



Various Sequence Types of *Enterobacteriaceae* Isolated from Commercial Chicken Farms in China and Carrying the *bla*_{NDM-5} Gene

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ABSTRACT A total of 108 meropenem-resistant *Enterobacteriaceae* isolates were obtained from 1,658 rectal swabs collected from 15 unrelated commercial chicken farms in China between 2014 and 2016. These samples yielded 16 *Escherichia coli* and 2 *Klebsiella pneumoniae* isolates of diverse sequence types carrying a *bla*_{NDM-5}-bearing IncX3 plasmid. *K. pneumoniae* strain sequence type 709 (ST709) has two *bla*_{NDM-5}-carrying plasmids that were transferred together to *E. coli*.

KEYWORDS *Escherichia coli*, *Klebsiella pneumoniae*, *bla*_{NDM-5}, IncX3 plasmid

Multidrug-resistant organisms, including those that are carbapenemase-producing *Enterobacteriaceae*, are becoming a challenging threat to public health worldwide (1). Gene *bla*_{NDM-5}, a variant of *bla*_{NDM}, was first identified in 2011 in an *Escherichia coli* sequence type 648 (ST648) isolate from a patient in the United Kingdom (2). Since then, it has been reported in various parts of the world, including South Korea (3), Denmark (4), Algeria (5), and China (6–8). The cooccurrence of NDM-5 and other carbapenemase enzyme isolates from the same patient is extremely worrisome because it might lead to therapeutic failure and death. The first cooccurrence of NDM-4- and NDM-5-producing *Klebsiella pneumoniae* in the same patient was reported in 2016 (9). In 2017, a carbapenem-resistant *K. pneumoniae* ST147 isolate harboring *bla*_{NDM-5} and *bla*_{OXA-181} from a hospitalized patient was found in the United States (10). In 2018, a *bla*_{NDM-5}- and *bla*_{OXA-48-like}-coproducing *E. coli* strain was first isolated in South Korea (11). Meanwhile, the first case of a clinical *Klebsiella michiganensis* isolate producing KPC-2, NDM-1, and NDM-5 was reported in China (12). A fusion plasmid (IncX3 and IncFIB) recoverable from an NDM-5-producing clinical *E. coli* isolate was recently characterized, and these types of recombination events presumably play a potential role in the development of new plasmids with extended resistance profiles (13). In this study, we identified the presence of various sequence types of *Enterobacteriaceae* carrying *bla*_{NDM-5} in chickens from multiple farms across seven Chinese provinces.

A total of 108 nonrepeated meropenem-resistant *Enterobacteriaceae* (97 *E. coli* and 11 *K. pneumoniae*) isolates (6.5%) were obtained from 1,658 rectal swabs collected from 15 unrelated commercial chicken farms in China between 2014 and 2016 (see Fig. S1 in the supplemental material). All of the *Enterobacteriaceae* isolates were selected on MacConkey agar plates supplemented with 2 μg/ml meropenem. Species identification was performed with the BD Phoenix-100 system (Becton Dickinson) and confirmed by 16S rRNA gene sequencing. Antimicrobial susceptibility testing was performed on Mueller-Hinton agar plates testing for 16 antimicrobials according to CLSI guidelines (14), except polymyxin B, for which European Committee on Antimicrobial Susceptibility Testing breakpoints were used (15).

These *Enterobacteriaceae* isolates were then subjected to screening for the presence

