Characterization of florfenicol resistance genes in the coagulase-negative *Staphylococcus* (CoNS) isolates and genomic features of a multidrug-resistant *Staphylococcus lentus* strain H29

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Abstract

Background: With the wide use of florfenicol to prevent and treat the bacterial infection of domestic animals, the emergence of the florfenicol resistance bacteria is increasingly serious. It is very important to elucidate the molecular mechanism of the bacteria's resistance to florfenicol.

Methods: The minimum inhibitory concentration (MIC) levels was determined by the agar dilution method, and polymerase chain reaction (PCR) was conducted to analyze the distribution of florfenicol resistance genes in 39 CoNS strains isolated from poultry and livestock animals and seafood. The whole genome sequence of one multidrug-resistant strain, *Staphylococcus lentus* H29, was characterized, and comparative genomics analysis of the resistance gene-related sequences was also performed.

Results: As a result, the isolates from the animals showed a higher resistance rate (23/28, 82.1%) and much higher MIC levels of florfenicol than those from seafood. Twenty-seven animal isolates carried 37 florfenicol resistance genes (including 26 *fexA*, 6 *cfr* and 5 *fexB* genes), of which 1 carried a *cfr* gene, 16 carried a *fexA* gene, 5 carried both *fexA* and *fexB* genes and 5 carried both *fexA* and *cfr* genes. On the other hand, all 11 isolates from seafood were sensitive to florfenicol, and only 3 carried a *fexA* gene each. The whole genome sequence of *S. lentus* H29 was composed of a chromosome and two plasmids (pH29-46, pH29-26) and harbored 11 resistance genes, including 6 genes [*cfr, fexA, ant(6)-Ia*, *aacA-aphD, mecA* and *mph(C)*] encoded on the chromosome, four genes [*cfr, fexA, aacA-aphD* and *tcaA*] on pH29-46 and one

gene (*fosD*) on pH29-26. It was interested to find that the *S. lentus* H29 genome carried two identical copies of the gene arrays of *radC-tnpABC-hp-fexA* (5,671 bp) and IS*256*-*cfr* (2,690 bp), of which one copy of the two gene arrays was encoded on plasmid pH29-46, while the other was encoded on the chromosome.

Conclusions: The current study revealed the wide distribution of florfenicol resistance genes (*cfr, fexA* and *fexB*) in animal bacteria, and to the best of our knowledge, this is the first report of one CoNS strain carrying two identical copies of florfenicol resistance-related gene arrays.

Keywords: Coagulase-negative staphylococci; *Staphylococcus lentus;* florfenicol resistance genes; whole genome; comparative genomics analysis

1. Background

Coagulase-negative *Staphylococcus* (CoNS) are opportunistic pathogens that are found not only in animals and humans but also widely in the environment, including dust, soil, water and air. CoNS are also considered a repository of resistance genes, highlighting their threat to public health[1]. In poultry, CoNS infection can lead to arthritis, cow mastitis, and even systemic infections[2]. Florfenicol (FF) is an antimicrobial widely used in veterinary medicine that acts by binding to the 50S ribosomal subunit, leading to inhibition of protein synthesis. Because of its broad antibacterial activity and few adverse effects, florfenicol has been licensed exclusively for use in veterinary medicine to treat infections caused by, for example, *Pasteurella multocida*, *Staphylococcus* sp. and *Streptococcus* sp. in companion animals, farm animals and fish[3]. However, the increasing use of the antibiotics for the treatment and prevention of infectious diseases in animals has contributed to the emergence and widespread of florfenicol resistance genes among bacteria of different species or genera. Reports of multidrug-resistant CoNS are also increasing, and this increased resistance of CoNS to antibiotics also limits the choice of drugs to treat infections[4]. To date, a variety of florfenicol resistance mechanisms have been characterized, including efflux pumps (*floR*, *fexA/fexB* and *pexA/pexB*)[5-9], rRNA methyltransferase (*cfr*)[10], chloramphenicol hydrolase (*estDL136*)[11], chloramphenicol acyltransferases (*catA* or *catC*)[12] and ribosomal protection proteins (*optrA* and *poxtA*)[13, 14]. In CoNS, only *cfr*, *optrA*, *poxtA* and *fexA/fexB* have been identified. The gene *cfr* was initially found on the 17.1-kb plasmid pSCFS1

