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# Nomenclature and Functionality of the So-Called *cfr* Gene from *Clostridium difficile*

**Stefan Schwarz,[a] Yang Wang[b]**

Institute of Farm Animal Genetics, Friedrich-Loeffler-Institut (FLI), Neustadt-Mariensee, Germany[a]; Department of Veterinary Pharmacology, College of Veterinary Medicine, China Agricultural University, Beijing, People's Republic of China[b]

We read with great interest the paper entitled "*Clostridium difficile* with High Linezolid MICs Harbor the Multiresistance Gene *cfr*" presented by Marín et al. (1). However, we have some concerns with regard to (i) the gene nomenclature used by the authors and (ii) the functionality of the gene.

It is important to understand that the Cfr protein belongs to the radical *S*-adenosylmethionine (SAM) superfamily, which includes proteins that generate a radical species by reductive cleavage of SAM (2). Members of the radical SAM superfamily are involved in a wide variety of reactions, including unusual methylations, isomerization, sulfur insertion, ring formation, anaerobic oxidation, and protein radical formation. They function in DNA precursor, vitamin, cofactor, antibiotic, and herbicide biosynthesis and in biodegradation pathways (2). Radical SAM proteins occur in a number of bacterial species. The Cfr protein is also a member of the radical SAM superfamily and represents the first radical SAM protein involved in antimicrobial resistance (3, 4). Although the *cfr* gene was initially identified in coagulase-negative staphylococci of animal origin (5), studies on the dissemination of the *cfr* gene conducted during recent years showed that it has spread to a number of Gram-positive bacterial species, including *Bacillus* spp., *Enterococcus* spp., *Macrococcus caseolyticus*, *Jeotgalicoccus pinnipedialis*, and *Streptococcus suis*, and also to Gram-negative bacterial species, such as *Proteus vulgaris* and *Escherichia coli* (6, 7). Despite the diverse origins of the proteins, it should be noted that all these Cfr proteins were either indistinguishable from the original Cfr protein or differed by at maximum 2 amino acids (99.4% identity). However, another study identified the genes *clbA* from *Bacillus amyloliquefaciens*, *clbB* from *Brevibacillus brevis*, and *clbC* from *Bacillus clausii* to also methylate A2305 in 23S rRNA and to confer the Cfr-associated resistance phenotype (8). The corresponding proteins, ClbA, ClbB, and ClbC, exhibited only 74.7%, 57.1%, and 62.2% identity, respectively, to the original Cfr protein from *Staphylococcus sciuri* (5). These data clearly showed that there are other genes whose products have the same resistance mechanism as Cfr but are structurally distantly related to Cfr. Thus, the occurrence of a *cfr*-related gene in an anaerobic species like *Clostridium difficile* might represent a novel and interesting observation. However, two aspects need to be taken into consideration.

First, the gene that the authors identified and termed *cfr* shows high identity to genes from *Clostridium* species and various *Bacillus* species whole-genome sequences but only limited identity to *cfr* genes with confirmed roles in linezolid resistance. The sequences of the 349-amino-acid proteins, deduced from the respective nucleotide sequences of the *cfr*-related genes from *C. difficile*, revealed only 75.1% identity to the original Cfr protein (GenBank accession no. NP_899167.1) from *Staphylococcus sciuri*. Moreover, they showed 84.5%, 58.2%, and 62.1% identity to the proteins ClbA, ClbB, and ClbC, respectively.

To harmonize the use of resistance gene designations, nomenclature centers for specific resistance genes have been established. These centers provide, upon request, unambiguous designations for newly identified antimicrobial resistance genes. Reviews that describe the currently known resistance genes and the rules on which gene designations are based have been published by the curators of these nomenclature centers (9–12). One such nomenclature center is also devoted to genes that confer resistance to macrolide-lincosamide-streptogramin (MLS) antibiotics (http://faculty.washington.edu/marilynr/). Since Cfr also mediates resistance to lincosamides and streptogramin A antibiotics, two classes of the MLS antibiotics, it is suggested that the authors submit their new *cfr*-related gene to this nomenclature center and ask for an appropriate gene designation.

Second, the manuscript provided by Marín et al. (1) does not contain any information about the functionality of their *cfr*-related gene as a linezolid resistance gene or a multiresistance gene that confers the Cfr-associated resistance phenotype. The observation that *C. difficile* isolates with elevated linezolid MICs harbor the gene in question might be considered a hint about the role of this gene but not a proof that this gene in fact plays a role in linezolid resistance. Moreover, two other *C. difficile* isolates from the same strain collection which showed elevated MICs of linezolid (16 μg/ml) and also elevated MICs of erythromycin, clindamycin, and chloramphenicol, did not harbor this *cfr*-related gene. This observation suggests that other, so-far-unidentified genes/mechanisms may account for the elevated linezolid MIC in *C. difficile*.

Taking these considerations together, we feel that it is absolutely important to confirm the role of this *cfr*-related gene in linezolid resistance by supplementing the observations made in the manuscript by Marín et al. (1) with adequate additional experiments. These experiments may include the cloning of the gene in question, transforming it into a susceptible host, showing that the gene is expressed in this host, and showing that it confers a Cfr-associated resistance phenotype in this new host. If these experi-

**Citation** Schwarz S, Wang Y. 2015. Nomenclature and functionality of the so-called *cfr* gene from *Clostridium difficile*. Antimicrob Agents Chemother 59:2476–2477. doi:10.1128/AAC.04893-14.

Address correspondence to Stefan Schwarz, stefan.schwarz@fli.bund.de.

***Ed. Note:*** *The authors of the published article declined to respond.*



doi:10.1128/AAC.04893-14

ments are successfully conducted and the role of this gene as a linezolid resistance gene is confirmed, the MLS nomenclature center should be approached for a unique resistance gene designation which, based on the comparatively low percentage of identity, should be other than *cfr*.

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