AMERICAN SOCIETY FOR MICROBIOLOGY | Antimicrobial Agents and Chemotherapy

LETTER TO THE EDITOR

Features of the *mcr-1* Cassette Related to Colistin Resistance

Katrin Zurfluh,[a] Nicolas Kieffer,[b] Laurent Poirel,[b,c] Patrice Nordmann,[b,c,d] Roger Stephan[a]

Institute for Food Safety and Hygiene, Vetsuisse Faculty, University of Zurich, Switzerland[a]; Emerging Antibiotic Resistance Unit, Medical and Molecular Microbiology, Department of Medicine, University of Fribourg, Fribourg, Switzerland[b]; INSERM European Unit (LEA Paris, France), University of Fribourg, Fribourg, Switzerland[c]; University of Lausanne and University Hospital Center, Lausanne, Switzerland[d]

The recent description of the plasmid-mediated colistin resistance gene, *mcr-1*, in strains isolated from food animals, food, and humans in China was the signal for an avalanche of retrospective and prospective studies investigating the occurrence of this specific gene (1). The *mcr-1* gene has been identified almost all over the world now, and the earliest evidence for its presence dates back to the 1980s (2). The *mcr-1* gene has so far been associated with nonrelated types of plasmid replicons such as IncI2, IncHI2, IncP, IncFIB, and IncX4 (1, 3–5) and was found only rarely to be carried on the chromosome (6). This gene is part of a 2,600-bp-long fragment designated as the *mcr-1* cassette that encompasses the likely promoter sequences for *mcr-1* expression (7). The *mcr-1* gene is most often located at the right-hand end of the insertion element IS*Apl1*, together with a 723-bp-long open reading frame (ORF) named *orf723* encoding a hypothetical protein. According to BLAST analysis, it is a putative phosphoesterase and shares 45% and 44% identities with those of *Corynebacterium durum* (GenBank accession no. WP_060996190.1) and *Psychrobacter arcticus* (GenBank accession no. WP_011280438.1), respectively. However, the putative contribution of this ORF to expression of the *mcr-1* gene and subsequently to colistin resistance remains unknown.

Our goal was to evaluate the role of *orf723* with respect to colistin resistance. Therefore, three *Escherichia coli* recombinant strains were constructed, with the same plasmid harboring either the *mcr-1* gene alone, *orf723* alone, or the entire *mcr-1* cassette, respectively. The primers used to amplify the *mcr-1* gene, the 723-bp ORF, and the whole *mcr-1* cassette are listed in Table 1, and the *mcr-1*-positive *E. coli* strain OW3E1 (GenBank accession no. KX129783) was used as the template. Amplicons were doubly digested with restriction enzymes BamHI and EcoRI and cloned into the low-copy vector pCCR9 (NCBI Taxonomy database, NCBI Nucleotide database accession no. 125570) digested with the respective enzymes, to create vectors pCCR9::*mcr-1*, pCCR9::*orf723*, and pCCR9::*mcr-1*::*orf723*. The constructs were transformed by electrotransformation into *E. coli* DH5α, giving rise to recombinant strains DH5α::pCCR9::*mcr-1*, DH5α::pCCR9::*orf723*, and DH5α::pCCR9::*mcr-1*::*orf723*, respectively. MICs of colistin were determined using broth dilution tests as recommended by EUCAST.

TABLE 2 Colistin MICs for each *E. coli* DH5α conjugant as well as the negative controls *E. coli* DH5α::pCCR9 and DH5α[a]

Conjugant or strain	Colistin MIC (mg/liter)
DH5α::pCCR9::*mcr-1*	4
DH5α::pCCR9::*orf723*	1
DH5α::pCCR9::*mcr-1*::*orf723*	4
DH5α::pCCR9	0.5
DH5α	1

[a] MICs were determined using broth dilution tests as recommended by EUCAST.

The MICs are summarized in Table 2. The MIC values of recombinant strains expressing MCR-1 with and without *orf723* were increased and identical. These results further confirm that expression of the *mcr-1* gene confers reduced susceptibility to colistin. However, they show that *orf723*, which encodes a hypothetical protein and which has likely been comobilized with the *mcr-1* gene from its original genetic context, does not impact colistin susceptibility.

