Presence and dissemination of the multiresistance gene \textit{cfr} in Gram-positive and Gram-negative bacteria

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The emergence of the multiresistance gene \textit{cfr} in staphylococci is of global concern. In addition to conferring resistance to phenicols, lincosamides, pleuromutilins, streptogramin A antibiotics and selected 16-membered macrolides, the \textit{cfr} gene also confers resistance to the oxazolidinone linezolid. Linezolid is a last-resort antimicrobial agent for the treatment of serious infections in humans caused by resistant Gram-positive bacteria. The \textit{cfr} gene is often located on plasmids and several \textit{cfr}-carrying plasmids have been described, which differ in their structure, their size and the presence of additional resistance genes. These plasmids are important vehicles that promote the spread of the \textit{cfr} gene not only among bacteria of the same species, but also among those of different species and genera. Moreover, the \textit{cfr} gene has been identified in close proximity to different insertion sequences, which most probably also play an important role in its dissemination. This review summarizes current knowledge on the genetic environment of the multiresistance gene \textit{cfr} with particular reference to mobile genetic elements and co-located resistance genes that may support its emergence.

Keywords: oxazolidinone resistance, plasmids, insertion sequences, recombination, food animals

Introduction

The \textit{cfr} gene encodes an RNA methyltransferase that modifies the adenine residue at position 2503 of the 23S rRNA gene and thereby confers combined resistance to phenicols, lincosamides, oxazolidinones, pleuromutilins and streptogramin A antibiotics (otherwise known as the PhLOPS A phenotype)$^{1,2}$ and decreased susceptibility to the 16-membered macrolides spiramycin and josamycin$^3$. These classes of protein biosynthesis inhibitors share overlapping binding sites at the peptidyltransferase centre of the ribosome. It is believed that the additional methylation of A2503 interferes with the correct positioning in the adenine residue of the ribosomal proteins L3 and L4$^4$. The \textit{cfr} gene is currently the only gene that confers transferable resistance to oxazolidinones in clinical isolates, although \textit{cfr}-like genes have recently been identified in environmental members of the order Bacillales and shown also to confer the PhLOPS A phenotype$^5$. Linezolid is considered to be a last-resort antimicrobial agent for the treatment of infections caused by vancomycin-resistant enterococci, methicillin-resistant \textit{Staphylococcus aureus} (MRSA) and penicillin-resistant pneumococci$^6$. Therefore, the transferable linezolid resistance conferred by the multiresistance gene \textit{cfr} poses a significant and interdisciplinary public health challenge$^7$.

Since its initial identification in a bovine \textit{Staphylococcus sciuri} isolate in 1997$^8$, the \textit{cfr} gene has been documented globally in linezolid-resistant clinical staphylococcal isolates of human and animal origin$^{4,7,9}$. Although most reports have identified this gene in staphylococci [$\textit{S. aureus}$ (MSSA) and MRSA, various coagulase-negative staphylococci (CoNS) and the coagulase-variable \textit{S. hyicus}$^9$, recent studies have described it in four other genera of Gram-positive bacteria, namely \textit{Bacillus}$^{10-12}$ \textit{Enterococcus}$^{13-16}$ \textit{Macrococcus} and \textit{Jeotgalicoccus}$^{17}$, as well as in members of two genera of Gram-negative bacteria, \textit{Proteus} and \textit{Escherichia}$^{18,19}$. This observation indicates that \textit{cfr}-mediated resistance is more widespread than initially thought. With the exception of the \textit{Enterococcus} isolates, the \textit{cfr}-positive isolates of the other five non-\textit{Staphylococcus} genera were obtained exclusively from livestock and related farm environments. Since the epidemiology and resistance mechanism of \textit{cfr} in staphylococci have been extensively reviewed elsewhere$^{4,7,9}$, this review will provide an update on the genetic environment of the multiresistance gene \textit{cfr} in the different host bacteria. This information will provide an insight into the processes that are likely to be involved in the dissemination of \textit{cfr} across strain, species and genus boundaries.
Genetic environment of cfr in Staphylococcus spp.

