Emergence of NDM-5- and MCR-1-Producing *Escherichia coli* Clones ST648 and ST156 from a Single Muscovy Duck (*Cairina moschata*)

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Two *Escherichia coli* clones (sequence type 648 [ST648] and ST156) that coproduce NDM-5 and MCR-1 were detected from a single fowl in China. The *bla*_{NDM-5} gene was found on the two indistinguishable IncX3 plasmids from the two different *E. coli* isolates, whereas the *mcr-1* gene was located on IncHI2 and IncI2 plasmids, respectively, suggesting that *bla*_{NDM-5} and *mcr-1* have spread in avian intestinal flora. Also, the two strains harbor *bla*_{TEM-1}, *bla*_{CTX-M-55}, *fosA3*, and *aac(6')-Ib*. The multiresistant *E. coli* strains (especially the epidemic clone ST648) might raise a potential threat to human health via food chain transmission.

Carbapenem-resistant *Enterobacteriaceae* (CRE) are a global public health problem. The New Delhi metallo- β -lactamase (NDM) was first described in 2008 (1) and has become one of the most widespread carbapenemases in the world (2–4). Meanwhile, since the recent discovery of the plasmid-mediated colistin resistance gene *mcr-1* in China (5), several studies have confirmed its dissemination in different humans and animals (6–10). In addition, the coproduction of carbapenemase and MCR-1 was detected in a few bacteria (6, 8, 11–13), which poses a serious concern to public health. Here, we report the first case of NDM-5- and MCR-1-producing *Escherichia coli* clones sequence type 648 (ST648) and ST156 from a single fowl.

In May 2015, a rectal swab was collected from a 1-month-old Muscovy duck (*Cairina moschata*) with colibacillosis to study carbapenemase-encoding genes from animals in China. The diseased duck was sent to the veterinary clinical diagnosis laboratory in South China Agricultural University from a duck farm in Guangdong Province, China. Carbapenem-producing isolates were selected in MacConkey medium supplemented with meropenem (1 μ g/ml). Two *E. coli* isolates (NDM131 and NDM132) with diverse morphological characteristics were isolated and were identified by the Axima matrix-assisted laser desorption ionization–time of flight (MALDI-TOF) mass spectrometer (Shimadzu-Biotech Corp., Kyoto, Japan).

Antibiotic susceptibility testing was performed using the agar dilution method (14) and interpreted according to the Clinical and Laboratory Standards Institute guidelines (15). The EUCAST breakpoints for *Enterobacteriaceae* for colistin and tigecycline were applied (16).

Both *E. coli* isolates (NDM131 and NDM132) were resistant to cefoxitin, ceftazidime, cefotaxime, imipenem, meropenem, ertapenem, gentamicin, tobramycin, ciprofloxacin, tetracycline, fosfomycin, trimethoprim-sulfamethoxazole, and colistin but susceptible to tigecycline (see Table S1 in the supplemental material). NDM131 was also resistant to aztreonam and amikacin. The double-disk synergy test and biochemical Carba NP test confirmed extended-spectrum β -lactamase (ESBL) and carbapenemase production in both isolates.

PCR analyses were performed to confirm carbapenemase, ESBLs, plasmid-mediated AmpC cephalosporinase-encoding

genes, 16S rRNA methyltransferase genes, and fosfomycin resistance genes. The complete coding sequence of the NDM gene was amplified with a previously reported primer pair (17). PCR products were sequenced on both strands using an ABI3700 instrument (Applied Biosystems, Foster City, CA).

The results indicated that both isolates contained *bla*_{NDM-5}, *bla*_{CTX-M-55}, and *bla*_{TEM-1}, but NDM131 also carried *bla*_{CMY-2}. Interestingly, both isolates carried *mcr-1*, which encodes a phosphoethanolamine transferase responsible for resistance to colistin. The screen for fosfomycin resistance genes identified the *fosA3* variant in both isolates, explaining the high-level fosfomycin resistance phenotype. Moreover, the 16S rRNA methylase gene *rmtB* was present in NDM131 but not in NDM132. This gene conferred high-level resistance to all aminoglycosides. In addition, both isolates harbored *aac(6')-Ib*.

Genetic relationships between the isolates were evaluated by pulsed-field gel electrophoresis (PFGE) (18) and multilocus sequence typing (MLST) (19). PFGE was successfully performed with *E. coli* NDM131 and NDM132, exhibiting significantly different patterns (Fig. 1a). MLST analyses showed that NDM131 and NDM132 belong to the two epidemic clones (ST156 and ST648). Phylogenetic typing was conducted as described previously, and NDM131 was assigned to phylogenetic group B1, while NDM132 was assigned to phylogroup D.

The transferability of the resistance phenotype was studied by a conjugation experiment using streptomycin-resistant *E. coli* strain

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FIG 1 (a) Clonal relationship of the NDM-5- and MCR-1-coproducing clones (*E. coli* NDM131 and *E. coli* NDM132) by PFGE. Lane 1, *E. coli* NDM131; lane 2, *E. coli* NDM132; lane M, XbaI-digested genomic DNA of reference *Salmonella enterica* serotype Braenderup, strain H9812. Numbers on the right are molecular masses in kilodaltons. (b) Schematic representations of the genetic organization surrounding *bla*_{NDM-5} on *E. coli* NDM131 and *E. coli* NDM132. (c) Schematic representations of the genetic organization surrounding *mcr-1* on *E. coli* NDM131 and *E. coli* NDM132. The arrows indicate open reading frames, and resistance genes, insertion sequences, and accessory genes are indicated by red, green, and gray arrows, respectively. The numbers in parentheses are accession numbers.