from an *S. sciuri* isolate and was shown to encode an rRNA methylase mediating resistance to phenicol by methylation of the 23S rRNA. In contrast, the gene *fexA*, which encodes an efflux protein within the major facilitator superfamily (MFS), was first identified on the 34-kb plasmid pSCFS2 from *S. lentus* and was shown to be part of the Tn*554*-like transposon Tn*558*. *fexB*, also a phenicol exporter gene, was first identified on the pEFM-1 (35 kb in size) of *E. faecium* and pEH-1 (25.3 kb in size) of *E. hirae*, both strains with swine origins. The genes *optrA* and *poxtA* encode ribosomal protection proteins of the ABC-F family. The gene *optrA* was first identified in *E. faecalis* and *E. faecium* and later found in various other gram-positive bacteria[15, 16], while *poxtA* was recently identified on the MRSA (methicillin-resistant *Staphylococcus aureus*) chromosome.

As a member of CoNS, *S. lentus* was traditionally considered to be an animal pathogen and has been isolated from a wide range of pets, farm animals, wild animals, and retail meats[17]. *S. lentus* has also been identified as the causative organism in several serious human infections, including endocarditis, peritonitis, septic shock, urinary tract infection, and wound infections, and its clinical significance is apparently increasing. In this work, in addition to detecting the florfenicol resistance levels and resistance genes of 39 *Staphylococcus* isolates from poultry and seafood, we also investigated the molecular mechanism of florfenicol resistance of a *S. lentus* strain with high level florfenicol resistance isolated from a hen. Through whole genome sequencing, we found, for the first time, two copies of the genes *cfr* and *fexA* colocalized on a plasmid as well as the chromosome of a bacterium.

2. Materials and Methods

2.1. Bacteria and antimicrobial susceptibility testing

CoNS strains were isolated from fresh fecal samples of ducks, cows, chickens and pigs collected from several farms in Sichuan, Zhejiang, Shanxi, Shandong and Henan provinces, China, in 2016 and from fresh seafood intestinal contents from Wenzhou, Zhejiang, China, in 2018. The isolates were identified by Gram's staining and serum coagulase testing in strict accordance with experimental procedures and verified by homology comparisons of the 16S rRNA genes. Antimicrobial susceptibility was evaluated by the agar dilution method following the guidelines recommended by the Clinical and Laboratory Standards Institute (CLSI, 2017: M100 https://clsi.org/ standards/). The MIC of linezolid was determined by the agar dilution method according to the European Committee on Antimicrobial Susceptibility Testing (EUCAST, www.eucast.org). *S. aureus* ATCC29213 was used as a control strain.

2.2. Clonal relationship analysis of the strains resistant to florfenicol

To examine the clonal relatedness of the florfenicol-resistant strains, we used PFGE to perform molecular typing for the 23 florfenicol resistance gene-positive strains (florfenicol MIC ≥32 μg/mL). Genomic DNA from 23 isolates was digested with 40 U of *Sma*I (Takara, Dalian, China). *Sma*I restriction patterns of the isolates were analyzed and interpreted according to initial criteria. The Bio-Rad Quantity One program was used to analyze the PFGE results, and a minimum spanning tree was constructed using a categorical coefficient with the unweighted pair group method with arithmetic mean (UPGMA) clustering.

