A Section 508–conformant HTML version of this article is available at https://doi.org/10.1289/EHP5251.

Inter-host Transmission of Carbapenemase-Producing *Escherichia coli* among Humans and Backyard Animals

Jiyun Li,[1,2] Zhenwang Bi,[3*] Shizhen Ma,[1*] Baoli Chen,[4*] Chang Cai,[5,6] Junjia He,[1] Stefan Schwarz,[1,7] Chengtao Sun,[1] Yuqing Zhou,[1] Jia Yin,[8,9] Anette Hulth,[10,11] Yongqiang Wang,[1] Zhangqi Shen,[1] Shaolin Wang,[1] Congming Wu,[1] Lennart E. Nilsson,[12] Timothy R Walsh,[1,13] Stefan Börjesson,[14,12] Jianzhong Shen,[1,15] Qiang Sun,[8,9] and Yang Wang[1,15]*

[1]Beijing Advanced Innovation Center for Food Nutrition and Human Health, College of Veterinary Medicine, China Agricultural University, Beijing, China
[2]College of Veterinary Medicine, Hunan Agricultural University, Changsha, Hunan, China
[3]Shandong Academy of Clinical Medicine, Shandong Provincial Hospital, Jinan, Shandong, China
[4]Shandong Provincial Key Laboratory of Infectious Disease Control and Prevention, Shandong Center for Disease Control and Prevention, Jinan, Shandong, China
[5]China Australia Joint Laboratory for Animal Health Big Data Analytics, College of Animal Science and Technology, Zhejiang Agricultural and Forestry University, Hangzhou, China
[6]Research and Innovation Office, Murdoch University, Murdoch, Australia
[7]Institute of Microbiology and Epizootics, Centre for Infection Medicine, Department of Veterinary Medicine, Freie Universität Berlin, Berlin, Germany
[8]School of Health Care Management, Shandong University, Jinan, China
[9]NHC Key Laboratory of Health Economics and Policy Research (Shandong University), Jinan, China
[10]Department of Public Health Sciences, Karolinska Institutet, Stockholm, Sweden
[11]Public Health Agency of Sweden, Stockholm, Sweden
[12]Department of Clinical and Experimental Medicine, Linköping University, Linköping, Sweden
[13]Department of Medical Microbiology and Infectious Disease, Division of Infection and Immunity, Cardiff University, Cardiff, UK
[14]Department of Animal Health and Antimicrobial Strategies, National Veterinary Institute (SVA), Uppsala, Sweden
[15]Beijing Laboratory of Food Quality and Safety, College of Veterinary Medicine, China Agricultural University, Beijing, China

Background: The rapidly increasing dissemination of carbapenem-resistant Enterobacteriaceae (CRE) in both humans and animals poses a global threat to public health. However, the transmission of CRE between humans and animals has not yet been well studied.

Objectives: We investigated the prevalence, risk factors, and drivers of CRE transmission between humans and their backyard animals in rural China.

Methods: We conducted a comprehensive sampling strategy in 12 villages in Shandong, China. Using the household [residents and their backyard animals (farm and companion animals)] as a single surveillance unit, we assessed the prevalence of CRE at the household level and examined the factors associated with CRE carriage through a detailed questionnaire. Genetic relationships among human- and animal-derived CRE were assessed using whole-genome sequencing–based molecular methods.

Results: A total of 88 New Delhi metallo-β-lactamases–type carbapenem-resistant *Escherichia coli* (NDM-EC), including 17 from humans, 44 from pigs, 12 from chickens, 1 from cattle, and 2 from dogs, were isolated from 65 of the 746 households examined. The remaining 12 NDM-EC were from flies in the immediate backyard environment. The NDM-EC colonization in households was significantly associated with *a*) the number of species of backyard animals raised/kept in the same household, and *b*) the use of human and/or animal feces as fertilizer. Discriminant analysis of principal components (DAPC) revealed that a large proportion of the core genomes of the NDM-EC belonged to strains from hosts other than their own, and several human isolates shared closely related core single-nucleotide polymorphisms and bla_{NDM} genetic contexts with isolates from backyard animals.

Conclusions: To our knowledge, we are the first to report evidence of direct transmission of NDM-EC between humans and animals. Given the rise of NDM-EC in community and hospital infections, combating NDM-EC transmission in backyard farm systems is needed. https://doi.org/10.1289/EHP5251

Introduction

Carbapenems, particularly imipenem, meropenem, and ertapenem, play a vital role in the treatment of human clinical infections caused by multidrug-resistant (MDR) Gram-negative bacteria (Nordmann et al. 2012). Carbapenems are not approved for use in animals in any part of the world. Carbapenem-resistant bacterial strains have been increasing rapidly worldwide (Johnson and Woodford 2013; Nordmann et al. 2012), and carbapenem-resistant Enterobacteriaceae (CRE) are classified as an urgent clinical threat by the Centers for Disease Control and Prevention (CDC 2013) and as priority pathogens for which new antibiotics are urgently needed by the World Health Organization (WHO 2017). New Delhi metallo-β-lactamases (NDM), *Klebsiella pneumoniae* carbapenemases (KPC), and carbapenem-hydrolyzing oxacillinase 48 type β-lactamases are emerging as the most commonly acquired carbapenemases among Enterobacteriaceae from health care settings worldwide (Iovleva and Doi 2017). NDM-1 was first identified in clinical *Escherichia coli* and *K. pneumoniae* isolates in India in 2008 (Yong et al. 2009), while in China, NDM-1 first appeared in clinical *Acinetobacter baumannii* isolates in 2011 (Chen et al. 2011) and in animal-derived *Acinetobacter lwoffii* isolates in 2012 (Wang et al. 2012).

NDM variants are increasingly becoming one of the main mechanisms of carbapenem resistance among *E. coli* isolates in both clinical and nonclinical settings in China. In humans, bla_{NDM} genes accounted for 48.5% [95% confidence interval (CI): 40.8, 56.3] of clinical carbapenem-resistant *E. coli* (CREC) isolates collected from hospitals in 25 Chinese provinces/municipalities from 2014 to 2015 (Zhang et al. 2017). In 2016, 2.4% (95% CI: 1.9, 2.9) of healthy people from 19 provinces in China harbored CREC as part of their physiological enteric microbiota, with 46.7% (95% CI:

*These authors contributed equally to this work.

Address correspondence to: Yang Wang, Beijing Advanced Innovation Center for Food Nutrition and Human Health, College of Veterinary Medicine, China Agricultural University, Beijing 100194, China. Email: Wangyang@cau.edu.cn; Qiang Sun, NHC Key Laboratory of Health Economics and Policy Research, School of Health Care Management, Shandong University, Jinan, Shandong 250100, China. Email: Qiangs@sdu.edu.cn

Supplemental Material is available online (https://doi.org/10.1289/EHP5251).

The authors declare they have no actual or potential competing financial interests.

Received 25 February 2019; Revised 25 September 2019; Accepted 1 October 2019; Published 23 October 2019; Corrected 3 January 2020.

Note to readers with disabilities: *EHP* strives to ensure that all journal content is accessible to all readers. However, some figures and Supplemental Material published in *EHP* articles may not conform to 508 standards due to the complexity of the information being presented. If you need assistance accessing journal content, please contact ehponline@niehs.nih.gov. Our staff will work with you to assess and meet your accessibility needs within 3 working days.

36.3, 57.4) of isolates testing positive for bla_{NDM} (Shen et al. 2018). Although carbapenems have never been approved for use in food-producing animals, sporadic cases of CREC from animal farms and surrounding environments, as well as the downstream meat production chain, have been reported (Wang et al. 2017; Zhang et al. 2015). Only four porcine NDM-type CREC (NDM-EC) isolates (three from Guangdong and one from Sichuan, China) were identified, and, in all cases, the genetic environment of bla_{NDM-1} was highly similar to that in human isolates from China and India (Kong et al. 2017; Zhang et al. 2015). Unlike the rare reports of NDM in pigs, NDM-EC were highly prevalent along the Chinese poultry production chain (commercial broiler farms, slaughterhouses, and supermarkets) and surrounding environments (flies and birds) (Wang et al. 2017). Moreover, NDM-EC isolates from poultry production showed the same genotypes as that from human infections in China and outside China (Wang et al. 2017). The similarity of CREC between animals and humans suggests that animals, although probably not the primary source of CRE in humans, still play an important role in NDM-EC transmission. However, established links between human- and animal-derived CRE isolates are still scarce (Köck et al. 2018).

Backyard farms that raise mainly poultry and swine on a small scale ($n < 50$) are a major source of food and income for villagers in low- and middle-income countries (Gao et al. 2015; Toro et al. 2018). For instance, approximately 43% of pigs worldwide are raised in small-scale farming operations (Robinson et al. 2011). Pig production by small backyard farms in 2010 accounted for one-third of the overall Chinese pig market (Gao et al. 2015). Notably, two or more types of animals are often raised in backyard farms, which are frequently associated with poor hygiene practices, such as frequent contact with other animals and humans (Correia-Gomes et al. 2017). Moreover, backyard farms with inadequate sanitation and poor management practices can add to environmental pollution with respect to MDR bacteria. Therefore, the spread of MDR bacteria among animals, humans, and the environment via direct contact with animal waste is more likely in backyard farms (Graham et al. 2017; Ngure et al. 2013). Herein, as part of the Sino-Swedish Integrated Multisectoral Partnership for Antibiotic Resistance Containment project (IMPACT) (Cars et al. 2016), we investigated the prevalence, risk factors, and drivers of CRE transmission between humans and animals in backyard farms and the surrounding environment in 746 households across 12 villages in rural areas of the Shandong Province, China. In addition, the two nearest commercial pig farms, located 20 km away from these villages, were also included in the study (Figure 1).

