Research
Antimicrobial Resistance—Article

Transmission of Carbapenem Resistance Between Human and Animal NDM-Positive *Escherichia coli* Strains

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ARTICLE INFO

ABSTRACT

Although carbapenem use is prohibited in animals in China, carbapenem-resistant *Escherichia coli* (CREC), especially New Delhi metallo-β-lactamase (NDM)-producing strains, are widely prevalent in food-producing animals. At present, the impact of livestock-associated CREC strains on human populations at the national level is unknown. Here, we conduct a retrospective cross-sectional study to investigate the prevalence of CREC from clinical settings across 22 Chinese provinces or municipalities and analyze anthropogenic factors associated with their presence. We also ascertain the bla_{NDM} and bla_{KPC} abundance among pig and chicken farms and present a detailed genomic framework for CREC of animal and human origin. Overall, 631/29 799 (2.1%) clinical *Escherichia coli* (*E. coli*) isolates were identified as CREC. Multivariable analysis revealed that being male, an age below 1, an age between 13 and 18, provinces with greater chicken production, and provinces with higher pig production were associated with higher odds of CREC infection. In general, 73.8% (*n* = 45/61) of pig farms and 62.2% (*n* = 28/45) of chicken farms had a bla_{NDM} abundance of 1×10^{-5} to 1×10^{-3} and 1×10^{-3} to 1×10^{-2}, respectively. Among all the Chinese NDM-positive *E. coli* (*n* = 463) available at the National Center for Biotechnology Information (NCBI), the genomic analysis revealed that bla_{NDM-5} and IncX3 were the predominant carbapenemase gene-plasmid combination, while a highly homogeneous relationship between NDM-positive isolates from humans and animals was demonstrated at the plasmid and core genome levels. All the findings suggest frequent CREC transmission between humans and animals, indicating that further discussions on the use of antibiotics in animals and humans are needed, both in China and across the globe.

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1. Introduction

Antibiotic resistance is one of the greatest global public health challenges of this century, and carbapenems remain one of our most powerful antibiotics to treat life-threatening multidrug-resistant infections [1]. Unfortunately, the rapid dissemination of

carbapenem-resistant *Enterobacterales* (CRE) is now a major concern among hospitalized patients, as CRE show high levels of resistance to carbapenems, cephalosporins, and penicillins, severely restricting treatment options [2]. As such, CRE have been referred to as "nightmare bacteria" with a higher burden [3] and are classified as an urgent public health threat [4] and as priority pathogens that urgently require new antibiotics [5].

At present, three dominant carbapenemases are produced by healthcare-associated CRE strains worldwide: New Delhi metallo-β-lactamases (NDM), *Klebsiella pneumoniae* carbapenemases (KPC), and carbapenem-hydrolyzing oxacillinase-48-type β-lactamases (OXA-48) [6]. NDM-producing strains are highly prevalent in over 50 countries, especially in South Asia (India, Pakistan, and Bangladesh) and China, while KPC-producing strains are more prevalent in North and South America (the United States, Argentina, and Brazil), as well as in China and Europe (Greece, Italy, and Israel) [7]. A multinational study involving 36 European countries revealed that 10.3% and 7.2% of hospital-acquired carbapenem-resistant *Escherichia coli* (*E. coli*) (CREC) were positive for NDM and KPC, respectively [8]. In China, NDM- and KPC-producing clinical isolates collected in 2014–2015 across 25 provinces respectively accounted for 49.4% and 39.6% of all CREC [9]. Conversely, in another study, bla_{NDM} was the only detected carbapenemase gene in 46.7% of CREC isolates from healthy individuals from 19 Chinese provinces [10]. Collectively, these data indicate that the majority of population-derived CREC isolates in China are mediated by NDM production.

In CREC isolates from food-producing, wild, and companion animals worldwide, NDM remains the most prevalent carbapenemase [11]. In China, NDM-producing isolates are becoming the most predominant carbapenemase-producing colonizers of food-producing animals and are contaminating subsequent meat products [12], in which bla_{OXA-48} and bla_{KPC} are sporadically detected [13]. Nevertheless, CREC of animal origin have not hitherto been generally considered to be a concern to human health [14], despite some evidence of direct transmission of CREC between animals and humans [15].

2. Materials and methods

2.1. Study design

Fig. 1 and Fig. S1 in Appendix A provide a schematic of the study design. We began by undertaking a retrospective cross-sectional study. Firstly, a total of 29 799 sequential *E. coli* isolates collected from outpatients and inpatients at 30 hospitals in China were obtained from the China Antimicrobial Surveillance Network (CHINET)[†] between 1 January 2016 and 31 December 2016. Most of these hospitals are large referral institutions; together, they cover 22 provinces or municipalities with a combined population of about 900 million people. To avoid duplicates, only one isolate from each clinical specimen was included per patient, based on the patient identification code. Species identification was performed using automated systems such as the Vitek 2 Compact (bioMérieux, France) and Phoenix-100 (Becton, Dickinson and Company, USA) systems or by matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS; Bruker Daltonics, Germany). The susceptibilities of the isolates to 19 clinical antimicrobial agents (ceftazidime, cefotaxime, cefepime, cefoxitin, cefoperazone/sulbactam, piperacillin/tazobactam, aztreonam, ertapenem, imipenem, meropenem, amikacin, gentamicin, ciprofloxacin, levofloxacin, trimethoprim/sulfamethoxazole, fosfomycin, colistin, nitrofurantoin, and tigecycline) were determined using the Vitek 2 Compact auto-

mated system (bioMérieux), as per the instrument specifications. The results were interpreted according to the Clinical and Laboratory Standards Institute criteria[††]. The strain showing resistance to at least one of the carbapenems was defined as CREC (Fig. 1 and Fig. S1).

Secondly, we collected samples in 22 pig and 16 chicken farms from each of three provinces (Liaoning, Hunan, and Shaanxi) during 1 April 2017 to 31 May 2017 to assess the general abundance of bla_{NDM} and bla_{KPC}; details on the sample collection and procedure have been published previously [16]. Farms were excluded if the extracted DNA was not of sufficient quality for further analyses (Fig. 1 and Fig. S1). DNA extracts were used as templates to assess the relative abundance of bla_{NDM} and bla_{KPC} using a quantitative polymerase chain reaction (PCR)-based assay with the 16S ribosomal RNA (rRNA) gene as a control, as described previously [17].

