Swine wastewater drives dissemination of high-risk *bla*$_{NDM}$-positive *Escherichia coli* clones

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ABSTRACT: Swine wastewater can accelerate the spread of antibiotic-resistant bacteria among animals, the environment, and humans. Therefore, the molecular epidemiological characteristics of carbapenemase-producing *E. coli* isolates from swine wastewater was investigated. In this study, a total of 100 no-cloning carbapenemase-producing ($bla_{\text{NDM-1}}$, $bla_{\text{NDM-5}}$ and $bla_{\text{OXA-48}}$-like) *E. coli* isolates were recovered from 316 swine wastewater samples collected from 29 swine farm in Shandong, China. Of which, two isolates that co-harbored $bla_{\text{OXA-48}}$-like and *mcr-1*, as well as five isolates that co-harbored $bla_{\text{NDM-5}}$ and *tet(X4)*. All isolates were multidrug-resistant (MDR), with a majority exhibiting resistance to meropenem, ciprofloxacin, imipenem and gentamicin. Whole genome sequencing (WGS) analysis indicated that these isolates were belonged to 12 distinct sequence types (STs), with the most prevalent STs being ST10 and ST5299. Additionally, Phylogenomic analysis revealed that clonal spread of bla_{NDM}-positive ST10 *E. coli* isolates at swine wastewater in multiple cities, including Binzhou, Dezhou, Heze and Weihai. Meanwhile, a notable divergence in SNPs was observed in isolates from this study and public database, which indicates that these bla_{NDM}-positive *E. coli* isolates have high genetic diversity. In addition, WGS analysis further revealed that bla_{NDM} co-existed with other antibiotic resistance genes, conferring resistance to twelve classes of antimicrobials. This study underscores the importance of surveillance for bla_{NDM}-harboring microbes in swine wastewater, and continuous monitoring in swine wastewater is essential for ensure human and environmental health.

IMPORTANCE: The study described the NDM-positive *E. coli* strains isolated from swine wastewater, where it was associated with antibiotic exposure. Notably, swine wastewater was increasingly considered to be related to CRE hosts. Pathogens originating from this source can be transmitted to humans via direct and indirect contact, as well as through the ingestion or inhalation of contaminated water or aerosols. In addition, under the selective pressure of antibiotics, the co-harbored of $bla_{\text{OXA-48}}$-like and *mcr-1*, $bla_{\text{NDM-5}}$ and *tet(X4)* genes within the same host bacterium signifies the emergence of an extremely MDR pathogen, posing a grave threat to public health. Consequently, the monitoring of bla_{NDM} in swine wastewater is critical for containing the transmission and persistence of carbapenem-resistant pathogens.

Key worlds: Swine wastewater, public health, *E. coli*, bla_{NDM}, MDR

48 The presence of carbapenem-resistant *Enterobacteriaceae* (CRE) in swine wastewater is
49 a cause of increasing concern due to its role in environmental contamination and potential
50 dissemination into human healthcare settings. These organisms often carry genes encoding
51 intra- and inter-cellular gene mobility, virulence, metal tolerance and antibiotics genes (1).
52 Such a gene pool can potentially interact with and modify the gene pool of environmental
53 bacteria, and vice versa (2), and microorganisms resulting from these interactions might pose
54 a health threat to livestock and poultry and, ultimately, back to humans. Accordingly, CRE in
55 swine wastewater need more attention.