Methods

Village and Household Selection

This study was conducted in Shandong Province in July 2015, which has a population of 96 million people spread across 17 cities and 140 counties, approximately half of which are rural. The study design and the selection of villages and households have been described in detail previously (Sun et al. 2018). Briefly, a specific town with 73 surrounding villages was chosen by considering the number of households with backyard farms and the degree of cooperation with the local township health centers. Based on the information provided by the local county CDC, we observed a long history and a high intensity of backyard livestock farming in this county, particularly pig breeding. Therefore, we used the backyard pig farms as a preliminary selection criterion for the villages. From all villages with at least 100 households, we specifically selected 12 villages with the most backyard pig farms. Considering the feasibility of the study, 65 households from each village were chosen based on registers from the local CDC. Our target was to include 35 households with backyard pig farms from each village, but not all villages contained this number of suitable households. We therefore selected all households with backyard pig farms per village if the number was 35 or less, randomly selected 35 households with backyard pig farms per village if the number was more than 35, and then used a matching sampling method (Sun et al. 2018) based on the number of families in the remaining households to bring the total number to 65 per village (Figure S1).

Sample Collection

In each household, one fecal sample from an adult resident and two pig fecal samples, if available, were collected by using the ESwab™ collection kit (Copan) as described previously (Sun

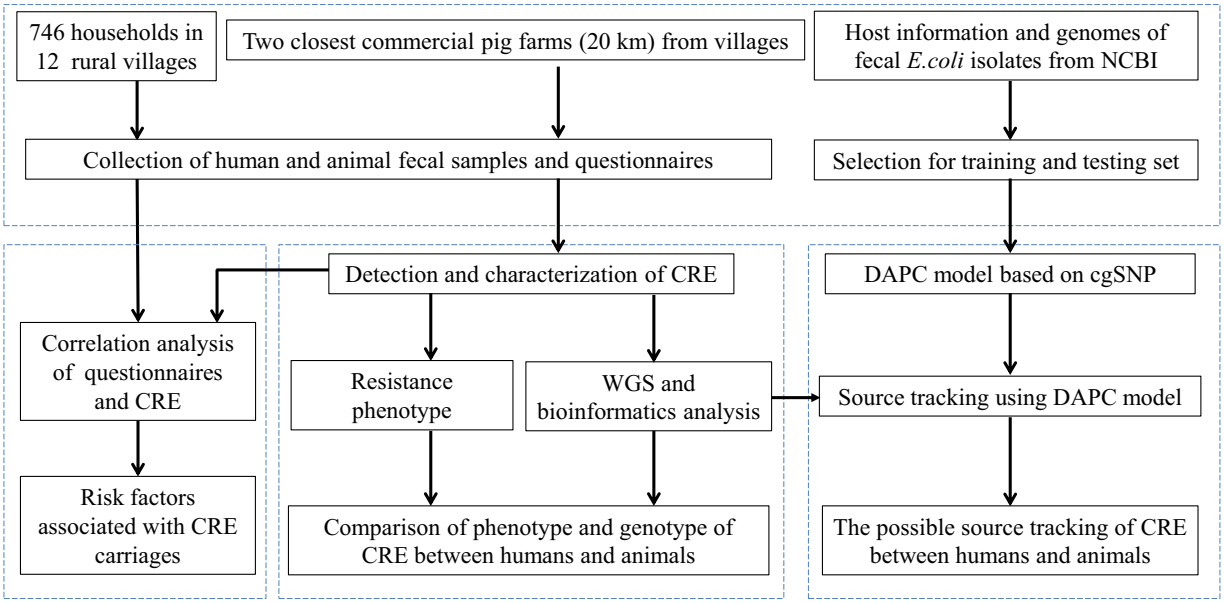

Figure 1. Schematic diagram of the research workflow. Note: cgSNP, core-genome single-nucleotide polymorphism; CRE, carbapenem-resistant Enterobacteriaceae; DAPC, discriminant analysis of principal components; WGS, whole-genome sequencing.

Table 1. Continuous variables evaluated as potential predictors of New Delhi metallo-β-lactamases–type carbapenem-resistant *Escherichia coli* (NDM-EC) carriage in households in rural China.

Continuous variables	Minimum	Maximum	Average	*p*-Value
Number of people living in the same household	2	9	4	0.94
Average age of the people living in the same household	22.3	98.5	49	0.26
Number of different animal species raised/kept in the same household	0	7	2	<0.001
Total number of backyard animals raised/kept in the same household	0	213	21	<0.001

et al. 2018). In addition, feces from other backyard animals, including chickens, cattle, goats, ducks, donkeys, dogs, and cats, were also sampled using the ESwabs™. Flies were captured from the backyard of 15 randomly selected households from each village by using the method described previously (Wang et al. 2017). We also selected the two nearest commercial pig farms with capacities of 3,000 (farm A) and 1,500 (farm B) pigs, respectively, located 20 km north of the villages. Nonduplicate fecal samples were randomly collected from 61 sows, 37 weaners and 35 growers in commercial pig farm A, and from 74 sows, 53 weaners and 29 growers in commercial farm B. In addition, fecal samples from 4 and 8 farm workers in the commercial farms A and B were respectively collected. None of these workers lived in any of the 12 villages included in the study. All samples from the villages and the commercial farms were kept in cool boxes with ice packs (4–8°C) upon collection and were transported to the local CDC laboratory for storage.

In total, we collected 180 flies and 1,599 fecal samples from 735 humans, 417 pigs, 305 chickens, 13 cattle, 10 goats, 7 ducks, 1 donkey, 92 dogs, and 19 cats across the 12 villages. In addition, 301 fecal samples from 289 pigs and 12 farm workers at the two commercial farms were investigated. Ethical approvals were given by the First Affiliated Hospital of Zhejiang University, China. All participants signed consent forms in Mandarin.

Bacterial Isolation and Identification

All samples were cultured on CHROMID® CARBA agar plates (bioMérieux) for 18 h at 37°C, and the red and green colonies were picked to identify presumptive CRE isolates according to the manufacturer's instructions. Species identification was carried out by matrix-assisted laser desorption ionization–time of flight mass spectrometry and 16S ribosomal RNA gene sequencing. All confirmed CRE isolates were screened for the presence of the carbapenemase genes bla_{VIM}, bla_{IMP}, bla_{KPC}, $bla_{\mathrm{OXA\text{-}48}}$, and bla_{NDM} by polymerase chain reaction (Poirel et al. 2011).

Questionnaire, Data Management, and Analysis

A cross-sectional household survey covering sociodemographics and the species and numbers of animals raised in the backyard farms, the hygiene habits (including washing hands before meals and after going to the toilet), and the source of daily used water in the household was set up as previously described (Sun et al. 2018). Participants providing fecal samples were invited to answer the questionnaire. Data from questionnaires were double-entered into Microsoft Access 2007 as previously described (Sun et al. 2018). Nine variables assumed to have an impact on the transmission of MDR bacteria between humans and animals were selected from the survey: *a*) the number and *b*) the average age of people living in the same household, the habit of washing hands *c*) before meals and *d*) after going to the toilet, *e*) the source of daily used water, *f*) the types of toilet, *g*) the number of different animal species and *h*) the total number of backyard animals raised/kept in the same household, and *i*) the use of human and/or animal feces as fertilizer, and were used for univariate statistical analysis to assess the potential association with CRE carriage in households. The four variables shown in Table 1 (i.e., the number and the average age of the household residents and the numbers and the species of backyard animals in the household) were used as continuous variables in the univariate analyses, while the other five variables (Table 2) were used as categorical variables. Variables with a significance level of $p \leq 0.20$ were entered into a multivariate logistic regression analysis using a backwards stepwise progress, of which variables with $p < 0.05$ were kept in the final model. All statistical analyses were carried out using SPSS Statistics (version 22; IBM Corporation).

Antimicrobial Susceptibility Testing

Susceptibility testing of CRE isolates was performed using the agar dilution method with twofold dilutions of imipenem, meropenem, tetracycline, tigecycline, cefotaxime, piperacillin-tazobactam, amoxicillin-clavulanate, gentamicin, ciprofloxacin, amikacin, nitrofurantoin, fosfomycin, trimethoprim-sulfamethoxazole, florfenicol, and colistin, the commonly used antimicrobial agents in the human clinic and/or animal husbandry. Results were inter-

Table 2. Categorical variables evaluated as potential predictors of New Delhi metallo-β-lactamases–type carbapenem-resistant *Escherichia coli* (NDM-EC) carriage in households in rural China.