Thirdly, we undertook a comprehensive worldwide genomic study of CREC of animal and human origin to determine their genomic characteristics and associations at the gene, plasmid, and strain level (Fig. 1 and Fig. S1). The detailed process is described below. Ethical permission for the study was granted on 24 November 2015 by the ethics committee of Huashan Hospital, Fudan University (project number: KY2015-285). Individual consent forms were translated into Mandarin, and consent was obtained from all inpatients either face to face or by phone. We excluded individuals who were not pleased to participate in this study, and all participants were informed of their right to withdraw from the study at any stage.

2.2. Data sources

Anthropogenic data for the 22 provinces or municipalities in China were obtained from the National Bureau of Statistics of China and the China Agriculture Yearbook[‡]. The six classified anthropogenic factors included: gross domestic product (GDP), population (number and density), antimicrobial usage, animal production (farm animals and freshwater aquaculture, mainly fish and shrimp), animal-derived food consumption, and daily animal-derived food intake. Most of the data was collected in 2015, although the antimicrobial usage data were from 2013 [18].

In the genomic analyses, we downloaded all available Chinese NDM-positive *E. coli* whole-genome sequences (*n* = 463) from the National Center for Biotechnology Information (NCBI) databases for comprehensive analysis (Fig. 1 and Table S1 in Appendix A). We also accessed the genomic sequences of 1852 NDM-negative *E. coli* isolates from four hosts (humans, pigs, chickens, and flies, corresponding to four times the total number of NDM-positive sequences) from the NCBI databases (Fig. 1 and Table S1). All strains were given an ordered natural number and were randomly selected using the RAND function in Excel for corresponding analysis.

2.3. Genomic analyses

The genome sequence analysis included genome assembly, multi-locus sequence typing, plasmid detection, and phylogenetic population prediction, according to the detailed description in our previous publication [19]. Contigs that did not contain plasmid replicon markers were analyzed using Bandage version 0.8.1 to identify possible types[‡‡]. A Sankey diagram was generated using the networkD3 package version 0.4 in R 3.6.1[†††]. All NDM-positive

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[†] www.chinets.com.

[††] https://clsi.org.
[‡] http://www.stats.gov.cn/tjsj/ndsj/2016/indexch.htm.
[‡‡] https://github.com/rrwick/Bandage.
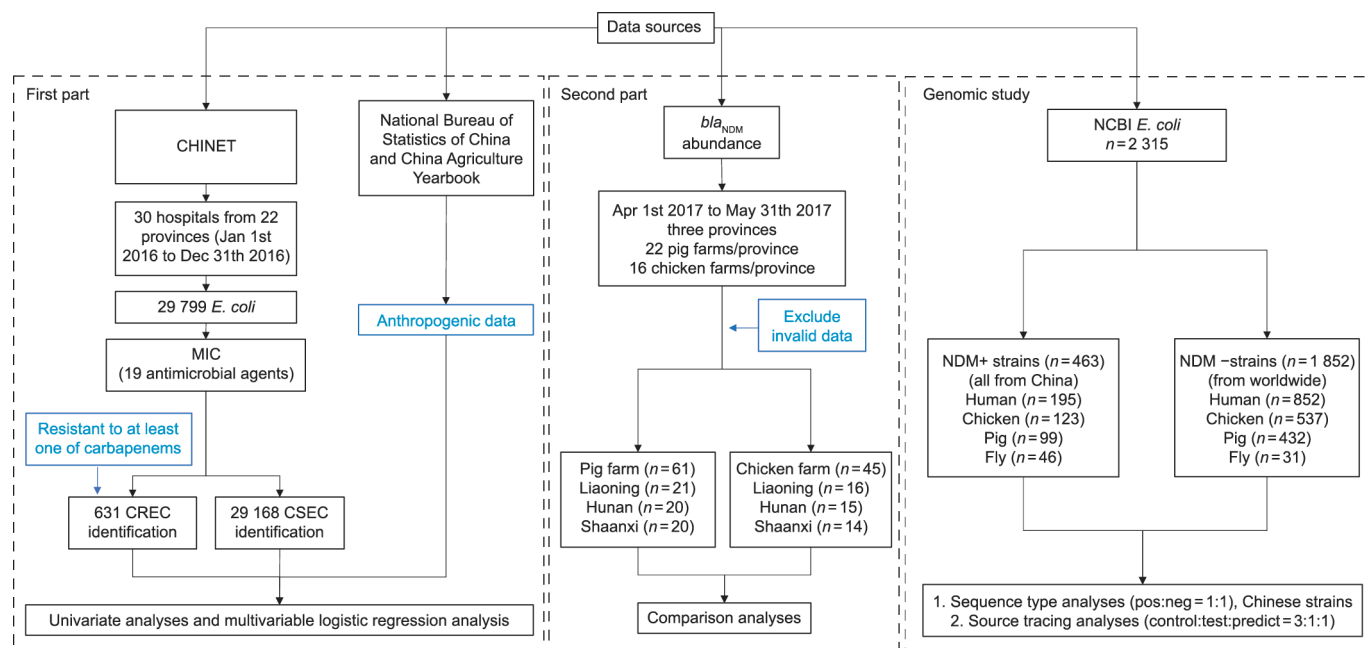[†††] https://CRAN.R-project.org/package=networkD3.

Fig. 1. Flowchart of data sources and the study process. MIC: minimum inhibitory concentration. CSEC: carbapenem-susceptible *E. coli*; pos: positive; neg: negative.

assembled genomes were used for core genome alignments to produce a phylogenetic tree via the RedDog pipeline[†]. The resulting tree was visualized using Interactive Tree of Life v5[††].

2.4. Source tracing

We used a discriminant analysis of principal components (DAPC) detectable model [20] to trace the potential origins of all NDM-positive *E. coli*. Core genome single-nucleotide polymorphisms (SNP) profiles for all isolates, including NDM-negative *E. coli* (total n = 2315), were generated using the snippy pipeline[‡]. The SNP matrix for NDM-negative *E. coli* used the DAPC model for 1389 isolates, by means of the Adegenet package implemented in R 3.6.1 [18]. The remaining 463 isolates were used to assess the fitness of the above model. Finally, the genetic origins of all Chinese NDM-positive isolates (n = 463) were predicted using the constructed DAPC model.

