Molecular Epidemiology of New Delhi Metallo-β-Lactamase-Producing *Escherichia coli* in Food-Producing Animals in China

Xu Kuang[1,2,3†], Yan Zhang[1,2,3†], Juan Liu[1,2,3], Run-Shi Yang[4], Zhi-Ying Qiu[1,2,3], Jian Sun[1,2,3], Xiao-Ping Liao[1,2,3], Ya-Hong Liu[1,2,3,5] and Yang Yu[1,2,3]*

[1]National Risk Assessment Laboratory for Antimicrobial Resistance of Animal Original Bacteria, South China Agricultural University, Guangzhou, China, [2]Guangdong Provincial Key Laboratory of Veterinary Pharmaceutics Development and Safety Evaluation, South China Agricultural University, Guangzhou, China, [3]National Reference Laboratory of Veterinary Drug Residues, College of Veterinary Medicine, South China Agricultural University, Guangzhou, China, [4]Guangdong Provincial Key Laboratory of Microbial Safety and Health, State Key Laboratory of Applied Microbiology Southern China, Institute of Microbiology, Guangdong Academy of Sciences, Guangzhou, China, [5]Guangdong Laboratory for Lingnan Modern Agriculture, Guangzhou, China

We conducted a molecular surveillance study for carbapenem-resistant *Enterobacteriaceae* (CRE) colonization in food-producing animals in China that included primarily swine and poultry for three consecutive years. A total of 2,771 samples from food-producing animals and their surrounding environments were collected from different regions in China from 2015 to 2017. Enrichment cultures supplemented with meropenem were used to isolate carbapenem non-susceptible isolates and these were subsequently identified by MALDI-TOF MS. Resistance phenotypes and genotypes were confirmed using antimicrobial susceptibility testing and molecular biological techniques. Genomic characteristics of the carbapenemase-producing isolates were investigated using whole genome sequencing (WGS) and bioinformatic analysis. In total, 88 NDM-positive *Enterobacteriaceae* were identified from 2,771 samples and 96.6% were *Escherichia coli*. The New Delhi metallo-β-lactamase (NDM)-positive *E. coli* displayed a diversity of sequence types (ST), and ST48 and ST165 were the most prevalent. Three variants of bla_{NDM} (bla_{NDM-1}, bla_{NDM-4}, and bla_{NDM-5}) were detected and WGS indicated that bla_{NDM-5} predominated and was carried primarily on IncX3 plasmids. All these isolates were also multiply-drug resistant. These results revealed that food-producing animals in China are an important reservoir for NDM-positive *E. coli* and pose a potential threat to public health.

Keywords: NDM, food-producing animals, epidemiology, antimicrobial resistance, the environment

INTRODUCTION

Antimicrobial resistance (AMR) is rising to high levels in both human and veterinary health. New threats arising from multidrug-resistant bacteria and the very limited number of antibiotics coming to the market and bring us perilously to the end of antibiotic era where common treatable infections can once again be fatal. The abuse of human antibiotics in food-producing animals as prophylactic agents or growth promoters has become an important contributing factor

to increasing AMR (Van et al., 2020). Food-producing animals could serve as reservoir for antibiotic-resistant bacteria that can be transmitted to humans through direct contact or the food chain (Founou et al., 2016; Wang et al., 2017). Therefore, the prevalence of AMR will continue to rise worldwide and become one of the most serious threats to human and animal health.

Among the major multidrug-resistant bacteria, the emergence and dissemination of carbapenem-resistant Enterobacteriaceae (CRE) is identified as one of the most serious threats to human and animal health worldwide (Mariana Huband et al., 2017; Zhang et al., 2017, 2018; Bonomo et al., 2018). Notably, the recent increase in CRE was primarily because of the acquisition of carbapenem hydrolyzing enzymes such as the New Delhi metallo-β-lactamase (NDM; Kumarasamy et al., 2010; Munoz-Price et al., 2013). Since the first report of NDM-1 in a *Klebsiella pneumoniae* isolated from a patient in 2009, NDM-positive *Enterobacteriaceae* have been rapidly spreading in hospitals as well as in food-producing animals around the world and especially in China (Yong et al., 2009; Logan and Weinstein, 2017). To date, NDM-positive *Enterobacteriaceae* have been found on swine, geese farms, cow and chicken in China (Kong et al., 2017a; Köck et al., 2018). The spread of NDM-positive *Escherichia coli* has been documented among chickens, swallows, farmers, dogs and even flies in a chicken farm (Berrazeg et al., 2014; Logan and Weinstein, 2017; Li et al., 2019).

However, the dissemination of NDM-positive *E. coli* among food-producing animals in China is currently poorly understood. A large-scale survey of the NDM-positive *Enterobacteriaceae* covering a large region of China has yet to be performed. In this study, we examined the long-term and large-scale prevalence and molecular epidemiological features of NDM-positive *E. coli* from livestock animals from northern to southern China for three consecutive years, 2015–2017.

MATERIALS AND METHODS

Sample Collection and Bacterial Isolation

A total of 2,771 samples, including 526 samples in 2015, 812 samples in 2016, and 1,433 samples in 2017 were collected from food-producing animals (fecal samples) on poultry and swine farms and their surrounding environments (**Supplementary Table S1**). Sixteen provinces that play important roles in the swine and poultry industry from north to south China were randomly selected for sampling. According to the north–south demarcation zone, eight provinces that include Jiangsu, Henan, Heilongjiang, Hebei, Jilin, Ningxia, Jiangsu, Inner Mongolia are situated in the north China while the remainder were located in south China (**Figure 1**).

