

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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Research

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

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43 **Abstract**

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

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

55 **Results:** As a result, the isolates from the animals showed a higher resistance rate
56 (23/28, 82.1%) and much higher MIC levels of florfenicol than those from seafood.
57 Twenty-seven animal isolates carried 37 florfenicol resistance genes (including 26
58 *fexA*, 6 *cfr* and 5 *fexB* genes), of which 1 carried a *cfr* gene, 16 carried a *fexA* gene, 5
59 carried both *fexA* and *fexB* genes and 5 carried both *fexA* and *cfr* genes. On the other
60 hand, all 11 isolates from seafood were sensitive to florfenicol, and only 3 carried a
61 *fexA* gene each. The whole genome sequence of *S. lentus* H29 was composed of a
62 chromosome and two plasmids (pH29-46, pH29-26) and harbored 11 resistance genes,
63 including 6 genes [*cfr*, *fexA*, *ant(6)-Ia*, *aacA-aphD*, *mecA* and *mph(C)*] encoded on
64 the chromosome, four genes [*cfr*, *fexA*, *aacA-aphD* and *tcaA*] on pH29-46 and one

65 gene (*fosD*) on pH29-26. It was interested to find that the *S. lentus* H29 genome
66 carried two identical copies of the gene arrays of *radC-tnpABC-hp-fexA* (5,671 bp)
67 and *IS256-cfr* (2,690 bp), of which one copy of the two gene arrays was encoded on
68 plasmid pH29-46, while the other was encoded on the chromosome.

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

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

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87 **1. Background**

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

109 from an *S. sciuri* isolate and was shown to encode an rRNA methylase mediating
110 resistance to phenicol by methylation of the 23S rRNA. In contrast, the gene *fexA*,
111 which encodes an efflux protein within the major facilitator superfamily (MFS), was
112 first identified on the 34-kb plasmid pSCFS2 from *S. lentus* and was shown to be part
113 of the Tn554-like transposon Tn558. *fexB*, also a phenicol exporter gene, was first
114 identified on the pEFM-1 (35 kb in size) of *E. faecium* and pEH-1 (25.3 kb in size) of
115 *E. hirae*, both strains with swine origins. The genes *optrA* and *poxA* encode
116 ribosomal protection proteins of the ABC-F family. The gene *optrA* was first
117 identified in *E. faecalis* and *E. faecium* and later found in various other gram-positive
118 bacteria[15, 16], while *poxA* was recently identified on the MRSA
119 (methicillin-resistant *Staphylococcus aureus*) chromosome.

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

131 **2. Materials and Methods**

132 2.1. Bacteria and antimicrobial susceptibility testing

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

144 2.2. Clonal relationship analysis of the strains resistant to florfenicol

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

153 2.3. Detection of florfenicol resistance genes

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

163

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

Primer	Sequence (5'-3')	Amplicon size (bp)	Annealing temperature (°C)	Reference
<i>floR1</i> -F	ATGACCACCACACGCCCCGCGTGGGC	1198	58	[7]
<i>floR1</i> -R	CTTCGATCCCGCGACGTTCTCCGAGA			
<i>fexA1</i> -F	CTCTTCTGGACAGGCTGGAA	332	57	[6]
<i>fexA1</i> -R	CCAGTTCCTGCTCCAAGGTA			
<i>fexB1</i> -F	ACTGGACAGGCAGGCTTAAT	319	57	[8]
<i>fexB1</i> -R	CCTGCCCCAAGATACATTGC			
	GGGAGGATTTAATAAATAATTTTGGAGAAACA			
<i>cfr1</i> -F	G	580	58	[7]
	CTTATATGTTTCATCGAGTATATTCATTACCTCAT			
<i>cfr1</i> -R	C			
<i>optrA1</i> -F	CTTATGGATGGTGTGGCAGC	309	56	[11]
<i>optrA1</i> -R	CCATGTGGTTTGTTCGGTTCA			