2.5. Statistical analyses

Demographic and non-clinical data were entered into Excel 2016 (Microsoft, USA). We divided the variable "age" into seven subgroups (baby, preschooler, school-aged child, teenager, youth, adult, and senior) and the variable "infection site" into five subgroups (blood, urine, sputum, cerebrospinal fluid, and other). Furthermore, based on a previous study on the distribution of chicken production [21] and the national pig production plan [22], we divided mainland China into two and four production regions for chickens and pigs, respectively, based on yearly production volumes [23]. Categorial data were tested by χ^2 analysis, and continuous data were tested using the Mann–Whitney U test. Univariable analysis was conducted in SPSS version 23.0 (IBM, USA), and variables with a P value $\leq$ 0.05 were subsequently assessed for collinearity using Cramer's coefficient phi (Φ). If a pair of variables were highly correlated (Φ > 4.0), we selected the more bio-

logically plausible variable for the multivariable logistic analysis. Significant variables identified by the univariable analyses were kept for the multivariable analysis. A forward stepwise (likelihood ratio) method was applied to the multivariable logistic regression analysis in order to control confounders. Variables with a P value of < 0.05 were kept in the final model. The goodness of fit to the logistic regression model was tested using the Hosmer–Lemeshow goodness-of-fit test. A forest plot was generated using the forest plot package in R[‡‡].

The abundance of bla_{NDM} was analyzed and visualized using R 3.6.1. Due to an excess of abundance values below the limit of detection (LOD), a pseudo-count of 1×10^{-7} (one order of magnitude lower than the lowest abundance) was defined as the abundance before the $\log_{10}$ scale for those values below the LOD [24]. Different groups were compared using a Mann–Whitney U test or Kruskal–Wallis H test at a significance level of P < 0.05.

3. Results

3.1. Association of CREC with clinical and non-clinical factors

A schematic of the study design is shown in Fig. 1 and Fig. S1. Among the 29 799 *E. coli* isolates derived from various infection samples from 22 provinces or municipalities in China, 631 isolates (2.1%) were defined as CREC. Sichuan had the highest prevalence of CREC isolates (4.7%), while no CREC isolates were recovered from the samples from Fujian (Fig. S2 in Appendix A). An analysis of the patients with CREC infections revealed that the ratio of females to males was 1.4:1; however, the prevalence of CREC among males was significantly higher than that among females according to the univariable analysis (P < 0.0001) (Fig. 2 and Table S2 in Appendix A). Infants (< 1 year) and teenagers (13–18 years) had the highest rates of CREC infection (3.5% and 3.2%, respectively). Urine accounted for the largest proportion of positive samples (42.1%) but displayed the lowest odds ratio (OR = 0.7), with a 95% confidence interval (CI) ranging from 0.6 to 0.9 (Table S2). In comparison, cerebrospinal fluid accounted for the lowest proportion of
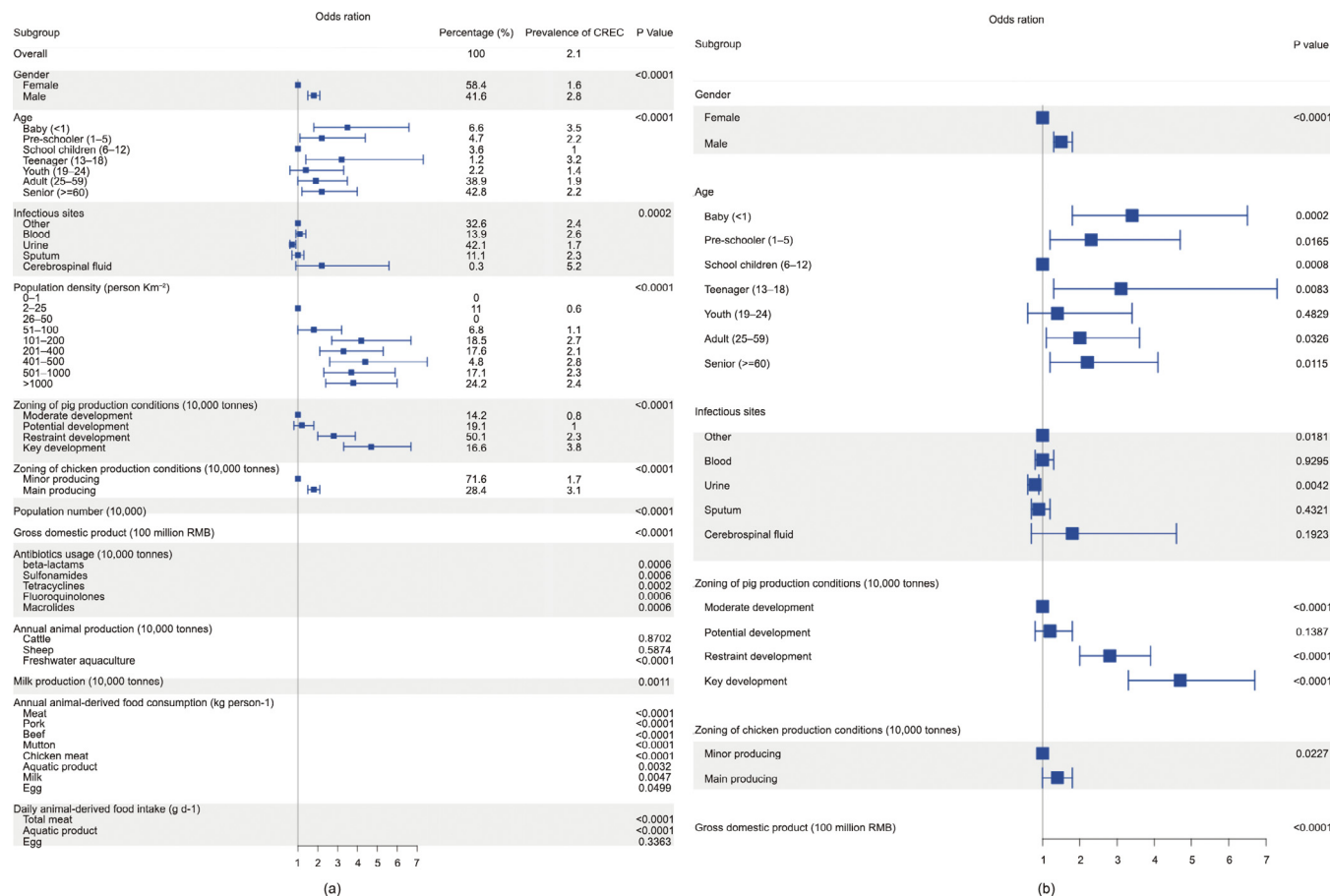
Fig. 2. Forest plots of (a) univariable and (b) multivariable analyses.

positive samples (0.3%) but had the highest prevalence (5.2%). In total, 26/29 variates were significantly associated with CREC ($P \leq 0.05$) (Table S2). After removing highly collinear variates, six non-clinical factors and three demographic variates were selected for multivariable logistic regression analysis (Fig. 2(b) and Table S2). In three clinical categories, males had higher ORs (OR = 1.6, 95% CI: 1.3–1.8) of CREC infections than females, with infants and teenagers also showing higher ORs (OR = 3.4, 95% CI: 1.8–6.5 and OR = 3.1, 95% CI: 1.3–7.5, respectively). Urinary tract infections had the lowest ORs (OR = 0.8, 95% CI: 0.6–0.9) for CREC. For the three non-clinical categories, populations in higher GDP zones (Table S2) or greater chicken production (OR = 1.4, 95% CI: 1.1–1.8; Table S2) and pig production (OR = 5.5, 95% CI: 3.5–8.7; Table S2) showed higher CREC infection rates. The Hosmer–Lemeshow test (χ^2 = 11.35, degree of freedom (d.f.) = 8, P = 0.182 > 0.05) indicated that the selected model was fit for an analysis of the data.