Most of the cfr-positive animal isolates originated from swine, although isolates of bovine, equine and—most recently—poultry origin have also been reported.20–25 So far, cfr has been discovered in nine staphylococcal species of porcine origin, including S. aureus (plasmid pSCFS3),20,21 S. lentus (pSCFS3),20 S. warneri (pSCFS6),22 S. simulans (pSCFS6),22 S. hyicus (chromosomal location),22 S. cohnii (pSS-01, pSS-03, pBS-01 and chromosomal location),23 S. arlettae (pSS-03 and chromosomal location),23 S. saprophyticus (pSS-01, pSS-02, pSS-03 and chromosomal location)23 and S. sciuri (pSS-02, pSS-03, pBS-01 and chromosomal location).20,23 It has also been detected in four staphylococcal species of bovine origin, namely S. lentus (pSCFS3), S. simulans and S. sciuri (both pSCFS1).8,20 The genetic context of the cfr gene in all these plasmids has been well characterized. Occasionally, indistinguishable or very closely related plasmids, such as pSCFS1, pSS-02 and pSS-03, have been found in isolates of different staphylococcal species. This observation may point towards the role of plasmids in the interspecies transfer of the cfr gene.

For isolates of human clinical origin, the first documented strain to harbour a chromosomally located cfr gene was MRSA strain CM05, isolated from a patient in Medellin, Colombia, in 2005.26 This gene has subsequently been detected among linezolid-resistant clinical staphylococci (mainly S. aureus and coagulase-negative S. epidermidis) of diverse geographical origin (USA, Belgium, Italy, Spain, Mexico and Ireland).27–35 Even though cfr has been reported in several human clinical staphylococcal isolates, its genetic context has only been characterized in MRSA plasmids p004-737X, pSCFS7 and pERGB, as well as on the chromosome of MRSA strain CM05 and plasmid p7LC of S. capitis strain MHZ, the 5.2 kb segment of pMHZ comprised the cfr gene and its flanking IS558-like elements and closely resembled (99.9% identity) the corresponding region of plasmid pSS-01. On plasmids pSCFS1, pSS-03 and pMSA16, the cfr gene was flanked in the downstream region by a pre/mob protein for plasmid recombination and mobilization whose deduced amino acid sequences showed >95% identity to each other (Figure 1). Similarly, a truncated copy of a pre/mob gene, whose deduced amino acid sequences showed >99% identity to the 271 amino acids at the C terminus of the Pre/mob protein from plasmid pSES2239 (GenBank accession no. CAJ43794), was located upstream of the cfr gene on plasmids pSS-03, pMSA16 and pBS-01.