<i>pexA1</i> -F	GTTGTGGTCTTTGGCCAGAG	318	56	[9]
<i>pexA1</i> -R	TCCATCAAGAGGACACCACC			

165 2.4. Sequencing and annotation of the *S. lentus* H29 genome

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

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

190 2.5. Comparative genomics analysis

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

201

202 3. Results and Discussions

203 3.1. Bacterial strains and antimicrobial susceptibility testing

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

207 *S. lentus* (2), *S. equorum* (6), *S. saprophyticus* (7), *S. sciuri* (4), *S. haemolyticus* (3), *S.*
208 *gallinarum* (2), *S. cohnii* (3), *S. warneri* (4) and 4 unclassified ones. The *S.*
209 *saprophyticus* strains, with the most isolates, were isolated from both the animals and
210 seafood, which was in accordance with the statistics reported[23]. *S. epidermis* is
211 most commonly isolated from humans[24], while in this work, it was present in the
212 animals as well as seafood. The results of the antimicrobial susceptibility testing of
213 the strains to 21 antimicrobial agents showed that the strains isolated from the animals
214 generally showed wider resistance spectra and higher MIC levels than those isolated
215 from seafood. More than 60% (17/28) of the animal strains showed resistance to 6
216 antibiotics, including FFC (82.1%, 23/28), CHL (85.8%, 24/28), CLI (75.0%, 21/28),
217 TET (67.9%, 19/28), STR (64.3%, 18/28) and ERY (60.7%, 17/28), while the seafood
218 bacteria were only resistant to ERY (63.6%, 7/11) (Table 2, Table S2). Although most
219 antibiotic resistance rates against the animal CoNS isolates were similar to those
220 previously reported, the resistance rates for CLR (39.3%, 11/28) and FD (36.7%,
221 10/28) were higher in this study than those in recent publications[25], which may
222 indicate the abused use of the drugs in local livestock husbandry. Meanwhile, more
223 than 90% of the animal isolates were sensitive to eight other antibiotics, especially
224 AMK, TMP and TGC with all the strains sensitive to them. However, the seafood
225 isolates only showed certain resistance rates to ERY (63.6%, 7/11) and CLI (36.4%,
226 4/11), and most strains were totally sensitive to some antibiotics, such as LZD, FOX,
227 VAN and NOR (Table 2).

228 Table 2 is in line 550, page 25.

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231 3.2. Identification of florfenicol resistance genes in the CoNS isolates

232 In *Staphylococcus*, florfenicol resistance has been reported to be mainly
233 mediated by *cfr*, *fexA*, *fexB*, *optrA*, and *poxtA*[26]. In this work, of all 6 florfenicol
234 resistance-related genes (*fexA*, *cfr*, *optrA*, *floR*, *fexB* and *pexA*), only 3 (*fexA*, *cfr* and
235 *fexB*) were identified in the 39 *Staphylococcus* strains. A total of 37 genes, including
236 26 *fexA*, 6 *cfr* and 5 *fexB* genes, were identified in 27 strains, with one (*S. cohnii* H19)
237 and 16 strains with a *cfr* and a *fexA* gene, respectively, 5 strains carrying both *fexA*
238 and *cfr* genes, and other 5 isolates harboring both *fexA* and *fexB* genes, while the
239 remaining twelve strains were free of the resistance gene. Many studies have reported
240 that *fexA* is the most common florfenicol resistance gene in household animals in rural
241 China[4, 9, 27]. In this study, the *fexA* gene occupied 70.3% (26/37) of the florfenicol
242 resistance genes. Strains from animals presented a much higher positive rate and
243 carried much more resistance genes, with 82.1% (23/28) of the strains carrying 91.9%
244 (34/37) of the resistance genes, while in the seafood bacteria, only three strains (3/11,
245 27.3%) carried one *fexA* gene each (3/37, 8.1%). All 23 florfenicol-resistant isolates
246 (florfenicol MIC level ≥ 32 $\mu\text{g/mL}$) were isolated from animals, and they all carried
247 two (*fexA* and *fexB*) or one (*fexA*) florfenicol resistance gene. Among the 16
248 florfenicol-sensitive isolates (MIC ≤ 1 $\mu\text{g/mL}$), 12 were free of the florfenicol
249 resistance gene, and 3 (HXM5, HXM10 and HXM13 all isolated from seafood)
250 carried a *fexA* gene and one strain from poultry with a *cfr* gene. Among the 5 isolates

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

259 3.3. Clonal relatedness of the florfenicol-resistant CoNS isolates

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

265 3.4. General features of the *S. lentus* H29 genome

266 To analyze the molecular characteristics of the florfenicol-resistant CoNS strains,
267 *S. lentus* H29, co-carrying *fexA* and *cfr* with a wide resistance spectrum and high MIC
268 values to the antibiotics tested, was chosen for whole genome sequencing (WGS)
269 analysis, and the general features of the H29 genome are shown in Table 3. The
270 complete genome of *S. lentus* H29 consists of one chromosome and two plasmids
271 (pH29-46 and pH29-26). The chromosome was 2,802,282 bp in length, encoded
272 2,683 ORFs and had a G+C content of 31.9%. pH29-46 was 46,167 bp in length and

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

282

283 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

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

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

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296 3.5. Comparative genomics analysis of the resistance plasmids and the *fexA*- and