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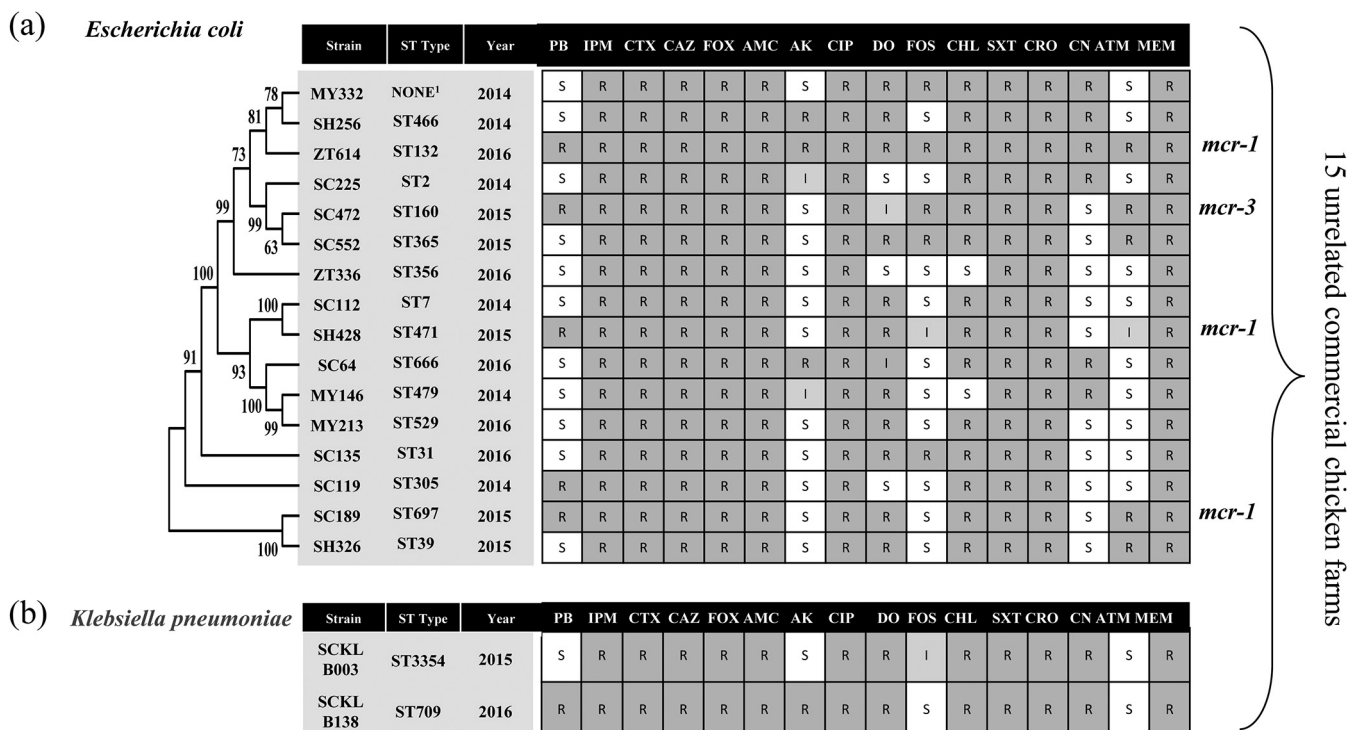


FIG 1 Phylogenetic relationship, antimicrobial resistance phenotypes, source of isolate, and sequence types of the 18 different *Enterobacteriaceae*: (a) *Escherichia coli* and (b) *Klebsiella pneumoniae*. A phylogenetic tree based on a maximum-likelihood method was built by MEGA6 with nucleotide sequences of 8 MLST genes to reveal a more detailed relationship among the analyzed strains. Bootstrap values (percentages of 1,000 replications) of >50% are shown at each node. 1, Failure to find any corresponding ST type with MLST database blasting. S, susceptible; I, intermediate; R, resistant; PB, polymyxin B; IPM, imipenem; CTX, cefotaxime; CAZ, ceftazidime; FOX, ceftoxitin; AMC, amoxicillin-clavulanate; AK, amikacin; CIP, ciprofloxacin; DO, doxycycline; FOS, fosfomycin; CHL, chloramphenicol; SXT, trimethoprim- sulfamethoxazole; CRO, ceftriaxone; CN, gentamicin; ATM, aztreonam; MEM, meropenem.

of *bla_{NDM}* and *mcr* genes by PCR assay (see Table S1 in the supplemental material) as previously described (16). Of 108 meropenem-resistant *Enterobacteriaceae* strains, 52 (48 *E. coli* and 4 *K. pneumoniae*; 3.14%) were found to harbor the *bla_{NDM-5}* gene. Multilocus sequence typing (MLST) was performed as previously described (<http://bigsd.bpasteur.fr/>); 16 different sequence types of *E. coli* isolates and 2 sequence types of *K. pneumoniae* isolates were found. Three of the 16 strains of *E. coli* were found to coharbor the *mcr-1* gene. Multidrug-resistant *E. coli* strain ZT614 ST132 was resistant to all 16 antimicrobials. Phylogenetic relationship, antimicrobial resistance phenotypes, and sequence types of the strains are shown in Fig. 1.