3.2. Susceptibility testing

Susceptibility testing indicated that resistances among CREC (n = 631) to cephalosporin, cefoperazone/sulbactam, piperacillin/-tazobactam, and fluoroquinolones (58.8%–95.7%) were significantly higher than those for carbapenem-susceptible *E. coli* (CSEC; n = 29 168, 2.9%–59%) ($P < 0.0001$) (Table 1). CREC showed resistance of 16.8% and 28.5% to amikacin and fosfomycin, versus CSEC showed resistance of 2.7% and 5.6% to amikacin and fosfomycin. Both CREC and CSEC showed high levels of susceptibility (> 96.7%) to colistin and tigecycline. Interestingly, 59% of CSEC showed resistance to cefotaxime (Table 1). Although the resistance

Table 1
Antimicrobial susceptibility profiles of clinical CREC and CSEC isolates.

Antimicrobial agents	CREC (n = 631)	CSEC (n = 29 168)	P value
Cefoperazone/sulbactam	413 (65.5%)	1 313 (4.5%)	< 0.0001
Piperacillin/tazobactam	371 (58.8%)	846 (2.9%)	< 0.0001
Ceftazidime	536 (84.9%)	7 380 (25.3%)	< 0.0001
Cefotaxime	604 (95.7%)	17 209 (59.0%)	< 0.0001
Cefepime	483 (76.5%)	7 671 (26.3%)	< 0.0001
Cefoxitin	413 (65.5%)	3 413 (11.7%)	< 0.0001
Aztreonam	481 (76.2%)	10 150 (34.8%)	< 0.0001
Ertapenem	569 (90.2%)	0	< 0.0001
Imipenem	395 (62.6%)	0	< 0.0001
Meropenem	487 (77.2%)	0	< 0.0001
Amikacin	106 (16.8%)	788 (2.7%)	< 0.0001
Gentamicin	339 (53.7%)	12 367 (42.4%)	< 0.0001
Ciprofloxacin	482 (76.4%)	16 247 (55.7%)	< 0.0001
Levofloxacin	454 (71.9%)	15 459 (53.0%)	< 0.0001
Trimethoprim/sulfamethoxazole	414 (65.6%)	16 392 (56.2%)	< 0.0001
Fosfomycin	180 (28.5%)	1 633 (5.6%)	< 0.0001
Colistin	11 (1.7%)	700 (2.4%)	0.2849
Nitrofurantoin	107 (17.0%)	1 108 (3.8%)	< 0.0001
Tigecycline	21 (3.3%)	117 (0.4%)	< 0.0001

Data indicate the number of CREC isolates (resistance rates, %). P values were calculated by χ^2 analysis.

genotype of the isolates from CHINET was unavailable, the sequences of Chinese clinical *E. coli* isolates (n = 195 and n = 494 of NDM-positive and -negative, respectively) from NCBI were utilized to detect the rates of resistance genes between these two groups. Overall, the prevalence of resistance genes to most antimicrobial agents—especially β-lactam resistance genes (bla_{CTX-M}, bla_{OXA}, and bla_{CMY})—in NDM-positive strains was significantly

higher than that in negative strains, except for the rates of *mcr* and *oqxAB* genes (Fig. S3 in Appendix A). The recently increasing number of *mcr*-positive strains without NDM that have been submitted to the NCBI might contribute to the higher prevalence of *mcr* in NDM-negative strains; this observation also coincided with our susceptibility testing, which showed higher resistance to colistin in NDM-negative strains than in positive strains.

3.3. Detectable abundance of bla$_{NDM}$ in samples from livestock farms

Because of the strong association between clinical CREC infection and livestock production at the provincial level, we conducted a study by collecting feces samples (pig (n = 22) and chicken (n = 16) farms) from Hunan, Liaoning, and Shaanxi to determine the abundance of *bla*$_{NDM}$ and *bla*$_{KPC}$. After excluding invalid DNA extracts, samples from 61 pig farms and 45 chicken farms were analyzed (Fig. S4(a) and Table S3 in Appendix A). All farms were negative for *bla*$_{KPC}$; however, *bla*$_{NDM}$ was detected in 73.8% (n = 45) and 62.2% (n = 28) of pig and chicken farms, respectively, with relative gene abundance values ranging from 1×10^{-5} to 1×10^{-3} and from 1×10^{-3} to 1×10^{-2} (*bla*$_{DNM}$ per 16 s rRNA), respectively (Fig. S4(b) and Table S3 in Appendix A). Overall, the median abundances of *bla*$_{NDM}$ among chicken and pig farms were similar (P = 0.0758); however, one pig farm in Hunan and one chicken farm in Liaoning had abnormally high *bla*$_{NDM}$ abundance values of 0.2467 and 0.2465, respectively (Fig. S4(c) and Table S3).

3.4. Conservation of sequencing types between animal- and human-derived NDM-positive E. coli isolates

Because NDM was the major carbapenemase among the CREC isolates, we further investigated the associations between NDM-positive *E. coli* of animal and human origin using genomic bioinformatics. A minimum spanning tree based on sequencing types (STs) was generated using 463 NDM-positive *E. coli* and 463 randomly selected matched NDM-negative *E. coli* isolates (1:1) from humans (195 vs 217), pig (99 vs 110), chickens (123 vs 136), and flies (46 vs 0) from China. Negative strains of fly origin were supplied with other origins of corresponding ratio. Overall, the NDM-positive *E. coli* isolates displayed 96 ST types, among which ST167 (n = 62, 13.4%), ST156 (n = 34, 7.3%), and ST48 (n = 31, 6.7%) were the most prevalent. In comparison, the NDM-negative *E. coli* belonged to 158 ST types, including ST10 (n = 47, 10.2%), ST101 (n = 16, 3.5%), and ST48 (n = 15, 3.2%) (Fig. S5 and Table S1 in Appendix A). Human NDM-positive *E. coli* isolates shared 16 (n = 111), 11 (n = 90), and 10 (n = 96) STs with those from chickens, pigs, and flies, respectively, while 25 (n = 93) and 19 (n = 77) STs associated with NDM-negative isolates from humans overlapped with those associated with chickens and pigs, respectively (Fig. S6 in Appendix A). NDM-positive isolates belonging to ST167, ST206, ST10, and ST48 were recovered from all four origins. In comparison, 85.9% (55/64) of ST167 and 62.1% (18/29) of ST10 isolates were recovered from humans and pigs, respectively (Fig. S6 and Table S1).