Second, cfr-carrying plasmids or chromosomal fragments often carry additional resistance genes. In addition to the coexistence of the Tn4001-associated aminoglycoside resistance gene aacA-aphD and cfr in pSS-01 and p7LC, the ~50 kb conjugative plasmid pERGB contains three resistance genes, namely the kanamycin/neomycin resistance gene aadD, the tetracycline resistance gene tet(L) and the trimethoprim resistance gene dfrK, located downstream of cfr and flanked by two divergently oriented copies of insertion sequence element IS431.15 These two copies of IS431, as well as the resistance genes, were highly similar to those previously described for the staphylococcal multiresistance plasmids pKKS825 and pKKS2187.45,46 In the 15.5 kb cfr insertion region that was found to disrupt the 23S rRNA allele 4 in the chromosomal DNA of S. aureus CM05, the IS21-SS element and the cfr gene were flanked by two >5 kb Tn917-derived repeated elements. These elements carry the rRNA methylase gene erm(B), which confers resistance to macrolide–lincosamide–streptogramin B (MLSb) antibiotics. Interestingly, a recombination event involving the two erm(B)-carrying segments can occur in CM05, leading to the deletion of cfr and IS21-SS.16 An intact Tn917 element harbouring the erm(B) gene was also located downstream of the cfr gene in plasmid pBS-01.23 Three other distinct MLSb resistance genes, erm(A), C and erm(33), were detected on plasmids pMSA16, pSS-03 and pSCFS1, respectively.3,24,25 As a novel MLSb resistance gene, erm(33) on pSCFS1 represented an in vivo derived ‘in-frame’ recombination product of the erm(C) and erm(A) genes. Moreover, pSCFS1 carries two additional resistance genes: the spectinomycin adenyltransferase gene spa as part of transposon Tn554 located downstream of erm(33), and an ABC
Figure 1. (a) Comparative analysis of the genetic environment of the cfr gene in plasmids and chromosomal DNA from staphylococci. (b) Comparative analysis of the genetic environment of the cfr gene in plasmids from non-staphylococcal Gram-positive bacteria. (c) Comparative analysis of the genetic environment of the cfr gene in plasmids and chromosomal DNA from Gram-negative bacteria. The arrows indicate the positions of the genes and their directions of transcription. Regions of >95% homology are shaded in grey. Different grey shadings are used in panels (a) and (c) to better illustrate the homologous regions. Target sites and direct target site duplications upstream and downstream of the IS elements (if present) are boxed. Δ indicates a truncated gene. A 1 kb distance scale is displayed in the upper right or left corner.

S. capitis pMHZ (JX232067)

S. epidermidis p7LC (JX910899)

S. cohnii pSS-01 (JQ041372)

S. aureus pSCFS7 (FR675942)

S. aureus p004-737X (EU598691)

S. aureus pSCFS3 (AM086211) and S. saprophyticus pSS-02 (JF834910)

S. warneri pSCFS6 (AM408573)

S. aureus pERGB (JN970908)

Chromosomal region of S. aureus CM05 (JN849634)

S. cohnii pBS-01 (GU591497)

S. aureus pMSA16 (JQ246438)

S. cohnii pSS-03 (JQ219851)

S. sciuri pSCFS1 (NC_005076)
Figure 1. Continued

- *J. pinnipedialis* pJP1 (JQ320084)
- *Bacillus* spp. pBS-01 (GU591497)
- *Bacillus* spp. pBS-02 (HQ128580)
- *Bacillus* spp. pBS-03 (JQ394981)
- *M. caseolyticus* pSS-03 (JQ219851)
- *E. faecalis* pEF-01 (NC_014508)
- *E. faecalis* pHOU-cfr (JQ660368)
- *E. faecalis* pW9-2 (JQ911741)
- *E. thailandicus* pW3 (JQ911739)
- *E. thailandicus* p3-38 (JQ911740)
S. aureus ST398 pKKS825 (NC_013034)

S. sciuri pSCFS1 (NC_005076)

Chromosomal fragment of P. vulgaris PV-01 (JF969273)

E. coli pEC-01 (JN982327)

C. rodentium ICC168 pCROD2 (NC_013718)

Figure 1. Continued
transporter gene Isa(B) conferring low-level clindamycin resistance and found immediately upstream of the cfr gene. It should be emphasized that the coexistence of cfr with additional resistance genes could allow for the persistence and co-selection of the cfr gene under selective pressure imposed by the use of the corresponding non-PhLOPSA antimicrobial agents.