Environmental samples (sewage and soil) were incubated overnight in Luria Bertani broth without antibiotics. Environmental cultures and diluted feces samples were directly plated on MacConkey agar with 1 mg/l meropenem and incubated at 37°C. Carbapenem non-susceptible isolates were identified using MALDI-TOF MS (Shimadzu Biotech) and 16S rDNA sequencing (Seng et al., 2009; Walsh and Duffy, 2013) Carbapenemase production was examined for all isolates using the Carbapenemase Nordmann-Poirel test

(Nordmann et al., 2012b). All the carbapenemase-producing isolates were screened using polymerase chain reaction (PCR) for the presence of bla_{NDM}, $bla_{OXA-48-like}$, bla_{VIM}, bla_{KPC}, and bla_{IMP} (Poirel et al., 2011). In addition, the entire bla_{NDM} amplicons were sequenced (Sanger) and the 813 bp ORF of bla_{NDM} was typed using BLAST.[1]

Antimicrobial Susceptibility Testing

The minimal inhibitory concentrations (MIC) of 14 antibiotics (amikacin, gentamicin, meropenem, imipenem, ertapenem, cefoxitin, cefotaxime, ceftazidime, aztreonam, cefoxitin, tetracycline, fosfomycin, trimethoprim/sulfamethoxazole, and florfenicol) for the isolates were determined by agar dilution and interpreted according to the clinical and laboratory standard institute (CLSI) documents M100-S28 and VET01-S2. The breakpoints of tigecycline and colistin for *Enterobacteriaceae* were interpreted according to the EUCAST criteria (Version 6.0). *E. coli* American Type Culture Collection (ATCC) 25922 served as a quality control strain for the testing.

Pulsed-Field Gel Electrophoresis (PFGE) Typing, Whole-Genome Sequencing and Conjugation Assay

The genetic relatedness of NDM-positive isolates was investigated using *Xba*I-PFGE for *E. coli*. PFGE patterns were analyzed with the Dice coefficient and the unweighted pair group with arithmetic mean clustering method using BioNumerics (Applied Maths, Ghent, Belgium), resulting in PFGE patterns with > 90% similarity between clusters. Based on different resistance phenotypes and genetic relationships, 76 strains were selected for Whole-Genome Sequencing (WGS). Genomic DNA was extracted from isolates using the Genomic DNA Purification Kit (Tiangen, Beijing, China). WGS was performed with the Illumina HiSeq 2,500 system (Novogene Guangzhou, China). Gene annotation and prediction were performed using RAST[2] and BLAST. MLST sequence types (ST), plasmid incompatibility (Inc) groups and antibiotic resistance genes (ARGs) were analyzed using software from IS finder[3] and the Center for Genomic Epidemiology.[4]

Streptomycin-resistant *E. coli* strain C600 was used as the recipient to determine the transferability of the resistance genes. The conjugation assays were performed using the filter mating method and the proportion of donor to recipient bacteria stands 1:1. Transconjugants were selected using MacConkey agar plates containing both 2,000 mg/l streptomycin and 1.0 mg/l meropenem. Selected transconjugants were confirmed by PCR.
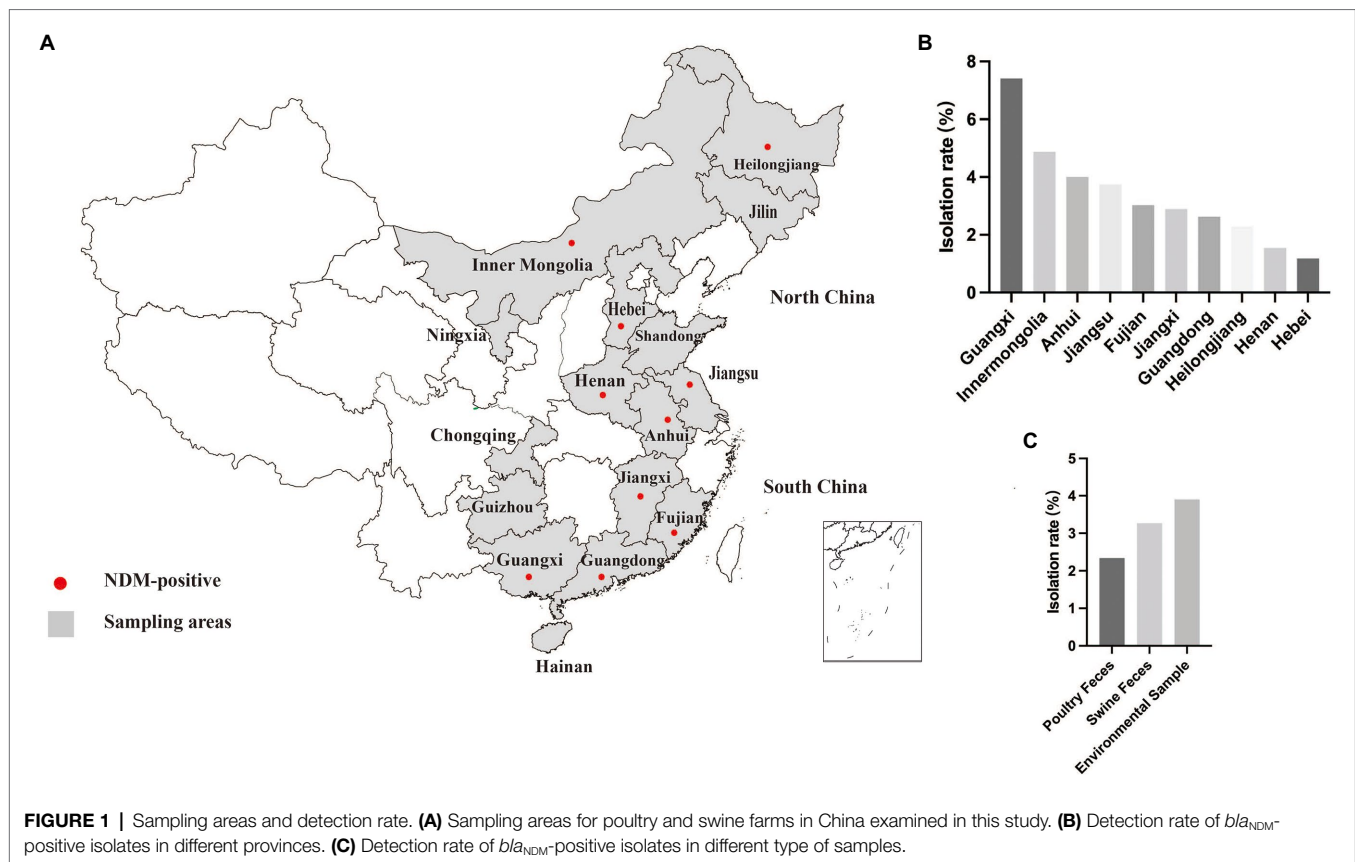
RESULTS