2.3. Detection of florfenicol resistance genes

The florfenicol resistance genes (*fexA, fexB, cfr, optrA, pexA* and *floR*) were detected by PCR with the primers previously reported (Table 1). Genomic DNA was extracted from each of the 39 isolates using the AxyPrep Bacterial Genomic DNA Miniprep kit (Axygen Scientific, Union City, CA, USA) and was used as the template for PCR amplification. Positive amplification products were verified by sequencing with an ABI 3730 automated sequencer (Shanghai Sunny Biotechnology Co., Ltd., Shanghai, China), and the sequencing results were compared with BLAST against the corresponding resistance gene sequences in NCBI nucleotide database (https://blast.ncbi.nlm.nih.gov/blast.cgi).

Table 1. Primer sequences and PCR product sizes of the florfenicol resistance genes

Primer	Sequence (5'-3')	Amplicon size (bp)	Annealing temperature (℃)	Reference
*floR*1-F	ATGACCACCACACGCCCCGCGTGGGC	1198	58	[7]
*floR*1-R	CTTCGATCCCGCGACGTTCCTTCCGAGA			
*fexA*1-F	CTCTTCTGGACAGGCTGGAA	332	57	[6]
*fexA*1-R	CCAGTTCCTGCTCCAAGGTA			
*fexB*1-F	ACTGGACAGGCAGGCTTAAT	319	57	[8]
*fexB*1-R	CCTGCCCCAAGATACATTGC			
*cfr*1-F	GGGAGGATTTAATAAATAATTTTGGAGAAACA G	580	58	[7]
*cfr*1-R	CTTATATGTTCATCGAGTATATTCATTACCTCAT C			
*optrA*1-F	CTTATGGATGGTGTGGCAGC	309	56	[11]
*optrA*1-R	CCATGTGGTTTGTCGGTTCA			

*pexA*1-F	GTTGTGGTCTTTGGCCAGAG	318	56	[9]
*pexA*1-R	TCCATCAAGAGGACACCACC			

2.4. Sequencing and annotation of the *S. lentus* H29 genome

Genomic DNA was extracted from *S. lentus* H29 as mentioned above and sequenced with Illumina HiSeq 2500 and Pacific Bioscience sequencers at Annoroad Gene Technology Co., Ltd. (Beijing, China). Pacific Bioscience sequencing reads of approximately 10-20 kb in length were assembled by Canu v1.2[18]. Two FASTQ sequence files corresponding to the reads derived from HiSeq 2500 sequencing were used to control assembly quality and to correct possible misidentified bases. Glimmer3.02 software with default parameters was used to predict potential open reading frames (ORFs). ORF annotation was determined by performing BLASTX comparisons with the NCBI nonredundant protein database. Comparisons of nucleotide sequences and amino acid sequences were performed by BLASTN and BLASTP, respectively[19]. BLASTp was applied to compare amino acid sequences with those in the Antibiotic Resistance Genes Database (ARDB https://card.mcmaster.ca/). The map of the plasmid with GC content and GC skew was drawn with the online CGView Server (http://stothard.afns.ualberta.ca/cgview_server/) and local GView 1.7 with a visual interface[20]. The plasmid sequences used in this study were downloaded from the NCBI database (http://www.ncbi.nlm.nih.gov). The rRNA gene sequences were annotated by the online tool RNAmmer (http://www.cbs.dtu.dk/services/RNAmmer/)[21], and the tRNA sequences were

annotated by the online tool tRNAscan-SE 2.0 (http://lowelab.ucsc.edu/tRNAscan-SE/)[22]. Promoter sites were predicted by using Soft Berry BPROM software (http://linux1.softberry.com/berry.phtmltopic=bprom&group=programs&subgroup=gfindb).

2.5. Comparative genomics analysis

Sequences containing resistance genes were obtained from the NCBI nucleotide database by the BLASTN program using the resistance gene sequences of *S. lentus* H29 as the query. The resulting sequences were filtered, and only sequences containing complete resistance genes were retained. CD-HIT was used to cluster the retained sequences using the genome sequence of *S. lentus* H29 as the reference with an identity of ≥ 90%. The sequence sharing the greatest similarity to the other sequences in each cluster was chosen as the candidate for ortholog analysis. Orthologous groups of the genes from the candidate sequences were identified using BLASTP[19]. Sequence retrieval, statistical analysis and other bioinformatics tools used in this study were applied with Perl and Bioperl scripts (http://www.perl.org/).