56 Swine wastewater, as a major recipient of substantial amounts of antimicrobial residues,
57 wastewater is recognized as both a primary source and transmission pathway for resistance
58 determinants (3). In reality, antimicrobial agents used by humans are not fully degraded, and
59 their metabolites are excreted into the environment, generating adverse impacts on
60 wastewater contamination and public health. Research has found that the overuse of β-lactam
61 antibiotics may lead to the rapid evolution and emergence of multidrug-resistant (MDR),
62 extensively drug-resistant (XDR), and even pandrug-resistant (PDR) Gram-negative
63 pathogens (4,5). Infact，although carbapenems are prohibited to be used with food-producing
64 animals, over 4000 tons of β-lactams (mainly amoxicillin) and β-lactamase inhibitors are used
65 in animals, which accounts for 12.5% (rank 3rd) of all animal antibiotics used in China
66 mainland in 2020 (6). However, the overuse of β-lactam antibiotics has promoted the
67 enrichment of CRE in livestock and poultry (5). The most common mechanism of CRE is the
68 expression of β-lactam-hydrolysing enzymes e.g, such as New NDM and KPC, encoded by
69 bla_{NDM} and bla_{KPC}, respectively. Unlike bla_{KPC} mainly found in bacteria from human
70 infections; bla_{NDM} can be found across both humans and animal sectors, the same as the swine
71 wastewater environment (7-9). In addition, the recent discoveries of plasmid-mediated bla_{NDM}
72 or $bla_{\mathrm{OXA\text{-}48}}$-like genes co-harboring *mcr* and/or the *tet(X)* among *Enterobacteriaceae*, predict
73 a return to the pre-antibiotic era and pose a severe threat to public health, which requires
74 urgent monitoring in terms of its prevalence.

75 In this context, the present study aimed to conduct the surveillance of MDR *E. coli* from
76 swine wastewater in Shandong, China through the phenotypic and molecular evaluation of the
77 co-harboring $bla_{\mathrm{NDM\text{-}5}}$ and *tet(X4)*, $bla_{\mathrm{OXA\text{-}48}}$-like and *mcr-1* gene cluster genes involved to

78 assess the possible role of the swine wastewater as a reservoir and dissemination pathway of
79 such resistance mechanisms.

RESULTS

Prevalence of carbapenemase-producing *E. coli* isolates

In this study, a total of 100 bla_{NDM}-positive *E. coli* isolates were recovered from 316 wastewater samples from swine wastewater in Shandong, China. Of these, two isolates carried both bla_{NDM} and bla_{OXA-48}, and no other carbapenemase-encoding genes were detected among these carbapenem-resistant isolates. Among the 2 bla_{NDM} variants, bla_{NDM-5} was the dominant, constituting (96.6%), followed by bla_{NDM-1} (3.44%). The highest detection rate of bla_{NDM}-positive *E. coli* isolates was observed in Dongying (100%), followed by Heze (77.8%), Weifang (57.1%) and Dezhou (26.8%). In contrast, Taian (21.4%), Yantai (21.4%), Binzhou (20%), Weihai (16.7%), Jinan (10.7%) and Liaocheng (7.8%) displayed the lowest detection rates (Figure 1).

