。若同一扁桃体分离的多个菌株属同一血清型，则仅保留其中 1 株作为冻存的分离株。由于普通 PCR 方法无法区分 2 型与 1/2 型、1 型与 14 型，对属于这 4 种血清型的菌株，参考 Matiasovic 等的方法[13]，使用基因 *cpsK* 特异性引物扩增(表 1)，反应完成后使用试剂盒 FastPure DNA Extraction Mini Kit (南京诺唯赞，货号：DC301)进行产物的纯化回收，将回收的片段进行 *BstN* Ⅰ限制性内切酶酶切(Themo Fisher，货号：FD0554)，跑胶鉴定。1 型和 1/2 型无法被内切酶识别，酶切后产物为 486 bp，2 型和 14 型可被识别，产物为 347 bp 和 139 bp 2 个片段。

表 1 猪链球菌分离鉴定引物信息

Table 1 The primers information for *Streptococcus suis* isolation and identification

Target genes	Sequence (5′→3′)	PCR product/bp	References
gdh	Forward: CCATGGACAGATAAAGATGG	689	[14]
	Reverse: GCAGCGTATTCTGTCAAACG		
recN	Forward: CTACAAACAGCTCTCTTCT	336	[15]
	Reverse: ACAACAGCCAATTCATGGCGTGATT		
SS 1&14	Forward: TCTTATAACAGGCGTCAAAACA	153	[16]
	Reverse: ATCGGTATAAAAGCAAGACACA		
SS 2&1/2	Forward: TTCGTATTAACTTACTTGGCGT	363	[16]
	Reverse: TAAATCCCCATATGCCAAATCC		
SS 3	Forward: ACATCCATTGCAGGAGTAGT	210	[16]
	Reverse: TGCAGTTCCAAAATTCTTCGT		
SS 4	Forward: TGATATTGGCTATCTTTTGGGG	542	[16]
	Reverse: TTCCCCCTTCAAATAAACTCTG		
SS 5	Forward: AGGTATGTCTTCTTATTCGCAG	428	[16]
	Reverse: ATAATCCCTCCTGATACTAGGC		
SS 6	Forward: TGGTGTCTTTCTACCTGCAA	705	[16]
	Reverse: TCACCAAGATACGTGAACCA		
SS 7	Forward: AAAATTCGTTCCATTGTAGGTG	609	[16]
	Reverse: TGAAGTTGAAGCTGGTGATAAA		
SS 8	Forward: ATCGCTTCAAATAAGGTAGGAG	268	[16]
	Reverse: TGTAGGCCGTAATATCAACAAA		
SS 9	Forward: TGAAAGTAGGTATATCTCAGCA	809	[16]
	Reverse: AAAGAATTGAATCCCACCTGAG		

(待续)

(续表 1)

SS 10	Forward: CTATCACTACCACGGAATGC	303	[16]
	Reverse: TAACCGTCCGTCTAGAATGT		
SS 11	Forward: ATTGTTACGATTTGGGCGAT	512	[16]
	Reverse: GAACCCCATGTAGTTATGGC		
SS 12	Forward: CATGGGAACTGTACAGGATAAG	171	[16]
	Reverse: CCACCTTACTACCTGTTTTACC		
SS 13	Forward: GCTTGTAGCGAATTTTGGTATT	741	[16]
	Reverse: CCATTAGATGTATTTGCTCCCA		
SS 15	Forward: ACCTACTCAAGAACATCCTTTC	458	[16]
	Reverse: GTAACTAAAACAGCAAACGTCA		
SS 16	Forward: ATCAACAAACATTTTCGAGGAC	223	[16]
	Reverse: GCTGAATAATAGATTCGTCCTGT		
SS 17	Forward: TTGCCGTATAAGGTCTTAGTTG	380	[16]
	Reverse: ATCTGACGGTAAATGTTCTCTG		
SS 18	Forward: ATAGGCTGTACTTTGATAACCG	310	[16]
	Reverse: AGCCTATCGCTCAAAAACTTAT		
SS 19	Forward: ATTATTATAGGGCAAAGCAGGG	674	[16]
	Reverse: ATCGTACACAACAAAACGATTC		
SS 21	Forward: TGGCAGACTTCTTTTCTCAC	858	[16]
	Reverse: CCTGTAGCGCCTCATAAAAC		
SS 23	Forward: TATTATAGTCCGATGCAAGCAG	461	[16]
	Reverse: ATGAGAACGAAACGGAATAGTT		
SS 24	Forward: GATAGCAATGTAATCCAATCGC	204	[16]
	Reverse: GTAGGTTCCCCTAGTAAGAAGT		
SS 25	Forward: ATTGAGTCCTTTTACTGGTAGC	390	[16]
	Reverse: TACTGAGCTACATAATCCCACA		
SS 27	Forward: GTGGTTTTGGAGGATATTTTCG	530	[16]
	Reverse: ATTGAGATAAACTACTCCGTGC		
SS 28	Forward: GGGCACTTGTTTTACTTCCT	896	[16]
	Reverse: GCCAAGTAATACCCTACCTG		
SS 29	Forward: AAAGTGCCTATTCTGGGATTTT	263	[16]
	Reverse: TAAAGGCAACTTCCACATTGTA		
SS 30	Forward: TTGGGCTTGTAAATAGTGAGAG	625	[16]
	Reverse: CGATTAGATAAGCGCATTTGTT		
SS 31	Forward: CATATGTTTTCGTGGGGAGT	1 006	[16]
	Reverse: GTGATGAAAACATCGTTGGTAG		
Chz	Forward: AATGAATAAGGAACTTGAACTA	424	[4]
	Reverse: CGTATCATCTGTATTAGCTAAA		
NCL 1	Forward: ATTCACATAGTAACATTGCGGA	254	[17]
	Reverse: CAACATTGCGCAGGAAATAATA		
NCL 2	Forward: TTCTTGATTATGCTGTTCTCGT	333	[17]
	Reverse: AAACACAACATCCTGTACTTCA		
NCL 3	Forward: ATTCAGGAGGTATTCAACCAAG	370	[17]
	Reverse: AATTCACTAGCATCAACAAACG		
NCL 4	Forward: TGCTTATTATGACTGTTGCCTT	293	[17]
	Reverse: ATCAGTTGATAAGGTTGCTGTT		