3. Results and Discussions

3.1. Bacterial strains and antimicrobial susceptibility testing

A total of 39 CoNS strains including 9 species were analyzed in this work (Table S1). Among them, 28 strains were isolated from animal feces and 11 strains were isolated from the seafood intestinal contents. The strains included *S. epidermidis* (4),

S. lentus (2), *S. equorum* (6), *S. saprophyticus* (7), *S. sciuri* (4), *S. haemolyticus* (3), *S. gallinarum* (2), *S. cohnii* (3), *S. warneri* (4) and 4 unclassified ones. The *S. saprophyticus* strains, with the most isolates, were isolated from both the animals and seafood, which was in accordance with the statistics reported[23]. *S. epidermis* is most commonly isolated from humans[24], while in this work, it was present in the animals as well as seafood. The results of the antimicrobial susceptibility testing of the strains to 21 antimicrobial agents showed that the strains isolated from the animals generally showed wider resistance spectra and higher MIC levels than those isolated from seafood. More than 60% (17/28) of the animal strains showed resistance to 6 antibiotics, including FFC (82.1%, 23/28), CHL (85.8%, 24/28), CLI (75.0%, 21/28), TET (67.9%, 19/28), STR (64.3%, 18/28) and ERY (60.7%, 17/28), while the seafood bacteria were only resistant to ERY (63.6%, 7/11) (Table 2, Table S2). Although most antibiotic resistance rates against the animal CoNS isolates were similar to those previously reported, the resistance rates for CLR (39.3%, 11/28) and FD (36.7%, 10/28) were higher in this study than those in recent publications[25], which may indicate the abused use of the drugs in local livestock husbandry. Meanwhile, more than 90% of the animal isolates were sensitive to eight other antibiotics, especially AMK, TMP and TGC with all the strains sensitive to them. However, the seafood isolates only showed certain resistance rates to ERY (63.6%, 7/11) and CLI (36.4%, 4/11), and most strains were totally sensitive to some antibiotics, such as LZD, FOX, VAN and NOR (Table 2).

Table 2 is in line 550, page 25.

3.2. Identification of florfenicol resistance genes in the CoNS isolates

In *Staphylococcus*, florfenicol resistance has been reported to be mainly mediated by *cfr*, *fexA*, *fexB, optrA*, and *poxtA*[26]. In this work, of all 6 florfenicol resistance-related genes (*fexA*, *cfr, optrA, floR, fexB* and *pexA*), only 3 (*fexA, cfr* and *fexB*) were identified in the 39 *Staphylococcus* strains. A total of 37 genes, including 26 *fexA, 6 cfr* and 5 *fexB* genes, were identified in 27 strains, with one (*S. cohnii* H19) and 16 strains with a *cfr* and a *fexA* gene, respectively, 5 strains carrying both *fexA* and *cfr* genes, and other 5 isolates harboring both *fexA* and *fexB* genes, while the remaining twelve strains were free of the resistance gene. Many studies have reported that *fexA* is the most common florfenicol resistance gene in household animals in rural China[4, 9, 27]. In this study, the *fexA* gene occupied 70.3% (26/37) of the florfenicol resistance genes. Strains from animals presented a much higher positive rate and carried much more resistance genes, with 82.1% (23/28) of the strains carrying 91.9% (34/37) of the resistance genes, while in the seafood bacteria, only three strains (3/11, 27.3%) carried one *fexA* gene each (3/37, 8.1%). All 23 florfenicol-resistant isolates (florfenicol MIC level ≥ 32 μg/mL) were isolated from animals, and they all carried two (*fexA* and *fexB*) or one (*fexA*) florfenicol resistance gene. Among the 16 florfenicol-sensitive isolates (MIC ≤ 1 μg/mL), 12 were free of the florfenicol resistance gene, and 3 (HXM5, HXM10 and HXM13 all isolated from seafood) carried a *fexA* gene and one strain from poultry with a *cfr* gene. Among the 5 isolates