Categorical variables	Percentage	NDM-EC carriage prevalence (95% CI)	Crude OR (95% CI)	*p*-Value
Source of daily-used water				
Well or river water	29.2%	13.3% (9.1, 18.5)	2.1 (1.3–3.6)	0.004
Tap water	70.8%	6.8% (4.8, 9.3)	1.0	
Type of toilet				
Dry	69.7%	9.6% (7.2, 12.5)	1.5 (0.8–2.7)	0.19
Flush	30.3%	6.6% (3.8, 10.7)	1.0	
Using human and/or animal faeces as fertilizer				
Yes	68.1%	11.6% (9.0, 14.7)	5.1 (2.2–11.9)	<0.001
No	31.9%	2.5% (0.9, 5.4)	1.0	
Washing hands before meal				
Always/often	95.3%	8.9% (6.9, 11.2)	1.6 (0.4–6.8)	0.74
Sometimes/never	4.7%	5.7% (0.7, 19.2)	1.0	
Washing hands after toilet				
Always/often	88.2%	8.7% (6.6, 11.1)	1.0 (0.4–2.1)	0.89
Sometimes/never	11.8%	9.1% (4.0, 17.1)	1.0	

Note: Odds ratios (ORs) for NDM-EC carriage were estimated using separate models for each predictor. CI, confidence interval.

preted according to the European Committee on Antimicrobial Susceptibility Testing (EUCAST) breakpoint tables for interpretation of minimum inhibitory concentrations (MICs) and zone diameters (version 8.0; http://www.eucast.org/fileadmin/src/media/PDFs/EUCAST_files/Breakpoint_tables/v_8.0_Breakpoint_Tables.pdf) and the Clinical and Laboratory Standards Institute (CLSI) documents VET08 (CLSI 2018a) and M100-S28 (CLSI 2018b). *E. coli* American Type Culture Collection (ATCC) 25922 served as the quality control strain.

Whole-Genome Sequencing and Molecular Analysis

DNA was extracted from all CRE isolates using a TIANamp Bacteria DNA Kit (Tiangen Biotech Co.) according to the manufacturer's instructions. DNA libraries were prepared using a KAPA HyperPrep Kit (Roche) and sequenced on a HiSeq X Ten platform (Illumina) with 150–base pair (bp) paired-end reads by Annoroad Genomics Co. The draft genomes were assembled using SPAdes (version 3.9.0; Center for Algorithmic Biotechnology CAB). Antibiotic resistance genes, virulence genes, and sequences suitable for multilocus sequence typing (MLST) were identified using the SRST2 toolkit (version 0.2.0; The University of Melbourne) (Inouye et al. 2014). A minimum spanning tree of all sequence types (STs) was generated by BioNumerics (version 7.0; Applied Maths) using the BURST algorithm. The phylogenetic groups (A, B1, B2, D, E, and F) of the *E. coli* isolates were determined as described previously (Beghain et al. 2018). All draft genomes were used for core-genome alignments to construct a phylogenetic tree using Parsnp in the Harvest package (version 1.1.2; National Biodefense Analysis and Countermeasures Center) (Treangen et al. 2014), and were visualized using the online tool Interactive Tree of Life (iTOL; http://itol.embl.de/) with the corresponding features of each isolate. The lineages of the phylogenetic tree were further defined using Bayesian analysis of the population structure (BAPS; version 6.0; Bayesian Statistics Group) (Cheng et al. 2013). All bla_{NDM}-carrying contigs retrieved from the draft genomes were annotated using the online automated PATRIC server (https://www.patricbrc.org). All whole-genome sequencing data in this study have been deposited in the GenBank and under BioProject accession no. PRJNA507915.

Source Predictions for Carbapenem-Resistant Enterobacteriaceae Isolates Using a Discriminant Analysis of Principal Components Model

Discriminant analysis of principal components (DAPC) is a multivariate method designed to identify and describe clusters of genetically related individuals and to assign individuals to groups using the adegenet package implemented in the free software R (R Development Core Team; version 3.4.1) (Jombart et al. 2010). To construct a detectable DAPC model, we downloaded 14,706 metadata files, including the source and sampling site information for *E. coli*, from the NCBI website (https://www.ncbi.nlm.nih.gov/pathogens). In total, 4,032 *E. coli* isolated from fecal samples of five groups (humans, pigs, chickens, cattle, and dogs) were screened (Figure S2). Twenty percent of the isolates were selected from groups containing >1,000 database entries, and 100 isolates were selected from other groups. In total, whole-genome sequencing data for 940 isolates from 440 humans, 200 cattle, 100 pigs, 100 chickens, and 100 dogs were downloaded from the NCBI databases. The core-genome single-nucleotide polymorphism (SNP) profiles of each of the 940 *E. coli* isolates were generated using Parsnp, and an SNP matrix based on these profiles was generated using HarvestTools (Treangen et al. 2014). The SNP matrix of the 940 strains from the five groups of hosts was then used to construct the DAPC model as previously described (Jombart et al. 2010). The remaining 228 whole-genome sequences from 28 isolates of dogs and 50 isolates each of humans, pigs, cattle, and chickens, respectively, were used as the testing set for the model (Table S1). Finally, the constructed model was used to predict the possible genetic sources of all CRE isolates from humans and backyard animals.

Results

Bacterial Isolation, Species Identification, and Detection of Carbapenemase Genes

Overall, 88 CREC isolates were identified from 1,779 samples collected in the 746 households across the12 villages, including 17 from humans (17 out of 735; 2.3%; 95% CI: 1.4, 3.7), 44 from pigs (44 out of 417; 10.6%; 95% CI: 7.9, 13.9), 12 from chickens (12 out of 305; 3.9%; 95% CI: 2.0, 6.8), 12 from flies (12 out of 180; 6.7%; 95% CI: 3.5, 11.4), 2 from dogs (2 out of 92; 2.2%; 95% CI: 0.3, 7.6), and 1 from cattle (1 out of 13; 7.7%; 95% CI: 0.2, 36.0) (Figure 2, Table S2), and each isolate was from individual samples. Excluding the 12 CREC isolates collected from flies, the other 76 isolates were collected from humans and/or backyard animals within 65 households. All samples from cats, goats, ducks, and the single sample from a donkey were negative for CRE (Table S2). Villages B and G displayed the highest prevalence of CREC (6.3 and 6.2%, respectively) among humans and had the highest CREC prevalence (27.3 and 25.9%, respectively) among backyard pigs as well. Almost half of the chicken-derived (5 out of 12) and dog-derived (1 out of 2) CREC isolates were recovered from samples collected in village B, which also had the highest prevalence of CREC (2 out of 9, 22.2%) among all fly-derived samples. None of the samples from residents from villages I or J contained CREC, and the pig samples from village I also had the lowest prevalence of CREC (1.7%) except for village K negative for CREC. (Figure 2). Similarly, no CREC isolates were recovered from workers ($n = 12$) or pigs ($n = 289$) of the two nearest commercial pig farms. All 88 CREC isolates contained bla_{NDM} carbapenemase genes and were negative for other tested carbapenemase genes.

Risk Factors Associated with NDM-EC Household Carriage in Villages

In total, we obtained 769 questionnaires from the 12 villages, of which 746 households also provided human and/or backyard animal fecal samples. The remaining 23 households without samples were excluded from this study (Figure S1). Humans and/or backyard animals in 65 households were positive for NDM-EC, while the remaining 681 households were negative. We analyzed the potential association of NDM-EC household carriage with sociodemographic indices and the production operations of the backyard farming. Four of the nine variables of interest (i.e., the number and the average age of people living in the same household and the habit of washing hands before meals and after going to the toilet) were excluded from further evaluation because they did not predict carriage (univariate $p < 0.20$) in the univariate analysis (Table 1 and Table 2). The five remaining variables met our criterion for inclusion in the initial multivariable model (univariate $p \leq 0.20$). However after backwards selection, only two variables were retained in the final model based on $p < 0.05$. These two variables were the number of different animal species raised/kept in the same backyard [odds ratio (OR) = 1.5; 95% CI: 1.2, 1.7 for each additional animal species] and the use of human or animal feces as fertilizer (OR = 2.7; 95% CI: 1.1, 6.6 for any use vs. no use) (Table 3). The Hosmer-Lemeshow goodness-of-fit test result of $\chi^2 = 9.975$ (8 degrees of freedom; $p = 0.267 > 0.05$), and a receiver operating