(待续)

(续表 1)

NCL 5	Forward: CAGATGAGTCAGCAAGTAATCA Reverse: AGGGAAGAGTAAGATTCAAGGT	262	[17]
NCL 6	Forward: TGATACGGGTACTGTTGAGTAT Reverse: ATTACTACTTCTGGTTGGGTCA	220	[17]
NCL 7	Forward: GTTGATTTATTTGCGGGACTAC Reverse: CAGAAAAACAATAGCAGTGACC	447	[17]
NCL 8	Forward: AAAATTTTCACTTCACCTCGAC Reverse: AATCTTCCAATCAATGCTACGA	390	[17]
NCL 9	Forward: GGGTAGATACGTTCTATTTGGG Reverse: GCGACGGTATATAGAGCTATTC	309	[17]
NCL 10	Forward: ACAAAAATAGTAGACGGGCTTT Reverse: TTGCACGCCAAGTATAAAATTC	231	[17]
NCL 11	Forward: GCCTTAATAATGGTGGGTTTTG Reverse: TACCTAAAACATTTTGCCCAGA	454	[17]
NCL 12	Forward: GTGAGAGATTTCGGTGTAGTTT Reverse: GCATCAGCATACATCTTTCCTA	380	[17]
NCL 13	Forward: TCTTGCTAGGTCTAATCGTAGT Reverse: CTCGCTTCCAATTAATAAACCG	179	[17]
NCL 14	Forward: AAAAGAAATGGAAAGCAGTGTG Reverse: TCTTTGCTCAGCTATTGAGTTT	178	[17]
NCL 15	Forward: TATGCTATTGTTACGATGTGGG Reverse: CTTGGAGAGTAAAACGATAGGG	169	[17]
NCL 16	Forward: AGGGTTATTCTTTTTGGTGGAT Reverse: TCTTGTAAGCAGAAAATCGTGA	200	[17]
NCL 17	Forward: ATGAAGGTTGTTTTTGATAATAGGG Reverse: TAGTGCGTCTTGAATAATAAAC	621	[17]
NCL 18	Forward: ATGATTTATATTATTCCGTCAATTATT Reverse: TATATTTGGCCCTGTCAAACTTTTA	276	[17]
NCL 19	Forward: ATGCGTACAAAAAATAGAGTTG Reverse: CATATTATTCCCACAAATATCT	414	[17]
NCL 20	Forward: ATGTTTATTTATTTATTCATTTTTATAG Reverse: ATAAGGATAGGACTTAGCAAAA	207	[17]
NCL 21	Forward: TGAGAAAGTATCCGTTTATGGC Reverse: TGGGCCTATTTCAAAGAATGTC	416	[17]
NCL 22	Forward: TAGAAAGTTCAATGTGCTCGGA Reverse: ACTGGTAAAGGAAAACTTGAAGCG	560	[17]
NCL 23	Forward: CAAAAATACACCCCTACTCAAC Reverse: ACCTAAGAGTGACGGAGTATGA	660	[17]
NCL 24	Forward: GTATTTAACTATGGCTACTCCT Reverse: TTAAGTAACAGTTGAATGGCCT	833	[17]
NCL 25	Forward: ATTATAGTAGTTGCGATGTTGT Reverse: TTTCCCACAGCGTAGCACTAAT	523	[17]
NCL 26	Forward: AATAGCGGAAATTCGGGCGGAG Reverse: ATGAGTGCTGAATAAATAATCC	737	[17]
cpsK	Forward: GTTGCTGGTTATGATAGGGTAG Reverse: AAGCTTCTTTTGCTGTTTGCTC	486	[13]