that carried both *fexA* and *cfr*, two strains (*S. sciuri* FC11 and *S. haemolyticus* FC24) showed an MIC value of 8 μg/mL to linezolid, which was interpreted as an intermediate for linezolid, while the other three strains showed MIC values of ≤ 0.25 μg/mL for linezolid. According to previous reports, linezolid resistance were related with ATP-binding cassette transporter gene *optrA* and it has been identified in bacteria of the animal origin[28, 29]. However, in this work, the *optrA* gene has not been identified in these strains. This may indicate that other mechanisms rather than *optrA* conferring the low-level linezolid resistance might exist in these two bacteria.

3.3. Clonal relatedness of the florfenicol-resistant CoNS isolates

Clonal relationship analysis for 23 florfenicol-resistant strains (MIC ≥ 32 μg/mL) revealed that no clonal relatedness was identified among them, including the strains of the same species (Fig. 1). The highest similarity of 63% was observed between two strains of different species, *S. equorum* (H37) and *S. haemolyticus* (FP36), which were isolated from different hosts (hen and pig, respectively).

3.4. General features of the *S. lentus* H29 genome

To analyze the molecular characteristics of the florfenicol-resistant CoNS strains, *S. lentus* H29, co-carrying *fexA* and *cfr* with a wide resistance spectrum and high MIC values to the antibiotics tested, was chosen for whole genome sequencing (WGS) analysis, and the general features of the H29 genome are shown in Table 3. The complete genome of *S. lentus* H29 consists of one chromosome and two plasmids (pH29-46 and pH29-26). The chromosome was 2,802,282 bp in length, encoded 2,683 ORFs and had a G+C content of 31.9%. pH29-46 was 46,167 bp in length and

encoded 46 ORFs, and pH29-26 was 26,210 bp in length and encoded 26 ORFs. At present, except for *S. lentus* H29, no complete genome sequence of *S. lentus* is available in the NCBI nucleotide database. The whole genome of *S. lentus* H29 encoded 11 resistance genes, of which 6 [*cfr, fexA, ant(6)-Ia*, *aacA-aphD, mecA* and *mph(C)*] were encoded on the chromosome, 4 [*cfr, fexA, aacA-aphD* and Δ*tcaA*] on pH29-46 and 1 (*fosD*) on pH29-26. The resistance phenotypes coincided with the resistance genotypes (Table 4). In addition to showing resistance to florfenicol (MIC of 256 μg/mL) and chloramphenicol (MIC of 256 μg/mL), *S. lentus* H29 was also resistant to erythromycin (>64 μg/mL) and macrolide antibiotics.

Table 3. General characteristics of the *S. lentus* H29 genome

	Chromosome	pH29-46	pH29-26
Size (bp)	2,802,282	46,167	26,210
GC content (%)	31.90	29.73	31.94
Predicted CDs	2,741	46	30
Known proteins	1,929	33	20
Hypothetical proteins	812	13	10
Protein coding sequences (%)	87.30	82.33	87.54
Average ORF length (bp)	892	719	878

Table 4. Antimicrobial resistance determinants in *S.lentus* H29

Antibiotics class	Antibiotics tested	MIC (μg/mL)	Interpretation	Resistance genes
Macrolide	erythromycin	>64	R	*erm(ABC)*
lincosamide	clindamycin	>64	R	
	clarithromycin	>64	R	
	streptomycin	64	R	
Aminoglycosides	gentamycin	4	S	*aac-aph*, *ant-Ia*
	amikacin	4	S	
	kanamycin	>64	R	
β-lactam	cefoxitin	2	R	*mecA*, *mecC*
	oxacillin	2	R	
Fusidic Acid	Fusidic Acid	1	S	
Rifampicin	Rifampin	>64	R	*rpoB*
FLuoroquinolones	norfloxacin	>64	R	*norA*
	levofloxacin	4	R	*gyrA*, *gyrB*
Phenicol	Chloramphenicol	256	R	*cml*
	Florfenicol	256	R	*cfr*, *fexA*
Sulfonamides/ Trimethoprim	Sulfonamides/ Trimethoprim	1	S	
Tetracycline	Tetracycline	64	R	*tet(K), tet(L)*
	Tigecycline	2	S	
oxazolidinones	Linezolid	<0.125	S	
Glycopeptides	Vancomycin	2	S	
	Teicoplanin	0.5	S	

