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MECHANISMS OF RESISTANCE

Plasmid-Mediated *mcr-1* Colistin Resistance in *Escherichia coli* from a Black Kite in Russia

Hassan Tarabai,[a] Adam Valcek,[a,b] Ivana Jamborova,[a,b] Sergey V. Vazhov,[c] Igor V. Karyakin,[d] Rainer Raab,[e] Ivan Literak,[a,b] Monika Dolejska[a,b]

[a]Department of Biology and Wildlife Diseases, University of Veterinary and Pharmaceutical Sciences Brno, Brno, Czech Republic
[b]Central European Institute of Technology, University of Veterinary and Pharmaceutical Sciences Brno, Brno, Czech Republic
[c]Shukshin Altai State University for Humanities and Pedagogy, Biysk, Russia
[d]LLC Sibecocenter, Novosibirsk, Russia
[e]Technisches Büro für Biologie, Deutsch-Wagram, Austria

ABSTRACT The gene *mcr-1* conferring resistance to last-line antibiotic colistin has been reported globally. Here, we describe the first detection of plasmid-mediated colistin resistance in Russian wildlife, an isolate of *Escherichia coli* sequence type 2280 from a black kite (*Milvus migrans*) scavenging raptor. Whole-genome sequencing and plasmid transferability experiments revealed that *mcr-1.1* was located on conjugative IncI2 plasmid pDR164 (59891 bp). Migratory black kites may contribute to the global spread of mobile colistin resistance.

KEYWORDS DNA sequencing, *Escherichia coli*, colistin, kite, landfill, plasmid, wildlife

Colistin is listed by World Health Organization as a critically important antibiotic of last resort for the treatment of severe human infections caused by multidrug-resistant Gram-negative bacteria (1). In veterinary medicine, apart from therapeutic purposes, colistin is used also as an in-feed growth promoter in livestock in some Asian countries, including India, Japan, and Vietnam (2). As a result of growing concern about emerging mobile colistin resistance, the use of colistin in food-producing animals is globally limited or banned (3). Plasmid-mediated colistin resistance gene *mcr-1* has been described in more than 40 countries, with the majority of reports coming from inpatients and domestic animals. Until now, only a few studies observed this resistance mechanism in bacteria from wildlife (4–12). In this study, we describe an *Escherichia coli* isolate with *mcr-1* from a migratory wild bird, the black kite (*Milvus migrans*), in Russia, which is to our best knowledge the first report of plasmid-mediated colistin resistance in wildlife in that country.

Black and red kites (*Milvus milvus*) are avian scavengers with different migration habits (13). Black kites of subspecies *M. migrans migrans* and *M. migrans lineatus* are summer residents in Europe and northern part of Asia that migrate to winter mainly in sub-Saharan Africa and southern parts of Asia, respectively. The red kite is essentially a European raptor (13). As a result of their frequent foraging in landfills, they may acquire antibiotic-resistant bacteria of human origin and spread them further over long distances (14). In 2018, a total of 168 cloacal swabs from nestlings of black and red kites were collected in Austria (6 samples), Belgium (12 samples), Czech Republic (93 samples), Germany (41 samples), and Russia (16 samples). Swabs were enriched in buffered peptone water and plated on SuperPolymyxin medium (15) to screen for *Enterobacteriales* isolates resistant to colistin. One single colony per plate/sample was selected, and species was identified and tested for the presence of *mcr-1* to *mcr-8*. One isolate positive for *mcr-1* was subjected to antibiotic resistance profiling and whole-genome sequencing on a MiSeq (Illumina) platform. Sequencing data were examined

Citation Tarabai H, Valcek A, Jamborova I, Vazhov SV, Karyakin IV, Raab R, Literak I, Dolejska M. 2019. Plasmid-mediated *mcr-1* colistin resistance in *Escherichia coli* from a black kite in Russia. Antimicrob Agents Chemother 63:e01266-19. https://doi.org/10.1128/AAC.01266-19.

Address correspondence to Monika Dolejska, monika.dolejska@gmail.com.

Received 21 June 2019
Returned for modification 9 July 2019
Accepted 11 July 2019
Accepted manuscript posted online 15 July 2019
Published 23 August 2019