Third, with a similar size of ~7.1 kb, the plasmids pSS-03 and pMSA16 are the smallest cfr-carrying plasmids known to date.2,3,24 The 6.3 kb cfr-carrying segment of pSS-03 from porcine CoNS closely resembles (99.6% identity; 6271/6298 bp) the corresponding region of plasmid pMSA16 from a bovine MRSA ST9 strain. Only the MLS\(_B\) resistance genes differ between the two plasmids, with the \(\text{erm}(A)\) and \(\text{erm}(C)\) genes located downstream of the recombination/mobilization gene \(\text{pre}/\text{mob}\) in pMSA16 and pSS-03, respectively. Moreover, similar to the widespread occurrence of plasmids pSCFS3/pSS-02, plasmid pSS-03 has been found in four different porcine staphylococcal species and recently in one porcine \textit{Macrococcus caseolyticus} strain.17,18 indicating that this small plasmid has a broad host range. In addition, a 4.6 kb cfr-carrying segment, including the cfr, \(\text{pre}/\text{mob}\) and partial \(\text{erm}\) genes from pSS-03, also shows high nucleotide sequence identity (96.4%; 4519/4689 bp) to the corresponding region [\(\text{cfr}, \text{pre}/\text{mob}, \text{and partial} \ \text{erm}(33)\) of plasmid pSCFS1 (Figure 1a). These observations suggest that (i) the gene cfr can be part of structurally diverse plasmids, (ii) small cfr-carrying plasmids can be integrated in part into larger plasmids and (iii) small cfr-carrying plasmids may be able to replicate in other bacterial hosts. The latter observation corresponds closely to previous observations that tet(L)- or \(\text{aad}\)D-carrying plasmids found in staphylococci can also replicate in \textit{Bacillus} hosts.30

**Genetic environment of cfr in other Gram-positive bacteria**

The immediate genetic environment of the cfr gene in other Gram-positive bacteria is much more diverse than that in \textit{Staphylococcus} (Figure 1b). To date, the cfr gene has been found to be plasmid-borne in four non-staphylococcal Gram-positive bacterial genera, namely three \textit{Bacillus} strains, five \textit{Enterococcus} strains, two \textit{Macrococcus} strains and one \textit{Jeotgalicoccus} strain.

**cfr in \textit{Bacillus}, \textit{Macrococcus} and \textit{Jeotgalicoccus} spp.**

A \textit{Bacillus} isolate of porcine origin was the first identified non-staphylococcal isolate harbouring the cfr gene.10 To date, three cfr-carrying plasmids, pBS-01 (16492 bp), pBS-02 (16543 bp) and pBS-03 (7446 bp), have been identified in \textit{Bacillus} isolates, all of which are of porcine origin and originate from the Shan-dong Province, China.10-12 Plasmid pBS-01 contains a complete copy of transposon Tn917, whereas plasmid pBS-02 contains an insertion that includes an IS256 element and the two genes \(\text{res}\) and \(\text{met}\) (Figure 1b). Whether the IS256, which is located immediately downstream of the cfr gene in pBS-02, has played a role in the acquisition of cfr and its dissemination among \textit{Bacillus} species remains to be answered.11 Plasmid pBS-03 is the smallest identified cfr-carrying plasmid from \textit{Bacillus} spp. and contains the novel streptomycin resistance gene \(\text{aad}\)Y.12 A 4516 bp segment in pBS-03 containing the truncated \(\text{pre}/\text{mob}\) gene and the intact \(\text{pre}/\text{mob}\) gene shares 99.7% nucleotide identity with the corresponding sequence of plasmid pSS-03, which has been identified in both \textit{Staphylococcus} and \textit{Macrococcus} isolates.

A novel ~53 kb cfr-carrying plasmid pJP1 was identified in an \textit{M. caseolyticus} isolate and in a \textit{J. pinnipedialis} isolate, both from pigs.17 Plasmid pJP1 is the only known non-staphylococcal plasmid that carries an IS21-558 element adjacent to the cfr gene. Moreover, the genes \(\text{aad}\)D, \(\text{aaca-aphD}\), \(\text{ble}\) and \(\text{erm}(C)\), the first two coding for aminoglycoside resistance, and the latter two for bleomycin and MLS\(_B\) resistance, respectively, were also identified on this plasmid.17 The occurrence of cfr-carrying plasmid pBS-01 in both \textit{Bacillus} and \textit{Staphylococcus} isolates, pJP1 in both \textit{Macrococcus} and \textit{Jeotgalicoccus} isolates and pSS-03 in both \textit{Staphylococcus} and \textit{Macrococcus} isolates suggests that these plasmids can spread between different Gram-positive genera.