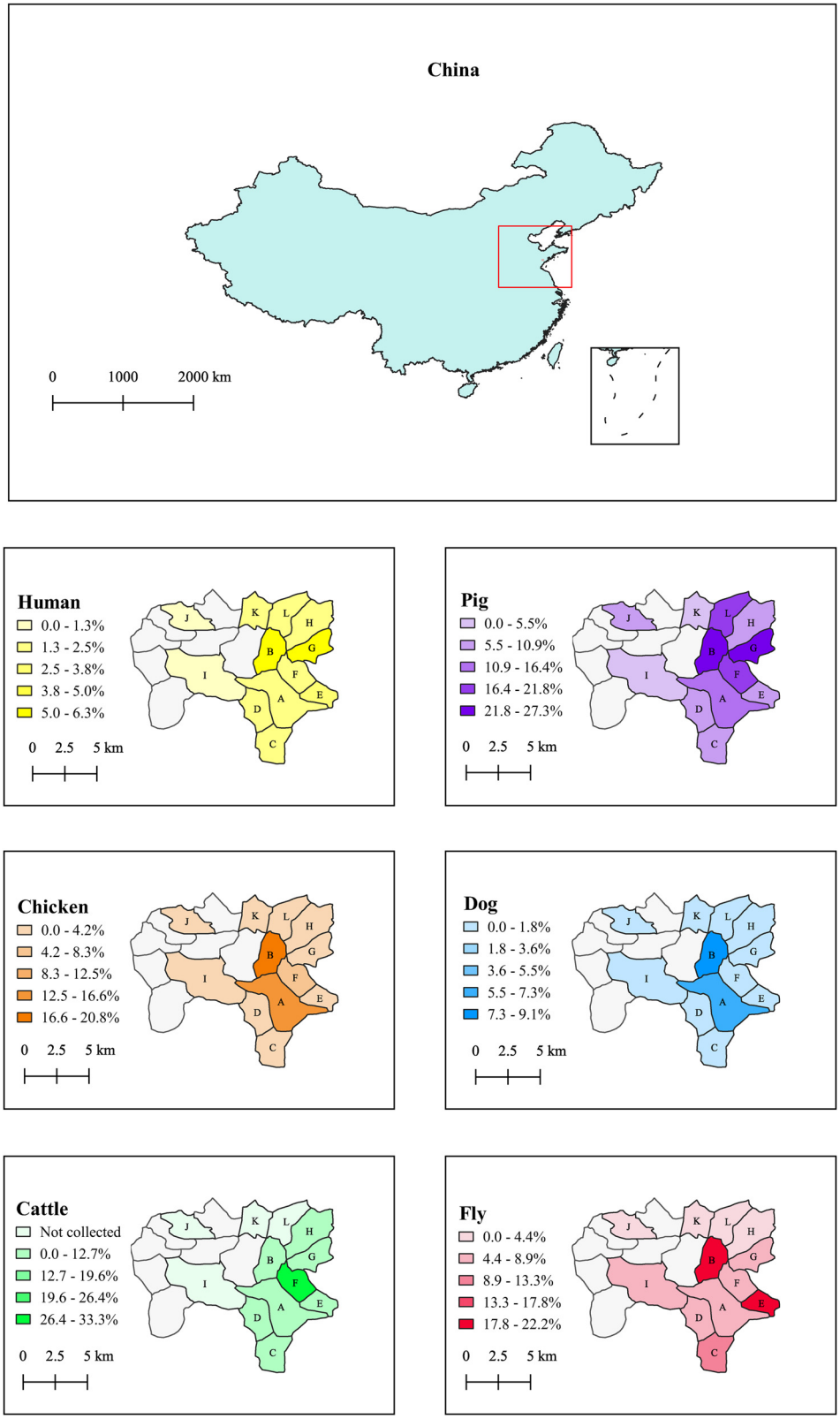

Figure 2. Map of the sampling locations and CREC prevalence among humans, pigs, chickens, dogs, cattle and flies in the 12 villages (from village A to L). The white areas represent the un-selected neighboring villages and the letters A-L indicate the 12 selected villages in this study. The color gradation stands for the prevalence of CREC in different villages in different sample types.

Table 3. Significant predictors ($p < 0.05$) in multivariable logistic regression model of New Delhi metallo-β-lactamases–type carbapenem-resistant *Escherichia coli* (NDM-EC) carriage in households in rural China.

Variables	OR (95% CI)	*p*-Value
Number of different animal species raised/kept in the same household	1.5 (1.2, 1.7)	0.001
Using human and/or animal faeces as fertilizer		
Yes	2.7 (1.1, 6.6)	
No	1.0	0.03

Note: Mutually adjusted model limited to significant predictors identified using backwards stepwise regression. CI, confidence interval.

characteristic curve value of 0.75 indicated that the model had good fit and predictive ability.

Antimicrobial Susceptibility Profiles of NDM-EC from Humans and Animals

All 88 NDM-EC isolates from humans and animals were resistant to cefotaxime, while >60% of these isolates exhibited resistance to amoxicillin-clavulanate, piperacillin-tazobactam, tetracycline, meropenem, florfenicol, and ciprofloxacin (Table 4). Resistance to meropenem, imipenem, and fosfomycin was significantly more prevalent ($p < 0.05$) among NDM-EC isolates from humans compared with isolates from animals, while isolates from animals showed higher rates of resistance ($p < 0.05$) to nitrofurantoin and amoxicillin-clavulanate than those from humans (Table 4, Figure S3). Of these 88 NDM-EC isolates, 81.8% ($n = 72$) and 62.5% ($n = 55$) were resistant to meropenem and imipenem, while the 6 and 19 isolates were considered intermediate (MIC = 2 mg/L) to meropenem and imipenem, and the remaining 10 and 14 isolates were classified as borderline susceptible (MIC = 0.5–1 mg/L) to meropenem and imipenem according to CLSI (Excel Table S1). All isolates were susceptible to tigecycline and showed relatively low percentages of resistance to colistin (23.5% in human isolates and 15.5% in animal isolates) (Table 4).

Genotypes of the NDM-EC Isolates

Most of the 88 NDM-EC isolates belonged to *E. coli* phylogroup A ($n = 79$), with only a few isolates belonging to groups B1 ($n = 5$), E ($n = 2$), and F ($n = 2$) (Figure 3). MLST analysis showed that 80 isolates were assigned to 36 known MLST types, while 8 isolates represented 6 novel STs. Overall, six (35.3%) human NDM-EC isolates displayed ST48 ($n = 3$), ST10 ($n = 1$), ST1114 ($n = 1$), or ST6910 ($n = 1$) shared by animal isolates. Of these, ST48 was the most prevalent ST among the NDM-EC isolates in this study (13 out of 88; 14.8%), and was associated with isolates from pigs ($n = 4$), humans ($n = 3$), chickens ($n = 3$), and flies ($n = 3$). ST206 ($n = 9$), ST744 ($n = 6$), and ST1178 ($n = 6$) were also associated with isolates from pigs, chickens, and flies (Figure 3; Figure S4). Core SNP–based phylogenetic analysis further supported the commonality of CREC isolates from humans and various animals with the same STs (Figure 3). The BAPS analysis revealed 7 lineages among the 88 NDM-EC isolates. Apart from lineage 6, each lineage comprised NDM-EC isolates from humans and their backyard animals (Figure 3). Furthermore, we found two human isolates (G018h and G028h) recovered from village G displayed only three SNPs with two pig isolates (G007p and G031p) from the same village.

Table 4. Rates of resistance based on minimum inhibitory concentrations of New Delhi metallo-β-lactamases–type carbapenem-resistant *Escherichia coli* (NDM-EC) isolates from humans and animals.

Antimicrobial agents	Human ($n = 17$)	Animal ($n = 71$)	*p*-Value
Imipenem	16 (94.1%)	39 (54.9%)	0.003
Meropenem	17 (100%)	55 (77.5%)	0.03
Colistin	4 (23.5%)	11 (15.5%)	0.43
Tetracycline	15 (88.2%)	69 (97.2%)	0.11
Tigecycline	0 (0%)	0 (0%)	—
Cefotaxime	17 (100%)	71 (100%)	—
Gentamicin	12 (70.6%)	36 (50.7%)	0.14
Ciprofloxacin	12 (70.6%)	48 (67.6%)	0.81
Amikacin	5 (29.4%)	8 (11.3%)	0.06
Nitrofurantoin	1 (5.9%)	25 (35.2%)	0.02
Fosfomycin	11 (64.7%)	27 (38.0%)	0.046
Florfenicol	14 (82.4%)	50 (70.4%)	0.32
Trimethoprim-sulfamethoxazole	14 (82.4%)	42 (59.2%)	0.07
Amoxicillin-clavulanate	16 (94.1%)	71 (100%)	0.04
Piperacillin-tazobactam	16 (94.1%)	70 (98.6%)	0.27

Note: Data are the number of resistant isolates (overall resistance rate as a percentage). The *p*-values (χ^2 test) are for comparisons of resistance rates between isolates from humans and animals (backyard animals and flies). —, no data.

Source Tracking for the NDM-EC Isolates

DAPC was used to investigate the genetic relationships among the 88 NDM-EC isolates from humans, backyard animals, and flies to identify the original host of each isolate. Overall, the first 407 principal components (99.2% of total variance) and four discriminant functions were retained to construct the training model. The five clusters of *E. coli* from five different hosts were well differentiated in the training model (Figure 4A). The prediction accuracy rate of the 228 selected *E. coli* for each host ranged from 53.6 to 92.0% (Figures 4B and 4C). Therefore, we concluded that the DAPC model, with its large and randomly selected data set, was suitable for analysis of our NDM-EC isolates. The 17 human NDM-EC isolates belonged to the human ($n = 4$; 23.5%), dog ($n = 5$; 29.4%), chicken ($n = 4$; 23.5%), pig ($n = 3$; 17.6%), and cattle ($n = 1$; 5.9%) clusters. The 44 pig NDM-EC isolates were assigned to the pig ($n = 11$; 25.0%), human ($n = 9$; 20.5%), cattle ($n = 12$; 27.3%), dog ($n = 6$; 13.6%), and chicken ($n = 6$; 13.6%) clusters (Figure 4D,E; Excel Table S2). The CREC isolated from flies, which interact with both animals and humans, were assigned to all five host clusters, with humans and dogs being the predominant origins of isolates. Surprisingly, the predominant origins of the chicken isolates were pigs and dogs, while the single cattle-derived isolate clustered with the chicken isolates.