Conjugation experiments were performed between 18 different isolates (Fig. 1) and *E. coli* J53 Az^r as the recipient. Transconjugants were selected on Mueller-Hinton agar (MHA; Oxoid) plates that contained 200 μg/ml sodium azide with 2 μg/ml imipenem. All of them could successfully transfer their carbapenem resistance genes to the recipient strain *E. coli* J53 Az^r. There was no cotransfer of carbapenem and colistin resistance phenotype transconjugant. The total plasmid DNA from 18 transconjugants was extracted using a Qiagen plasmid minikit following manufacturer's recommendations (Qiagen, Hilden, Germany). Whole-genome sequencing was performed on the Illumina MiSeq platform (Majorbio, Shanghai) using a 350-bp paired-end TruSeq library with a 2 × 300 run. A draft assembly of the plasmids was made with plasmidSPAdes (17). Predicted gaps were closed by PCR and Sanger sequencing using specifically designed primers listed in Table S1. Identification of antibiotic resistance genes was done by ResFinder 3.0 (<http://www.genomicepidemiology.org/>), and plasmid replicon types were determined by using the PlasmidFinder tool (<http://genomicepidemiology.org/>).

Sequence analysis revealed that all of the transconjugants harbored a 46-kb *bla_{NDM-5}*-bearing IncX3 plasmid. BLASTN results showed that all 18 IncX3 plasmids had almost