**cfr in \textit{Enterococcus} spp.**

The identification of cfr in the human MRSA strain CM05 suggested enterococci as a possible source of this multiresistance gene.26 However, the presence of cfr in human clinical enterococcal isolates was not reported until 2010,27,28 and an in-depth characterization of these isolates for their cfr regions has yet to be published. Recently, a linezolid-resistant \textit{E. faecalis} isolate belonging to the multilocus sequence type (MLST) ST16 was recovered from a patient in Thailand.18 In this isolate, the cfr gene was located on a ~97 kb conjugative plasmid, designated pHOU-cfr. The cfr gene was flanked by IS256-like sequences.18 A recent surveillance study also revealed three conjugative cfr-carrying plasmids in enterococci in 2020:32 from swine and their associated farm environment in China.16 These included the ~55 kb plasmid pW9-2 from \textit{E. faecalis} ST469 isolate W9-2, and also the ~75 kb plasmid pW3 and the ~72 kb plasmid p3-38, both from \textit{E. thailandicus} (Table 1). Sequence analysis revealed that these conjugative plasmids had a 4447 bp central region in common, which comprised the cfr gene bracketed by copies of the novel insertion sequence IS\textit{Enfa}4 located in the same orientation (Figure 1b). This IS element is most closely related to IS1542 from \textit{E. faecium}, with an 81% nucleotide sequence identity of 91.6% (1213/1324 bp) and 93.8% amino acid sequence identity (366/390 amino acids) in the transposase protein.16 This newly designated IS element and the associated cfr gene showed a high degree of nucleotide sequence identity (>99%) to the IS256-like element and cfr gene found in both the \textit{Enterococcus} plasmid pHOU-cfr and the \textit{Staphylococcus} plasmid pSS-01. This observation might suggest that the cfr gene and IS\textit{Enfa}4 on staphylococcal plasmid pSS-01 have originated from an enterococcal species. Further analysis of the three conjugative enterococcal plasmids of veterinary origin revealed the presence of a \(\omega\)-\textit{e} toxin–antitoxin module, which exhibited >95% nucleotide sequence identity to that of the conjugative plasmid pAM\text{B}1 from \textit{E. faecalis}.16

In addition, a 32 kb non-conjugative plasmid (pEF-01) carrying cfr has been described in \textit{E. faecalis} isolate EF-01, which was isolated from bovine faeces in the Sichuan province, China.13 The genetic environment of the cfr gene on this plasmid was distinct from that of other cfr-carrying plasmids or chromosomal segments (Figure 1b). Three copies of the insertion sequence IS1216 were identified, and the detection of cfr- and IS1216-containing amplicons by inverse PCR suggested that IS1216 might have played a role in the dissemination of cfr in
enterococci via recombination processes. Moreover, a novel phenicol exporter gene, fexB, was present on this plasmid.47

Genetic environment of cfr in Gram-negative bacteria

cfr in Proteus vulgaris

Although cloning experiments showed that cfr is functionally active in enterobacterial hosts, such as Escherichia coli,67 there was no information available regarding the presence of cfr in naturally occurring Gram-negative bacteria. This situation changed with the identification of the Proteus vulgaris isolate PV-01, obtained from a porcine nasal swab in China in 2011, which carried cfr and the florfenicol exporter gene floR.18 An ~7 kb segment that contained the cfr gene was found to be inserted into the chromosomal fimD gene of PV-01. The region flanking the cfr gene showed partial homology to plasmid pKK825 from MRSA strain ST398.40 In addition, this inserted segment was flanked by IS26 elements located in the same

Table 1. Overview of the currently identified cfr-carrying plasmids in different Gram-positive and Gram-negative bacteria