3.5. Comparative genomics analysis of the resistance plasmids and the *fexA*- and *cfr*-related sequences in the *S. lentus* H29 genome

Three plasmids, pSX01 (NZ_KP890694.1) of *Staphylococcus xylosus* 378, pSR01 (NZ_CP019564.1) of *Staphylococcus aureus* strain SR434 and pLRSA417 (KJ922127.1) of *Staphylococcus aureus* 417, sharing the highest nucleotide sequence similarities (coverage > 70%, identities ≥ 97%) with pH29-46 were retrieved from the NCBI nucleotide database. According to the structure and function of the genes encoded on the plasmid, pH29-46 could be divided into two regions (Regions A and B, Fig. 2). Region A was about 26 kb in size encoding the backbone genes, mainly including a replication gene *repA*, a segregation gene *parM*, 16 T4SS genes and several hypothetical protein genes, and it displayed 98~100% identity to the corresponding regions of the plasmids pSR01 and pLRSA417. Region B, about 20 kb in length, harbored five resistance genes, which could be divided into two segments. One segment (about 7.5 kb in length) included the *tnpABC* and *fexA* genes, which were not present in the three plasmids from the database. The other segment was a 12.5 kb sequence encoding the resistance genes of *cfr*, *aacA-aphD* and *tcaA*, and three copies of IS*256* showing 99% identity and 80% coverage to the sequence on pSR01 and pLRSA417.

It was interested to find that the *S. lentus* H29 genome carried two identical copies of the gene arrays of *radC-tnpABC*-hp-*fexA* (5,671 bp) and IS256-*cfr* (2,690 bp), of which one copy was encoded on plasmid pH29-46, while the other was encoded on the chromosome. To the best of our knowledge, this is the first case that the combination of the mobile genetic element related *cfr* (IS*256-cfr)* and *fexA* (*tnpABC-hp-fexA*) was identified in both the plasmid (pH29-46) and the chromosome

of an isolate *S. lentus* H29, respectively, even though this combination has been identified in several other plasmids such as pSS-01 of *S. cohnii.* (JQ041372.1) and either IS*256-cfr* or *tnpABC-hp-fexA*) has been identified encoded in plasmids or chromosomes in other *Staphylococcus* strains of different sources(Fig. 3). These findings indicate that the *cfr* and *fexA* genes encoded on pH29-46 and the MGEs carrying them can be horizontally transferred between strains of different species, causing the spread of drug resistance. On the other hand, these MGE-related florfenicol resistance genes identified in CoNS of different origins (such as those isolated from animals and humans) demonstrate the threat of the use of antibiotics in animals to human health.

Conclusions

In this work, the animal CoNS isolates showed resistance to multiple antibiotics, including florfenicol, chloramphenicol, tetracycline, erythromycin, streptomycin, clindamycin and other common veterinary antibiotics, while seafood-derived isolates were much less resistant to these antibiotics. The main molecular mechanism that makes the CoNS isolates resistant to florfenicol is the *fexA, fexB* and *cfr* genes they carry. It was interesting to find that one isolate *S. lentus* H29 harbored two identical copies of the gene arrays that carried either a *fexA* or a *cfr* gene, with one copy on a plasmid and the other on the chromosome. Genetic structure analysis of the *fexA* and *cfr* gene-related sequences indicated that these florfenicol resistance genes were related to mobile genetic elements and located on both plasmids and chromosomes

among different Staphylococci species. These findings indicate that the resistance genes in Staphylococci may be transmitted between different Staphylococci species through horizontal gene transfer, causing widespread florfenicol and chloramphenicol resistance.