1.7 药敏试验

参照美国临床和实验室标准协会(M100-Ed31)推荐的肉汤稀释法进行猪链球菌药敏试验。以金黄色葡萄球菌 ATCC 29213 作为质控菌株，测定每种抗生素的 MIC，耐药折点参考前期发表的论文[9]。共选用 11 类 23 种抗生素：β-内酰胺类(青霉素、阿莫西林、头孢噻肟)、利福霉素类(利福平)、糖肽类(万古霉素)、噁唑烷酮类(利奈唑胺)、喹诺酮类(恩诺沙星、马波沙星)、酰氨醇类(氯霉素、氟苯尼考)、林可酰胺类(林可霉素、克林霉素)、截短侧耳类(泰妙菌素、沃尼妙林)、氨基糖苷类(庆大霉素、卡那霉素、链霉素、壮观霉素)、大环内酯类(替米考星、红霉素、阿奇霉素)、四环素类(多西环素、四环素)。

1.8 耐药基因检测

将冻存的猪链球菌分离株划线至血平板复苏，挑取单菌落至 THB 液体培养基中，置于 37 °C 摇床 180 r/min 培养至菌液浑浊。检测猪链球菌常见的 19 个耐药基因：大环内酯类/林可酰胺类耐药基因[*erm(A)*、*erm(B)*、*erm(C)*、*mefA*、*msr(D)*、*lsa(E)*]、四环素类耐药基因[*tet(O)*、*tet(M)*、*tet(O/W/32/O)*、*tet(L)*]、酰氨醇类耐药基因(*fexA*、*fexB*)、噁唑烷酮类耐药基因(*optrA*)、氨基糖苷类耐药基因[*aac(6')-aph(2'')*、*aph(2')-Ib*、*aph(2')-Ic*、*aph(2')-Id*、*aph(3')-IIIa*、*ant(6')-Ia*]进行 PCR 反应，出现阳性则鉴定菌株有此耐药基因，上述耐药基因检测引物详见表 2。

1.9 斑马鱼毒力实验

选取规格相近的成年斑马鱼(约为 3 cm)，饲养 1 周。按分离批次选择菌株，将冻存的猪链球菌菌株划线至 THB 血平板复苏，挑取单菌落至 THB 液体培养基中，置于 37 °C 摇床 180 r/min 培养至对数期。将菌液以 5 000 r/min 离心 10 min，倒掉上清后用 PBS 重悬，重复 3 次。将重悬菌液注射至斑马鱼腹腔，每尾 20 μL，8–12 h 后观察到斑马鱼濒死或死亡后，立刻吸取脑脊液，并均匀划线至 THB 血平板，置于 37 °C 培养箱中培养 8 h。挑取猪链球菌疑似菌株至 THB 液体培养基，培养至菌液浑浊，将菌液进行猪链球菌鉴定，确定攻毒菌株。将攻毒菌液按 1:100 转接至 THB 液体培养基中，置于 37 °C 摇床 180 r/min 培养至对数期，将菌液以 5 000 r/min 离心 10 min，倒掉上清后用 PBS 重悬，重复 3 次，最后一次重悬至 OD_{600}=0.6。每株菌攻毒 15 尾斑马鱼，每尾腹腔注射 20 μL，其攻毒菌量控制在 3×10^6 CFU/尾，攻毒菌液进行平板计数，次日确定攻毒菌量。实验选取猪链球菌血清 2 型 SC070731 为强毒对照，血清 9 型 SH040917 为弱毒对照，每隔 12 h 观察并记录斑马鱼死亡情况，连续观察一周。每株菌攻毒结果均与强毒对照组 SC070731 进行比较，采用 Log rank (Mantel Cox) test 进行统计学分析[9]。

2 结果与分析

2.1 猪链球菌分离鉴定

分别于 2020 年 10、11 月及 2021 年 3、4 月采集浙江某市屠宰场健康猪扁桃体共 131 份，猪链球菌阳性样品为 82 份，占比 62.59%，共分离到猪链球菌 68 株。2020 年 11 月采集的样品阳性率最高，为 93.33% (28/30)，共分离 23 株；其次为 2021 年 3 月的样品，为 70.97% (22/31)，共分离 15 株。各批次样品猪链球菌的血清型分布各不相同，2020 年 10 月采集的样品分离 2 株血清 2 型菌株，而其余批次均未发现 2 型菌株，结果见表 3。所有批次的分离株中，16 型(16.18%，11/68)分离率最高，其次为 31 型(11.76%，8/68)、9 型(7.35%，5/68)、3 型