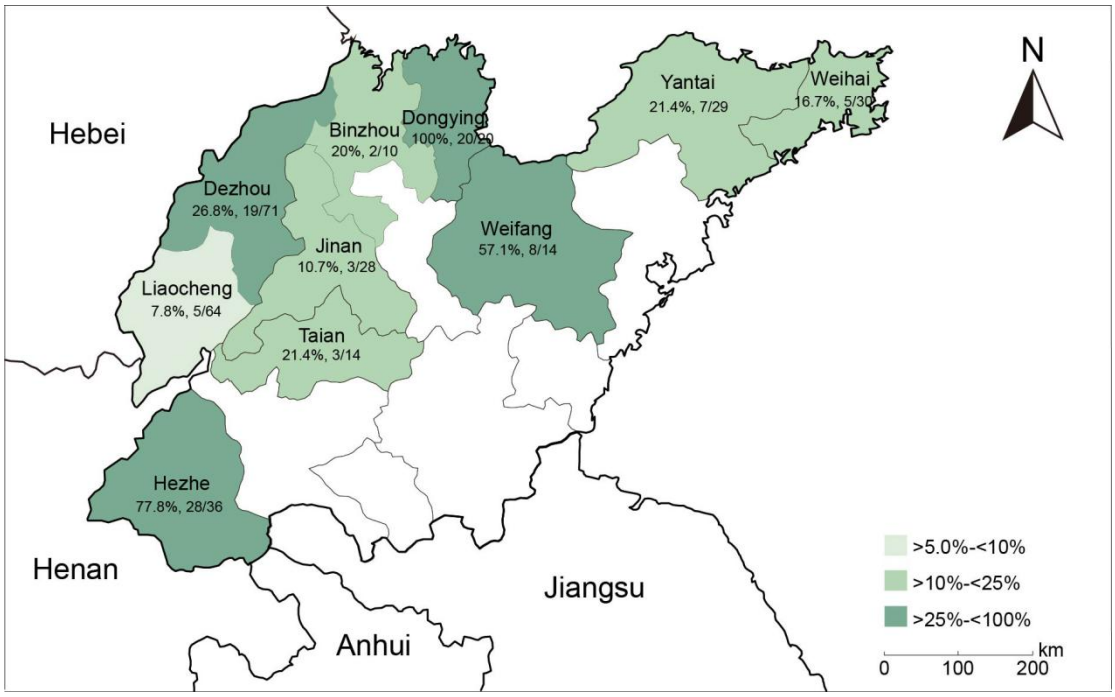
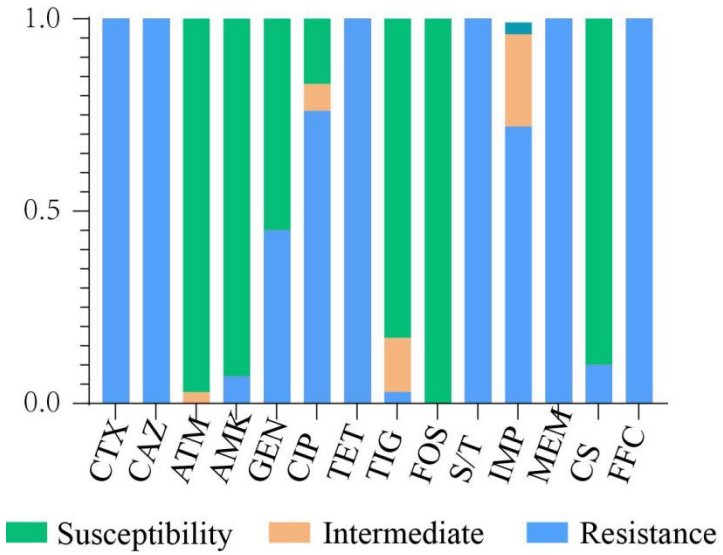


Figure 1. Geographical distribution of Carbapenemase-positive *E. coli* isolates in Shandong Province, China.

Antibiotic resistance phenotypes

We obtained all of 100 non-clonal carbapenemase-producing *E. coli* strains exhibited resistance to meropenem, cefotaxime, ceftazidime, tetracycline, and trimethoprim-

99 sulfamethoxazole (Figure 2). In addition, the majority of these isolates remained resistant to
100 meropenem (100%), ciprofloxacin (75.9%), imipenem (72.4%) and gentamicin (44.8%). In
101 contrast, lower prevalence of resistance phenotypes was observed for colistin (10.3%),
102 amikacin (6.9%), tigecycline (1%), of note, none of isolates showed resistance to amtronam
103 and fosfomycin.



104

105 Figure 2. Minimum inhibitory concentrations of tested antimicrobial agents for the
106 studied bacterial isolates.
107 Note: CTX, cefotaxime; CAZ, ceftazidime; ATM, aztreonam; AMK, amikacin; GEN,
108 gentamicin; CIP, ciprofloxacin; TET, tetracycline; TIG, tigecycline; FOS, fosfomycin; S/T,
109 sulfamethoxazole/trimethoprim; IMP, imipenem; MEM, meropenem; CS, colistin; FFC,
110 florfenicol.

111

112 **Phylogenetic analysis of NDM and OXA-48-like-positive *E. coli* isolates**

113 Twenty-nine non-clonal bla_{NDM}-positive *E. coli* isolates were picked for whole-genome
114 sequencing (WGS), and results revealed that these isolates were categorized into 12 distinct
115 STs, with 4 isolates classified as unclassified STs. Overall, ST10 (20.7%, 6/29) was the most
116 prevalent in Binzhou, Dezhou, Heze and Weihai, followed by ST5299 (17.2%, 5/29) in
117 Dongying and Dezhou. These findings suggest a distinct predilection for specific geographic
118 distributions (Figure 3). A phylogenetic tree was established using these carbapenemase-
119 producing *E. coli* isolates, and revealed that all the *E. coli* isolates were classified into 3