Plasmid Profiles and Genetic Context of bla_{NDM} Genes

All 88 CREC isolates carried bla_{NDM} genes, of which 69 (78.4%) harbored bla_{NDM-5}, 12 (13.6%) carried bla_{NDM-1}, and 7 (8.0%) were positive for bla_{NDM-9}. The gene bla_{NDM-5} was widely distributed among pig ($n = 33$), human ($n = 12$), fly ($n = 11$), chicken ($n = 10$), dog ($n = 2$), and cattle ($n = 1$) isolates. Although only a small percentage of isolates carried bla_{NDM-1} or bla_{NDM-9}, these two genes were present in isolates from both household residents (three bla_{NDM-1} and two bla_{NDM-9}) and backyard animals (nine bla_{NDM-1} and five bla_{NDM-9}). The complete genome sequences of 75 of the isolates (66 bla_{NDM-5} and 9 bla_{NDM-1}) from backyard animals ($n = 50$), humans ($n = 14$), and flies ($n = 11$) contained regions showing >99% nucleotide sequence identity to the reference plasmid pNDM5_IncX3 (GenBank accession no. KU761328), suggesting that most of these bla_{NDM} genes are likely to be located on IncX3-type plasmids (Figure S5A). The lengths of the 88 bla_{NDM}-carrying contigs ranged from 3,421–48,161 bp, with the genomic backbones classified into types A ($n = 75$), B ($n = 8$), and C ($n = 5$) (Figure S6). The conserved region (bla_{NDM}-ble_{MBL}-*trpF*) was found in all three genetic types. The type A backbones contained the typical type IV secretion system, and the type B backbones carried resistance genes *mph*(E), *msr*(E), *armA*, *aph(3')-IV*, *aadA2*, *sul1*, and *dfrA12* in addition to bla_{NDM}, while the type C genomic backbone contained only the bla_{NDM} resistance gene.

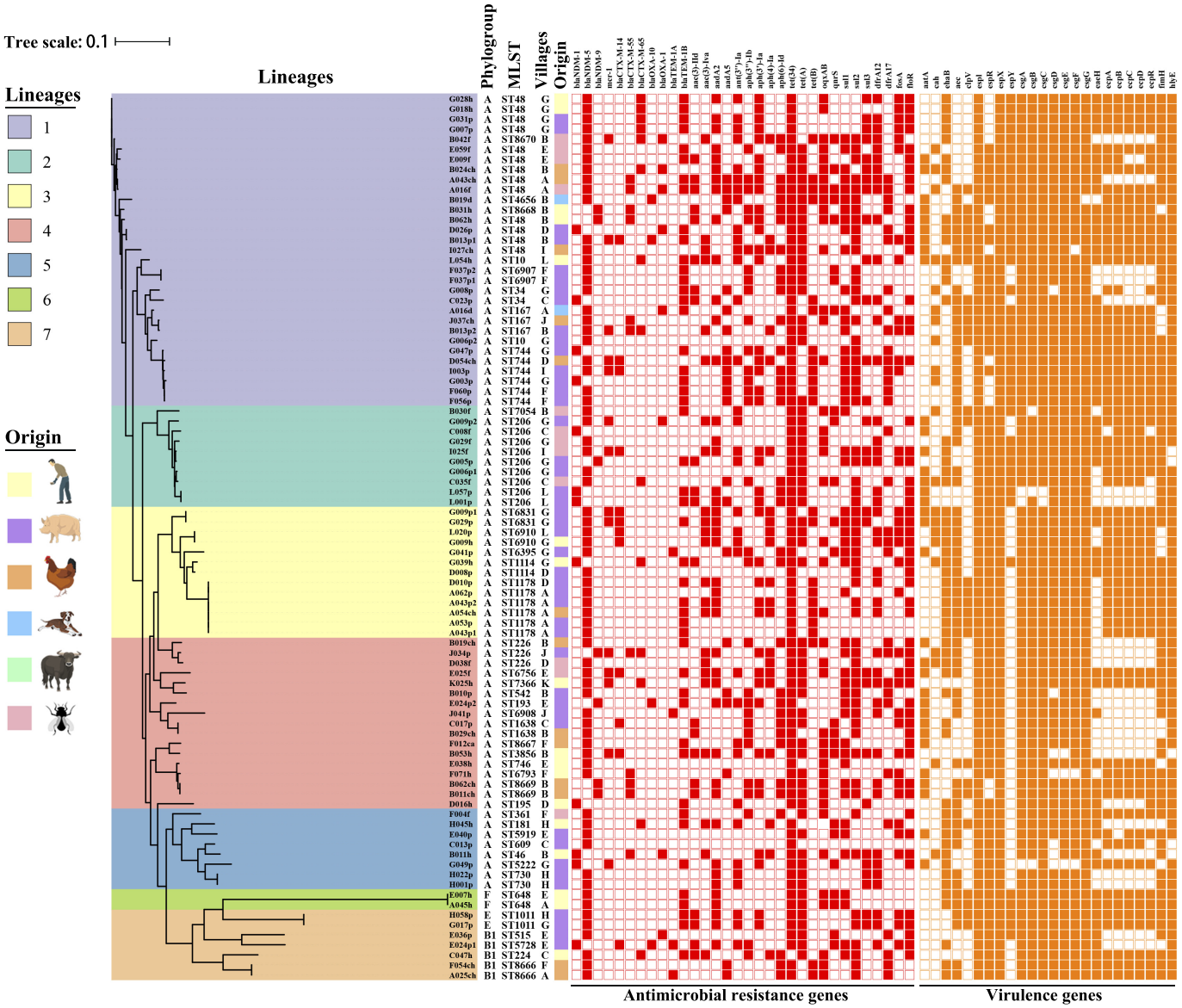

Figure 3. Distribution of *Escherichia coli* phylogroups, multilocus sequence typing (MLST), antimicrobial-resistance, and virulence-associated genes among New Delhi metallo-β-lactamases–type carbapenem-resistant *E. coli* (NDM-EC) isolates from humans and animals across the phylogenetic tree.

Distribution of Antimicrobial Resistance- and Virulence-Associated Genes among the NDM-EC Isolates

In addition to the bla_{NDM} genes, further β-lactamase genes ($bla_{\mathrm{CTX\text{-}M}}$, bla_{TEM}, and bla_{OXA}), plasmid-mediated quinolone resistance genes (*oqxAB* and *qnrS*), tetracycline resistance genes [(*tet*(34), *tet*(A), and *tet*(B)], aminoglycoside resistance genes [*aac(3)-IId*, *aac(3)-IVa*, *aadA2*, *aadA5*, *ant(3")-Ia*, *aph(3')-Ia*, *aph(3")-Ib*, *aph(4)-Ia* and *aph(6)-Id*], sulfonamide resistance genes (*sul1*, *sul2*, and *sul3*), trimethoprim resistance genes (*dfrA12* and *dfrA17*), a fosfomycin resistance gene (*fosA*), and a florfenicol resistance gene (*floR*) were commonly present in both human and animal isolates (Figure 3). Notably, 14 NDM-EC isolates from pigs ($n = 8$), flies ($n = 3$), humans ($n = 2$), and chicken ($n = 1$) were positive for *mcr-1*, the first-identified mobile colistin resistance gene (Liu et al. 2016). All of these 14 *mcr-1*-bla_{NDM}-carrying isolates belonged to 11 ST types, suggesting nonclonal dissemination was responsible for cotransmission of bla_{NDM} and *mcr-1* genes. In addition, both human and animal isolates exhibited no difference in virulence factor-encoding genes, including the genes coding for autotransporters (*aatA*, *cah*, and *ehaB*), secretion systems (*aec*, *clpV*, and *espL/R/X/Y*), curli fibers (*csgA/B/C/D/E/F/G*), common pili (*eaeH*, *ecpA/B/C/D/R*), type I fimbriae (*fimH*), and a hemolysin (*hlyE*) (Figure 3).