<table>
<thead>
<tr>
<th>Bacteria</th>
<th>Origin</th>
<th>Plasmid or chromosomal location of cfr (size)</th>
<th>Accession no. (size of submitted sequence)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Staphylococcus spp.</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>S. sciuri and S. simulans</td>
<td>calf and cattle origin, Germany, 2000–03</td>
<td>pSCFS1 (17.108 kb)8,20 NC_005076 (17.108 kb)</td>
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<tr>
<td>S. lentus, S. aureus, ST9-MRSA, ST398-MRSA-V</td>
<td>cattle and swine origin, Germany, 2000–03</td>
<td>pSCFS3 (~35.7 kb)20,21 AM086211 (9.497 kb)</td>
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<tr>
<td>S. warneri and S. simulans</td>
<td>swine origin, Denmark, 2005</td>
<td>pSCFS6 (~43 kb)22 AM408573 (22.010 kb)</td>
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<td>S. aureus 004-737X</td>
<td>human with a skin abscess, Ireland, 2005</td>
<td>pSCFS7 (~45 kb)33 FR675942 (4.043 kb)</td>
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<tr>
<td>S. epidermidis 426-3147L</td>
<td>blood culture of a human patient in a long-term care facility, USA; LEADER Program, 2007</td>
<td>p004-737X (~55 kb)30 EU598691 (3.699 kb)</td>
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<tr>
<td>S. saprophyticus and S. cohnii</td>
<td>pig nasal swab, China, 2010</td>
<td>p7LC (~30.5 kb)37 JX910899 (5.882 kb)</td>
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<td>S. saprophyticus and S. sciuri</td>
<td>pig nasal swab, China, 2010</td>
<td>pSS-01 (~40 kb)23 JQ041372 (15.702 kb)</td>
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<tr>
<td>S. saprophyticus, S. cohnii, S. sciuri, and S. arlettae</td>
<td>pig nasal swab, China, 2010</td>
<td>pSS-02 (~35.4 kb)23 JF834910 (8.580 kb)</td>
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<tr>
<td>S. saprophyticus and S. cohnii ST125-MRSA-1Vc</td>
<td>two humans with respiratory infections, Spain, 2010 bovine mastitis, China, 2010</td>
<td>pBS-01 (16.492 kb)23 GU591497 (16.492 kb)</td>
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</tr>
<tr>
<td>S. saprophyticus and S. cohnii ST9-MRSA-1Vc</td>
<td>sewage from pig farm, China, 2011</td>
<td>pERGB (~50 kb)16 JN970906 (15.259 kb)</td>
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<tr>
<td>S. saprophyticus and S. cohnii S. capitis MHZ</td>
<td>sewage from pig farm, China, 2011</td>
<td>pMA16 (7.054 kb)24 JQ246438 (7.054 kb)</td>
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<tr>
<td>S. saprophyticus and S. cohnii S. capitis MHZ</td>
<td>sewage from pig farm, China, 2011</td>
<td>pMHZ JX232067 (5.247 kb)</td>
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<tr>
<td>MRSA CM05</td>
<td>sputum cultures of a patient with a fatal ventilator-associated pneumonia, Colombia, 2005</td>
<td>insertion into chromosomal 235 rRNA allele 425,35 JN849634 (15.711 kb)</td>
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<td>Entercoccus spp.</td>
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<td>E. faecalis EF-01</td>
<td>cattle, faecal sample, China, 2009</td>
<td>pEF-01 (32.388 kb)13 NC_014508 (32.388 kb)</td>
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<tr>
<td>E. faecalis 603-50427X</td>
<td>skin sample of a diabetic human, Thailand, 2010</td>
<td>pHOU-cfr (~97 kb)42 Q660368 (3.494 kb)</td>
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<td>E. faecalis W9-2</td>
<td>sewage from pig farm, China, 2011</td>
<td>pW9-2 (~55 kb)16 JQ911741 (21.116 kb)</td>
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<td>E. thailandicus W3</td>
<td>sewage from pig farm, China, 2011</td>
<td>pW3 (~75 kb)16 JQ911739 (27.360 kb)</td>
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<tr>
<td>E. thailandicus 3-38</td>
<td>pig rectal swab, China, 2011</td>
<td>p3-38 (~72 kb)16 JQ911740 (21.116 kb)</td>
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<tr>
<td>Bacillus spp.</td>
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<tr>
<td>Bacillus BS-01</td>
<td>pig rectal swab, China, 2008</td>
<td>pBS-01 (16.492 kb)10 GU591497 (16.492 kb)</td>
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<td>Bacillus BS-02</td>
<td>pig rectal swab, China, 2010</td>
<td>pBS-02 (16.543 kb)11 HQ128580 (16.543 kb)</td>
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<td>Bacillus BS-03</td>
<td>pig nasal swab, China, 2011</td>
<td>pBS-03 (7.446 kb)12 JQ94981 (7.446 kb)</td>
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<td>Jeotgalicoccus spp.</td>
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<td>J. pinnipedialis 102</td>
<td>pig nasal swab, China, 2010</td>
<td>pJP1 (~53 kb)17 JQ320084 (8.896 kb)</td>
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<tr>
<td>Macroccoccus spp.</td>
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<tr>
<td>M. caseolyticus 207</td>
<td>pig nasal swab, China, 2010</td>
<td>pJP1 (~53 kb)17 JQ320084 (8.896 kb)</td>
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<td>M. caseolyticus K3</td>
<td>pig nasal swab, China, 2010</td>
<td>pSS-03 (7.057 kb)17 JQ219851 (7.057 kb)</td>
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<td>Proteus spp.</td>
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<tr>
<td>P. vulgaris PV-01</td>
<td>pig nasal swab, China, 2010</td>
<td>insertion into the chromosomal fimD gene18 JF969273 (11.228 kb)</td>
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<td>Escherichia spp.</td>
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<tr>
<td>E. coli EC-01</td>
<td>pig nasal swab, 2010</td>
<td>pEC-01 (~110 kb)19 JN982327 (12.390 kb)</td>
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</table>
orientation, which are believed to have played a role in the integration process (Figure 1c). Stability testing via inverse PCR approaches showed that the integration was not entirely stable, and that the cfr-carrying centre region plus one IS26 copy could be looped out via IS26-mediated recombination.18