Accepted manuscript posted online 15 August 2016

Citation Zurfluh K, Kieffer N, Poirel L, Nordmann P, Stephan R. 2016. Features of the *mcr-1* cassette related to colistin resistance. Antimicrob Agents Chemother 60:6438–6439. doi:10.1128/AAC.01519-16.

Address correspondence to Roger Stephan, stephanr@fsafety.uzh.ch.

TABLE 1 Primers used for cloning of *mcr-1*, the hypothetical protein product, and the whole *mcr-1* cassette

Primer name	Sequence (5′→3′)[a]	T_m (°C)[b]	Location
mcr_BamHI_up	TTTTTTGGATCCGCCGCAATTATCCCACCG	53	22 bp upstream of *mcr-1* start codon[c]
mcr_EcoRI_dn	TTTTTTGAATTCCCACCGCCCATAATACGAATGG	56	36 bp downstream of *mcr-1* stop codon
orf723_BamHI_up2	TTTTTTGGATCCGCACACTCCATTCGTATTATGGGC	57	18 bp upstream of 723-bp ORF start codon
orf723_EcoRI_dn	TTTTTTGAATTCCCGTTCCTATTGGTAGTTTCCAGG	56	81 bp downstream of 723-bp ORF stop codon

[a] The restriction sites are underlined.
[b] T_m, melting temperature.
[c] Downstream of putative promoter region.

ACKNOWLEDGMENT

The authors have no conflicts of interest to declare.

FUNDING INFORMATION

This work, including the efforts of Katrin Zurfluh, was funded by University of Zurich. This work, including the efforts of Nicolas Kieffer, was funded by University of Fribourg. This work, including the efforts of Katrin Zurfluh, was funded by Swiss Federal Office of Public Health.

REFERENCES

1. **Liu YY, Wang Y, Walsh TR, Yi LX, Zhang R, Spencer J, Doi Y, Tian G, Dong B, Huang X, Yu LF, Gu D, Ren H, Chen X, Lv L, He D, Zhou H, Liang Z, Liu JH, Shen J.** 2016. Emergence of plasmid-mediated colistin resistance mechanism MCR-1 in animals and human beings in China: a microbiological and molecular biological study. Lancet Infect Dis **16:**161–168. http://dx.doi.org/10.1016/S1473-3099(15)00424-7.
2. **Schwarz S, Johnson AP.** 2016. Transferable resistance to colistin: a new but old threat. J Antimicrob Chemother **71:**2066–2070. http://dx.doi.org/10.1093/jac/dkw274.
3. **Nordmann P, Lienhard R, Kieffer N, Clerc O, Poirel L.** 2016. Plasmid-mediated colistin-resistant *Escherichia coli* in bacteremia in Switzerland. Clin Infect Dis **62:**1322–1323. http://dx.doi.org/10.1093/cid/ciw124.
4. **Zhi C, Lv L, Yu LF, Doi Y, Liu JH.** 2016. Dissemination of the *mcr-1* colistin resistance gene. Lancet Infect Dis **16:**292–293. http://dx.doi.org/10.1016/S1473-3099(16)00063-3.
5. **Zurfluh K, Klumpp, J, Nüesch-Inderbinen M, Stephan, R.** 20 June 2016. Full-length nucleotide sequences of *mcr-1* harboring plasmids isolated from extended-spectrum β-lactamase (ESBL)-producing *Escherichia coli* of different origins. Antimicrob Agents Chemother http://dx.doi.org/10.1128/AAC.00935-16.
6. **Zurfluh K, Tasara T, Poirel L, Nordmann P, Stephan R.** 2016. Draft genome sequence of *Escherichia coli* S51, a chicken isolate harboring a chromosomally-encoded *mcr-1* gene. Genome Announc **4:**e00796-16. http://dx.doi.org/10.1128/genomeA.00796-16.
7. **Poirel L, Kieffer N, Brink A, Coetze J, Jayol A, Nordmann P.** 2016. Genetic features of MCR-1-producing colistin-resistant *Escherichia coli* isolates in South Africa. Antimicrob Agents Chemother **60:**4394–4397. http://dx.doi.org/10.1128/AAC.00444-16.