3.5. Genomic association among NDM-positive isolates/plasmids between animals and humans

We established a core genome phylogenetic tree of 463 NDM-positive *E. coli* isolates using 362 650 SNPs to investigate associations at the isolate and plasmid level (Figs. 3 and 4). All isolates from the four origins were clustered into 11 lineages by means of Bayesian analysis of population structure (BAPS). Human isolates were distributed across all lineages, with the L4 lineage
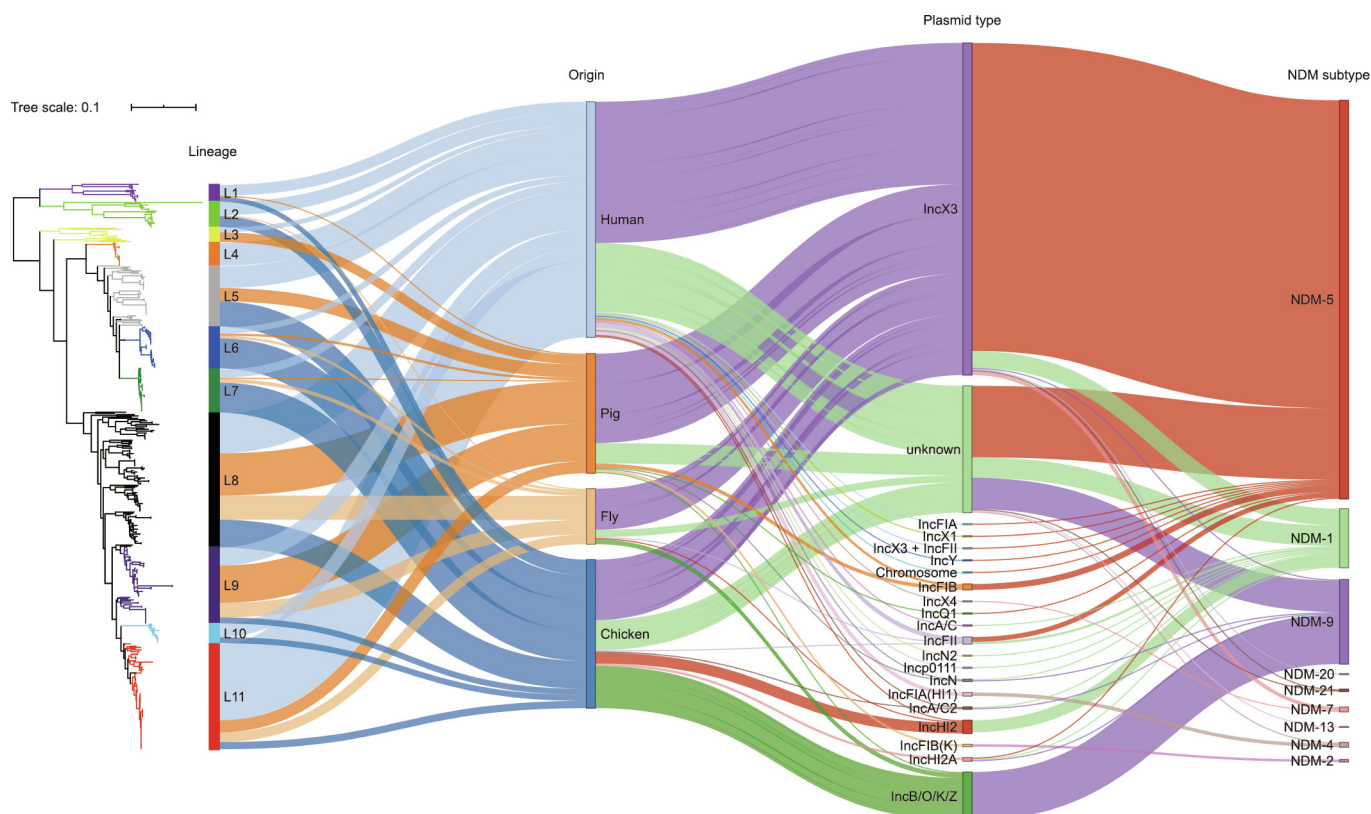
Fig. 3. Phylogenetic tree of 463 NDM-positive isolates. The lineages are indicated by differently colored tree branches and stripes to the right of the tree. The distribution and linkages among the lineages, isolate origins, *bla*$_{NDM}$-carrying plasmid types, and NDM protein subtypes are indicated in the Sankey diagram. All stripes are labeled with corresponding items.
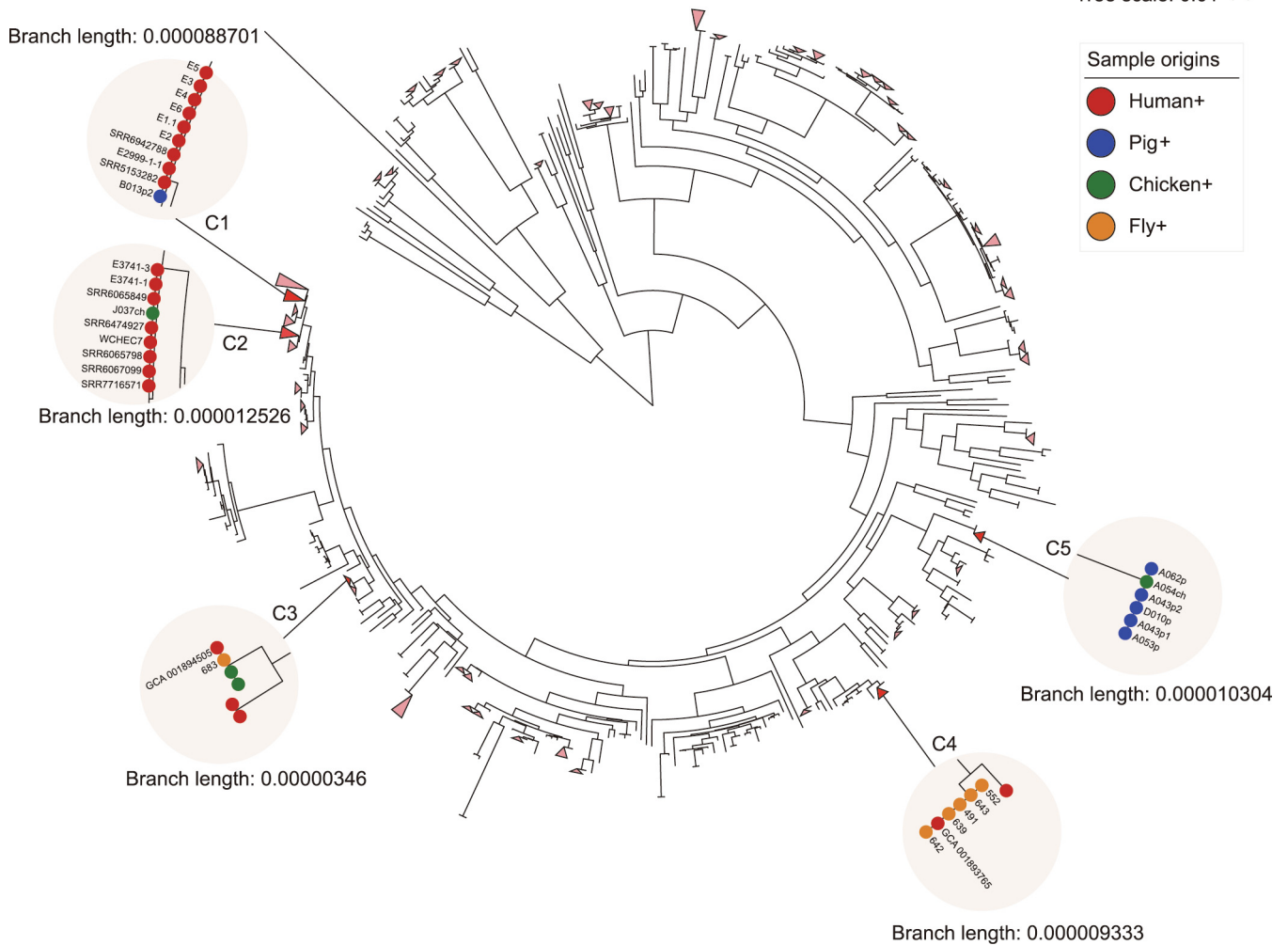
**Fig. 4.** Tree showing the closest genetic relationships among various hosts of the five major clades, C1–C5. The midpoint rooted circular phylogenetic tree of all 463 NDM-positive isolates based on SNP differences was generated using the maximum likelihood method. Clades with an average branch length < 0.0005, corresponding to 99.95% similarity, were collapsed and are indicated with pink triangles. The size of the triangle is proportional to the number of isolates within the clades. Close genetic relationships among the isolates from various hosts are indicated by red triangles, with the five clades labeled C1–C5. Isolates belonging to each clade are shown in pink circles, and the exact branch length is provided. The names and hosts of these isolates are indicated with labels and differently colored circular dots, respectively.