Bacterial Isolation and Detection of Carbapenemase Genes

In this study, 654 carbapenem non-susceptible bacteria (≥ 1 mg/l) were recovered from 2,771 fecal and environmental

---

[1] https://blast.ncbi.nlm.nih.gov/Blast.cgi

[2] http://rast.nmpdr.org/

[3] https://www-is.biotoul.fr/

[4] http://www.genomicepidemiology.org/

FIGURE 1 | Sampling areas and detection rate. **(A)** Sampling areas for poultry and swine farms in China examined in this study. **(B)** Detection rate of bla_{NDM}-positive isolates in different provinces. **(C)** Detection rate of bla_{NDM}-positive isolates in different type of samples.

samples that included sewage and soil samples from 16 provinces in China from 2015 to 2017. We identified the presence of the bla_{NDM} gene in 88 isolates. In 2015, the prevalence of NDM-positive *Enterobacteriaceae* detected in samples was 1.52% (8/526) and the positive samples were primarily from Jiangsu and Guangdong. The prevalence for 2016 was almost identical (1.48%, 12/812). Interestingly, the prevalence increased dramatically to 4.82% (69/1433) in 2017 (**Supplementary Table S1**). Among all the provinces, bla_{NDM} was the most prevalent in Guangxi. Notably, samples collected from six provinces, including Jilin, Shandong, Ningxia, Chongqing, Guizhou and Hainan did not detected NDM-positive strain (**Figure 1**). Species identification and WGS analysis indicated that the 88 bla_{NDM}-carrying isolates belonged to three *Enterobacteriaceae* species, including *E. coli* ($n = 82$), *K. pneumoniae* ($n = 5$) and *Citrobacter freundii* ($n = 1$).

Antimicrobial Susceptibility Testing and Conjugation Assays

Our collection of 88 NDM-positive isolates were found to be resistant to imipenem (MIC 4 to > 64 mg/l), meropenem (MIC 4 to > 64 mg/l) and ertapenem (MIC 16 to > 64 mg/l) as well as concurrently resistant to the other β-lactams cefotaxime, cefoxitin and ceftazidime. Moreover, most were also resistant to trimethoprim/sulfanilamide (96.6%; $n = 85$; MIC 40 to > 320 mg/l), tetracycline (94.3%; $n = 83$; MIC 16 to > 256 mg/l), florfenicol (80.6%; $n = 71$; MIC 16 to

> 256 mg/l), ciprofloxacin (72.7%; $n = 60$; MIC 4 to > 256 mg/l), and gentamicin (59.1%; $n = 52$; MIC 16 to > 256 mg/l). However, we found relatively a lower prevalence for resistance to aztreonam (45.5%), amikacin (34.1%), Fosfomycin (29.5%), colistin (28.4%) and tigecycline (11.4%). These results showed that NDM-positive isolates displayed reduced susceptibilities to β-lactam antibiotics, but remained susceptible to colistin and tigecycline. Conjugation experiments using these isolates resulted in the transfer of bla_{NDM}-carrying plasmids from 76 isolates (86.5%) to *E. coli* C600str recipient (**Supplementary Table S2**).

Phylogenetic Analysis of NDM-Positive *Escherichia coli*

PFGE was successfully performed on these 77 bla_{NDM}-carrying *E. coli* and resulted in 74 PFGE patterns. There were four groups of isolates that shared similar PFGE patterns including FS21-4 and FSCK25-1, HB14-1 and HB14-3, JZ33 and JZ41-2; most isolates of each PFGE group were from the same area, while HN68-2 and FCG47-1 were from different provinces and years but shared > 90% similarity in PFGE patterns. The remaining 69 isolates belonged to different phylogenetic groups (**Supplementary Figure S1**). Based on PFGE profiles, a total of 72 isolates were selected for WGS and this analysis demonstrated that these *E. coli* isolates had 38 distinct STs. The most prevalent STs were ST48 (10/72; 13.9%), ST165 (6/72; 8.3%) and ST405 (4/72; 5.6%).

Resistance Profiles

Whole genome sequencing analysis revealed that NDM-positive *E. coli* isolates possessed >30 distinct ARGs conferring resistance to nearly all currently available antibiotics. Among the other 24 ARG types, 11 ARGs were common with a prevalence >50% and these included *bla*$_{TEM}$ ($n = 43$), *aph* ($n = 64$), *ant* ($n = 36$), *aadA* ($n = 49$), *aac* ($n = 40$), *cmlA* ($n = 37$), *dfrA* ($n = 68$), *sul* ($n = 65$), *tet* ($n = 65$), *mdf* ($n = 72$), and *flor* ($n = 53$). These genes conferred resistance to seven classes of antibiotics; β-lactams, sulphonamides, quinolones, tetracyclines, chloramphenicol, aminoglycosides and macrolides. Notably, gene *mcr-1* mediating resistance to the last-resort antibiotic colistin (Poirel et al., 2017; Li et al., 2020) was detected in 19 NDM-positive *E. coli* isolates.

Additional analysis of plasmid sequences indicated that 72 NDM-positive *E. coli* isolates in this study carried 14 types of Inc. plasmids. IncX3 predominated and IncFIB (65.3%, 47/72) and IncFII (34.7%, 25/72) were also highly represented (**Figure 2**).

