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.

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Transparency declarations

None to declare.

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

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Sir,

Liu *et al.*[1] recently reported the first transferrable colistin resistance gene *mcr-1* with prevalence in animals, foodstuffs and human beings in China. The *mcr-1* gene found in *Escherichia coli*,[2,3] *Klebsiella pneumoniae*[4] and *Salmonella* spp.[5] has been proved subsequently to be disseminated worldwide. The present study investigated the presence of the *mcr-1* gene among 53 ESBL-positive *Salmonella* spp. isolates, and obtained the complete sequence of an IncI2-type conjugative plasmid co-harbouring *mcr-1* and $bla_{CTX\text{-}M\text{-}55}$ genes in a *Salmonella enterica* isolate.

Fifty-three ESBL-positive *Salmonella* spp. isolates were collected between 2014 and 2015 from diseased chickens in eight provinces in China (Anhui, Hebei, Heilongjiang, Henan, Hubei, Ningxia, Shandong and Sichuan). All isolates collected from different samples were identified by using an automated system (BD Diagnostic Systems, Sparks, MD, USA). The *mcr-1* and $bla_{CTX\text{-}M}$ genes were detected by PCR amplification, and the corresponding primers amplifying the whole *mcr-1* gene are listed in Table S1 (available as Supplementary data at *JAC* Online). We found a total of four (7.5%) *mcr-1*-positive isolates with colistin MICs ranging from 4 to 8 mg/L (Table S2), and DNA sequencing of the whole *mcr-1* gene revealed 100% identity compared with the originally published gene sequence.[1]

Conjugation experiments and S1-PFGE demonstrated the co-occurrence of *mcr-1* and $bla_{CTX\text{-}M\text{-}55}$ on a conjugative plasmid of about 65 kb (Figure S1) in one isolate named SC23. SC23 belongs to the ST292 *S. enterica* serovar Albany, which was isolated from the intestinal contents of a sick chicken in Sichuan province on 13 January 2014. The plasmid was successfully transferred from *S. enterica* to *E. coli* J53 Az^r with a frequency of 3×10^{-4} per recipient cell. The MICs of colistin and cefotaxime for transconjugants were 8 and >128 mg/L, respectively, showing that the plasmid conferred a >32-fold and >256-fold increase in MIC compared with the recipient *E. coli* J53 Az^r (Table S2).