表 2　耐药基因检出率及其引物信息

Table 2　Detection rate and primers information of antimicrobial resistance genes

Classes (rate)	Resistance genes	Rate	Primer sequences (5′→3′)	PCR product/bp	References
Macrolides/ lincosamides 82.35% (56/68)	*ermA*	5.88% (4/68)	Forward: TCTAAAAAGCATGTAAAAGAA Reverse: CTTCGATAGTTTATTAATATTAGT	645	[18]
	ermB	80.88% (55/68)	Forward: TCATCTATTCAACTTATCGTC Reverse: CTGTGGTATGGCGGGTAAG	639	[19]
	ermC	0.00% (0/68)	Forward: TCAAAACATAATATAGATAAA Reverse: GCTAATATTGTTTAAATCGTCAAT	642	[20]
	mefA	2.94% (20/68)	Forward: AGTATCATTAATCACTAGTGC Reverse: TTCTTCTGGTACTAAAAGTGG	348	[21]
	msr(D)	4.41% (3/68)	Forward: CCTTATCGGCACAGGTTCAT Reverse: GCCTTCCGGAGCTCCTACTT	494	/
	lsa(E)	0.00% (0/68)	Forward: TTGTACGGAATGTATGG Reverse: TTCGCTTCTATTAAGCACTCTT	675	[22]
Tetracyclines 82.35% (56/68)	*tetO*	67.65% (46/68)	Forward: AACTTAGGCATTCTGGCTCAC Reverse: TCCCACTGTTCCATATCGTCA	519	[21]
	tetM	17.65% (12/68)	Forward: GTGGACAAAGGTACAACGAG Reverse: CGGTAAAGTTCGTCACACAC	406	[23]
	tet(O/W/32/O)	1.47% (1/68)	Forward: GGAGGAAAATACCGACATA Reverse: CTCTTTCATAGCCACGCC	750	[24]
	tetL	11.76% (8/68)	Forward: ATAAATTGTTTCGGGTCGGTAAT Reverse: AACCAGCCAACTAATGACAATGAT	1 077	[25]
Amphenicols 0.00% (0/68)	*fexA*	0.00% (0/68)	Forward: GTACTTGTAGGTGCAATTACGGCTGA Reverse: CGCATCTGAGTAGGACATAGCGTC	1 272	[26]
	fexB	0.00% (0/68)	Forward: TTCCCACTATTGGTGAAAGGAT Reverse: GCAATTCCCTTTTATGGACGTT	787	/
Oxazolidinones 33.82% (23/68)	*optrA*	33.82% (23/68)	Forward: AGGTGGTCAGCGAACTAA Reverse: ATCAACTGTTCCCATTCA	1 395	[27]
Aminoglycosides 51.47% (35/68)	*aph(2')-Ib*	1.47% (1/68)	Forward: CTTGGACGCTGAGATATATGAGCAC Reverse: GTTTGTAGCAATTCAGAAACACCCTT	867	[28]
	aph(2')-Ic	0.00% (0/68)	Forward: CCACAATGATAATGACTCAGTTCCC Reverse: CCACAGCTTCCGATAGCAAGAG	444	[28]
	aph(2')-Id	0.00% (0/68)	Forward: GTGGTTTTTACAGGAATGCCATC Reverse: CCCTCTTCATACCAATCCATATAACC	641	[28]
	aph(3')-IIIa	17.65% (12/68)	Forward: GGCTAAAATGAGAATATCACCGG Reverse: CTTTAAAAAATCATACAGCTCGCG	523	[28]
	ant(6')-Ia	16.18% (11/68)	Forward: ACTGGCTTAATCAATTTGGG Reverse: GCCTTTCCGCCACCTCACCG	596	[28]
	aac(6')-aph(2'')	33.82% (23/68)	Forward: CAGGAATTTATCGAAAATGGTAGAAAAG Reverse: CACAATCGACTAAAGAGTACCAATC	369	[28]

表 3　浙江某地屠宰场健康猪扁桃体猪链球菌分离鉴定

Table 3　The isolation and identification of *S. suis* from healthy pigs' tonsils collected from a slaughterhouse in an area of Zhejiang Province

Collection month	Sample number	Number of positive sample	Isolate number	Number of serotypes															
				2	3	4	5	7	8	9	10	12	15	16	21	28	31	NCL4	NCL17	Unknown
2020-10	40	18	16	2	4	0	0	0	1	0	0	0	1	2	1	1	1	0	0	3
2020-11	30	28	23	0	1	2	1	0	3	2	3	1	0	4	1	0	2	0	1	2
2021-03	31	22	15	0	0	0	0	1	0	1	0	0	0	1	0	0	3	1	0	8
2021-04	30	14	14	0	0	0	0	0	0	2	1	0	0	4	1	0	2	0	0	4