Genetic Environments of *bla*$_{NDM}$

A sequence comparison analysis revealed that all 64 IncX3 plasmids were similar to a *bla*$_{NDM-5}$-carrying IncX3 plasmid pNDM-MGR194 (Acc. No. NC_022740) from a *K. pneumoniae* isolate. These 64 plasmids shared an identical and conserved IncX3 backbone, and their inserted *bla*$_{NDM}$ sites were all near the *umuD* gene. Differences between these plasmids were

FIGURE 2 | MLST, Resistance Phenotype, ARG and plasmid replicons of 72 NDM-positive *Escherichia coli* isolates from food-producing animals in China. The heatmap was generated after aligning the contigs of sequenced genomes of each strain to MLST, Resfinder and PlasmidFinder. ARGs and plasmid Inc. types are indicated by yellow and blue squares, respectively. AMK, amikacin; GEN, gentamicin; MEM, meropenem; IMP, imipenem; ERT, ertapenem; CTX, cefotaxime; CAZ, ceftazidime; FOX, cefoxitin; ATM, aztreonam; FOS, fosfomycin; CS, colistin; TET, tetracycline; TGC, tigecycline; CIP, ciprofloxacin; SXT, sulfamethoxazole-trimethoprim. FFC, florfenicol.

primarily contained within the *bla*NDM-5 region between the *par*A and IS*3000* genes. The diversity within this region was used to separate these 64 plasmids into four groups (Types A–D). Type A plasmids (*n* = 47) shared the highest similarity with the reference plasmid. Types B (*n* = 5), C (*n* = 11), and D (*n* = 1) omitted IS*5*, ΔISA*ba125* and ΔISA*ba125*-IS*3000*-ΔTn*2*, respectively (**Figure 3A**). In the Type B group, *bla*NDM was located in the structure IS*26*-*tat*-*trp*F-*ble*MBL-*bla*NDM-ISA*ba125*-IS*3000*-ΔTn*2*. The structure was inserted into the plasmid pEC14-35 (Acc. No. NC_019083) and therefore formed the genetic environments of *bla*NDM for Type B. Type A most likely was formed by IS*5* insertion into the region between *bla*NDM and ISA*ba125*. Types C and D emerged after losing ISA*ba125* and ISA*ba125*-IS*3000*-ΔTn*2* most likely due to homologous recombination (**Figure 3B**).

In addition, the genetic environments of a *bla*NDM-4-bearing IncA/C plasmid (pHB141-NDM4) demonstrated that the ARGs *sul1*, *aadA2* and *dfrA12* were packaged in Class I integrons located within the structure of ISA*ba125*-*bla*NDM-4-*ble*MBL-*trp*F-*tat*-ΔcutA. There were also a series of genes mediating

Hg resistance downstream of *bla*NDM-4. Two IS*26* elements flanking the *aphA1* gene were also downstream of *bla*NDM-4. A sequence comparison analysis revealed that all the 64 IncX3 plasmids were similar to a *bla*NDM-9-carrying IncK2 plasmid pHNTH02-1 from *E. coli* in China (Acc. No. NZ_MG196294). The reference sequence featured a composite transferable structure of IS*26*-ΔISA*ba125*-*bla*NDM-*ble*MBL-*trp*F-*tat*-ΔcutA-ISCR1-*sul1*-*aad2*-*gcuF*-*dfrA12*-*intI1*-IS*26*, with two inverted repeat sequences ΔTn*1721* and ΔTn*5393* (**Figure 3C**).

DISCUSSION

The presence of NDM-positive *Enterobacteriaceae* in livestock animals is of concern because this may facilitate expansion of the gene pool from which pathogenic bacteria can acquire ARGs and consumers may subsequently be exposed *via* the food chain (Woodford et al., 2014; Mollenkopf et al., 2017). For this reason, we conducted a long-term and large-scale characterization of the prevalence and genetic relationships
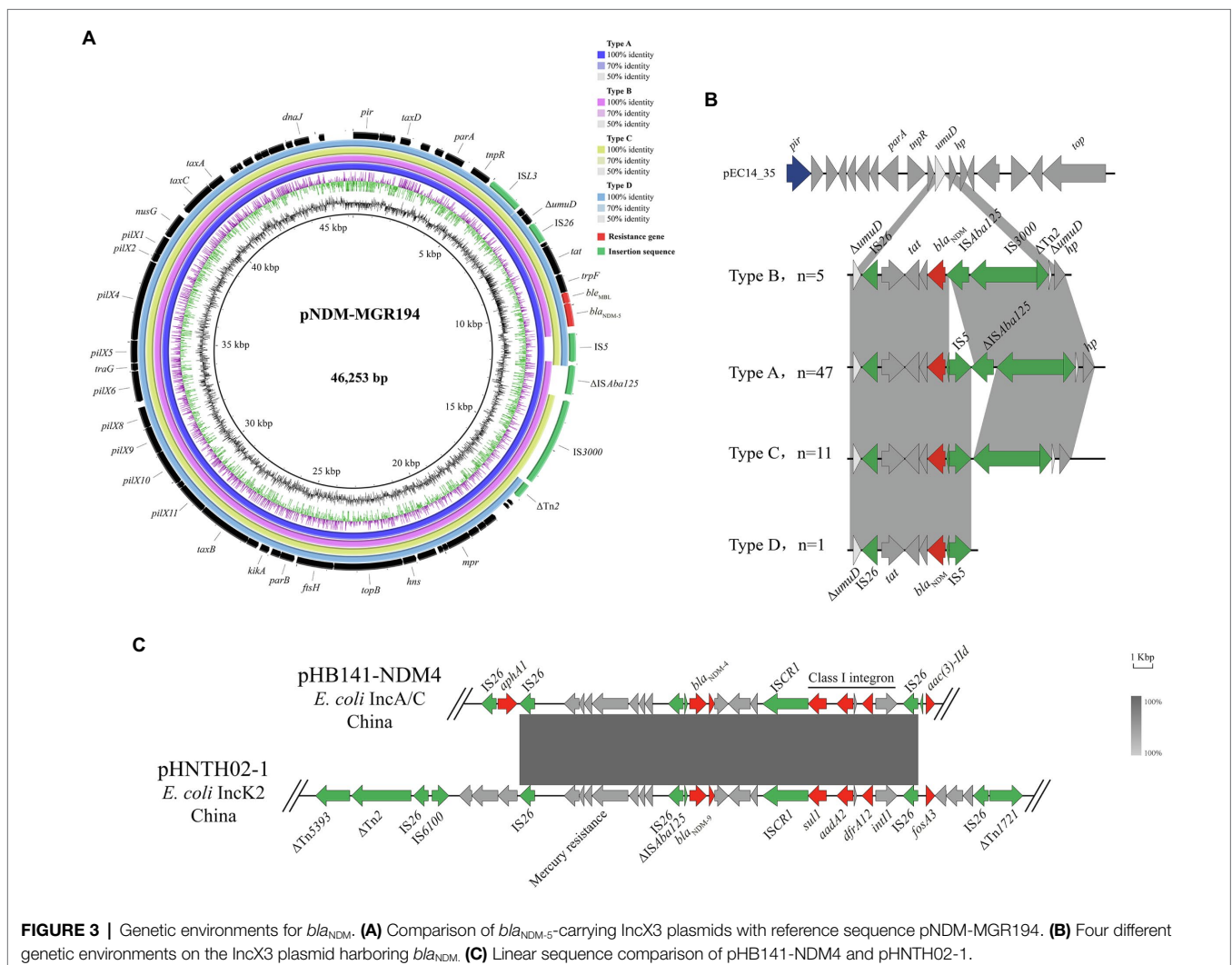