**cfr in E. coli**

Following the discovery of the cfr gene in a naturally occurring *P. vulgaris* isolate, a survey was conducted to investigate the presence and genetic environment of this multiresistance gene in *E. coli* isolates from food-producing animals. In total, 1230 *E. coli* isolates, collected from pigs, chickens and ducks in China between 2008 and 2010, were screened for the presence of cfr. Only a single isolate, *E. coli* EC-01, obtained from the nasal swab of a pig from a slaughterhouse, harboured the cfr gene.19 The cfr gene was located on the ~110 kb plasmid pEC-01, which contains an IS26-flanked 1545 bp cfr-carrying segment identical to the corresponding region of the staphylococcal plasmid pSCFS1.19 Similarly to the situation in *P. vulgaris*, this cfr-carrying segment plus one IS26 copy could also be looped out via IS26-mediated recombination. Moreover, the IS26 transposition created a new hybrid promoter in which the −35 region was part of the left inverted repeat of IS26, while the −10-like sequence was part of the original cfr upstream region.19 The regions flanking the two IS26 elements on pEC-01 showed a high degree of homology (≥96%) to plasmid pCROD2 (GenBank accession no. NC_013718) from *Citrobacter rodentium* ICC168,18 and to plasmid pBS512_33 (GenBank accession no. NC_010657) from *Shigella boydii* CDC 3083-94, suggesting that a 3849 bp element containing the cfr gene, both IS26 elements and the transposase gene *tnpA* had integrated into a pCROD2-like or pBS512_33-like backbone.19

Although cfr has so far been identified only in single isolates of *P. vulgaris* and *E. coli*, the insertion element IS26 appears to play an important role in the transfer of this multiresistance gene in Gram-negative bacteria. Moreover, IS26 is widespread among Gram-negative bacterial plasmids, and also shares significant homology with the staphylococcal insertion sequences IS431 and IS257,59 which are present on various staphylococcal plasmids as well as within certain SCCmec cassettes of MRSA strains. Thus the cfr gene may not only be disseminated within Gram-negative bacteria via IS26-mediated recombination events, but may also be re-introduced into the Gram-positive gene pool.