297 *cfr*-related sequences in the *S. lentus* H29 genome

298 Three plasmids, pSX01 (NZ_KP890694.1) of *Staphylococcus xylosus* 378,
299 pSR01 (NZ_CP019564.1) of *Staphylococcus aureus* strain SR434 and pLRSA417
300 (KJ922127.1) of *Staphylococcus aureus* 417, sharing the highest nucleotide sequence
301 similarities (coverage > 70%, identities \geq 97%) with pH29-46 were retrieved from the
302 NCBI nucleotide database. According to the structure and function of the genes
303 encoded on the plasmid, pH29-46 could be divided into two regions (Regions A and
304 B, Fig. 2). Region A was about 26 kb in size encoding the backbone genes, mainly
305 including a replication gene *repA*, a segregation gene *parM*, 16 T4SS genes and
306 several hypothetical protein genes, and it displayed 98~100% identity to the
307 corresponding regions of the plasmids pSR01 and pLRSA417. Region B, about 20 kb
308 in length, harbored five resistance genes, which could be divided into two segments.
309 One segment (about 7.5 kb in length) included the *tnpABC* and *fexA* genes, which
310 were not present in the three plasmids from the database. The other segment was a
311 12.5 kb sequence encoding the resistance genes of *cfr*, *aacA-aphD* and *tcaA*, and three
312 copies of IS256 showing 99% identity and 80% coverage to the sequence on pSR01
313 and pLRSA417.

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

320 of an isolate *S. lentus* H29, respectively, even though this combination has been
321 identified in several other plasmids such as pSS-01 of *S. cohnii*. (JQ041372.1) and
322 either IS256-*cfr* or *tnpABC-hp-fexA*) has been identified encoded in plasmids or
323 chromosomes in other *Staphylococcus* strains of different sources(Fig. 3). These
324 findings indicate that the *cfr* and *fexA* genes encoded on pH29-46 and the MGEs
325 carrying them can be horizontally transferred between strains of different species,
326 causing the spread of drug resistance. On the other hand, these MGE-related
327 florfenicol resistance genes identified in CoNS of different origins (such as those
328 isolated from animals and humans) demonstrate the threat of the use of antibiotics in
329 animals to human health.

330

331 **Conclusions**

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

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

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348 **Abbreviations**

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

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354 contributed for the study.

355 **Authors' contributions**

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

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366 **Availability of data and materials**

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

371 **Ethics approval and consent to participate**

372 Not applicable.

373 **Consent for publication**

374 Not applicable.

375 **Competing interests**

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

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518 Figure legends

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

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

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

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

544

545 **Supplementary Materials**

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

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

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

Antibiotics	Animal (N=28)			Seafood (N=11)			Total (N=39)		
	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,