distinct lineages. In the lineages I mainly belonged to ST5409 and were sourced from Taian, with these isolates sharing only 0~2 core-genome SNP (cgSNP) among themselves. It is worth noting that all isolates in the lineages II mainly belonged to ST5229 and were sourced from Dongying and Dezhou. However, they exhibited significant SNP differences (1314 SNP), and the same as the strains from Dezhou carried $bla_{\text{OXA-48}}$-like gene sharing 36 cgSNP. In addition, isolates in lineages III mainly belonged to ST10 and originated from Weifang, Bingzhou, Dezhou and Heze, and 3 new strains that failed to be classified as ST sharing only one SNP from the strains in Tai'an and Weifang. Our phylogenomic analysis demonstrated that the majority of bla_{NDM}-positive *E. coli* isolates exhibited a significant degree of variation in core-genome sequences, suggesting a high genetic diversity of bla_{NDM}-positive *E. coli* isolates from swine wastewater in Shandong Province, China.

To further assess the relationship between the isolates from the current study and other resources in China. A total of 397 bla_{NDM}-positive *E. coli* isolates, obtained from 32 provincial-level administrative divisions across China, were randomly collected via the National Center for Biotechnology Information (NCBI) database (as April 2024) (Figure 4). Then, a maximum likelihood phylogenetic tree was constructed using these 426 bla_{NDM}-positive *E. coli* isolates, which were grouped into 6 clades, among which the 29 isolates from this study distributed across 5 clades. In addition, a notable divergence in SNPs was observed in bla_{NDM}-positive *E. coli* isolates between those analyzed in this study and those sourced from the NCBI database, which indicates that these bla_{NDM}-positive *E. coli* isolates have high genetic diversity.

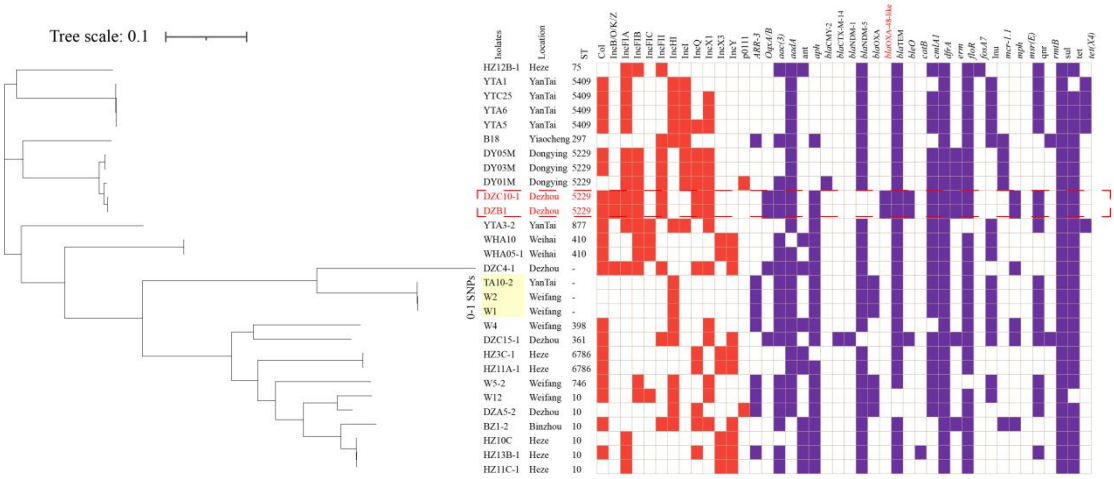


Figure 3. Phylogenetic analysis of Carbapenemase-producing *E. coli* isolates in this study. Bayesian evolutionary tree was constructed using core-genome SNPs. Each isolate is labeled with the city of isolation year and ST. The red-filled squares indicate the possession of the indicated ARGs.