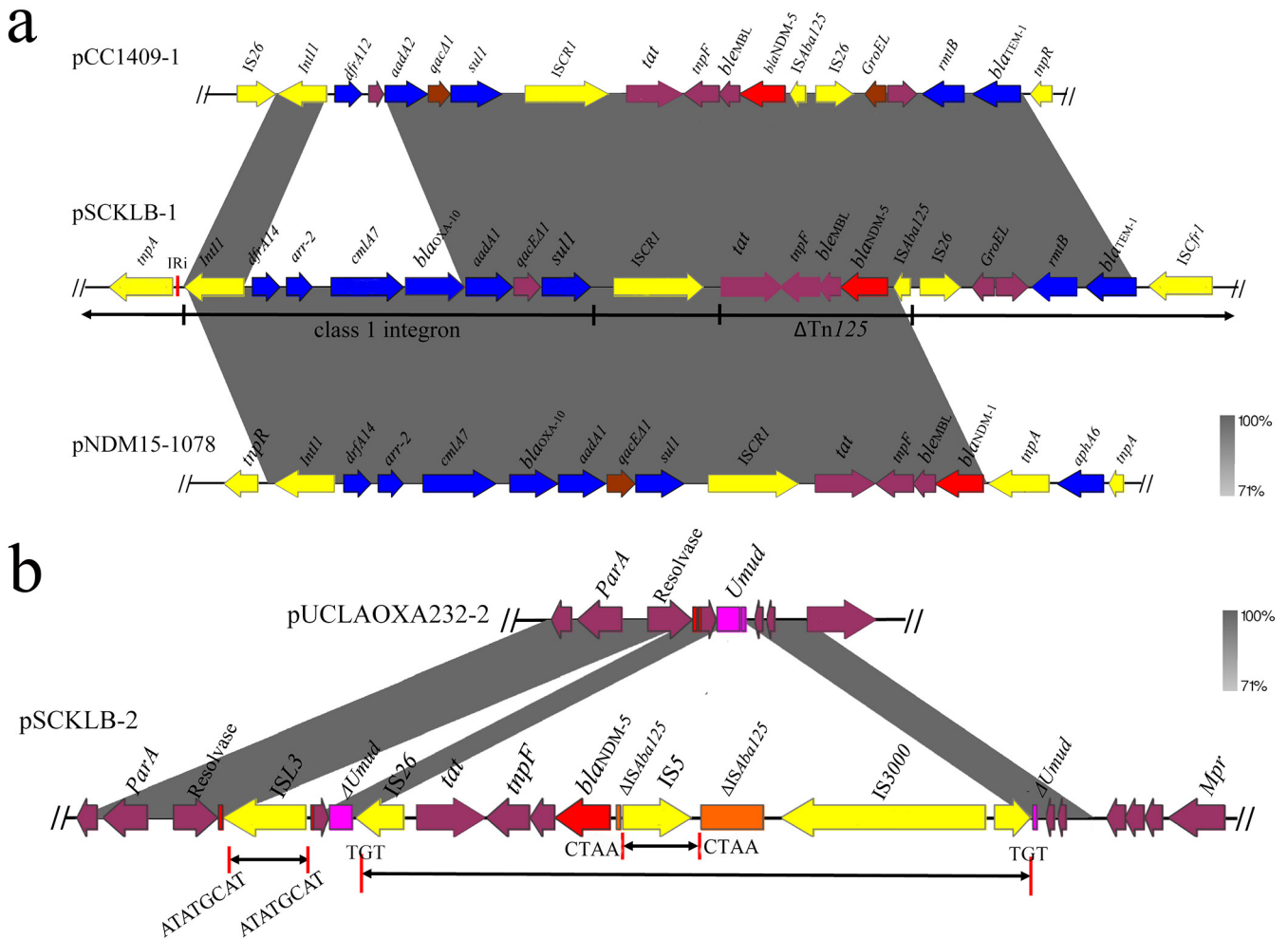


FIG 2 The genetic context of *bla*_{NDM-5} on pSCKLB138-1 and pSCKLB138-2 compared with other plasmids. (a) Genetic structure of *bla*_{NDM-5} gene on pSCKLB138-1 compared with pCC1409-1 and pNDM15-1078. (b) Genetic structure of *bla*_{NDM-5} gene on pSCKLB138-2 compared with pUCLAOXA232-2.

100% identity to the 46,161-bp plasmid pBJ114-46 (GenBank accession no. [MF679143](#)) with only 1 to 4 single-base changes (data not shown), indicating that the *bla*_{NDM-5}-bearing IncX3 plasmid was an important vector responsible for the dissemination of NDM-5 among *Enterobacteriaceae* isolates originating from chicken farms in China. Recently, a study identified the occurrence of similar IncX3 plasmids carrying *bla*_{NDM-5} in pigs originating from multiple farms across China (18), confirming that this mobile NDM vector is widespread in animal production.

Interestingly, a transconjugant of the ST709 *K. pneumoniae* strain named SCKLB138 harbored two *bla*_{NDM-5}-bearing plasmids, pSCKLB-1 and pSCKLB-2 (see Fig. S2 in the supplemental material). Plasmid pSCKLB-1 was found to comprise the IncFIB and IncFII replicons; the *bla*_{NDM-5}, *bla*_{OXA-10}, *rmtB*, *aadA1*, and *bla*_{TEM-1} genes; and some other resistance gene cassettes bounded by various insertion sequences. *bla*_{NDM-5} together with the bleomycin resistance gene *ble*_{MBL}, *tat*, and *tnpF* form part of the transposon Tn125 (Δ Tn125). Upstream of the *IS*_{Aba125}, *IS*₂₆ and *IS*_{Cfr1} were identified bracketing *bla*_{TEM-1} and the 16S rRNA methylase gene *rmtB*, conferring resistance to aminoglycosides. The same arrangement of Δ Tn125 and *rmtB* was found in ST147 *K. pneumoniae* plasmid pCC1409-1 (GenBank accession no. [KT725789](#)), except that the upstream of the *bla*_{TEM-1} gene of pCC1409-1 was truncated by Tn2 resolvase rather than *IS*_{Cfr1} (Fig. 2a). The *bla*_{OXA-10} gene was localized downstream of *bla*_{NDM-5} in a class 1 integron with the *dfrA14-arr-2-cmlA7-bla*_{OXA-10}-*aadA1* cassette array and the *IS*_{CR1} element behind the 3'-conserved segments. The region bracketed by *bla*_{NDM-1} and *tnpR* in plasmid pHN-