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

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Figures

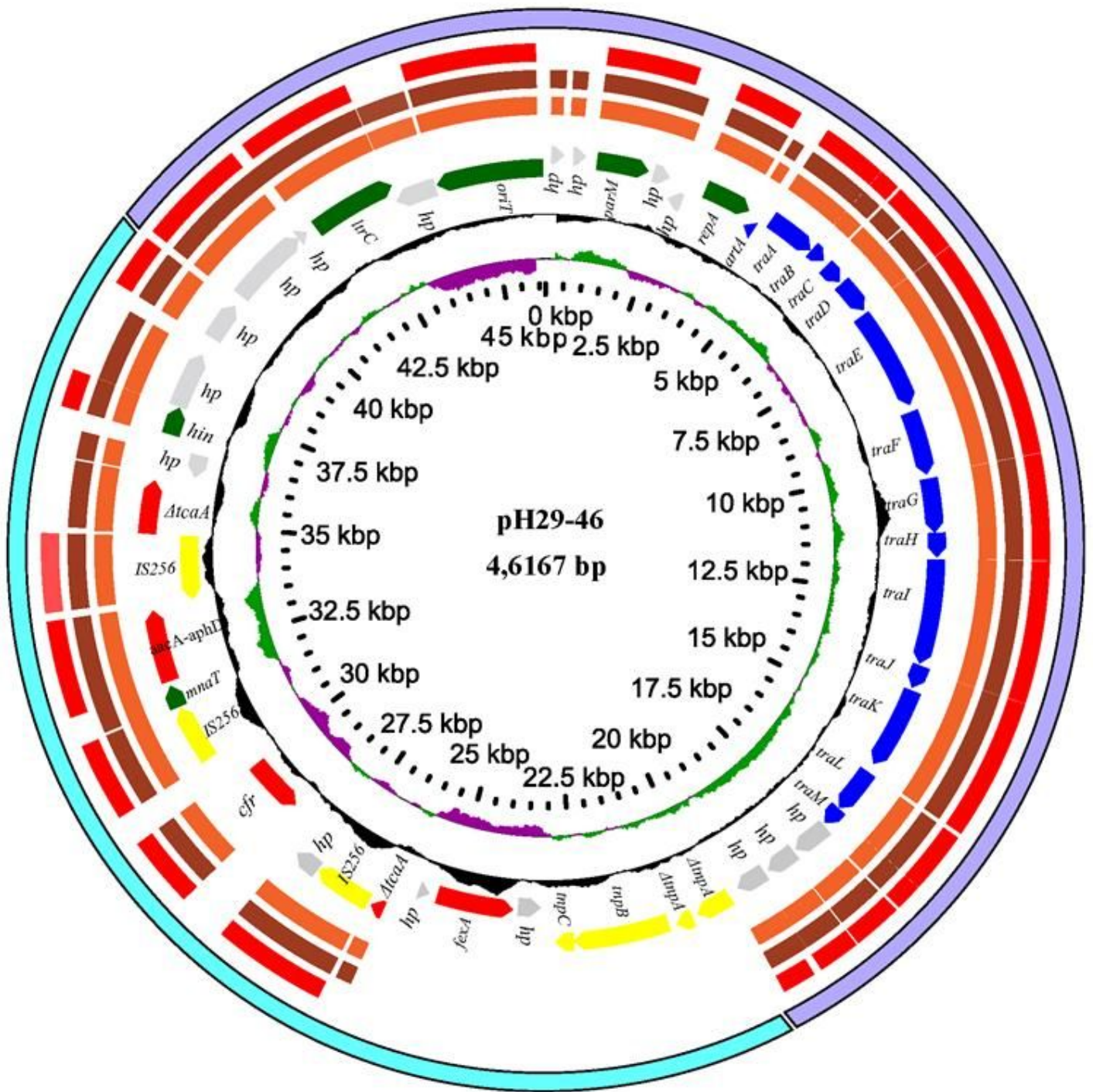


Figure 1

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.*

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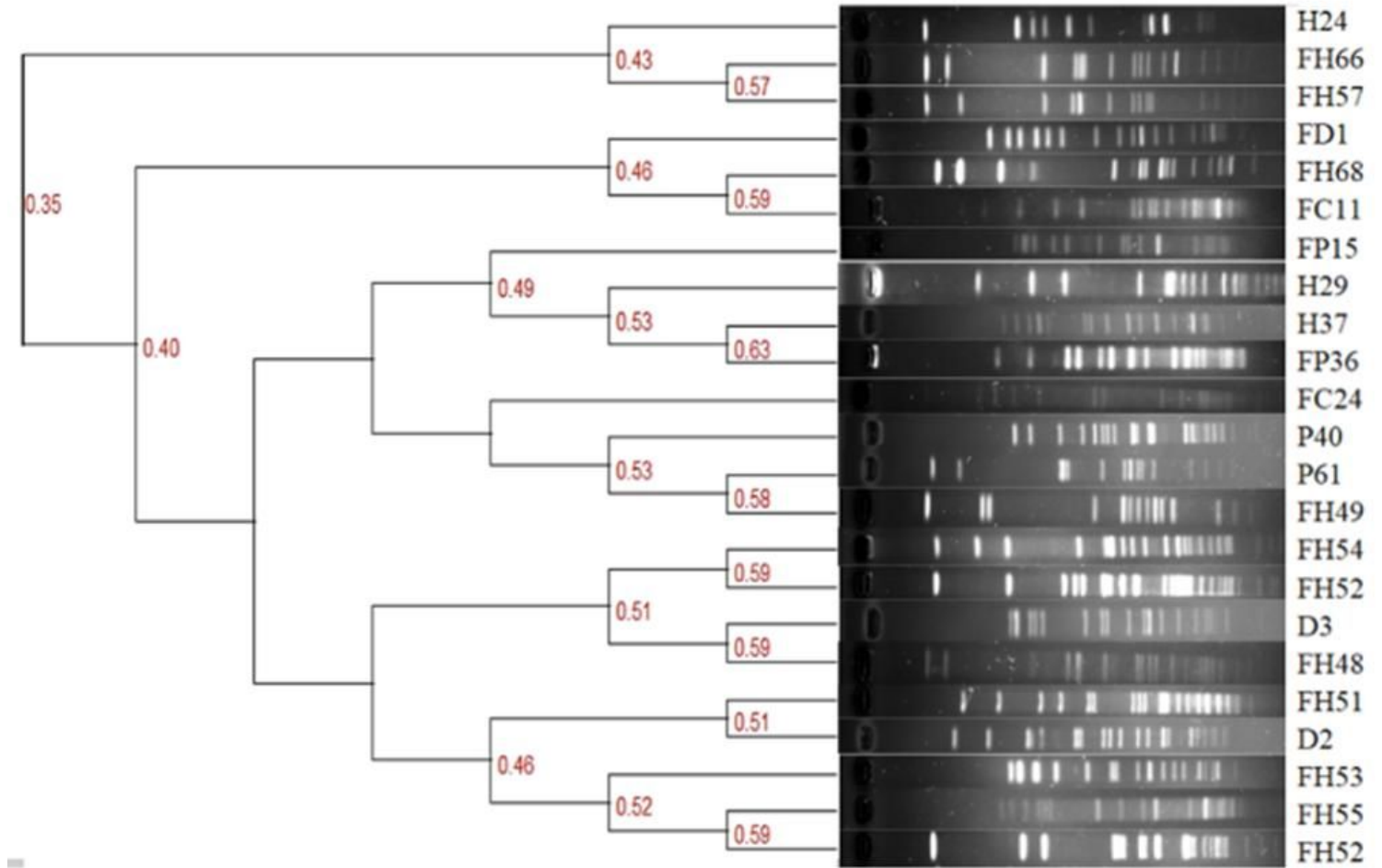