FIGURE 3 | Genetic environments for *bla*NDM. **(A)** Comparison of *bla*NDM-5-carrying IncX3 plasmids with reference sequence pNDM-MGR194. **(B)** Four different genetic environments on the IncX3 plasmid harboring *bla*NDM. **(C)** Linear sequence comparison of pHB141-NDM4 and pHNTH02-1.

between NDM-positive bacteria from livestock animals in China from 2015 to 2017. Since its first report in British, bla_{NDM-5} has been detected in clinical isolates from Europe, Asia, South America, the United States of America and Australia (Nakano et al., 2014; Wailan et al., 2015; Baek et al., 2019; López-Hernández et al., 2020; Ramadan et al., 2020; Costa et al., 2021). Notably, the prevalence of bla_{NDM-5} isolates from food-producing animals has been sporadic in aboard (Ghatak et al., 2013), although there are more relevant reports in China (Yang et al., 2016; Kong et al., 2017a; Yao et al., 2020; Zhao et al., 2021; Lv et al., 2022). In the current study, our results revealed a wide contamination of NDM-positive *Enterobacteriaceae* in ten different provinces. In particular, the prevalence of bla_{NDM}-carrying *Enterobacteriaceae* in swine and poultry in Guangxi province (10.3%, 32/312) was much higher than for 2017 and suggested that food-producing animals in Guangxi province have been severely contaminated by carbapenem-resistant *Enterobacteriaceae*. Fortunately, three northern provinces Jilin, Ningxia and Shandong, and three southern provinces Chongqing, Guizhou and Hainan had not been contaminated by NDM-positive *Enterobacteriaceae* over our 3-year study period.

While carbapenem use is prohibited in swine and poultry production chains, different *Enterobacteriaceae* species and various bla_{NDM} variants have been found on livestock farms (Zhai et al., 2020; Wang et al., 2021). In the current study, we identified 88 NDM-positive isolates belonging to three *Enterobacteriaceae* species including *E. coli*, *K. pneumoniae* and *C. freundii*. For the NDM-positive *E. coli* isolates, the predominant variant of bla_{NDM} was bla_{NDM-5}. There are to date seven bla_{NDM} variants (bla_{NDM-1}, bla_{NDM-4}, bla_{NDM-5}, bla_{NDM-7}, bla_{NDM-9}, bla_{NDM-17}, and bla_{NDM-20}) that have been detected in food-producing animals in China and bla_{NDM-5} was the most prevalent and was found in poultry, swine, geese and ducks (Liu et al., 2017, 2018; Pruthvishree et al., 2017; Cen et al., 2021; Wang et al., 2021). Furthermore, our PFGE analysis illustrated that the genetic background of NDM-positive *E. coli* varied considerably across the country although there were a small number of closely related strains in the same regions. Therefore, clonal spread was not the primary mode of bla_{NDM} gene transmission and was consistent with the results of previous studies (Mantilla-Calderon et al., 2016; Zhang et al., 2018). Our WGS results also demonstrated that these *E. coli* isolates had 38 distinct STs and could be distinguished by their geographical distribution. For instance, ST48, ST165 and ST405 were more prevalent than the others. ST48 *E. coli* isolates have been linked to bla_{NDM} spread in livestock farms and in retail meat in China (Liu et al., 2019; Zhang et al., 2019b).

IncX3 plasmids have been identified as the primary vectors for the horizontal transfer of bla_{NDM-5} on farms (Ho et al., 2018; Williams et al., 2018) and our study further confirms this. IncX3 plasmids normally encode a type IV secretion system (pilX1-11) that allows the exchange of genetic material within bacteria. IncX3 plasmids are also highly conjugatable and stable and exert no fitness costs on their bacterial hosts

(Zhai et al., 2020). This characteristic may explain why NDM-positive bacteria still have been found in these settings even though carbapenem use is prohibited on livestock farms. In addition to the IncX3 plasmids, IncFIB and IncFII were also highly represented. IncFIB plasmids have been frequently detected in *E. coli* and has already been described as an ESBL gene carrier (Zhang et al., 2019a). IncFII plasmids are important in ARGs spread and are also the primary *mcr*-1 vector for *E. coli* (Xavier et al., 2016; Sugawara et al., 2019). Therefore, the three types of plasmids discussed above may serve as an important vehicles for the spread of ARGs among animals and humans.

CONCLUSION

In this study we identified 88 NDM-positive isolates from animal farms and their surrounding environments in China. Notably, this is a large-scale survey of CRE covering a large region of China over three consecutive years. Phylogenetic analysis indicated considerable diversity for this population of CRE isolates taken from livestock farms. WGS analysis further determined that bla_{NDM-5} coexisted with other ARGs and also demonstrated a great diversity in the plasmid population; the latter provides important epidemiological information for the global dissemination of bla_{NDM-5}. These results indicated that continuous monitoring of NDM-positive *E. coli* in livestock farms and their surrounding environments is required to ensure public health safety.