NDM0711 exhibited 99% identity to the corresponding region of pSCKLB-2 (Fig. 2a). Plasmid pSCKLB-2 was a 46-kb IncX3 *bla*_{NDM-5}-bearing plasmid. An IS5 was inserted with IS*Aba125* upstream of *bla*_{NDM-5} and the *ble*, *trpF*, and *tat* genes downstream from *bla*_{NDM-5}. Comparison of the genetic characteristics of pSCKLB-2 and pUCLAOXA232-2 (GenBank accession no. NZ_CP012563) showed that an ISL3 was inserted downstream of the resolvase gene, leading to the flanking 8-bp direct repeats (ATATGCAT). The *bla*_{NDM-5}-carrying region bracketed by IS26 and *tnpA* was inserted into the *umuD* gene, resulting in a pair of 3-bp direct repeats (TGT). The IS*Aba125* gene was interrupted by IS5 and split into two fragments, resulting in a pair of 4-bp direct repeats (CTAA) (Fig. 2b).

In conclusion, this study identified a self-transmissible IncX3 plasmid carrying *bla*_{NDM-5} that was an important vector responsible for the dissemination of NDM-5 among *Enterobacteriaceae* isolates originating from chicken farms in China. The co-occurrence of *bla*_{NDM-5} and other resistance *rmtB* and *mcr-1* genes in *Enterobacteriaceae* isolated in chicken farms strongly suggests a potential food chain dissemination pathway, which warrants further attention. To the best of our knowledge, this is the first report of two *bla*_{NDM-5}-carrying plasmids coexisting in a *K. pneumoniae* strain isolated from commercial chicken farms in China. The results highlight that the chicken farms are an important reservoir of *Enterobacteriaceae* carrying *bla*_{NDM-5} gene.

Accession number(s). The complete nucleotide sequences of plasmids pSCKLB-1 and pSCKLB-2 characterized in this study were submitted to the GenBank database and assigned accession numbers MH161191 and MH161192.

SUPPLEMENTAL MATERIAL

Supplemental material for this article may be found at <https://doi.org/10.1128/AAC.00779-18>.

SUPPLEMENTAL FILE 1, PDF file, 0.7 MB.

