J Antimicrob Chemother 2016
doi:10.1093/jac/dkw262
Advance Access publication 20 June 2016

The colistin resistance *mcr-1* gene is going wild

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Sir,

Since the first report by Liu *et al.*[1] of the plasmid-encoded *mcr-1* gene conferring colistin resistance, its presence has been documented worldwide in food and food-producing animals, the environment and humans.[2] However, the presence of the *mcr-1* gene in wildlife, i.e. animals that do not naturally come into contact with antibiotics, has rarely been documented. In the present study, we determined the occurrence and the molecular characteristics of *mcr-1*-carrying isolates among extended-spectrum cephalosporin-resistant (ESC^R) Enterobacteriaceae recovered from Kelp gulls (*Larus dominicanus*).

During November 2012, faecal specimens ($n=50$) were collected from Kelp gulls in Ushuaia, Argentina. All samples were enriched in brain heart infusion broth (Becton–Dickinson, Franklin Lakes, NJ, USA), supplemented with 16 mg/L vancomycin for 18–24 h at 37°C, and subsequently inoculated on ChromID™ ESBL (bioMérieux, Solna, Sweden). The recovered isolates were identified using MALDI-TOF MS (Bruker, Coventry, UK), while their antibiotic susceptibility was assessed by broth microdilution (EUVSEC, Sensititre, Thermo Fischer, Basingstoke, UK). Overall, we recovered five non-duplicate ESC^R *E. coli* isolates exhibiting reduced susceptibility to colistin (MICs varying from 4 to 8 mg/L) from an equal number of faecal samples. Apart from colistin, all isolates exhibited non-WT MICs of ampicillin, cefotaxime, ciprofloxacin, nalidixic acid, tetracycline and sulfamethoxazole. For the remaining agents tested non-WT MICs were found for 0% to 80% of the isolates. See Table 1.

Genes conferring ESC^R and the plasmid-mediated colistin resistance (*mcr-1*) phenotype were sought, as previously described.[1,3] The plasmid content of each strain was determined by PCR-based replicon typing (DIATHEVA, Fano, Italy) with the addition of single PCRs for IncX4 and ColE plasmids, as previously described.[4,5] The plasmid location of the *mcr-1* gene was assessed by S1-PFGE assay and Southern-blot analysis using DIG-labelled probes (DIG DNA Labeling and Detection Kit, Roche, Mannheim, Germany) targeting the *mcr*-1 gene and the different plasmid replicons present in each strain. The presence of the

Table 1. Characteristics of *mcr-1*-carrying *E. coli* isolates recovered from Kelp gulls in Ushuaia, Argentina, 2012

	MIC (mg/L)												Characteristics of *mcr*-1-encoding plasmid				
Strain ID	AMP	AZM	CAZ	CHL	CIP	CST	CTX	GEN	MEM	NAL	SMX	TET	TGC	TMP	ST/CC	plasmid type	size (kb)	transferability	Upstream region	ESBL gene
wb2	>64	8	≤0.5	128	>8	4	>4	1	≤0.03	>128	>1024	>64	≤0.25	>32	744	IncI2	57	ND	ISApl1	$bla_{CTX-M-14}$
wb6	>64	4	8	≤8	>8	8	>4	≤0.5	≤0.03	>128	>1024	16	≤0.25	≤0.25	101/101	IncI2	57	2.4×10^{-6}	ISApl1	$bla_{CTX-M-2}$
wb15	>64	8	1	>128	8	4	>4	≤0.5	≤0.03	>128	>1024	>64	≤0.25	>32	744	IncI2	57	ND	ISApl1	$bla_{CTX-M-14}$
wb32	>64	8	≤0.5	>128	>8	4	>4	1	≤0.03	>128	>1024	>64	≤0.25	>32	744	IncI2	57	2.3×10^{-6}	ISApl1	$bla_{CTX-M-14}$
wb38	>64	8	≤0.5	128	8	4	>4	≤0.5	≤0.03	>128	>1024	>64	≤0.25	>32	744	IncI2	57	ND	ISApl1	$bla_{CTX-M-14}$