DATA AVAILABILITY STATEMENT

The datasets presented in this study can be found in online repositories. The names of the repository/repositories and accession number(s) can be found at: "NCBI BioProject—PRJNA826982."

ETHICS STATEMENT

The Institutional Review Board of South China Agricultural University (SCAU-IRB) approved the samples and bacteria protocols. All animal faeces were sampled under authorization from Animal Research Committees of South China Agricultural University (SCAU-IACUC).

AUTHOR CONTRIBUTIONS

YY and Y-HL conceived of this study. R-SY, XK, YZ, and JL performed the experiments and collected the data. XK, R-SY, YZ, and Z-YQ analyzed and interpreted the data. XK drafted the manuscript. YY revised the manuscript. X-PL and JS coordinated the whole project. All authors contributed to the article and approved the submitted version.

FUNDING

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fmicb.2022.912260/full#supplementary-material

SUPPLEMENTARY FIGURE S1 | PFGE analysis of 77 *bla*_{NDM}-positive *Escherichia coli* isolates from food-producing animals. Triangles represent that the corresponding isolates sharing similar PFGE patterns.

REFERENCES

Baek, J. Y., Cho, S. Y., Kim, S. H., Kang, C. I., Peck, K. R., Song, J. H., et al. (2019). Plasmid analysis of *Escherichia coli* isolates from South Korea co-producing NDM-5 and OXA-181 carbapenemases. *Plasmid* 104:102417. doi: 10.1016/J.PLASMID.2019.102417

Berrazeg, M., Diene, S. M., Medjahed, L., Parola, P., Drissi, M., Raoult, D., et al. (2014). New Delhi metallo-beta-lactamase around the world: An ereview using google maps. *Eur. Secur.* 19:20809. doi: 10.2807/1560-7917.ES2014.19.20.20809

Bonomo, R. A., Burd, E. M., Conly, J., Limbago, B. M., Poirel, L., Segre, J. A., et al. (2018). Carbapenemase-producing organisms: a global scourge. *Clin. Infect. Dis.* 66, 1290–1297. doi: 10.1093/cid/cix893

Cen, D. J., Sun, R. Y., Mai, J. L., Jiang, Y. W., Wang, D., Guo, W. Y., et al. (2021). Occurrence and transmission of blaNDM-carrying Enterobacteriaceae from geese and the surrounding environment on a commercial goose farm. *Appl. Environ. Microbiol.* 87, 1–13. doi: 10.1128/AEM.00087-21

Costa, A., Figueroa-Espinosa, R., Gaudenzi, F., Lincopan, N., Fuga, B., Ghiglione, B., et al. (2021). Co-occurrence of NDM-5 and RmtB in a clinical isolate of *Escherichia coli* belonging to CC354 in Latin America. *Front. Cell. Infect. Microbiol.* 11:654852. doi: 10.3389/FCIMB.2021.654852/PDF

Founou, L. L., Founou, R. C., and Essack, S. Y. (2016). Antibiotic resistance in the food chain: a developing country-perspective. *Front. Microbiol.* 7:1881. doi: 10.3389/fmicb.2016.01881

Ghatak, S., Singha, A., Sen, A., Guha, C., Ahuja, A., Bhattacharjee, U., et al. (2013). Detection of New Delhi metallo-beta-lactamase and extended-spectrum beta-lactamase genes in *Escherichia coli* isolated from mastitic milk samples. *Transbound. Emerg. Dis.* 60, 385–389. doi: 10.1111/TBED.12119

Ho, P. L., Wang, Y., Liu, M. C. J., Lai, E. L. Y., Law, P. Y. T., Cao, H., et al. (2018). IncX3 epidemic plasmid carrying blaNDM-5 In *Escherichia coli* from swine in multiple geographic areas in China. *Antimicrob. Agents Chemother.* 62, e02295–17. doi: 10.1128/AAC.02295-17

Köck, R., Daniels-Haardt, I., Becker, K., Mellmann, A., Friedrich, A. W., Mevius, D., et al. (2018). Carbapenem-resistant Enterobacteriaceae in wildlife, food-producing, and companion animals: a systematic review. *Clin. Microbiol. Infect.* 24, 1241–1250. doi: 10.1016/j.cmi.2018.04.004

Kong, L. H., Lei, C. W., Ma, S. Z., Jiang, W., Liu, B. H., Wang, Y. X., et al. (2017a). Various sequence types of *Escherichia coli* isolates coharboring blaNDM-5 and mcr-1 genes from a commercial swine farm in China. *Antimicrob. Agents Chemother.* 61, e02167–16. doi: 10.1128/AAC.02167-16

Kumarasamy, K. K., Toleman, M. A., Walsh, T. R., Bagaria, J., Butt, F., Balakrishnan, R., et al. (2010). Emergence of a new antibiotic resistance mechanism in India, Pakistan, and the UK: a molecular, biological, and epidemiological study. *Lancet Infect. Dis.* 10, 597–602. doi: 10.1016/S1473-3099(10)70143-2

Li, J., Bi, Z., Ma, S., Chen, B., Cai, C., He, J., et al. (2019). Inter-host transmission of carbapenemase-producing *Escherichia coli* among humans and backyard animals. *Environ. Health Perspect.* 127:107009. doi: 10.1289/EHP5251

Li, B., Yin, F., Zhao, X., Guo, Y., Wang, W., Wang, P., et al. (2020). Colistin resistance gene mcr-1 mediates cell permeability and resistance to hydrophobic antibiotics. *Front. Microbiol.* 10, 1–7. doi: 10.3389/fmicb.2019.03015

Liu, Z., Li, J., Wang, X., Liu, D., Ke, Y., Wang, Y., et al. (2018). Novel variant of New Delhi metallo-β-lactamase, NDM-20, in *Escherichia coli. Front. Microbiol.* 9:497. doi: 10.3389/fmicb.2018.00248