EC600 as a recipient. Transconjugants were selected on MacConkey agar plates supplemented with streptomycin (1,000 mg/liter) and meropenem (1 mg/liter), and streptomycin (1,000 mg/liter) and colistin (2 mg/liter) for NDM131 and NDM132, respectively. NDM131 harbored multiple plasmid replicon types, including IncP, FIB, FIA, A/C, HI2, I2, and X3, but only the *bla*_{NDM-5}-carrying IncX3 plasmid was present in its transconjugant (NDM131T-N). In contrast, NDM132 harbored IncN, HI2, and X3, and its transconjugant (NDM132T-N) also possessed a *bla*_{NDM-5}-carrying IncX3 plasmid. As the conjugal transfer of *mcr-1* failed, electrotransformation assays were carried out using plasmid DNA from the two isolates. Only the *mcr-1*-carrying plasmid in NDM131 was successfully transformed into electrocompe-

tent *E. coli* DH5 α (NDM131E-M), which belonged to the IncI2 incompatibility group.

Plasmid sizes in the donor isolates NDM131, NDM132, and their transconjugants/transformants were estimated by pulsed-field gel electrophoresis analysis of S1 nuclease-digested DNA (S1-PFGE), followed by Southern blotting (20), using the appropriate probes. *bla*_{NDM-5} was located on a unique IncX3 plasmid in both donor isolates and their transconjugants (see Fig. S2b in the supplemental material), which ranged from 33.3 to 54.7 kb, while *mcr-1* was on a 54.7- to 76.8-kb IncI2 plasmid in NDM131 and its transformant (see Fig S2c). The *mcr-1* in donor isolate NDM132 was located on a 216.9- to 244.4-kb IncHI2 plasmid. The IncX3 plasmids from the two transconjugants yielded restriction frag-

ment length polymorphism (RFLP) patterns that were indistinguishable by the naked eye using EcoRI and BglII digestion (see Fig. S1 in the supplemental material). The complete sequence of the *bla*_{NDM-5}-carrying plasmid pNDM_MGR194 (GenBank accession no. [KF220657](#)) (21) was taken as the reference sequence for PCR mapping of the *bla*_{NDM-5} genetic environment. The *bla*_{NDM-5} genetic contexts on both pNDM131T-N and pNDM132T-N were identical to that in pNDM-MGR194 and were also highly similar to that of another NDM-4 IncX3 plasmid, pJEG027 (GenBank accession no. [KM400601](#)). In such a context, *bla*_{NDM-5} was adjacent to a truncated *ISAbal25* sequence flanked by an IS5 immediately upstream, as well as the genes *ble* (mediating bleomycin resistance), *trpF* (encoding a phosphoribosylanthranilate isomerase), *dsbC* (encoding an oxidoreductase), and a remnant of *ctuA1* (encoding an ion-tolerant protein) downstream, which is truncated by the insertion of IS26.

The published sequences (pHNSHP45, pMR0516mcr, and pHNSHP45-2) (5, 22, 23) were used as reference sequences for PCR mapping of the *mcr-1* genetic environment. The *mcr-1* genes were found to be located within diverse genetic contexts in both strains. For NDM131 and its transformant, the *mcr-1* genetic contexts were identical to that in pHNSHP45. While in NDM132, the *mcr-1* genetic context was highly similar to that in pMR0516mcr, except the *mcr-1* element was flanked by two *ISAp11* elements, and the second *ISAp11* was truncated by *ISKpn26*. Compared to another IncHI2 plasmid, pHNSHP45-2, the *ISAp11-mcr-1* transposition unit was in a different location and orientation.

To our knowledge, this is the first description of two *E. coli* clones (ST648 and ST156) that coproduce NDM-5 and MCR-1 from a single fowl. *E. coli* ST648 is a predominant multidrug-resistant clone observed worldwide in humans, companion animals, livestock, and wild birds (24, 25) and is frequently associated with various β -lactamases, including ESBLs, NDM, and KPCs (26, 27). Recently, *E. coli* ST648 was also found to carry *mcr-1*, which brought a more serious threat (28). In contrast, *E. coli* ST156 was not common as ST648, but it was also related to the distribution of *bla*_{NDM} and *bla*_{CTX-M} in humans and animals (27, 29). Two *E. coli* strains that shared an indistinguishable plasmid carrying *bla*_{NDM-5} indicate that this plasmid has *in vivo* transfer. Colistin is considered to be the most active *in vitro* agent against carbapenem-resistant *Enterobacteriaceae* (30). NDM131 and NDM132 possess two diverse *mcr-1*-carrying plasmids, which revealed that *mcr-1*-harboring plasmid reservoirs are diverse in avian intestinal flora. The cooccurrence of *bla*_{NDM-5} and *mcr-1* within the two *E. coli* isolates from the same fowl poses a potential threat to human public health following possible entry and spread through the food chain. Thus, it is important to monitor food animals for the emergence and prevalence of such carbapenem and colistin resistance genes.

Accession number. The annotated sequence of the *mcr-1* genetic environment from strain NDM132 has been submitted to GenBank under accession no. [KX458104](#).

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