Abbreviations

CoNS, Coagulase-negative *Staphylococcus*; BLAST, The Basic Local Alignment Search Tool; MIC, Minimum Inhibitory Concentration; PFGE, Pulsed-field gel electrophoresis. PCR: polymerase chain reaction.

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Authors' contributions

CW, XZ, JL, QL, HL, CL, WL, XL and HZ collected the strains and performed the experiments. JL, HL, DZ, ZS, KL and TX analyzed the experimental results. JL, ZS, TX and JL performed the bioinformatics analysis. CW, XZ and QB co-led the writing of the manuscript. TX, QB and JL designed the work. All authors read and approved the final manuscript

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Availability of data and materials

All data generated or analyzed during this study are included in this published article and its supplementary information files. The data related to the paper are deposited in the NCBI GenBank. The accession numbers (available soon) for the chromosome, pH29-46 and pH29-26 are XXXX, XXX and XXX, respectively.

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Competing interests

The authors declare that there are no conflicts of interest in this work.

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Figure legends

Figure 1. PFGE patterns of 23 florfenicol-resistant CoNS isolates.

Figure 2. Genetic map of pH29-46 and its comparison with other plasmids of the highest nucleotide sequence similarities. From the outside to the inside: circle 1, pH29-46 region A in purple and region B in green; circle 2, pSX01 (the plasmid of *S. xylosus* strain 378 isolated from pig, NZ_KP890694.1); circle 3, pSR01 (the plasmid of *S. aureus* strain SR434 isolated from human, NZ_CP019564.1); circle 3, pLRSA417 (*S. aureus* strain 417 isolated from human, KJ922127.1); circle 4, pH29-46 with genes encoded on the two strands. The red arrows indicate drug-resistant genes, blue arrows indicate transfer genes and the gray arrows indicate the genes encoding hypothetical proteins.

Figure 3. Genetic environments of the *fexA* and *cfr* genes encoded in plasmids or chromosomes. The sequences and their origins are: *S. lentus* S. LQQ24 chr (the chromosome of *S. lentus* S. LQQ24 isolated from chichen in China, KF029594.1), *S. sciuri* wo227 chr (the chromosome of *S. sciuri* wo227 isolated from swine, KX982170.1), *S. lentus* H29 chr (the chromosome of H29 isolated from hen of this work, XXXXX), *S. lentus* H29 pH29-46 (the plasmid of pH29-46 isolated from a hen of this work, XXXX), *S. cohnii* pSS-01 (the plasmid of S. cohnii SS-01 isolated from swine, JQ041372.1), *S.aureus* BA01611 chr (the chromosome of *S.aureus* BA01611 isolated from bovine, CP019945.1), *S.aureus* QD-CD9 chr (the chromosome of

S.aureus QD-CD9 isolated from in swine, CP031838.1). Antimicrobial resistance genes are in red, transposase or integrase genes are in blue and other genes are in gray. Gray-shaded areas represent regions with > 95% nucleotide sequence identities. The arrows indicate the positions and orientations of the genes.

Supplementary Materials

Supplementary Table S1. Resistance phenotype and florfenicol resistance genes of the CoNS isolates.

Supplementary Table S2. Antibiotics resistance profile of all 39 CoNS isolates.