Liu, Z., Wang, Y., Walsh, T. R., Liu, D., Shen, Z., Zhang, R., et al. (2017). Plasmid-mediated novel blaNDM-17 gene encoding a carbapenemase with enhanced activity in a sequence type 48 *Escherichia coli* strain. *Antimicrob. Agents Chemother.* 61. doi: 10.1128/AAC.02233-16

Liu, X., Xiao, X., Li, Y., Liu, Y., Li, R., and Wang, Z. (2019). Emergence of IncX3 plasmid-harboring blaNDM–5 dominated by *Escherichia coli* ST48 in a goose farm in Jiangsu. *China. Front. Microbiol.* 10:2002. doi: 10.3389/fmicb.2019.02002

Logan, L. K., and Weinstein, R. A. (2017). The epidemiology of Carbapenem-resistant enterobacteriaceae: the impact and evolution of a global menace. *J. Infect. Dis.* 215, S28–S36. doi: 10.1093/infdis/jiw282

López-Hernández, I., García Barrionuevo, A., Díaz de Alba, P., Clavijo, E., and Pascual, A. (2020). Characterization of NDM-1- and CMH-3-producing *Enterobacter cloacae* complex ST932 in a patient transferred from Ukraine to Spain. *Enfermedades Infecc. y Microbiol. Clin. (English ed.)* 38, 327–330. doi: 10.1016/J.EIMC.2019.10.007

Lv, L. C., Lu, Y. Y., Gao, X., He, W. Y., Gao, M. Y., Mo, K. B., et al. (2022). Characterization of NDM-5-producing Enterobacteriaceae isolates from retail grass carp (*Ctenopharyngodon idella*) and evidence of Bla NDM-5-bearing IncHI2 plasmid transfer between ducks and fish. *Zool. Res.* 43, 255–264. doi: 10.24272/J.ISSN.2095-8137.2021.426

Mantilla-Calderon, D., Jumat, M. R., Wang, T., Ganesan, P., Al-Jassim, N., and Hong, P. Y. (2016). Isolation and characterization of NDM-positive *Escherichia coli* from municipal wastewater in Jeddah, Saudi Arabia. *Antimicrob. Agents Chemother.* 60, 5223–5231. doi: 10.1128/AAC.00236-16

Mariana Huband, M. D., Mendes, R. E., and Flamm, R. K. F. (2017). Meropenem-vaborbactam tested against contemporary gram-negative isolates collected worldwide during 2014, including carbapenem-resistant, KPC-producing, multidrug-resistant, and extensively drug-resistant Enterobacteriaceae. *Am. Soc. Microbiol.* 61, 1–12. doi: 10.1128/AAC.00567-17

Mollenkopf, D. F., Stull, J. W., Mathys, D. A., Bowman, A. S., Feicht, S. M., Grooters, S. V., et al. (2017). Carbapenemase-producing Enterobacteriaceae recovered from the environment of a swine farrow-to-finish operation in the United States. *Antimicrob. Agents Chemother.* 61, e01298–16. doi: 10.1128/AAC.01298-16

Munoz-Price, L. S., Poirel, L., Bonomo, R. A., Schwaber, M. J., Daikos, G. L., Cormican, M., et al. (2013). Clinical epidemiology of the global expansion of *Klebsiella pneumoniae* carbapenemases. *Lancet Infect. Dis.* 13, 785–796. doi: 10.1016/S1473-3099(13)70190-7

Nakano, R., Nakano, A., Hikosaka, K., Kawakami, S., Matsunaga, N., Asahara, M., et al. (2014). First report of metallo-β-lactamase NDM-5-producing *Escherichia coli* in Japan. *Antimicrob. Agents Chemother.* 58, 7611–7612. doi: 10.1128/AAC.04265-14

Nordmann, P., Boulanger, A. E., and Poirel, L. (2012a). NDM-4 metallo-β-lactamase with increased carbapenemase activity from *Escherichia coli. Antimicrob. Agents Chemother.* 56, 2184–2186. doi: 10.1128/AAC.05961-11

Nordmann, P., Poirel, L., and Dortet, L. (2012b). Rapid detection of carbapenemase-producing enterobacteriaceae. *Emerg. Infect. Dis.* 18, 1503–1507. doi: 10.3201/eid1809.120355

Poirel, L., Jayol, A., Nordmann, P., Aurélie, J., and Nordmann, P. (2017). Polymyxins: antibacterial activity, susceptibility testing, and resistance mechanisms encoded by plasmids or chromosomes Laurent Poirela,b,c, Aurélie Jayola,b,c and Patrice Nordmann. *Clin. Microbiol. Rev.* 30, 557–596. doi: 10.1128/CMR.00064-16

Poirel, L., Walsh, T. R., Cuvillier, V., and Nordmann, P. (2011). Multiplex PCR for detection of acquired carbapenemase genes. *Diagn. Microbiol. Infect. Dis.* 70, 119–123. doi: 10.1016/j.diagmicrobio.2010.12.002

Pruthvishree, B. S., Vinodh Kumar, O. R., Sinha, D. K., Malik, Y. P. S., Dubal, Z. B., Desingu, P. A., et al. (2017). Spatial molecular epidemiology of carbapenem-resistant and New Delhi metallo beta-lactamase (blaNDM)-producing *Escherichia coli* in the piglets of organized farms in India. *J. Appl. Microbiol.* 122, 1537–1546. doi: 10.1111/jam.13455

Ramadan, H., Gupta, S. K., Sharma, P., Ahmed, M., Hiott, L. M., Barrett, J. B., et al. (2020). Circulation of emerging NDM-5-producing *Escherichia coli* among humans and dogs in Egypt. *Zoonoses Public Health* 67, 324–329. doi: 10.1111/ZPH.12676

Seng, P., Drancourt, M., Gouriet, F., Scola, B.La, Fournier, P. E., Rolain, J. M., et al. (2009). Ongoing revolution in bacteriology: routine identification of bacteria by matrix-assisted laser desorption ionization time-of-flight mass spectrometry. *Clin. Infect. Dis.* 49, 543–551. doi:10.1086/600885.