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REFERENCES

- Nordmann P, Naas T, Poirel L. 2011. Global spread of carbapenemase-producing *Enterobacteriaceae*. *Emerg Infect Dis* 17:1791–1798. <https://doi.org/10.3201/eid1710.110655>.
- Hornsey M, Phee L, Wareham DW. 2011. A novel variant, NDM-5, of the New Delhi metallo- β -lactamase in a multidrug-resistant *Escherichia coli* ST648 isolate recovered from a patient in the United Kingdom. *Antimicrob Agents Chemother* 55:5952–5954. <https://doi.org/10.1128/AAC.05108-11>.
- Cho SY, Huh HJ, Baek JY, Chung NY, Ryu JG, Ki CS, Chung DR, Lee NY, Song JH. 2015. *Klebsiella pneumoniae* co-producing NDM-5 and OXA-181 carbapenemases, South Korea. *Emerg Infect Dis* 21:1088–1089. <https://doi.org/10.3201/eid2106.150048>.
- Hammerum AM, Hansen F, Olesen B, Struve C, Holzknrecht BJ, Andersen PS, Thye AM, Jakobsen L, Roder BL, Stegger M, Hansen DS. 2015. Investigation of a possible outbreak of NDM-5-producing ST16 *Klebsiella pneumoniae* among patients in Denmark with no history of recent travel using whole-genome sequencing. *J Glob Antimicrob Resist* 3:219–221. <https://doi.org/10.1016/j.jgar.2015.05.003>.
- Yousfi M, Mairi A, Bakour S, Touati A, Hassissen L, Hadjadj L, Rolain JM. 2015. First report of NDM-5-producing *Escherichia coli* ST1284 isolated from dog in Bejaia, Algeria. *New Microbes New Infect* 8:17–18. <https://doi.org/10.1016/j.nmni.2015.09.002>.
- Zhang F, Xie L, Wang X, Han L, Guo X, Ni Y, Qu H, Sun J. 2016. Further spread of *bla*_{NDM-5} in *Enterobacteriaceae* via IncX3 plasmids in Shanghai, China. *Front Microbiol* 7:424.
- Zhang LP, Xue WC, Meng DY. 2016. First report of New Delhi metallo-beta-lactamase 5 (NDM-5)-producing *Escherichia coli* from blood cultures of three leukemia patients. *Int J Infect Dis* 42:45–46. <https://doi.org/10.1016/j.ijid.2015.10.006>.
- Yang P, Xie Y, Feng P, Zong Z. 2014. *bla*_{NDM-5} carried by an IncX3 plasmid in *Escherichia coli* sequence type 167. *Antimicrob Agents Chemother* 58:7548–7552. <https://doi.org/10.1128/AAC.03911-14>.
- Khalifa HO, Soliman AM, Ahmed AM, Shimamoto T, Shimamoto T. 2016. NDM-4- and NDM-5-producing *Klebsiella pneumoniae* coinfection in a 6-month-old infant. *Antimicrob Agents Chemother* 60:4416–4417. <https://doi.org/10.1128/AAC.00479-16>.
- Rojas LJ, Hujer AM, Rudin SD, Wright MS, Domitrovic TN, Marshall SH, Hujer KM, Richter SS, Cober E, Perez F, Adams MD, van Duin D, Bonomo RA. 2017. NDM-5 and OXA-181 β -lactamases, a significant threat continues to spread in the Americas. *Antimicrob Agents Chemother* 61:e00454-17. <https://doi.org/10.1128/AAC.00454-17>.
- Jhang J, Wang HY, Yoo G, Hwang GY, Uh Y, Yoon KJ. 2018. NDM-5 and OXA-48 co-producing uropathogenic *Escherichia coli* isolate: first case in Korea. *Ann Lab Med* 38:277–279. <https://doi.org/10.3343/alm.2018.38.3.277>.
- Zheng B, Xu H, Yu X, Lv T, Jiang X, Cheng H, Zhang J, Chen Y, Huang C, Xiao Y. 2018. Identification and genomic characterization of a KPC-2-

- NDM-1- and NDM-5-producing *Klebsiella michiganensis* isolate. *J Antimicrob Chemother* 73:536–538. <https://doi.org/10.1093/jac/dkx415>.
13. Xie M, Li R, Liu Z, Chan EWC, Chen S. 2018. Recombination of plasmids in a carbapenem-resistant NDM-5-producing clinical *Escherichia coli* isolate. *J Antimicrob Chemother* 73:1230–1234. <https://doi.org/10.1093/jac/dkx540>.
 14. Clinical and Laboratory Standards Institute. 2018. Performance standards for antimicrobial susceptibility testing; 28th informational supplement. CLSI document M100-S28. Clinical and Laboratory Standards Institute, Wayne, PA.
 15. European Committee on Antimicrobial Susceptibility Testing. 2017. Breakpoint tables for interpretation of MICs and zone diameters, version 7.1. http://www.eucast.org/fileadmin/src/media/PDFs/EUCAST_files/Breakpoint_tables/v_7.1_Breakpoint_Tables.pdf.
 16. Mushtaq S, Irfan S, Sarma JB, Doumith M, Pike R, Pitout J, Livermore DM, Woodford N. 2011. Phylogenetic diversity of *Escherichia coli* strains producing NDM-type carbapenemases. *J Antimicrob Chemother* 66: 2002–2005. <https://doi.org/10.1093/jac/dkr226>.
 17. Laczny CC, Galata V, Plum A, Posch AE, Keller A. 2017. Assessing the heterogeneity of in silico plasmid predictions based on whole-genome-sequenced clinical isolates. *Brief Bioinform*. <https://doi.org/10.1093/bib/bbx162>.
 18. Ho PL, Wang Y, Liu MC, Lai EL, Law PY, Cao H, Chow KH. 2018. IncX3 epidemic plasmid carrying bla_{NDM-5} in *Escherichia coli* from swine in multiple geographic areas in China. *Antimicrob Agents Chemother* 62: e02295-17. <https://doi.org/10.1128/AAC.02295-17>.