(n = 19) belonging exclusively to human (Fig. 3). L8 contained the largest number of NDM-positive *E. coli* (111, 24.0%), consisting of 33 human (29.7%), 35 pig (31.5%), 23 chicken (20.7%), and 20 fly (18.0%) isolates (Fig. 3). The 87 isolates in L11 were derived from four origins, including 63 (72.4%) from humans (Fig. 3). Isolates from the same origin often formed clades with > 99.95% similarity (indicated by collapsed pink triangles in Fig. 4), while closely related isolates from two or more different origins were observed in clades C1–C5 (Fig. 4).

Except for 105 short bla_{NDM}-carrying contigs with uncertain plasmid types and one contig with chromosomally located bla_{NDM-5}, the remaining 357 bla_{NDM}-carrying plasmids belonged to 19 different Inc types (Fig. 3 and Table S1); of these, bla_{NDM-5} (332, 71.7%) was the most prevalent and was mainly located on IncX3-type plasmids (255, 76.8%). Interestingly, the IncB/O/K/Z plasmids (n = 40, 8.6%) only carried bla_{NDM-9}. Overall, IncX3 (275, 59.4%) was the predominant plasmid type across all origins and lineages, and was found in 117 human (42.5%), 74 pig (26.9%), 50 chicken (18.2%), and 34 fly (12.4%) isolates. NDM-positive IncB/O/K/Z plasmids were mainly found in chicken isolates (35, 87.5%) and were absent from human isolates (Fig. 3 and Table S1).