Figure 1

PFGE patterns of 23 florfenicol-resistant CoNS isolates.

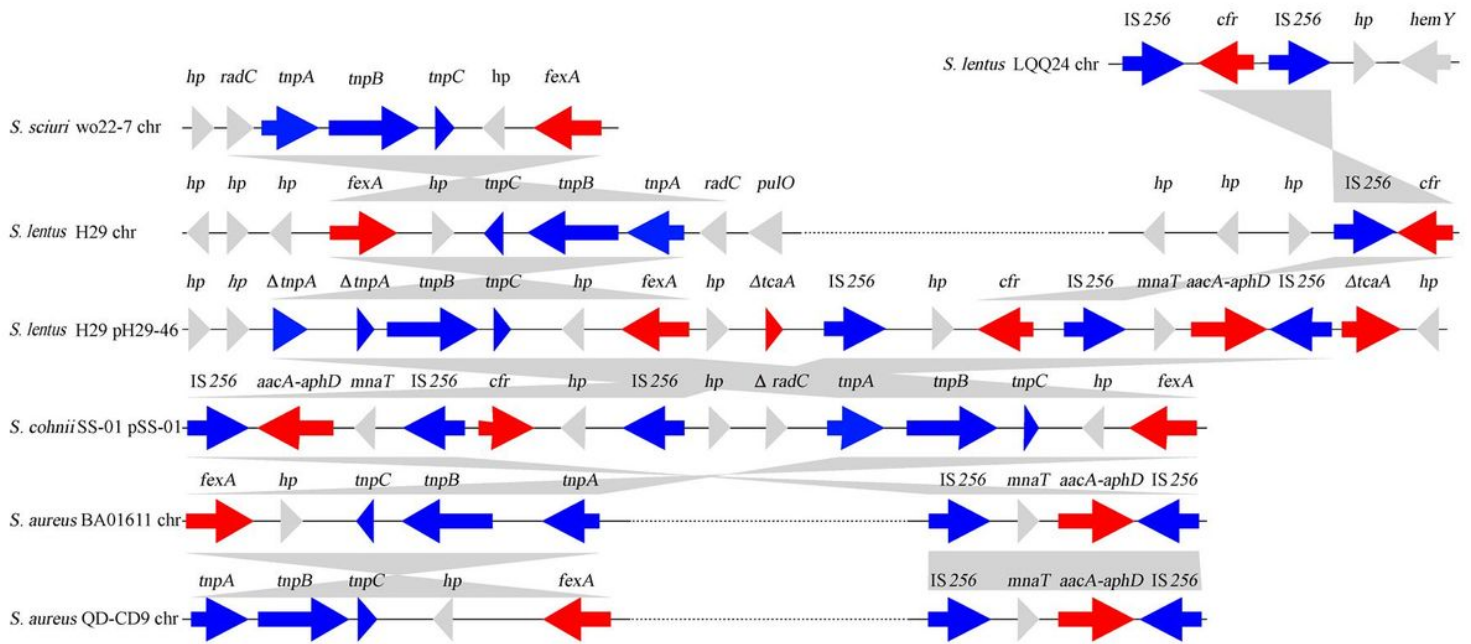


Figure 1

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