(7.35%, 5/68)、10 型(5.88%, 4/68)、8 型(5.88%, 4/68)、21 型(4.41%, 3/68)、2 型(2.94%, 2/68)、4 型(2.94%, 2/68)、5 型(1.47%, 1/68)、7 型(1.47%, 1/68)、12 型(1.47%, 1/68)、15 型(1.47%, 1/68)、28 型(1.47%, 1/68)、NCL 4 型(1.47%, 1/68)、NCL 17 型(1.47%, 1/68)以及未知血清型(25.00%, 17/68)，结果见图 1。属于感染人的 10 种血清型(2、4、5、7、9、14、16、21、24、31)占所有菌株的 48.53% (33/68)。此外，含 2 种及以上血清型菌株的扁桃体占猪链球菌阳性扁桃体 15.85% (13/82)。

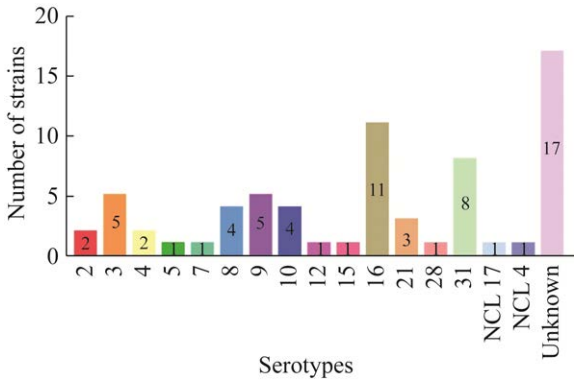

图 1　浙江某地屠宰场健康猪扁桃体猪链球菌血清型分布

Figure 1　Distribution of *S. suis* serotypes from healthy pigs' tonsils collected from a slaughterhouse in an area of Zhejiang Province. The number on the *Y*-axis refers to the number of *S. suis* isolates, and the symbol or number on the *X*-axis refer to isolates' serotypes or genotypes.

2.2　药敏试验

选择 11 类 23 种抗生素进行药敏试验，结果见表 4。100% (68/68)分离株对四环素类抗生素(四环素及多西环素)、林可酰胺类抗生素(林可霉素及克林霉素)耐药；98.53% (67/68)分离株对大环内酯类抗生素耐药，其中替米考星及阿奇霉素耐药率均为 98.53% (67/68)、红霉素为 95.59% (65/68)；67.65% (46/68)分离株对氨基糖苷类抗生素耐药，庆大霉素和卡那霉素耐药率分别为 54.41% (37/68)和 52.94% (36/68)，链霉素和壮观霉素分别为 32.35% (22/68)和 17.65% (12/68)；36.76% (25/68)分离株对酰氨醇类抗生素耐药，其中氟苯尼考耐药率为 33.82% (23/68)、氯霉素为 5.88% (4/68)；26.47% (18/68)分离株对 β-内酰胺类抗生素耐药，其中青霉素耐药率为 26.47% (18/68)、头孢噻肟为 4.41% (3/68)。值得关注的是，2 株菌对利福平耐药，11 株对利奈唑胺耐药。对三类及三类以上抗生素耐药可判定为多重耐药[29]，根据此判定标准，68 株分离株均属于多药耐药，结果如图 2 所示。12 株对三类抗生素耐药，占比 17.65%；23 株对四类抗生素耐药，占比 33.82%；13 株对五类抗生素耐药，占比 19.12%；12 株对六类抗生素耐药，占比 17.65%；4 株对七类抗生素耐药，占比 5.88%；2 株对八类抗生素耐药，占比 2.94%；2 株对九类抗生素耐药，占比 2.94%。

表 4　猪链球菌分离株对 23 种抗生素耐药率

Table 4　Resistance rate of *S. suis* isolates to 23 antimicrobials tested

Classes (rate)	Antimicrobials	Rate
β-lactam antibiotics (26.47%, 18/68)	Penicillin	26.47% (18/68)
	Amoxicillin	0.00% (0/68)
	Cefotaxime	4.41% (3/68)
Rifamycins (2.94%, 2/68)	Rifampin	2.94% (2/68)
Glycopeptides (0.00%, 0/68)	Vancomycin	0.00% (0/68)
Oxazolidinones (16.18%, 11/68)	Linezolid	16.18% (11/68)
Quinolones (13.24%, 9/68)	Enrofloxacin	11.76% (8/68)
	Marbofloxacin	13.24% (9/68)
Amphenicols (36.76%, 25/68)	Chloramphenicol	5.88% (4/68)
	Florfenicol	33.82% (23/68)
Lincosamides (100.00%, 68/68)	Lincomycin	100.00% (68/68)
	Clindamycin	100.00% (68/68)
Pleuromutilin antibiotics (19.12%, 13/68)	Tiamulin	19.12% (13/68)
	Valnemulin	13.24% (9/68)
Aminoglycosides (67.65%, 46/68)	Gentamycin	54.41% (37/68)
	Kanamycin	52.94% (36/68)
	Streptomycin	32.35% (22/68)
	Spectinomycin	17.65% (12/68)
Macrolides (98.53%, 67/68)	Erythromycin	95.59% (65/68)
	Tilmicosin	98.53% (67/68)
	Azithromycin	98.53% (67/68)
Tetracyclines (100.00%, 68/68)	Tetracycline	100.00% (68/68)
	Doxycycline	100.00% (68/68)