3.6. Prediction of host shuffling by NDM-positive E. coli

We employed a DAPC method to track the origins of the 463 NDM-positive isolates, with a testing group of 463 NDM-negative isolates and a control group of 1389 NDM-negative isolates (ratio = 1:1:3, Fig. S1). Although the DAPC model predicted a close genetic relationship among the isolates from humans, chickens, and pigs, a distinguishable boundary was observed (Fig. 5(a)), and the first 600 principal components representing the highest successful outcome prediction (a total of 1200) were selected for analysis (Fig. 5(b)). The predicted origins of the isolates using this model showed 87.4%, 83.9%, 88.3%, and 83.5% accuracy for chicken, fly, human, and pig, respectively, with an overall mean accuracy of 56.2% (standard deviation = 1.9%) in the testing group (Fig. 5(c) and Table S4 in Appendix A). Using this method, 19.5% (n = 24), 8.1% (n = 10), and 1.6% (n = 2) of chicken NDM-positive *E. coli* isolates (n = 123) were predicted to originate from humans, pigs, and flies, respectively, while 27.3% (27/99) of pig NDM-positive *E. coli* isolates were predicted to originate from humans (Fig. 5(d) and Table S5 in Appendix A). Although 30.8% (60/195) of human-derived isolates did not appear to have changed hosts, a further
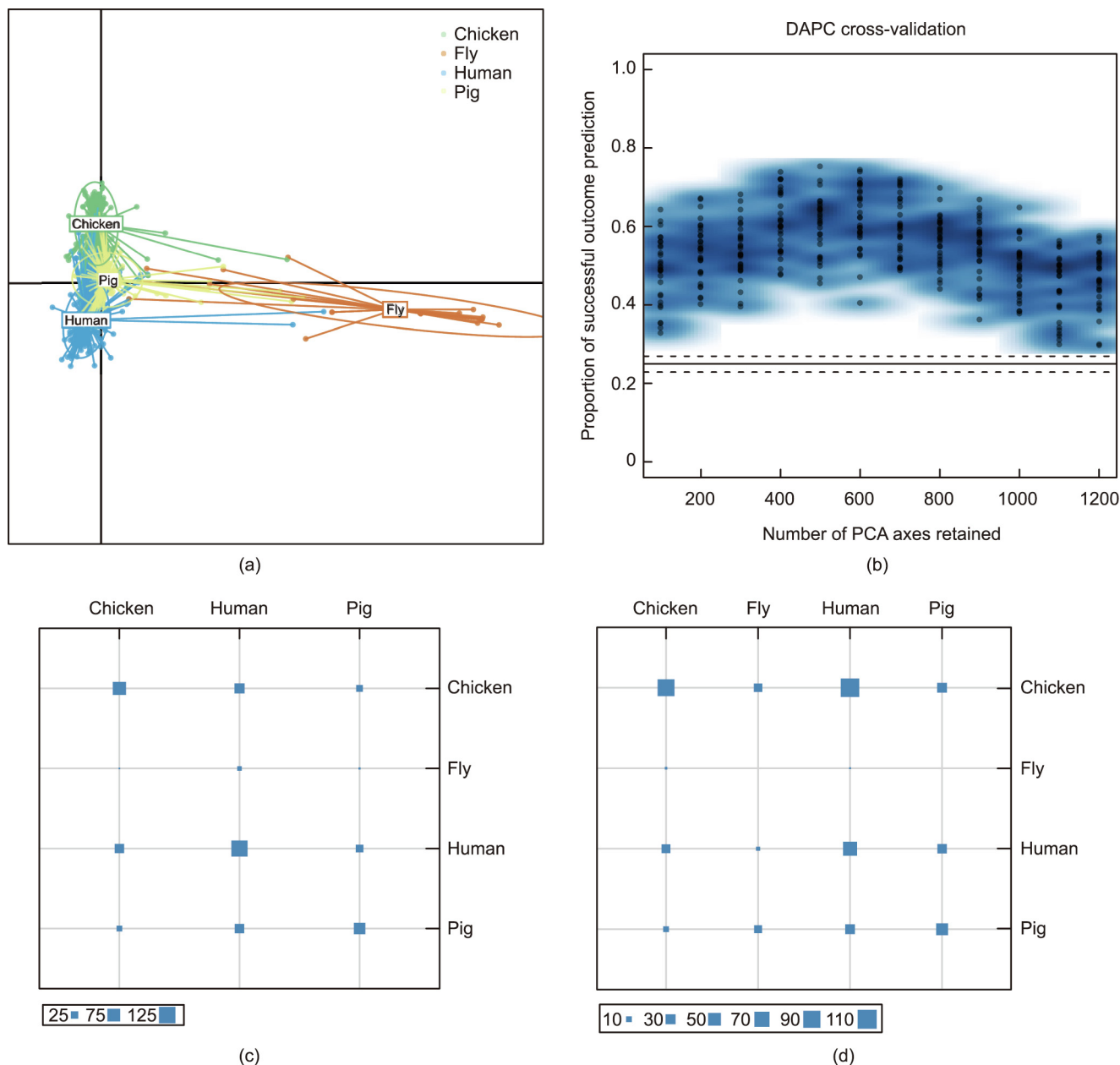
Fig. 5. Source trace of NDM-positive *E. coli* isolates generated using a DAPC detectable model. (a) Scatterplot of training set. Each dot represents one individual isolate, with different origins indicated by different colors. The ellipse indicates the 95% CI for each origin. (b) Cross-validation of the DAPC model. (c) Table-plot of testing set. (d) Table-plot of prediction set. Rows and columns represent reported and predicted origins, respectively, while the size of each square indicates the number of corresponding isolates. PCA: principal components analysis.

53.8% (n = 105) and 14.9% (n = 29) of human isolates were predicted to have originated from chickens and pigs, respectively (Fig. 5(d) and Table S5). All fly-derived isolates (n = 46) were predicted to have originated from humans (n = 5, 10.9%), chickens (n = 22, 47.8%), and pigs (n = 19, 41.3%) (Fig. 5(d) and Table S5).

4. Discussion

Our data represents the largest clinical CREC surveillance study in China, encompassing samples from 22 provinces or municipalities. The overall prevalence of CREC (2.1%, 631/29 799, CHINET, 2016) among infection-associated *E. coli* isolates from 30 hospitals in China was higher than that in Europe (0.1%, not provided/

121 582, EARS-Net, 2016) [25] and the United States (0.3%, 5/1916, 30 hospitals, 2015–2017) [26]. The positive association between CREC infection and male sex in the current study was in accordance with previous reports [27]. The finding that the highest ORs of CREC infection were observed among infants (< 1 year old) in our current study reflects the recent CRE rates among children in the United States [28]. The microbial communities of infants are particularly dynamic and vulnerable to perturbation by antibiotics, possibly providing opportunities for CRE colonization, which has been shown to be a risk factor for endogenous infections in older intensive care unit (ICU) patients [29]. Interestingly, the emission of β-lactams used in humans and animals [18] is not significantly associated with CREC infection, implying that the discharge of β-lactams into the environment might not be a

major driver of CREC dissemination. Studies have shown that β-lactams are rapidly degraded at room temperature [30], which likely contributes to them exerting little selective pressure in the environment.

This study is the first to identify a positive association between livestock production and human CREC infections. Our microcosmic genome analysis provided several pieces of evidence in support of this linkage. Firstly, we observed a high abundance of bla_{NDM}—but not bla_{KPC}—in samples from animal farms across three Chinese provinces. Secondly, isolates with similar bla_{NDM}-carrying IncX3 plasmids were identified from human, chicken, pig, and fly isolates, while bla_{KPC} was rarely identified in isolates from animals in both the current and previous studies [11]. Thirdly, we identified a close relationship among the core genome sequences of NDM-positive *E. coli* from humans and animals. Fourthly, the source-tracing analysis revealed indistinct boundaries between human- and animal-derived NDM-positive *E. coli*. Collectively, these observations suggest that NDM-positive CREC isolates pose a significant transmission risk between animals and humans. Notably, the consumption of animal-derived food products was significantly associated with CREC infections in the univariable analysis but showed a non-significant association in the multivariable logistic regression analysis, suggesting that other possible transmission routes (e.g., fomite transmission) could also be a factor in the spread of bla_{NDM}-carrying plasmids or CREC. Supporting these observations, bla_{NDM} has been detected in samples from several environmental sources, including hospital sewage, wastewater, drinking water, natural waterways, and agricultural environments [31], as well as in companion animals, flies, and wildlife [11]. Furthermore, transmission of CREC between animals and humans via environmental sources has been previously reported [12,32].