Sugawara, Y., Akeda, Y., Hagiya, H., Sakamoto, N., Takeuchi, D., Shanmugakani, R. K., et al. (2019). Spreading patterns of NDM-producing Enterobacteriaceae in clinical and environmental settings in Yangon. *Myanmar. Antimicrob. Agents Chemother.* 63, e01924–18. doi: 10.1128/AAC.01924-18

Van, T. T. H., Yidana, Z., Smooker, P. M., and Coloe, P. J. (2020). Antibiotic use in food animals worldwide, with a focus on Africa: pluses and minuses. *J. Glob. Antimicrob. Resist.* 20, 170–177. doi: 10.1016/j.jgar.2019.07.031

Wailan, A. M., Paterson, D. L., Kennedy, K., Ingram, P. R., Bursle, E., and Sidjabat, H. E. (2015). Genomic characteristics of NDM-producing Enterobacteriaceae isolates in Australia and their blaNDM genetic contexts. *Antimicrob. Agents Chemother.* 60, 136–141. doi: 10.1128/AAC.01243-15

Walsh, F., and Duffy, B. (2013). The culturable soil antibiotic Resistome: a community of multi-drug resistant bacteria. *PLoS One* 8:e65567. doi: 10.1371/journal.pone.0065567

Wang, Y., Zhang, R., Li, J., Wu, Z., Yin, W., Schwarz, S., et al. (2017). Comprehensive resistome analysis reveals the prevalence of NDM and MCR-1 in Chinese poultry production. *Nat. Microbiol.* 2:16260. doi: 10.1038/nmicrobiol.2016.260

Wang, M. G., Zhang, R. M., Wang, L. L., Sun, R. Y., Bai, S. C., Han, L., et al. (2021). Molecular epidemiology of carbapenemase-producing *Escherichia coli* from duck farms in south-east coastal China. *J. Antimicrob. Chemother.* 76, 322–329. doi: 10.1093/jac/dkaa433

Williams, S. H., Che, X., Paulick, A., Guo, C., Lee, B., Muller, D., et al. (2018). New York City house mice (*Mus musculus*) as potential reservoirs for pathogenic bacteria and antimicrobial resistance determinants. *mBio* 9, e00624–18. doi: 10.1128/mBio.00624-18

Woodford, N., Wareham, D. W., Guerra, B., and Teale, C. (2014). Carbapenemase-producing Enterobacteriaceae and non-Enterobacteriaceae from animals and the environment: an emerging public health risk of our own making? *J. Antimicrob. Chemother.* 69, 287–291. doi: 10.1093/jac/dkt392

Xavier, B. B., Lammens, C., Butaye, P., Goossens, H., and Malhotra-Kumar, S. (2016). Complete sequence of an IncFII plasmid harbouring the colistin resistance gene mcr-1 isolated from Belgian pig farms. *J. Antimicrob. Chemother.* 71, 2342–2344. doi: 10.1093/jac/dkw191

Yang, R. S., Feng, Y., Lv, X. Y., Duan, J. H., Chen, J., Fang, L. X., et al. (2016). Emergence of NDM-5- and MCR-1-producing *Escherichia coli* clones ST648 and ST156 from a single Muscovy duck (*Cairina moschata*). *Antimicrob. Agents Chemother.* 60, 6899–6902. doi: 10.1128/AAC.01365-16

Yao, H., Li, A., Yu, R., Schwarz, S., Dong, H., and Du, X. D. (2020). Multiple copies of Bla NDM-5 located on conjugative Megaplasmids from porcine *Escherichia coli* sequence type 218 isolates. *Antimicrob. Agents Chemother.* 64, e02134–19. doi: 10.1128/AAC.02134-19

Yong, D., Toleman, M. A., Giske, C. G., Cho, H. S., Sundman, K., Lee, K., et al. (2009). Characterization of a new metallo-beta-lactamase gene, Bla(NDM-1), and a novel erythromycin esterase gene carried on a unique genetic structure in *Klebsiella pneumoniae* sequence type 14 from India. *Antimicrob. Agents Chemother.* 53, 5046–5054. doi: 10.1128/AAC.00774-09

Zhai, R., Fu, B., Shi, X., Sun, C., Liu, Z., Wang, S., et al. (2020). Contaminated in-house environment contributes to the persistence and transmission of NDM-producing bacteria in a Chinese poultry farm. *Environ. Int.* 139:105715. doi: 10.1016/j.envint.2020.105715

Zhang, R., Liu, L., Zhou, H., Chan, E. W., Li, J., Fang, Y., et al. (2017). Nationwide surveillance of clinical Carbapenem-resistant Enterobacteriaceae (CRE) strains in China. *EBioMedicine* 19, 98–106. doi: 10.1016/j.ebiom.2017.04.032

Zhang, Q., Lv, L., Huang, X., Huang, Y., Zhuang, Z., Lu, J., et al. (2019b). Rapid increase in carbapenemase-producing Enterobacteriaceae in retail meat driven by the spread of the blaNDM-5-carrying IncX3 plasmid in China from 2016 to 2018. *Antimicrob. Agents Chemother.* 63, e00573–19. doi: 10.1128/AAC.00573-19

Zhang, P., Wang, J., Wang, X., Bai, X., Ma, J., Dang, R., et al. (2019a). Characterization of five *escherichia coli* isolates co-expressing ESBL and mcr-1 resistance mechanisms from different origins in China. *Front. Microbiol.* 10, 1–9. doi: 10.3389/fmicb.2019.01994

Zhang, Y., Wang, Q., Yin, Y., Chen, H., Jin, L., Gu, B., et al. (2018). Epidemiology of carbapenem-resistant enterobacteriaceae infections: report from the China CRE network. *Antimicrob. Agents Chemother.* 62, 1–11. doi: 10.1128/AAC.01882-17

Zhao, W., Li, S., Schwarz, S., Li, A., Yao, H., and Du, X. D. (2021). Detection of a NDM-5-producing *Klebsiella pneumoniae* sequence type 340 (CG258) high-risk clone in swine. *Vet. Microbiol.* 262:109218. doi: 10.1016/J.VETMIC.2021.109218