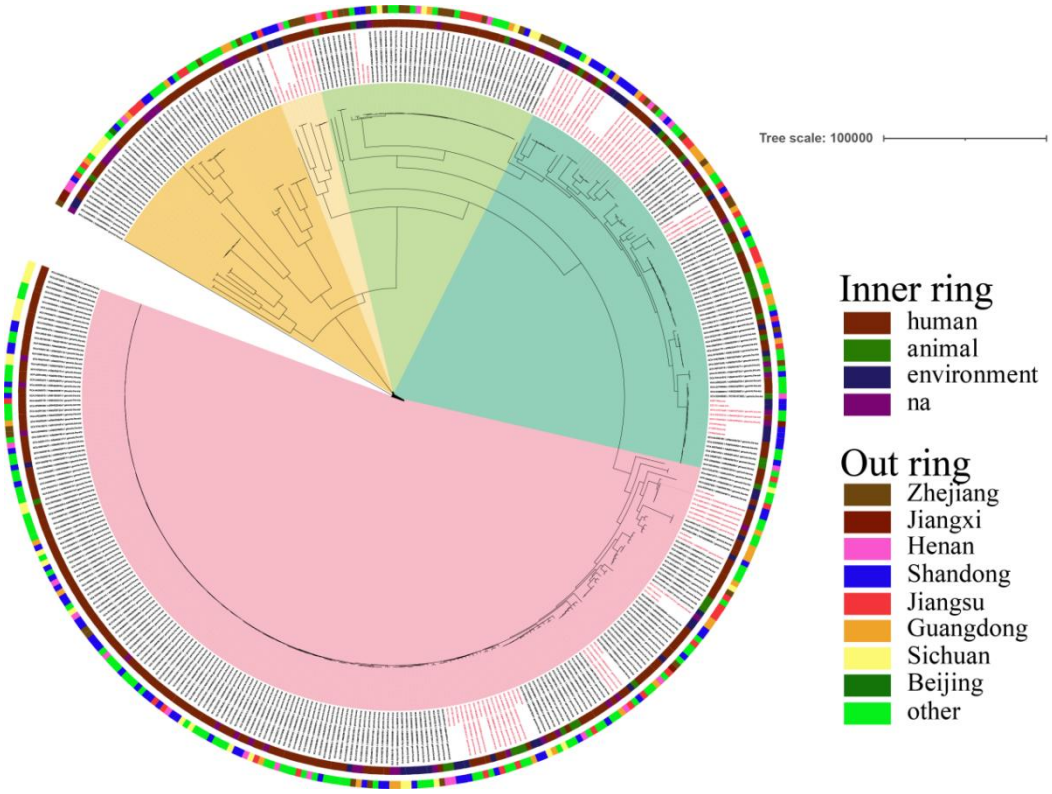


Figure 4. Phylogenetic structures of the$bla_{\text{NDM-}}$ and $bla_{\text{OXA-48}}$-like- positive *E. coli* isolates from this study and the GenBank database. Strain hosts and country of origin are indicated in the inner and outer rings, respectively.

153 **The genetic environments of *bla*$_{NDM}$ and *bla*$_{OXA-48}$-like**

154 A total of 5 genetic contexts (type I to type V) were found in 27 *bla*$_{NDM}$-positive isolates
155 (Figure 5). The type IV genetic contexts was novel in the GenBank database. Notably, *bla*$_{NDM-}$
156 $_{5}$ was found in 2 out of the 3 genetic context types. In all types, *bla*$_{NDM-5}$ was directly
157 associated with the *ble*$_{MBL}$-*trpF*-*tat* genes. In addition, we found that the type I genomic
158 context was the most prevalent *bla*$_{NDM}$ genetic environment in this study (63%, 17/27), which
159 is identical to a *bla*$_{NDM-5}$-carrying plasmid of carbapenem-resistant *Enterobacteriaceae*
160 isolates from humans in China (10). Meanwhile, the backbone structure of *bla*$_{NDM-5}$ including
161 the IS*Aba125*-IS*5*-*bla*$_{NDM-5}$-*ble*$_{MBL}$-*trpF*-*tat*-IS*6* was usually carrying the IncX3-type plasmid
162 and highly conserved (11). In contrast, *bla*$_{NDM-1}$ was linked to IS*5* and IS*3000* (Type IV and
163 V). In type IV, *bla*$_{NDM-1}$ is flanked by IS*3000* and IS*5* within the backbone region of IncY
164 plasmids. In type V, the backbone carrying *bla*$_{NDM-1}$ simultaneously harbors multiple virulence
165 genes (*virB1-11* and virD4). Type II and Type III share a core resistance gene cluster (*ant(3")-*
166 *Ia-qacE-sul-bla*$_{NDM-5}$). However, Type III acquires unique evolutionary traits due to the
167 insertion of the *yheS* gene (ABC transporter, ATP-binding protein) mediated by the Tn*3*
168 transposon. In addition, two OXA-48-like-positive bacterial strains identified in this study
169 share an identical genetic environment (*bla*$_{OXA-48}$-like-IS*Kpn19*).