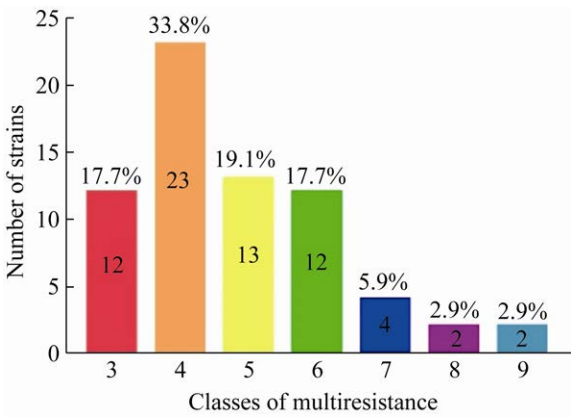

图 2　猪链球菌分离株多重耐药情况

Figure 2　Multidrug resistance of *S. suis* isolates. The number on the *Y*-axis refers to the number of *S. suis* isolates, and the number on the *X*-axis refers to the class number of multidrug-resistant isolates.

2.3　耐药基因检测

选择 19 种猪链球菌常见耐药基因，对 68 株猪链球菌进行 PCR 反应，检测结果见表 2。82.35% (56/68)分离株存在大环内酯类/林可酰胺类耐药基因，而 98.53% (67/68)分离株对大环内酯类抗生素耐药，其中 11 株菌未检出大环内酯类耐药基因；此外，所有分离株对林可酰胺类抗生素耐药，其中 12 株菌未检出林可酰胺类耐药基因。82.35% (56/68)分离株存在四环素类耐药基因，所有分离株对这类抗生素耐药，其中 12 株菌未检出相应耐药基因。51.47% (35/68)分离株存在氨基糖苷类耐药基因，而 67.65% (46/68)分离株对这类抗生素耐药，其中 12 株菌

未检出相应耐药基因，1 株菌存在耐药基因，但无耐药表型。33.82% (23/68)分离株存在噁唑烷酮类耐药基因 *optrA*，但仅 16.17% (11/68)分离株对利奈唑胺耐药，其中 2 株菌未检出相应耐药基因，12 株菌存在耐药基因，但无耐药表型。上述有耐药表型但无相应耐药基因，可能这些菌株存在其他耐药基因未检测到；而部分菌株出现氨基糖苷类、噁唑烷酮类耐药基因，但无耐药表型，可能这些耐药基因无法正常表达或存在突变。

2.4 斑马鱼毒力实验

按不同批次分离的菌株，选择 25 株进行斑马鱼毒力实验，结果见表 5。菌株 2020WUSS020、2021WUSS006、2021WUSS009、2021WUSS010、2021WUSS014 对斑马鱼致病力强，攻毒量为 3×10^6 CFU/尾时死亡率为 80%–100%，与强毒株 SC070731 相当；菌株 2020WUSS019、2020WUSS025、2021WUSS028 对斑马鱼致死率为 40%–60%；其余菌株对斑马鱼致病力弱，致死率低于 33.3%。