Discussion

Over the past decade, numerous studies have examined possible links between the occurrence of carbapenem-resistant microorganisms in animals and in humans. However, evidence of the direct transfer of CRE between animals and humans through either close contact or via the food chain is scarce (Grönthal et al. 2018). Hence, CRE of animal origin has not been considered a major threat to human health (Madec et al. 2017; Poirel et al. 2014). Here, for the first time, we have applied a One Health approach and provide strong evidence of direct transmission of NDM-EC among household family members, their backyard animals, and the immediate environment in backyard farm ecosystems from China. This evidence includes the following observations: *a*) the greater

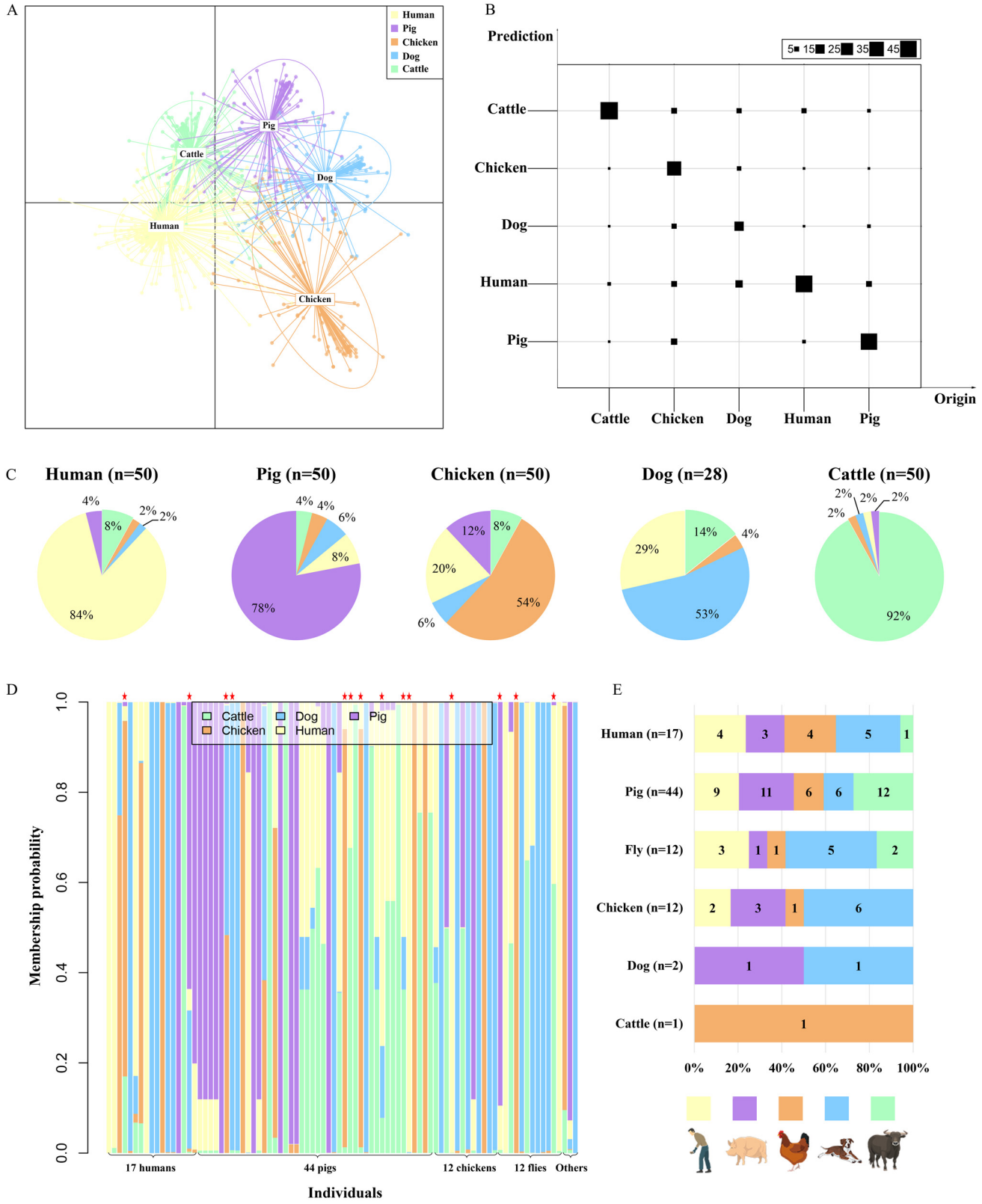

Figure 4. Source predictions for New Delhi metallo-β-lactamases–type carbapenem-resistant *Escherichia coli* (NDM-EC) isolates using the DAPC model. (A) Scatterplot represents individuals as dots, and groups as inertia ellipses. (B) Testing validation: rows correspond to actual trait, and columns correspond to inferred trait. (C) Successful assignment rates for prediction of testing group. (D) Membership probability of individuals. Others include one cattle and two dogs. Red pentagram stands for *mcr-1*-positive NDM-EC. (E) Probable source of NDM-EC.

the number of different species of animals in the backyard farm, the greater the likelihood of NDM-EC being detected from members (residents and animals) of the household. In addition, the application of manure originating from humans and/or animals as fertilizer significantly increases the risk of NDM-EC household colonization; *b*) the MLST types of >35% of the NDM-EC

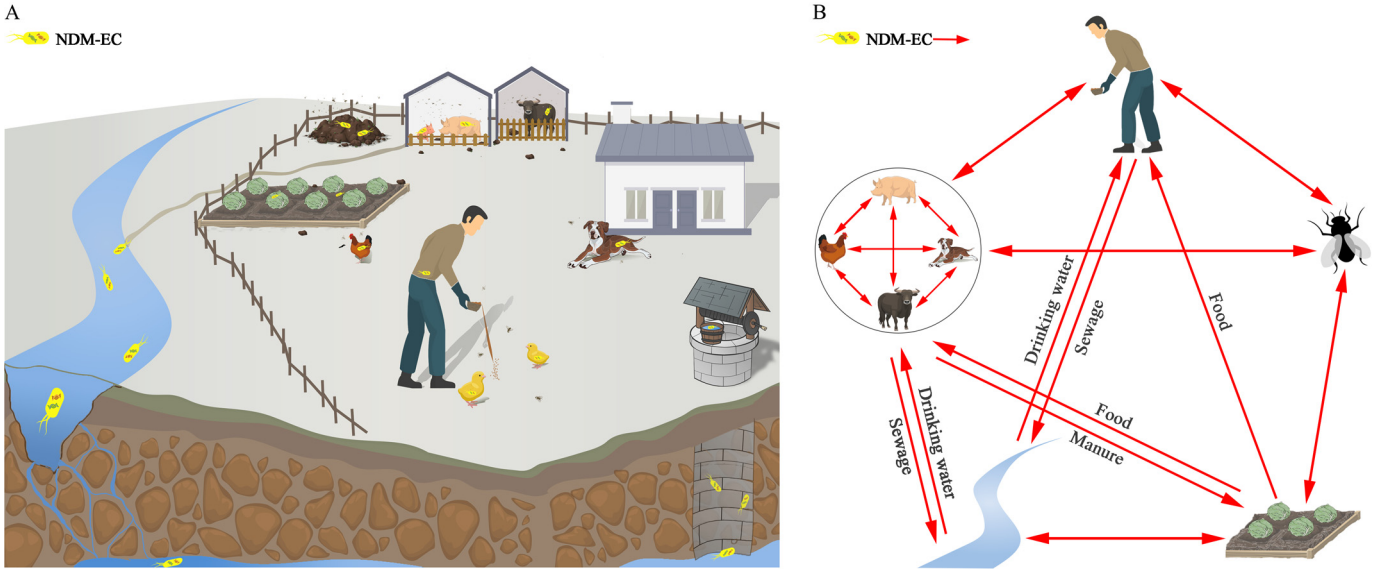

Figure 5. Diagram showing possible transmission routes for New Delhi metallo-β-lactamases–type carbapenem-resistant *Escherichia coli* (NDM-EC) among humans, animals, and environmental sources in the backyard farm. (A) The production mode in backyard farm in rural China. (B) Possible NDM-EC transmission routes.

isolates from humans were shared by isolates from backyard animals and flies; *c*) DAPC analysis showed that a large proportion of NDM-EC isolates from humans, backyard animals (pigs, chickens, cattle, and dogs), and flies originated from hosts other than those from which they were isolated in this study; *d*) some NDM-EC isolates from the household residents showed the same genotypes as those from pigs, chickens, cattle, dogs, or flies based on core-genome SNP-based phylogenetic analysis; and *e*) the bla_{NDM}-carrying regions/plasmids (IncX3) in NDM-EC isolates from humans exhibited >99% nucleotide sequence identity to those in isolates from backyard animals and flies. Therefore, these observations suggest that backyard farming is a previously underestimated risk for the transfer of MDR bacteria between humans and animals (Figure 5).