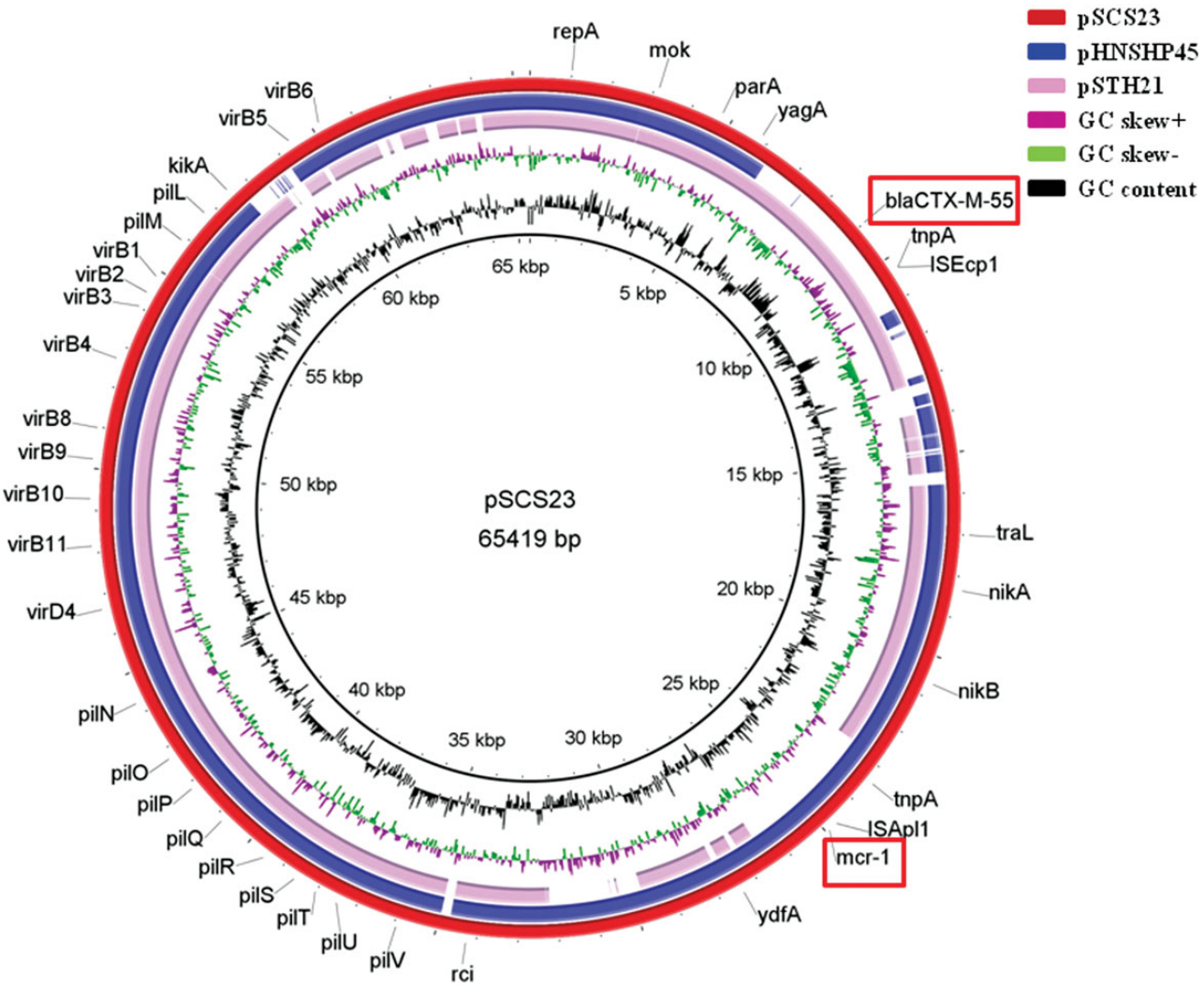

Figure 1. Circular genetic map of pSCS23. Sequence comparison of pSCS23 (KU934209) with other completely sequenced plasmids: pHNSHP45, KP347127; and pSTH21, LN623683. The *mcr-1* and $bla_{CTX\text{-}M\text{-}55}$ genes are highlighted by red boxes. The map was drawn using BRIG (http://sourceforge.net/projects/brig/). This figure appears in colour in the online version of *JAC* and in black and white in the print version of *JAC*.

The plasmid co-harbouring the *mcr-1* and $bla_{CTX\text{-}M\text{-}55}$ genes was extracted from the positive transconjugant with a Qiagen Midi kit (Qiagen, Hilden, Germany), and sequencing was carried out with an Illumina MiSeq using a 350 bp paired-end library (Majorbio Company, Shanghai, China). The sequences obtained were assembled into a unique scaffold and the existing gaps were closed by PCR using specific primers listed in Table S3. The complete sequence of the 65 419 bp plasmid, designated pSCS23, was found to encode 57 ORFs and the overall GC content was 42.26% (Figure 1). A BLAST comparison of our plasmid and pHNSHP45[1] revealed a 99% similarity (86% query coverage), and both plasmids share the same IS*Apl1* transposable element (3676 bp) that contains *mcr-1* and *tnpA* genes. In contrast, other studies showed that the *mcr-1* gene is flanked by either a truncated IS*Apl1* element without the *tnpA* gene[6] or sequences without the whole IS*Apl1* element.[7] This indicates that the genetic environment of the *mcr-1* gene is more mobile than we expected. Notably, pSCS23 harbours the $bla_{CTX\text{-}M\text{-}55}$ gene, which is flanked by the IS*Ecp1* mobile element within the structure of IS*Ecp1*–*tnpA*–$bla_{CTX\text{-}M\text{-}55}$ (2600 bp) (Figure S2). Furthermore, two recent studies demonstrated a plasmid co-harbouring either $bla_{CTX\text{-}M\text{-}1}$[8] or *tetA*–*tetR*–*sul1*–*aadA1*–*dfrA1*–*strA*–*strB*[6] accompanied by the *mcr-1* gene in *E. coli*. For other species, a recent study identified a plasmid carrying *mcr-1* and $bla_{CTX\text{-}M\text{-}15}$ and $bla_{TEM\text{-}1}$ in *Enterobacter aerogenes* from a patient in China.[9] It seems that the *mcr-1* gene easily exists together with $bla_{CTX\text{-}M}$ genes, and we speculate that the selection pressure on the *mcr-1* gene may select for broad-spectrum cephalosporin resistance, especially by IS*Ecp1*–*tnpA*–$bla_{CTX\text{-}M}$. The coexistence of *mcr-1* and $bla_{CTX\text{-}M}$ genes on a single plasmid presents more severe challenges in terms of controlling the election and transmission of *mcr-1*. Another characteristic of pSCS23 is that it lacks the IS*683* mobile element (2703 bp) that exists on pHNSHP45, which is consistent with other plasmids harbouring the *mcr-1* gene but lacking IS*683*.[6] We postulate that the IS*683* element should be present on pHNSHP45 occasionally, although complete sequences of plasmids carrying the *mcr-1* gene are very scarce at present. Moreover, pSCS23 showed a 99% similarity (83% query coverage) to plasmid pSTH21 identified in *S. enterica* isolated from a human in China, which also carries the $bla_{CTX\text{-}M\text{-}55}$ gene flanked by IS*Ecp1*.[10]

Here we report for the first time the co-occurrence of *mcr-1* and $bla_{CTX\text{-}M\text{-}55}$ on a single plasmid in *S. enterica.* Strong evidence exists for the ongoing transmission of *mcr-1* on a global scale and its co-occurrence on plasmids with $bla_{CTX\text{-}M}$ genes. This evidence is of grave concern and merits immediate action to limit the selection and transmission of *mcr-1* in order to preserve the efficacy of polymyxins.

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Transparency declarations

None to declare.

Supplementary data

Tables S1–S3 and Figures S1 and S2 are available as Supplementary data at *JAC* Online (http://jac.oxfordjournals.org/).

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Detection of an *mcr-1*-encoding plasmid mediating colistin resistance in *Salmonella enterica* from retail meat in Portugal

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