

# Occurrence of the Plasmid-Borne *mcr-1* Colistin Resistance Gene in Extended-Spectrum-β-Lactamase-Producing *Enterobacteriaceae* in River Water and Imported Vegetable Samples in Switzerland

Katrin Zurfluh,<sup>a</sup> Laurent Poirel,<sup>b</sup> Patrice Nordmann,<sup>b,c</sup> Magdalena Nüesch-Inderbinnen,<sup>a</sup> Herbert Hächler,<sup>a</sup> Roger Stephan<sup>a</sup>

Institute for Food Safety and Hygiene, Vetsuisse Faculty, University of Zurich, Zurich, Switzerland<sup>a</sup>; Emerging Antibiotic Resistance Unit, Medical and Molecular Microbiology, Department of Medicine, Faculty of Science, University of Fribourg,<sup>b</sup> and HFR - Hôpital Cantonal,<sup>c</sup> Fribourg, Switzerland

The recent identification of members of the family *Enterobacteriaceae* harboring the plasmid-mediated transferable colistin resistance *mcr-1* gene is of great concern to public health (1–4). Here, we report on the occurrence of *mcr-1*-harboring extended-spectrum β-lactamase (ESBL)-producing members of *Enterobacteriaceae* from river water in Switzerland and ready-to-eat imported vegetables.

For this study, 74 ESBL-producing members of *Enterobacteriaceae* isolated from 21 rivers and lakes sampled in 2012 in Switzerland (5) and 60 ESBL-producing members of *Enterobacteriaceae* isolated from 42 imported vegetable samples (11 from the Dominican Republic, 13 from India, 11 from Thailand, and 8

from Vietnam) (6) were screened by PCR for the presence of the *mcr-1* gene.

The *mcr-1* gene was detected in 1 out of 74 water strains (an isolate from the river Birs) and 2 out of 60 vegetable strains (products from Thailand and Vietnam), and sequencing of the amplicons showed a 100% identity with the published *mcr-1* sequence (1). The colistin resistance was transferable by transformation experiments into *Escherichia coli* DH5-α. All strains were *Escherichia coli* and belonged to different multilocus sequence types (MLSTs), harbored different *bla*<sub>ESBL</sub> genes, and showed a multiresistance phenotype (Table 1). The diversity of ESBL genes and MLSTs identified among *mcr-1*-positive isolates suggests that the *mcr-1* gene might be carried on different plasmids.

The spread of *mcr-1*-harboring, ESBL-producing members of the *Enterobacteriaceae* in surface water suggest environmental contamination. Appropriate measures urgently need to be enforced in order to reduce the anthropogenic burden of antibiotic resistance in the environment, such as the judicious use of antibiotics in human and veterinary medicine as well as in agriculture. In addition, improvement of water status is of major concern. New strategies for the treatment of wastewaters, e.g., the use of sand filters or more-stringent chlorine disinfection, need to be taken into consideration to prevent resistant bacteria from being released into the aquatic environment. Moreover, these data show that the international production and trade of fresh vegetables constitute a possible route for the spread of antibiotic-resistant, and particularly colistin-resistant, *Enterobacteriaceae*.

TABLE 1 Characteristics and resistance profiles of three ESBL-producing and *mcr-1*-positive strains of *Enterobacteriaceae*<sup>a</sup>

Characteristic or antimicrobial agent	Strain OW3E1	Strain H226B	Strain 2SK1
Characteristic			
Sample type	River water sampled in Switzerland	Cha-om imported from Thailand	Basil leaves imported from Vietnam
Yr	2012	2014	2014
Species	<i>E. coli</i>	<i>E. coli</i>	<i>E. coli</i>
Phylogroup	B1	A	B1
Sequence type	ST359	ST167	ST4683
<i>bla</i> <sub>ESBL</sub> type	SHV-12	CTX-M55	CTX-M-65
MIC for colistin (μg/ml)	6	6	6
Antimicrobial agent			
AM	R	R	R
AMC	S	S	R
CF	R	R	R
CTX	R	R	R
CIP	R	R	R
GM	S	R	R
TE	R	R	R
S	R	R	R
C	R	S	R
K	S	R	S
NA	R	R	R
SMZ	R	R	R
TMP	R	S	R

<sup>a</sup> The characteristics and resistance profiles of three strains are shown. Whether the strain was resistant (R) or susceptible (S) to the antimicrobial agents is shown. Antimicrobial agent abbreviations: AM, ampicillin; AMC, amoxicillin-clavulanic acid; CF, cephalothin; CTX, cefotaxime; CIP, ciprofloxacin; GM, gentamicin; TE, tetracycline; S, streptomycin; C, chloramphenicol; K, kanamycin; NA, nalidixic acid; SMZ, sulfamethoxazole; TMP, trimethoprim.

## 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. 18 November 2015. 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* [http://dx.doi.org/10.1016/S1473-3099\(15\)00424-7](http://dx.doi.org/10.1016/S1473-3099(15)00424-7).
2. Arcilla MS, van Hattem JM, Matamoros S, Melles DC, Penders J, de Jong MD, Schultsz C, COMBAT Consortium. 17 December 2015. Dissemination of the *mcr-1* colistin resistance gene. *Lancet Infect Dis* [http://dx.doi.org/10.1016/S1473-3099\(15\)00541-1](http://dx.doi.org/10.1016/S1473-3099(15)00541-1).

3. Webb HE, Granier SA, Marault M, Millemann Y, den Bakker HC, Nightingale KK, Bugarel M, Ison SA, Scott HM, Loneragan GH. 17 December 2015. Dissemination of the *mcr-1* colistin resistance gene. *Lancet Infect Dis* [http://dx.doi.org/10.1016/S1473-3099\(15\)00538-1](http://dx.doi.org/10.1016/S1473-3099(15)00538-1).
4. Hasman H, Hammerum AM, Hansen F, Hendriksen RS, Olesen B, Agersø Y, Zankari E, Leekitcharoenphon P, Stegger M, Kaas RS, Cavaco LM, Hansen DS, Aarestrup FM, Skov RL. 10 December 2015. Detection of *mcr-1* encoding plasmid-mediated colistin-resistant *Escherichia coli* isolates from human bloodstream infection and imported chicken meat, Denmark 2015. *Euro Surveill* 20(49). <http://dx.doi.org/10.2807/1560-7917.ES.2015.20.49.30085>.
5. Zurfluh K, Hächler H, Nüesch-Inderbinnen M, Stephan R. 2013. Characteristics of extended-spectrum beta-lactamase (ESBL)- and carbapenemase-producing Enterobacteriaceae isolated from rivers and lakes in Switzerland. *Appl Environ Microbiol* 79:3021–3026. <http://dx.doi.org/10.1128/AEM.00054-13>.
6. Zurfluh K, Nüesch-Inderbinnen M, Morach M, Zihler Berner A, Hächler H, Stephan R. 2015. Extended-spectrum  $\beta$ -lactamase-producing Enterobacteriaceae in vegetables imported from the Dominican Republic, India, Thailand, and Vietnam. *Appl Environ Microbiol* 81:3115–3120. <http://dx.doi.org/10.1128/AEM.00258-15>.