表 5 猪链球菌分离株斑马鱼毒力实验结果

Table 5 The results of zebrafish infection experiments

Strains	Serotypes	Death in each period/h								Total deaths	Mortality rate/%	*P* value	Significance
		12	24	36	48	60	72	84	96				
SC070731	2	9	1	0	1	3	0	1	0	15	100.00	–	–
SH040917	9	0	0	0	0	0	0	0	0	0	0.00	<0.000 1	****
2020WUSS016	16	0	0	0	0	0	0	0	0	0	0.00	<0.000 1	****
2020WUSS019	2	1	3	1	1	0	0	0	0	6	40.00	0.000 4	***
2020WUSS020	2	8	7	0	0	0	0	0	0	15	100.00	0.184 8	*ns*
2020WUSS025	3	1	3	0	2	0	0	0	0	6	40.00	0.000 3	***
2020WUSS030	8	0	0	0	0	0	0	0	0	0	0.00	<0.000 1	****
2020WUSS036	21	0	0	0	0	0	0	0	0	0	0.00	<0.000 1	****
2020WUSS043	8	1	2	0	2	0	0	0	0	5	33.33	<0.000 1	****
2020WUSS047	12	0	0	1	0	0	0	0	0	1	6.67	<0.000 1	****
2020WUSS048	10	0	0	0	0	0	0	0	0	0	0.00	<0.000 1	****
2020WUSS049	10	0	0	0	0	0	0	0	0	0	0.00	<0.000 1	****
2020WUSS051	5	0	1	0	0	0	0	0	0	1	6.67	<0.000 1	****
2020WUSS055	9	0	4	0	0	0	0	0	0	4	26.67	<0.000 1	****
2021WUSS001	16	0	1	0	0	0	0	0	0	1	6.67	<0.000 1	****
2021WUSS002	16	0	0	0	0	0	0	0	0	0	0.00	<0.000 1	****
2021WUSS006	7	11	0	0	0	0	0	0	1	12	80.00	0.208 5	*ns*
2021WUSS009	31	5	5	2	0	0	0	0	0	12	80.00	0.293 1	*ns*
2021WUSS010	31	1	7	5	0	0	0	0	0	13	86.67	0.238 4	*ns*
2021WUSS013	9	0	2	1	0	1	0	0	0	4	26.67	<0.000 1	****
2021WUSS014	9	6	6	0	0	0	0	0	0	12	80.00	0.279 6	*ns*
2021WUSS015	21	0	0	0	0	0	0	1	0	1	6.67	<0.000 1	****
2021WUSS016	NCL 4	0	0	0	0	0	0	1	1	2	13.33	<0.000 1	****
2021WUSS020	Unknown	0	0	0	0	0	0	0	0	0	0.00	<0.000 1	****
2021WUSS025	Unknown	0	0	0	0	0	0	0	0	0	0.00	<0.000 1	****
2021WUSS027	Unknown	0	0	0	0	0	0	0	0	0	0.00	<0.000 1	****
2021WUSS028	10	5	3	0	0	0	0	1	0	9	60.00	0.013 3	*

The virulence of each strain was compared with that of the virulent strain SC070731 (as a positive control) in the same batch, and the Log-rank (Mantel-Cox) test was used for statistical analysis. *, ***, and **** indicate $P<0.05$, $P<0.001$, and $P<0.000\ 1$, respectively. *ns* indicates no significance.

3 讨论

屠宰场表观健康猪的猪链球菌携带率较高，血清型多而复杂，是潜在的传染源。Ngo等[30]发现，在越南南部，屠宰场健康猪的扁桃体是人感染猪链球菌病的重要来源，且血清2型是优势血清型。Dong等对1 634株分离自人和猪的猪链球菌进行大规模系统进化分析，将具有感染人潜能的菌株归为一簇，属于该簇的健康猪来源的菌株具有较强的致病性，提示健康猪携带的猪链球菌是人的重要传染源[31]。因此，对健康猪开展猪链球菌流行病学调查，具有重要的公共卫生学意义。迄今，感染人的血清型共有10种，除血清14型、24型外，其他血清型菌株在本研究中均能分离到。此外，该地区屠宰场不同批次样品的猪链球菌阳性率和血清型分布有所不同，这可能与各批次屠宰的猪群不同有关。本课题组2019–2020年于江苏某市屠宰场健康猪扁桃体猪链球菌分离情况比较发现[12,32]，江苏地区108份健康猪扁桃体样品中，猪链球菌阳性率为51.85% (56/108)，而本次浙江地区为62.59% (82/131)，均超过50%；江苏地区分离的菌株中，感染人的10种血清型占比为55.17% (48/87)，浙江为48.53% (33/68)，均比较高；两地区均为16型分离率最高，提示16型可能为两地区健康猪的优势血清型，其次为31型；两地区均存在同一份扁桃体中含2种及以上血清型菌株，江苏占比为35.71% (20/56)，浙江为15.85% (13/82)。陆亚男等对上海地区种猪场健康猪进行了猪链球菌检测，阳性率为58.11%，优势血清型为7型(分离率为42.86%)[33]，而本研究仅分离1株血清7型菌株。Thongkamkoon等从泰国180份健康猪扁桃体中分离猪链球菌196株，23型(10.2%)为优势血清型[2]，而本研究未检出23型菌株。上述结果提示，健康猪的猪链球菌携带率较高，且较多健康猪中含有2种及以上血清型猪链球菌，但不同地区呈现不同优势血清型。