170

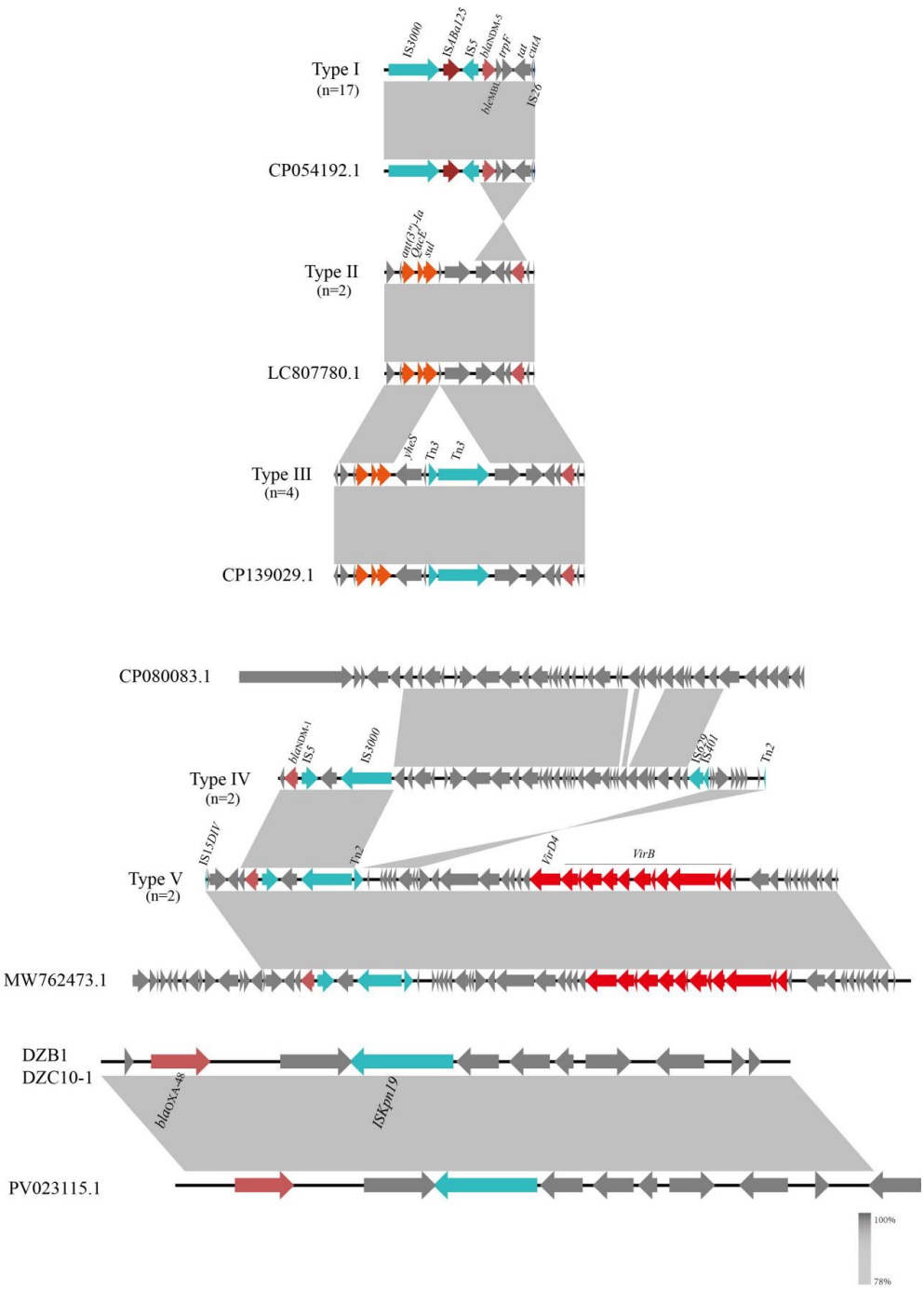


Figure 5. Genomic environments of *bla*$_{NDM}$ and *bla*$_{OAX-48}$-like of *E. coli* isolates. The figure was generated using Easyfig. Regions of homology are marked by shading and regions of ≥ 99.0 % nucleotide sequence identity are shaded grey. Arrows indicate the direction of transcription of the genes. In addition, red represents resistance gene, blue represents mobile genetic elements, black represents transfer gene tra clusters and yellow represents other coding sequence (CDS).