Unlike the observed NDM-EC transmission within the backyard farming system, all samples collected from pigs and farm workers at the two nearest commercial farms were negative for CRE. We propose four possible reasons for this discrepancy. First, in commercial pig farms, good hygiene practices were followed, which included that pigs were raised separately in the sows, weaners, and growers pens at different growth stages and the "all-in and all-out" strategy was obeyed (Sun and Wang 2015). In contrast, backyard farms often accommodate many different species of animals. Free-ranging animals such as chickens and dogs increase the likelihood of direct and frequent contact between humans and animals, enhancing the dissemination of NDM-EC in the backyard farms (Figure 5). Second, we observed that the disinfection practice was well implemented in the commercial farms, e.g., all the transfer vehicles were disinfected by wheel washes before entering and before leaving the farms. Moreover, a disinfecting footbath and protective clothing were provided for workers and visitors before entering the pigpen. Unlike commercial farm workers, household family members are less likely to have professional training in rearing food-producing animals, and they have less impetus to implement good management practices, resulting in less hygienic conditions. Combined with nonstandard disinfection procedures, decreased hygiene standards means that animals on backyard farms are likely to come into contact with MDR bacteria–carrying vectors, such as flies. This was confirmed by the identification of 12 NDM-EC isolates from flies in the backyard farm environment, with most of the isolates traced back to the human, dog, and cattle clusters (Figure 4D,E). Third, on commercial farms, the use of antimicrobial agents is restricted and carefully managed. On the contrary, the villagers in our study area did possess low levels of knowledge about antimicrobial agents, yet it is still common practice among backyard farmers to apply antimicrobial agents without prior consultation of a veterinarian (Dyar et al. 2018). The inappropriate and probable overuse of antimicrobial agents in backyard animals could have resulted in a high selection pressure, leading to the acquisition of corresponding resistance genes by bacterial strains. All NDM-EC isolates in the current study exhibited MDR profiles, and bla_{NDM} genes commonly coexisted with other resistance genes, including *qnr* and *oqxAB*. Once introduced into the animal sector, NDM-EC isolates were likely subject to further selection pressure from other antibiotics, such as fluoroquinolones (Madec et al. 2017). Fourth, the two commercial farms adopted anaerobic digestion processes to treat swine waste, during which the relative abundance of Enterobacteriaceae significantly decreased (Xia et al. 2019). Therefore, the bla_{NDM} gene may be largely reduced or eliminated following anaerobic digestion, as Enterobacteriaceae are the predominant family carrying this gene (Wu et al. 2019). However, unlike the pretreated manure in the commercial farms, backyard animal farmers may discharge untreated manure and sewage into the environment, due to the lack of awareness of environmental protection practices. As a result, antimicrobial residues, resistant bacteria, and resistance genes are released into rivers and introduced into soil through the use of manure for irrigation (Sun et al. 2017). We previously identified various fluoroquinolone residues in water, sediment, manure, and soil samples from this same area (Hanna et al. 2018), and confirmed the presence of CRE isolates in local well water (Sun et al. 2017). Additionally, we also recovered two $bla_{\mathrm{NDM-5}}$-positive isolates from the pig manure in villages A and C. These two strains displayed ST10 and ST1114; both ST types were shared between NDM-EC isolates from humans and animals (data not shown). All of these antimicrobial residues, resistant bacteria, and genes in environmental samples suggested that environmental sources may also be involved in MDR bacteria transmission between humans and animals.

Although we provide evidence of the transfer of CRE between animals and humans in the backyard farms, we acknowledge several limitations in this study. First, although we involved 65 households in each of these 12 villages, we assumed that there were no correlation among households within one village when analyzing the potential risk factors associated with the CRE carriage. Therefore, we treated all the households as independent and did not calculate the village-level variances. Second, only the two nearest typical large-scale pig farms were included to compare the presence of CRE in the commercial pig farms and in the backyard pig farms. However, these two farms are managed by one company, the production modes and hygienic practices of which are almost consistent with that of other commercial farms in Shandong Province and across China. Furthermore, all farm workers and representative pigs at different growing stage in these two commercial farms were sampled and analyzed for the presence of CRE. Third, we collected the samples in the commercial pig farms in the early spring, when the maximum temperature was about 15°C, and no flies were captured in these two commercial farms. Therefore, we could not analyze whether flies are associated with NDM-EC in commercial farms.

The medium-sized and industrial pig production facilities in China increased rapidly from 1990 onwards after the economic reform and free-trade agreements (Bai et al. 2014). However, backyard farming, which existed for thousands of years, still accounts for a third of the pig production in China, providing both food for the family and a household income in rural and underdeveloped areas (Bai et al. 2014). On a global level, backyard farming is recognized as one of the most common types of animal production, especially in low- and middle-income countries and areas. Backyard farming accounts for 40 to 96% of the pig production in some countries in Europe (Bulgaria and Romania), South Asia (Vietnam, Laos, Cambodia, Indonesia, and the Philippines), and South America (Chile) (Abao et al. 2014; Bravo-Vasquez et al. 2016; Leslie et al. 2015; Martínez-López et al. 2014). Although the scale of backyard farm systems continues to shrink, backyard farming is likely to persist for some time in China and other parts of the world. For instance, in our IMPACT project, the number of households raising animals in the backyard has slightly decreased from 537 (69.8%) out of the selected 769 households in the 12 villages in 2015 to 373 (59.3%) out of 629 households in 2017. Therefore, more efforts should be made to control the transmission of CRE in backyard farm ecosystems and to support the sustainable development of this type of farming. To achieve this, policies to improve backyard farming should be developed and implemented, followed by a better education of people in rural communities on professional farming practices, hygiene management, and infectious disease control.

Acknowledgments

This study is funded by the National Natural Science Foundation of China (grants 81361138021, 81861138051, and 81661138002), National Key Research and Development Program of China (2018YFD0500300), the Swedish Research Council (Grant D0879801), and the Medical Research Council (MR/P007295/1). The funders had no role in study design, data collection and interpretation, or the decision to submit the work for publication.

References

Abao LN, Kono H, Gunarathne A, Promentilla RR, Gaerlan MZ. 2014. Impact of foot-and-mouth disease on pork and chicken prices in Central Luzon, Philippines. Prev Vet Med 113(4):398–406, PMID: 24433637, https://doi.org/10.1016/j.prevetmed.2013.12.005.

Bai ZH, Ma L, Qin W, Chen Q, Oenema O, Zhang FS. 2014. Changes in pig production in China and their effects on nitrogen and phosphorus use and losses. Environ Sci Technol 48(21):12742–12749, PMID: 25292109, https://doi.org/10.1021/es502160v.

Beghain J, Bridier-Nahmias A, Le Nagard H, Denamur E, Clermont O. 2018. ClermonTyping: an easy-to-use and accurate in silico method for Escherichia genus strain phylotyping. Microb Genom 4(7):e000192, PMID: 29916797, https://doi.org/10.1099/mgen.1090.000192.

Bravo-Vasquez N, Di Pillo F, Lazo A, Jiménez-Bluhm P, Schultz-Cherry S, Hamilton-West C. 2016. Presence of influenza viruses in backyard poultry and swine in El Yali wetland, Chile. Prev Vet Med 134:211–215, PMID: 27726887, https://doi.org/10.1016/j.prevetmed.2016.10.004.

Cars O, Xiao Y, Stålsby Lundborg C, Nilsson LE, Shen J, Sun Q, et al. 2016. Building bridges to operationalise one health - a Sino-Swedish collaboration to tackle antibiotic resistance. One Health 2:139–143, PMID: 28616488, https://doi.org/10.1016/j.onehlt.2016.09.002.

CDC (Centers for Disease Control and Prevention). 2013. *Antibiotic Resistance Threats in the United States, 2013*. Atlanta, GA: CDC.

Chen Y, Zhou Z, Jiang Y, Yu Y. 2011. Emergence of NDM-1-producing *Acinetobacter baumannii* in China. J Antimicrob Chemother 66(6):1255–1259, PMID: 21398294, https://doi.org/10.1093/jac/dkr082.

Cheng L, Connor TR, Sirén J, Aanensen DM, Corander J. 2013. Hierarchical and spatially explicit clustering of DNA sequences with BAPS software. Mol Biol Evol 30(5):1224–1228, PMID: 23408797, https://doi.org/10.1093/molbev/mst028.

CLSI (Clinical and Laboratory Standards Institute). 2018a. *Performance Standards for Antimicrobial Disk and Dilution Susceptibility Tests for Bacteria Isolated from Animals*. 4th ed. Wayne, PA: CLSI.

CLSI. 2018b. *Performance Standards for Antimicrobial Susceptibility Testing: Twenty-Fifth Informational Supplement M100-S28*. Wayne, PA: CLSI.

Correia-Gomes C, Henry MK, Auty HK, Gunn GJ. 2017. Exploring the role of small-scale livestock keepers for national biosecurity-the pig case. Prev Vet Med 145:7–15, PMID: 28903877, https://doi.org/10.1016/j.prevetmed.2017.06.005.

Dyar OJ, Yin J, Ding L, Wikander K, Zhang T, Sun C, et al. 2018. Antibiotic use in people and pigs: a One Health survey of rural residents' knowledge, attitudes and practices in Shandong province, China. J Antimicrob Chemother 73(10):2893–2899, PMID: 30053125, https://doi.org/10.1093/jac/dky240.

Gao LL, Liu YM, Pan CL. 2015. Development of large scale pig farm in China and its impact. Swine Ind Sci 32:130–131.

Graham JP, Eisenberg JNS, Trueba G, Zhang L, Johnson TJ. 2017. Small-scale food animal production and antimicrobial resistance: mountain, molehill, or something in-between? Environ Health Perspect 125(10):104501, PMID: 29038091, https://doi.org/10.1289/EHP2116.

Grönthal T, Österblad M, Eklund M, Jalava J, Nykäsenoja S, Pekkanen K, et al. 2018. Sharing more than friendship-transmission of NDM-5 ST167 and CTX-M-9 ST69 *Escherichia coli* between dogs and humans in a family, Finland, 2015. Euro Surveill 23(27):1700497, PMID: 29991384, https://doi.org/10.2807/1560-7917.ES.2018.23.27.1700497.