Table 2. Characterization of the sensitivity of 39 CoNS isolates to 21 antibiotics

	Animal (N=28)			Seafood (N=11)			Total (N=39)		
Antibiotics	S	I	R	S	I	R	S	I	R
LZD	24 (85.8%)	2 (7.1%)	2 (7.1%)	11 (100%)	0 (0)	0 (0%)	35（89.8%）	2（5.1%）	2（5.1%）
FD	18 (64.3%)	0 (0)	10 (36.7%)	8 (72.7%)	0 (0)	3 (27.3%)	26（66.7%）	0 (0)	13（33.3%）
CLI	7 (25.0%)	0 (0)	21(75.0%)	7 (63.6%)	0 (0)	4 (36.4%)	14（35.9%）	0 (0)	25（64.1%）
AMK	28 (100%)	0 (0)	0 (0)	11 (100%)	0 (0)	0 (0)	39（100%）	0 (0)	0 (0)
ERY	11 (39.3%)	0 (0)	17 (60.7%)	4 (36.4%)	0 (0)	7 (63.6%)	15（38.5%）	0 (0)	24（61.5%）
GEN	27 (96.4%)	0 (0)	1 (4.6%)	11 (100%)	0 (0)	0 (0)	38（97.4%）	0 (0)	1（2.6%）
OXA	24(86.%)	0 (0)	4 (14%)	9 (81.8%)	0 (0)	2 (18.2%)	33（84.6%）	0 (0)	6（15.4%）
FOX	26 (93%)	0 (0)	2 (7%)	11 (100%)	0 (0)	0 (0)	37（94.9%）	0 (0)	2（5.1%）
RIF	24 (85.8%)	0 (0)	4 (14.2%)	11 (100%)	0 (0)	0 (0)	35（89.8%）	0 (0)	4（10.2%）

TMP	28 (100%)	0 (0)	0 (0%)	11 (100%)	0 (0)	0 (0)	39（100.0%）	0 (0)	0 (0)
TET	9 (32.1%)	0 (0)	19 (67.9%)	9 (81.8%)	0 (0)	2 (18.2%)	18（46.2%）	0 (0)	21（53.8%）
VAN	27 (96.4%)	0 (0)	1 (3.6%)	11 (100%)	0 (0)	0 (0)	38（97.4%）	0 (0)	1（2.6%）
CLR	17 (60.7%)	0 (0)	11 (39.3%)	8 (72.7%)	0 (0)	3 (27.2%)	25（64.1%）	0 (0)	14（35.9%）
CHL	4 (14.2%)	0 (0)	24 (85.8%)	10 (90.9%)	0 (0)	1 (9.1%)	14（35.9%）	0 (0)	25（64.1%）
LVX	21 (75.0%)	0 (0)	7 (25.0%)	10 (90.9%)	0 (0)	1 (9.1%)	31（79.5%）	0 (0)	8（20.5%）
NOR	23 (82.1%)	0 (0)	5 (17.9%)	11 (100%)	0 (0)	0 (0)	34（87.2%）	0 (0)	5（12.8%）
KAN	21 (75.0%)	0 (0)	7 (25.0%)	9 (81.8%)	0 (0)	2 (18.2%)	30（76.9%）	0 (0)	9（23.1%）
TGC	28 (100%)	0 (0)	0 (0)	11 (100%)	0 (0)	0 (0)	39（100%）	0 (0)	0 (0)
TEC	27 (96.4%)	0 (0)	1 (4.6%)	11 (100%)	0 (0)	0 (0)	38（97.4%）	0 (0)	1（2.6%）
STR	10 (35.7%)	0 (0)	18 (64.3%)	10 (90.9%)	0 (0)	1 (9.1%)	20（51.3%）	0 (0)	19（48.7%）
FFC	5 (17.9%)	0 (0)	23 (82.1%)	11(100%)	0 (0)	0 (0)	16（41.0%）	0 (0)	23（59.0%）

552 LZD, Linezolid; FD, Fusidic Acid; OXA, Oxacillin; TGC, Tigecycline; LVX, Levofloxacin; FOX, Cefoxitin; TMP, Trimethopim; CHL, Chloramphenicol; TEC,

teicoplanin; FFC, Florfenicol; CLR, Clarithromycin; CLI, Clindamycin; RIF, Rifampin; NOR, Norfloxacin; VAN, Vancomycin; GEN, Gentamycin; TET, Tetracycline; STR, Streptomycin; AMK, Amikacin; KAN, Kanamycin; ERY, Erythromycin.