**Conclusions and perspectives**

It is still uncertain whether the cfr gene originally evolved to protect its hosts against certain antimicrobial agents that inhibit protein biosynthesis, or whether it also has some other functions. Phylogenetic comparisons have shown that Cfr is similar to the RimN methyltransferase that adds a methyl group at the C-2 position of 23S rRNA nucleotide A2503, the same nucleotide that is methylated by Cfr.50 Recently, an interesting study verified the functionality of three cfr-like genes (>50% amino acid identity to Cfr) from Bacillales hosts, indicating that cfr-like genes are present in the environment and may be acquired by environmental bacteria.5

There may be several reasons for the emergence and dissemination of the cfr gene in clinical, veterinary and environmental bacteria. Unlike the reduced fitness observed among linezolid-resistant isolates that carry mutations in their 23S rRNA genes, the transferable cfr gene appears to have a low fitness cost in Gram-positive bacteria.51 Under the selective pressure of antimicrobial agents to which cfr confers resistance, e.g. oxazolidinones and lincosamides in human medicine as well as phenicols, lincosamides and pleuromutilins in veterinary medicine, this low fitness cost may not have a negative impact on the acquisition and further dissemination of the cfr gene.4,7 Moreover, many plasmids that carry the cfr gene—especially those found in staphylococci—harbour additional resistance genes, which may enable the co-selection of the cfr gene and its persistence even in the absence of a direct selective pressure. Among them, aminoglycoside and MLSB resistance genes have been most frequently observed. It is also noteworthy that the simultaneous presence of cfr and erm genes results in resistance to clinically used streptogramin A-B combinations, such as quinupristin/dalfopristin, as cfr confers resistance to the streptogramin A compound and the erm genes confer resistance to the streptogramin B compound.

The location of cfr on plasmids, including conjugative plasmids, is a key factor for its dissemination across strain, species and genus boundaries. This has been confirmed not only by the identification of the same plasmids in different strains of the same staphylococcal species,23 but also by the presence of the same plasmid in different staphylococcal species20,23 and even in different Gram-positive bacteria.17,23 Another aspect is that several different insertion sequences are involved in the mobility of the cfr gene. Such insertion sequences, including IS21-558, ISEnfo4, IS2126 and IS26, are widely disseminated in the different Gram-positive and Gram-negative bacteria and allow translocation of the cfr gene between different plasmids, as well as mediating its integration into chromosomal DNA. Knowledge of the genetics of cfr-mediated resistance—as summarized in this review—is essential for understanding how this gene could have emerged over the past decade in different parts of the world.

Based on the flexibility of bacteria to accept foreign resistance genes and the mobility of the cfr gene, it is likely that this gene will in future also be found in bacteria other than the ones listed in this review. The most efficient way of limiting the spread of this multiresistance gene is to reduce the selective pressure for cfr and other coexisting resistance genes. This can only be achieved by the prudent use of phenicols, lincosamides, and pleuromutilins, and also macrolides and aminoglycosides, in animal production and veterinary medicine. The same applies to the use of oxazolidinones, lincosamides and streptogramins, as well as macrolides and aminoglycosides, in human medicine. In addition, continued surveillance and molecular epidemiologic analysis of this multiresistance gene among Gram-positive and Gram-negative bacteria in both human and veterinary medicine are warranted to identify the origins of cfr-positive bacteria and limit their dissemination.

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Transparency declarations

None to declare.

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