Hanna N, Sun P, Sun Q, Li X, Yang X, Ji X, et al. 2018. Presence of antibiotic residues in various environmental compartments of Shandong province in eastern China: its potential for resistance development and ecological and human risk. Environ Int 114:131–142, PMID: 29501851, https://doi.org/10.1016/j.envint.2018.02.003.

Inouye M, Dashnow H, Raven LA, Schultz MB, Pope BJ, Tomita T, et al. 2014. SRST2: rapid genomic surveillance for public health and hospital microbiology labs. Genome Med 6(11):90, PMID: 25422674, https://doi.org/10.1186/s13073-014-0090-6.

Iovleva A, Doi Y. 2017. Carbapenem-resistant Enterobacteriaceae. Clin Lab Med 37(2):303–315, PMID: 28457352, https://doi.org/10.1016/j.cll.2017.01.005.

Johnson AP, Woodford N. 2013. Global spread of antibiotic resistance: the example of New Delhi metallo-beta-lactamase (NDM)-mediated carbapenem resistance. J Med Microbiol 62(Pt 4):499–513, PMID: 23329317, https://doi.org/10.1099/jmm.0.052555-0.

Jombart T, Devillard S, Balloux F. 2010. Discriminant analysis of principal components: a new method for the analysis of genetically structured populations. BMC Genet 11:94, PMID: 20950446, https://doi.org/10.1186/1471-2156-11-94.

Köck R, Daniels-Haardt I, Becker K, Mellmann A, Friedrich AW, Mevius D, et al. 2018. Carbapenem-resistant Enterobacteriaceae in wildlife, food-producing, and companion animals: a systematic review. Clin Microbiol Infect 24(12):1241–1250, PMID: 29654871, https://doi.org/10.1016/j.cmi.2018.04.004.

Kong LH, Lei CW, Ma SZ, Jiang W, Liu BH, Wang YX, et al. 2017. Various sequence types of *Escherichia coli* isolates coharboring bla_{NDM-5} and *mcr-1* genes from a commercial swine farm in China. Antimicrob. Agents Chemother 61(3): e02167–e02216, PMID: 27993847, https://doi.org/10.1128/AAC.02167-16.

Leslie EE, Geong M, Abdurrahman M, Ward MP, Toribio JA. 2015. A description of smallholder pig production systems in eastern Indonesia. Prev Vet Med 118(4):319–327, PMID: 25572130, https://doi.org/10.1016/j.prevetmed.2014.12.006.

Liu YY, Wang Y, Walsh TR, Yi LX, Zhang R, Spencer J, et al. 2016. Emergence of plasmid-mediated colistin resistance mechanism MCR-1 in animals and human beings in China: a microbiological and molecular biological study. Lancet Infect Dis 16(2):161–168, PMID: 26603172, https://doi.org/10.1016/S1473-3099(15)00424-7.

Madec JY, Haenni M, Nordmann P, Poirel L. 2017. Extended-spectrum beta-lactamase/AmpC- and carbapenemase-producing Enterobacteriaceae in animals: a threat for humans? Clin Microbiol Infect 23(11):826–833, PMID: 28143782, https://doi.org/10.1016/j.cmi.2017.01.013.

Martínez-López B, Alexandrov T, Mur L, Sánchez-Vizcaíno F, Sánchez-Vizcaíno JM. 2014. Evaluation of the spatial patterns and risk factors, including backyard pigs, for classical swine fever occurrence in Bulgaria using a Bayesian model. Geospat Health 8(2):489–501, PMID: 24893026, https://doi.org/10.4081/gh.2014.38.

Ngure FM, Humphrey JH, Mbuya MN, Majo F, Mutasa K, Govha M, et al. 2013. Formative research on hygiene behaviors and geophagy among infants and young children and implications of exposure to fecal bacteria. Am J Trop Med Hyg 89(4):709–716, PMID: 24002485, https://doi.org/10.4269/ajtmh.12-0568.

Nordmann P, Dortet L, Poirel L. 2012. Carbapenem resistance in Enterobacteriaceae: here is the storm! Trends Mol Med 18(5):263–272, PMID: 22480775, https://doi.org/10.1016/j.molmed.2012.03.003.

Poirel L, Stephan R, Perreten V, Nordmann P. 2014. The carbapenemase threat in the animal world: the wrong culprit. J Antimicrob Chemother 69(7):2007–2008, PMID: 24576948, https://doi.org/10.1093/jac/dku054.

Poirel L, Walsh TR, Cuvillier V, Nordmann P. 2011. Multiplex PCR for detection of acquired carbapenemase genes. Diagn Microbiol Infect Dis 70(1):119–123, PMID: 21398074, https://doi.org/10.1016/j.diagmicrobio.2010.12.002.

Robinson TP, Thornton P, Franceschini G, Kruska RL, Chiozza F, Notenbaert A, et al. 2011. *Global Livestock Production Systems*. Rome, Italy: Food and Agriculture Organization of the United Nations (FAO) and International Livestock Research Institute (ILRI).

Shen Z, Hu Y, Sun QL, Hu F, Zhou H, Shu L, et al. 2018. Emerging carriage of NDM-5 and MCR-1 in *Escherichia coli* from healthy people in multiple regions in China: a cross sectional observational study. EClinicalMedicine 6:11–20, PMID: 31193653, https://doi.org/10.1016/j.eclinm.2018.11.003.

Sun P, Bi Z, Nilsson M, Zheng B, Berglund B, Stalsby Lundborg C, et al. 2017. Occurrence of $bla_{\text{KPC-2}}$, $bla_{\text{CTX-M}}$, and *mcr-1* in Enterobacteriaceae from well water in rural China. Antimicrob Agents Chemother 61(4):e02569–e02616, PMID: 28115344, https://doi.org/10.1128/AAC.02569-16.

Sun H, Wang YM. 2015. The disinfection measures of large-scale farms. Chinese Abstracts of Animal Husbandry and Veterinary Medicine 31:63–64.

Sun Q, Wang Y, Hulth A, Xiao Y, Nilsson LE, Li X, et al. 2018. Study protocol for One Health data collections, analyses and intervention of the Sino-Swedish integrated multisectoral partnership for antibiotic resistance containment (IMPACT). BMJ Open 8(1):e017832, PMID: 29358424, https://doi.org/10.1136/bmjopen-2017-017832.

Toro M, Rivera D, Toledo V, Campos-Vargas R, Allard MW, Hamilton-West C, et al. 2018. Genomics of *Salmonella* contaminating backyard production systems reveals persistence and transmission of genetically related *Salmonella* on a farm basis. Zoonoses Public Health 65(8):1008–1014, PMID: 30264433, https://doi.org/10.1111/zph.12526.

Treangen TJ, Ondov BD, Koren S, Phillippy AM. 2014. The Harvest suite for rapid core-genome alignment and visualization of thousands of intraspecific microbial genomes. Genome Biol 15(11):524, PMID: 25410596, https://doi.org/10.1186/s13059-014-0524-x.

Wang Y, Wu C, Zhang Q, Qi J, Liu H, Wang Y, et al. 2012. Identification of New Delhi metallo-beta-lactamase 1 in *Acinetobacter lwoffii* of food animal origin. PLoS One 7(5):e37152, PMID: 22629360, https://doi.org/10.1371/journal.pone.0037152.

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, PMID: 28165472, https://doi.org/10.1038/nmicrobiol.2016.260.

WHO (World Health Organization). 2017. WHO publishes list of bacteria for which new antibiotics are urgently. http://www.who.int/news-room/detail/27-02-2017-who-publishes-list-of-bacteria-for-which-new-antibiotics-are-urgently-needed [accessed June 18 2018].

Wu W, Feng Y, Tang G, Qiao F, McNally A, Zong Z. 2019. NDM metallo-beta-lactamases and their bacterial producers in health care settings. Clin Microbiol Rev 32(2):e00115–e00118, PMID: 30700432, https://doi.org/10.1128/CMR.00115-18.

Xia X, Wang Z, Fu Y, Du XD, Gao B, Zhou Y, et al. 2019. Association of colistin residues and manure treatment with the abundance of *mcr-1* gene in swine feedlots. Environ Int 127:361–370, PMID: 30954722, https://doi.org/10.1016/j.envint.2019.03.061.

Yong D, Toleman MA, Giske CG, Cho HS, Sundman K, Lee K, et al. 2009. Characterization of a new metallo-beta-lactamase gene, $bla_{\text{NDM-1}}$, and a novel erythromycin esterase gene carried on a unique genetic structure in *Klebsiella pneumoniae* sequence type 14 from India. Antimicrob Agents Chemother 53(12):5046–5054, PMID: 19770275, https://doi.org/10.1128/AAC.00774-09.

Zhang R, Liu L, Zhou H, Chan EW, Li J, Fang Y, et al. 2017. Nationwide surveillance of clinical carbapenem-resistant Enterobacteriaceae (CRE) strains in China. EBioMedicine 19:98–106, PMID: 28479289, https://doi.org/10.1016/j.ebiom.2017.04.032.

Zhang R, Wang Y, Liu Z, Li J, Yin W, Lei L, et al. 2015. Characterization of NDM-1-producing carbapenemase in *Acinetobacter spp.* and *E. coli* isolates from diseased pigs. Front Agr Sci Eng 2(3):223–229, https://doi.org/10.15302/J-FASE-2015065.