The successful circulation of bla_{NDM} between animals and humans could include the following evidences. Firstly, with a prevalence of 76.8% and occurrence within CREC from each major lineage (Fig. 3), bla_{NDM}-IncX3 plasmid appears to be a non-specific host plasmid; moreover, bla_{NDM}-IncX3 plasmid is likely to be underestimated in this study, as the large number (n = 105) of NDM-carrying contigs were too short for their plasmid type to be confirmed, and many are likely to have been IncX3. Secondly, bla_{NDM}-carrying plasmid—particularly bla_{NDM}-IncX3—confers little or no fitness cost to the host bacterium, which adds to its persistence and stability in different ecosystems [33,34]. Thirdly, other than the veterinary-prohibited carbapenems, β-lactam antibiotics, including penicillins and cephalosporins, are the second highest class of antimicrobial agents used in farm animals in China (3192 tonnes in 2018) [35] and in 116 other World Organization for Animal Health member countries from 2015–2017 (16.1% of total antimicrobial usage) [36]. These frequently used β-lactams, which are mostly administered in drinking water, may play a vital role in the selection and persistence of NDM-positive bacteria in livestock. Fourthly, the coexistence of bla_{NDM} and other resistance genes, including *mcr-1* [10], *floR*, and *tet*(A) [37], on transmissible plasmids can lead to the co-selection of bla_{NDM} through the use of colistin, florfenicol, and tetracyclines in livestock, respectively.

Despite the evidence supporting CREC transmission between animals and humans, our study has several limitations. First, our sampling sectors might not perfectly align in terms of their time span; however, we used large datasets to provide strong evidence of CREC transmission between animals and humans via statistical analysis and concomitant epidemiological and molecular datasets. Second, data on the types of carbapenemases produced by the clinical CREC isolates was unavailable, although (based on previous large-scale surveillance studies [8,10]) it is likely that NDM was the most prevalent type of carbapenemase. Third, we directly analyzed associations between animal and human infections but did

not analyze colonization in patients and corresponding animal-derived food. However, previous studies have shown that pathogens from bloodstream and urinary tract infections are frequently derived from the gut or from foodborne infections [38,39]. Fourth, *Enterobacterales*, including *E. coli*, *Klebsiella pneumoniae*, and *Enterobacter cloacae*, are major hosts of bla_{NDM} in clinical settings [7]. However, we only focused on NDM-positive *E. coli* because it is the predominant carbapenem-resistant bacterium in animals [11]. Fifth, parts of the genomic data were collected from other studies and were missing information (e.g., time and location of isolation, inconsistencies between true and reported origin), which may have introduced systematic errors into our DAPC model. However, strong associations were revealed in several clusters, and identical plasmids were found in different hosts, unequivocally indicating the transmission of CREC between animals and humans.

To combat the transmission of CREC between animals and humans from the "One Health" perspective, the following actions should be considered: ① As drug revocation has been shown to reduce the prevalence of antibiotic resistance [16,40], a decrease in the prevalence of bla_{NDM}-carrying bacteria on animal farms may be achieved by more judicious use of β-lactams and other antibiotics leading to the co-selection of bla_{NDM}-carrying plasmids/CREC. ② A transition toward large-scale industrial livestock farming and a decrease in backyard farming systems in China† will facilitate the tighter regulation of hygiene, antibiotic-consumption, and infection-control practices. Regulation of these practices should also diminish the use of drugs solely for growth promotion or disease prevention. ③ The food supply chain, the environment, and hospitals play important roles in the transmission of drug-resistant pathogens; therefore, active surveillance systems for both animals (farms and surrounding environments) and humans (colonization, infection, and living environment) are necessary to restrict the spread of CREC. ④ A large proportions of bla_{NDM} genes (> 70%) were associated with IncX3 plasmid in both animals and humans (Fig. 3); thus, host preferences, persistence, and interventions to eliminate bla_{NDM}-IncX3 plasmids require investigation. ⑤ Severing CREC transmission routes through efficient cleaning and disinfection procedures should be implemented at farms, slaughterhouses, food-processing plants, and retail outlets to reduce CREC contamination of non-biological surfaces. In addition, as flies and birds play an important role in the transmission of CREC, their contribution to CREC transmission needs to be fully understood [12]. Finally, following the withdrawal of all antimicrobials as growth promoters by the Chinese livestock industry in July 2020, alternative approaches such as clustered regularly interspaced short palindromic repeat (CRISPR)-associated protein 9 (Cas9) [41], phages [42], and traditional Chinese medicine [43] are promising new weapons to combat this ubiquitous pathogen.

5. Conclusions

This study revealed significant associations between CREC of clinical and provincial animal-production origins at a national scale, and a general prevalence of bla_{NDM} abundance in fecal samples from both chicken and pig farms. Moreover, we identified a persistence of bla_{NDM}-positive *E. coli* on pig farms, and demonstrated host shuffling among the isolates. Taken together, this evidence indicates associations and transmission of CREC between animals and humans. We hypothesize that CREC first arose in clinical settings and was then introduced into livestock animals, which are favorable hosts for the persistence of CREC. This led to the circulation of CREC between humans and animals, either via the food chain or through environmental vectors (biological and/or non-biological).

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† http://baogao.chinabaogao.com/xumuye/291653291653.html.

Acknowledgments

Authors' Contribution

Yang Wang, Fupin Hu, Jianzhong Shen, and Timothy R. Walsh designed the study. Yingbo Shen, Fupin Hu, Dandan Yin, Lu Yang, Yiqiang Chen, Chunyan Xu, Jiyun Li, Junyao Jiang, Xueyang Wang, Yulin Fu, Dongyan Shao, Zhangqi Shen, Shaolin Wang, Juan Li, Rong Zhang, and Congming Wu collected the data. Yingbo Shen, Fupin Hu, Yongqiang Wang, Lu Yang, Dejun Liu, Tengfei Ma, Chang Cai, Timothy R. Walsh, Jianzhong Shen, and Yang Wang analyzed and interpreted the data. Yingbo Shen, Yang Wang, and Timothy R. Walsh wrote the manuscript. All authors reviewed, revised, and approved the final report.

Compliance with ethics guidelines

Yingbo Shen, Fupin Hu, Yongqiang Wang, Dandan Yin, Lu Yang, Yiqiang Chen, Chunyan Xu, Jiyun Li, Junyao Jiang, Xueyang Wang, Yulin Fu, Dongyan Shao, Dejun Liu, Tengfei Ma, Chang Cai, Zhangqi Shen, Shaolin Wang, Juan Li, Rong Zhang, Yuebin Ke, Congming Wu, Jianzhong Shen, Timothy R. Walsh, and Yang Wang declare that they have no conflict of interest or financial conflicts to disclose.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.eng.2021.07.030.

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