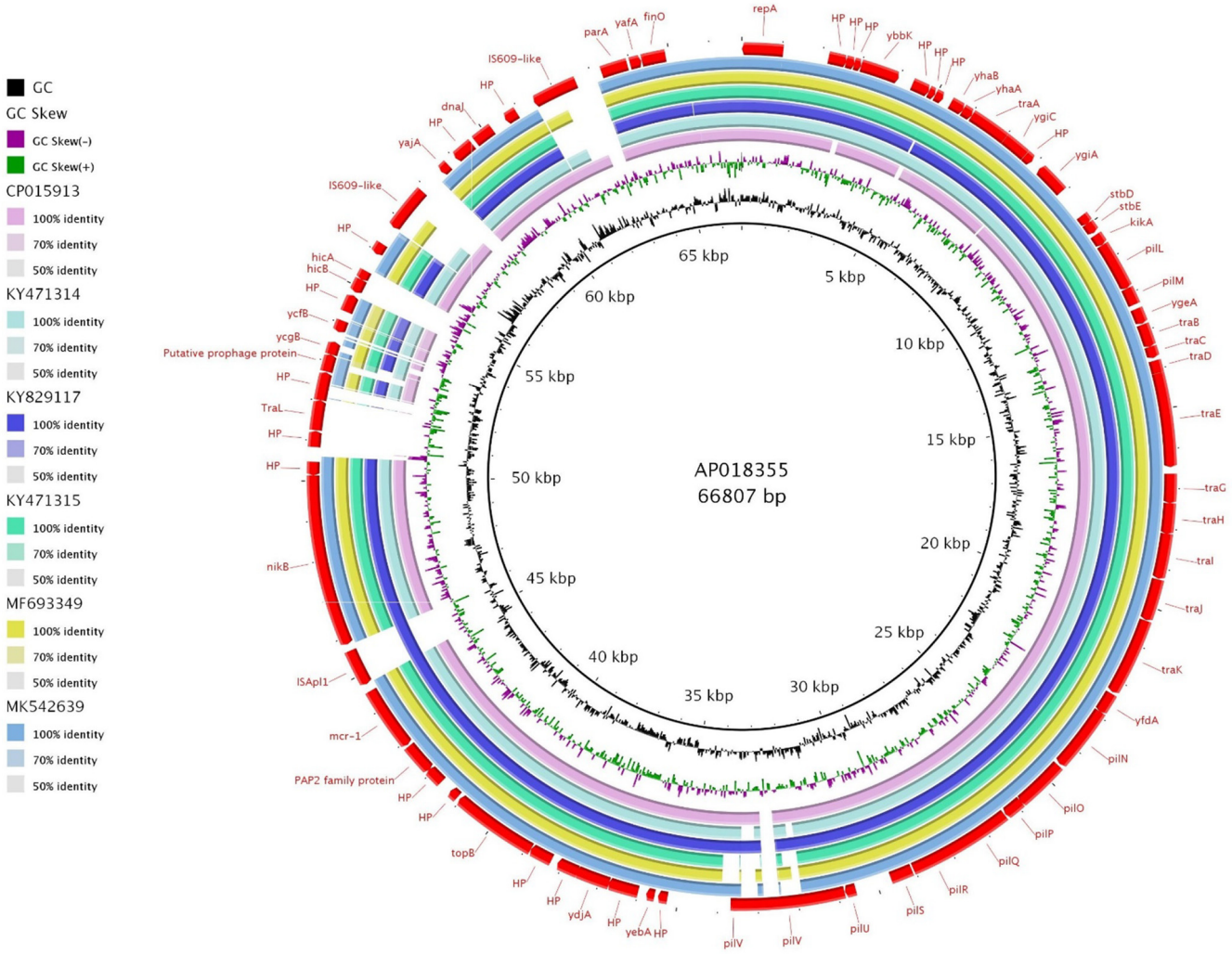

FIG 1 BRIG comparison of *mcr-1*-positive IncI2 plasmid pDR164 (GenBank accession no. MK542639 [17]) with similar plasmid sequences retrieved from GenBank. Plasmids used for comparison include pMCR-M19736 (25), pMCR-M19855 (KY471315 [16]), and pG3216 (MF693349 [26]) originating from human clinical samples in Argentina, pSLy1 (CP015913 [27]) from a swine sample in the United States, pVE362 (AP018355 [19]) from poultry in Vietnam, and pMCR_WCHEC1604-IncI2 (KY829117 [18]) from sewage in China.

to identify the genetic context of *mcr-1*. Horizontal transfer of colistin resistance into a recipient *E. coli* strain was tested using the filter mating method. See the supplemental material for detailed description of sampling and methods.

One isolate with *mcr-1.1* identified as *E. coli* sequence type (ST) 2280 was recovered from a black kite sampled in Semenovod, Altaysky Kray, Russia (52°33′N, 85°24′E) on 7 July 2018. The isolate was susceptible to all tested antibiotics apart from colistin (MIC $>$ 4 mg liter^{-1}). Whole-genome sequencing analysis and transferability experiments showed that the *mcr-1* gene was located on a conjugative IncI2 plasmid pDR164 (Fig. 1). The complete circular plasmid sequence was annotated according to pMCR-M19855 (GenBank accession no. KY471315 [16]) and submitted to GenBank under accession no. MK542639 (17). pDR164 sequence consists of 59,891 bp with GC content of 42.4% and 80 predicted open reading frames with a typical IncI2 backbone, including regions for stability, replication, maintenance, and horizontal gene transfer.