斑马鱼毒力实验表明，虽然大多菌株对斑马鱼致病力较低，但有5株毒力较强：血清31型菌株2021WUSS009和2021WUSS010、血清7型菌株2020WUSS006、血清2型菌株2020WUSS020、血清9型菌株2021WUSS014，这4种血清型都属感染人的血清型。本课题组最近研究发现，47株血清31型的代表菌株中有7株致病性较强，分离自健康猪[9]；21株血清7型的代表菌株中有7株致病性较强，分离自健康猪，而且其中2株菌与人源株GX69同属于ST373，提示ST373的血清7型菌株值得关注[10]。

猪链球菌可作为耐药基因储库，促进耐药基因在其他致病性链球菌间传播[34]。由于抗生素的大量使用，在抗生素的选择性压力下，猪链球菌的耐药现象较为严重。本研究中，所有猪链球菌分离株对林可酰胺类抗生素耐药，大环内酯类的耐药率也高达98.53% (67/68)。猪链球菌对大环内酯类抗生素的耐药由甲基化酶Erm和外排泵蛋白Mef介导，主要由*ermB*和*mefA*编码，其中耐药基因*ermB*可产生MLSB耐药表型，即大环内酯类、林可酰胺类和链阳菌素B类抗生素联合耐药[35]。*ermB*在分离株中分布率达80.88% (55/68)，这可能是分离株对大环内酯类、林可酰胺类抗生素耐药率高的主要原因。此外，82.35% (56/68)的分离株分布有四环素类耐药基因，*tetL*编码跨膜泵将四环素排出细菌外，*tetO*、*tetM*、*tet(O/W/32/O)*编码核糖体保护蛋白阻止四环素与细菌核糖体30S亚基的结合[36]，上述耐药基因可能是导致所有分离株对这类抗生素耐药的主要原因。链球菌属细菌本身就对氨基糖类抗生素呈现低水平耐药，

而氨基糖苷类耐药基因则可导致高水平的耐药[34]。51.47% (35/68)分离株有氨基糖苷类耐药基因，该类耐药基因可引起酶促失活反应[36]，从而使猪链球菌对这类抗生素高水平耐药。噁唑烷酮类抗生素利奈唑胺常用于治疗由耐甲氧西林金黄色葡萄球菌或耐万古霉素肠球菌引起人的感染[37],通常是临床治疗中的最后手段[38]。*optrA* 耐药基因可编码 ABC 转运子使猪链球菌对利奈唑胺、酰氨醇类、林可酰胺类抗生素耐药[34]。Huang 等检测了 107 株江苏地区的猪链球菌分离株对利奈唑胺的耐药性，其耐药率为 38%，且均携带 *optrA* 基因[39]。本次浙江地区分离株中 *optrA* 分布率为 33.82% (23/68)，但仅有 16.18% (11/68)的分离株对利奈唑胺耐药，推测有部分菌株 *optrA* 基因可能存在突变或无法正常表达。不同地区分离的猪链球菌对青霉素、头孢噻肟敏感性有所不同，Petrocchi-Rilo 等对 207 株西班牙猪链球菌分离株进行药敏试验，青霉素和头孢噻肟的耐药率分别为 26.2%和 17.5%[40]，O'Dea 等调查了近 7 年澳大利亚 143 株猪链球菌分离株，青霉素耐药率仅为 8.1%[41]。猪链球菌对 β-内酰胺类抗生素产生耐药，主要是由于编码青霉素结合蛋白(PBPs)相关基因发生突变，从而使这类抗生素与 PBPs 结合能力减弱[42]。本研究有 18 株菌对 β-内酰胺类抗生素耐药，其中 18 株菌对青霉素耐药，3 株菌对头孢噻肟耐药，但其耐药机制有待深入研究。利福平主要用于结核分枝杆菌及部分非结核分枝杆菌(肺炎链球菌、金黄色葡萄球菌)的治疗[43]。*rpoB* 基因的突变是链球菌属细菌对利福平产生抗性的主要原因，Chen 等发现 23 株肺炎链球菌利福平耐药菌株中，22 株菌存在 *rpoB* 基因的突变,有 2 株菌呈现高水平的利福平耐药[44]。猪链球菌对利福平耐药率较低，Wasteson 等对 21 株猪链球菌进行药敏试验，未发现利福平耐药[45]，最近本课题组在 75 株猪链球菌血清 31 型及血清 7 型菌株的药敏试验中，仅发现 1 株利福平耐药株[12,32]。本研究有 2 株分离株对利福平耐药。值得关注的是，本次分离的菌株都属于多药耐药，其中菌株 2020WUSS038 和 2021WUSS001 甚至表现出对 9 类抗生素耐药，但所有菌株都对阿莫西林及万古霉素敏感，这也为猪链球菌病的临床治疗提供了参考。

综上所述，该地区屠宰场来源的健康猪不仅猪链球菌携带率高，而且存在多药耐药和致病性强的菌株，这为了解该地区猪链球菌病的发病规律和制定相关的防控策略提供参考。

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