AMP, ampicillin; AZM, azithromycin; CAZ, ceftazidime; CHL, chloramphenicol; CIP, ciprofloxacin; CST, colistin; CTX, cefotaxime; GEN, gentamicin; MEM, meropenem; NAL, nalidixic acid; SMX, sulfamethoxazole; TET, tetracycline; TGC, tigecycline; TMP, trimethoprim; ND, not determined; CC, clonal complex.

mcr-1 gene was confirmed in all five *E. coli* isolates with amplicon sequences being 100% identical to that reported by Liu *et al.*,[1] while they co-carried either $bla_{CTX\text{-}M\text{-}2}$ ($n=1$) or $bla_{CTX\text{-}M\text{-}14}$ ($n=4$) genes. In all five isolates the *mcr*-1 probe was hybridized with an ~57 kb plasmid and subsequent hybridization with rep probes showed that it belonged to the IncI2 family, whereas the $bla_{CTX\text{-}M}$ genes were located on different plasmids (data not shown).

The presence of IS*Apl1* upstream of the *mcr-1* gene was sought by PCR using BioMix Red (Bioline, London, UK) according to the manufacturer's instructions and the primer pair IS*Apl1*-*mcr*-F (5′-TGGACATTGGGAAGCCGATA-3′) and IS*Apl1*-*mcr*-R (5′-GCCACAAGAACAAACGGACT-3′), and subsequent sequencing analysis. The PCR conditions were as follows: 1 cycle of denaturation at 95°C for 5 min, followed by 30 cycles of denaturation at 95°C for 1 min, annealing at 60°C for 1 min and elongation at 72°C for 3 min, followed by 1 cycle at 72°C for 5 min. We confirmed the presence of IS*Apl1* upstream and in the same orientation as the *mcr-1* gene in all five *E. coli* isolates (Table 1), as previously described for *mcr-1*-carrying IncI2 plasmids.[1]

The genetic relatedness of *E. coli* isolates was assessed by MLST, as previously described.[6] Among the five *E. coli* isolates that carried *mcr*-1, two different STs were identified, namely ST101 ($n=1$) and ST744 ($n=4$), associated with the co-carriage of $bla_{CTX\text{-}M\text{-}2}$ and $bla_{CTX\text{-}M\text{-}14}$ genes, respectively (Table 1). Interestingly, an *E. coli* isolate belonging to ST744 and encoding *mcr*-1 on an IncI2 plasmid has been previously documented from human bloodstream infection in Denmark.[7]

Transfer of the *mcr*-1 gene from representative isolates for ST101 and ST744 to the recipient chloramphenicol-resistant *E. coli* MG1655 YFP was attempted by liquid mating assays in a 1:1 ratio. Transconjugants were selected on LB agar supplemented with a combination of chloramphenicol (25 mg/L) and colistin (2 mg/L). Positive transconjugants were confirmed by PCR amplification for the *mcr-1* and *yfp* genes. Plasmids carrying the *mcr*-1 gene conjugated at a transfer frequency of $\sim 2\times10^{-6}$ transconjugants per donor cell (Table 1).

To the best of our knowledge, this is the first report of the dissemination of the *mcr-1* gene in Kelp gulls. The fact that gull species migrate, sometimes even between continents, indicates that they may play a role in the global dissemination of these clinically relevant bacteria. The association of the *mcr-1* gene with conjugative IncI2 plasmids also among gulls illustrates a successful plasmid–gene combination, resulting in the emergence and spread of this gene. Having now documented the presence of *mcr-1*-carrying strains in wildlife, we emphasize the need for surveillance studies in different ecological niches to identify reservoirs and potential transmission routes of this gene.

Acknowledgements

We gratefully acknowledge Alieda van Essen-Zandbergen for excellent technical assistance, Dr Charlotte Berg and Dr Badrul Hasan for their contribution with sampling and isolation of the strains included in the study, and Dr Daniela Ceccarelli for critically reading the manuscript prior to submission. We are also thankful to Quark Expeditions for supporting the fieldtrip in Ushuaia, Argentina.

Funding

This work was funded by the Dutch Ministry of Economic Affairs (BO-22.04-008-001).

Transparency declarations

None to declare.

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J Antimicrob Chemother 2016
doi:10.1093/jac/dkw243
Advance Access publication 20 June 2016

Co-occurrence of *mcr-1* and ESBL on a single plasmid in *Salmonella enterica*

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