179 **Analysis of antibiotic resistance genes**

180 We conducted a comprehensive antimicrobial analysis on all of the *E. coli* isolates that
181 revealed the presence of the β-lactam resistance genes (bla_{NDM-1}, bla_{NDM-5}, bla_{OXA-10}, bla_{OXA-}
182 $_{48}$-like, bla_{CTX-M}, ble_{O}, bla_{CMY-2} and $bla_{TEM,}$). Other important resistance determinants that
183 conferring resistance to quinolones (*oqxA/B*, *qnr*), aminoglycosides (*rmtB*, *aadA, aph*, *ant* and
184 *aac*), fosfomycin (*fosA*), chloramphenicol/florfenicol (*floR* and *catB*), sulfonamides (*sul*),
185 macrolide (*erm*, *msr(E)* and *mph(A)*), rifampicin (*ARR-3*), phenicols (*cmlA*), lincomycin (*lnu*),
186 tetracycline (*tet*) and trimethoprim (*dfrA*). Additionally, we identified two isolates that co-
187 harbored bla_{OXA-48}-like and *mcr-1*, as well as five isolates that co-harbored bla_{NDM-5} and *tet(X4)*
188 (Figure 3).

189

190 **Plasmid analysis**

191 A total of 13 incompatible group plasmid replicon types were detected among the 29
192 carbapenemase-producing *E. coli* isolates including Col (69%, 20/29), IncB/O/K/Z (10%,
193 3/29), IncFIA (52%, 15/29), IncFIB (41%, 12/29), IncFIC (14%, 4/29), IncFII (34%, 10/29),
194 IncHI (52%, 15/29), IncI (31%, 9/29), IncQ (41%, 12/29), IncXI (52%, 15/29), IncX3 (24%,
195 7/29), IncY (34%, 10/29) and p0111 (7%, 2/29). It is worth noting that the cloning strains W1,
196 W2 and TA10-2 from Weifang and Yantai only carry the IncHI plasmid, suggesting that this
197 plasmid type represents a multidrug-resistant (MDR) plasmid harboring resistance
198 determinants including bla_{NDM}.

199 **DISCUSSION**

200 In the present study, we investigated the prevalence of carbapenemases-producing *E. coli*
201 isolates from swine wastewater in Shandong, China. Our results revealed a wide
202 contamination of bla_{NDM}- and bla_{OXA-48}-like-producing *Enterobacteriaceae* from the
203 environmental. Previous studies have also shown the detection of these species causing
204 infections in the livestock production environment (12,13). Researches show that gram-
205 negative bacteria, including *Enterobacteriaceae*, are able to survive on abiotic surfaces which
206 present a pathogenic transmitter from one surface to another, or even from the environmental
207 area to patients and to staff (14). and most likely exacerbate the transmission of bla_{NDM} and
208 bla_{OXA-48}-like.