For the comparison of pDR164 with similar plasmid sequences, a BLAST search was performed (on 15 March 2019), and complete plasmid sequences with identity thresholds of ≥99% and query coverage thresholds of ≥96% were selected. The five IncI2 plasmid sequences that met the criteria are listed in Table S1, all of which carried *mcr-1*

with the absence of any other antibiotic resistance gene. A high level of similarity within the conserved IncI2 plasmid backbone and the variable region carrying *mcr* was observed (Fig. 1). pDR164 did not contain insertion sequence IS*Apl1* flanking the *mcr-1* region, unlike pMCR_WCHEC1604-IncI2 (KY829117 [18]) and pVE362 (AP018355 [19]). The *mcr-1* gene can be mobilized in a conjugative plasmid with two IS*Apa1* sequences flanking it as a composite transposon (Tn*6330*) or with one IS*Apl1*. However, the absence of IS*Apl1* from the vicinity of the *mcr-1* gene is common and may occur after the insertion of *mcr-1* in a conjugative plasmid and following subsequent recombination events (20).

Seven reports of *mcr-1*-positive *E. coli* isolates in wildlife were published. Most of them originated from migrating birds such as European herring gull (*Larus argentatus*) in Lithuania (4), kelp gull (*Larus dominicanus*) in Argentina (5), Eurasian coot (*Fulica atra*) from Pakistan (6), and swallows from China (10). The *mcr-1* gene was commonly detected on conjugative IncI2 and IncX4 plasmids in human, domestic animal (21), and wildlife *E. coli* isolates of diverse sequence types and antibiotic resistance profiles. As in our study, IncI2 plasmids carrying *mcr-1* were reported in wild birds from Argentina (5) and Pakistan (6). IncX4 plasmids, which appear to be a globally major source of mobility and dissemination of *mcr-1* gene (2), were described in *Enterobacterales* in penguins in Brazil (7), common blowflies from urban and rural communities in Thailand (11), and stable flies in Germany (12). At the time of writing this article, eleven *mcr-1*-positive *E. coli* isolates from clinical sources with phenotypic colistin resistance (MIC $>$ 4 mg liter^{-1}) have been reported in Russia (22).

Black kites have a wide range of human-made and natural diets (14). Based on the available food opportunities and the reported food diet of black kites in the vicinity of the sampled nestling (23), two possible sources of *mcr-1* isolates can be hypothesized. The first source could be Biysk municipal landfill located 16 km northwest of the sampled nest, which is commonly used by local black kites for foraging, while the second source could be natural food (carrions, rodents, birds, or small mammals) of black kites from flooded area along the Biya River where the nest was located.

In conclusion, we describe the first report of mobile colistin resistance from wildlife in Russia and present the first complete sequence of an *mcr-1*-harboring IncI2 plasmid from wildlife. Detection of a plasmid-mediated gene for resistance to a last-line drug in black kites is worrisome. Black kites can act as reservoirs and vectors of antibiotic-resistant bacteria that can be disseminated through their long migratory pathways. Since its discovery in 2015 (24), the *mcr-1* gene appears to be spread globally in veterinary, clinical, and environmental sectors (2). Based on our finding and on previous reports of colistin-resistant *E. coli* in wildlife, it appears that *mcr-1* found its way to the environment. Thus, continued surveillance of bacteria with transferrable colistin resistance mediated by *mcr* genes in wildlife is desirable.

(Part of this work was presented at the Central European Institute of Technology [CEITEC] PhD retreat, Kouty, Czech Republic, 2019 [talk number T18], and the 8th Symposium on Antimicrobial Resistance in Animals and the Environment [ARAE], Institut national de la recherche agronomique, Tours, France, 2019 [talk number O7].)

SUPPLEMENTAL MATERIAL

Supplemental material for this article may be found at https://doi.org/10.1128/AAC.01266-19.

SUPPLEMENTAL FILE 1, PDF file, 0.4 MB.

ACKNOWLEDGMENTS

We thank L. Cavaco (Technical University of Denmark, Kongens Lyngby, Denmark), B. B. Xavier (University of Antwerp, Antwerp, Belgium), M. Ugarte Ruiz (Foodborne Zoonoses and Antimicrobial Resistance Unit, Complutense University, Madrid, Spain), A. Carattoli (Istituto Superiore di Sanita, Rome, Italy), and Marcela Krutova (Charles University, 2nd Faculty of Medicine, Motol University Hospital, Prague, Czech Republic) for providing positive-control strains. We also thank Katerina Rehakova and Martin Klvana

from the University of Veterinary and Pharmaceutical Sciences Brno, Czech Republic, for their help in the laboratory. We thank Karel Makon, Hynek Matusik, and Roman Bachtin for the cooperation in the field.

The project was supported by Czech Health Research Council (grant number NV18-09-00605) and the National Sustainability Programs II (NPU II; grant LQ1601) provided by the Ministry of Education Youth and Sports of the Czech Republic. The work was partially financed by Internal Grant Agency of University of Veterinary and Pharmaceutical Sciences Brno (204/2018/FVHE) to M.D. The funders had no role in study design, data collection and interpretation, or the decision to submit the work for publication.

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