209 More importantly, we detected *bla*$_{NDM}$-positive *E. coli* with relatively high prevalence
210 from environmental samples (31%, 98/316), and 2 OXA-48-like-producing *E. coli*. A high
211 prevalence of *bla*$_{NDM}$-positive and *bla*$_{OXA-48}$-like positive was reported for all food animals
212 with prevalence rates of chicken, cattle, and swine (3,15). Together with the current findings,
213 these data strongly indicated that the Chinese poultry farm environment is likely to be an
214 important reservoir for *bla*$_{NDM}$ and *bla*$_{OXA-48}$-like carrying bacteria. Importantly, carbapenems
215 are not used in food animal production. Consequently, we surmise that the high prevalence of
216 *bla*$_{NDM}$-carrying bacteria in the present study may have originated from the use of amoxicillin
217 on the farm, which probably provided a direct selection pressure for *bla*$_{NDM}$ maintenance.
218 This hypothesis has been supported by recent research evidence (5). In addition, the swine
219 wastewater was an open space suitable, and this made it difficult to perform complete
220 disinfection and sterilization of the farm environment. There was widespread contamination
221 by *bla*$_{NDM}$-positive *Enterobacteriaceae* in farm environments. In this study, *bla*$_{NDM-5}$ was the
222 predominant variant, consistent with previous reports identifying *E. coli* as the primary
223 *Enterobacteriaceae* species carrying *bla*$_{NDM-5}$ in both farm animals and their environments
224 (8,16).
225 The genetic background analysis shows In type VI, was found a fusion plasmid was
226 found that was recombined from an IncX3 plasmid carrying *bla*$_{NDM-5}$ and an IncF plasmid
227 carrying *ant(3")-Ia*, *qacE* and *sul*.This finding is consistent with our recent study, with the
228 difference being that the previous study sampled retail chicken markets (17). It's worth noting
229 that *bla*$_{NDM-1}$ is flanked by IS3000 and IS5 within the backbone region of IncY and IncX3
230 plasmids. A few reports of *bla*$_{NDM-1}$ transmission via IncY plasmids, and there was only one
231 report of *bla*$_{NDM-1}$ transmission via IncY plasmids (18).
232 WGS analysis revealed that *bla*$_{NDM}$ coexisted with other 29 other types of ARGs, 12 of
233 which ARGs were highly prevalent with detection rates >50%. This diversity of ARGs
234 confirms their role as potential sources of determinants of drug resistance (19). These ARGs
235 encoded resistance to aminoglycosides, β-Lactam and tetracycline and can further exacerbate
236 the spread of carbapenemases-resistant isolates in swine wastewater as well as among
237 surrounding animals, humans and environments in pig farm. To date, indeed, there are only
238 very limited findings on OXA-48 and OXA-48-like producing and *mcr-1* producing *E. coli* in

239 livestock (20,21). In this study, WGS analysis revealed that *bla*$_{OXA-48}$-like and *mcr-1* coexisted
240 with other 14 other types of ARGs. The widespread presence of carbapenem-resistant
241 *Enterobacteriaceae* (CRE) and *mcr*-positive *E. coli* poses a huge threat to both animal and
242 human health (22). In fact, for now, the *tet(X4)*-positive isolates were mostly reported in
243 animals such as pigs, chickens, cows and ducks, and *tet(X4)* has been previously identified in
244 pigs and chickens at slaughters, from soil and dust in animal farms, and even pork from
245 markets (23-25). However, despite the *tet(X4)* and *bla*$_{NDM-5}$ genes being reported as co-
246 harboured in *E coli* (26), it is unknown whether *tet(X4)* and *bla*$_{NDM-5}$ could co-exist in ST5409
247 and ST877. Here we report the first identification of a carbapenem- and tigecycline-resistant *E.*
248 *coli* isolate recovered from pig farm in China harbouring the *tet(X4)* and *bla*$_{NDM-5}$ genes on
249 different ST. This strain of *E. coli* carries a large number of plasmids. The *tet(X4)*-positive *E.*
250 *coli* carries different replicon types including Col, IncFIA, IncFIC, IncHI, IncI and IncX1, and
251 harbouring multiple antimicrobial resistance genes, including *tet(X4)* and *bla*$_{NDM-5}$ along with
252 *aadA*, *bla*$_{TEM-1B}$, *sul*, *floR* and *qnrS1 et, al.*.

253 **CONCLUSION**

254 As far as we know, this study identified the ST5299 *E. coli* strain co-harboring *bla*$_{OXA-48}$-
255 like and *mcr-1*, and the ST5409 and ST877 *E. coli* strain co-harboring *bla*$_{NDM-5}$ and *tet(X4)*
256 gene cluster for the first time. Regardless of their low prevalence rate in environment-
257 associated sources, the mobile plasmid-mediated resistance genes in such superbugs can pose
258 a significant threat to public health. Therefore, continuous monitoring of such MDR bacteria
259 in humans, animals, and the environment should be considered and to guide the deployment
260 of public health interventions before clinical cases increase.

261 **MATERIALS AND METHODS**

262 **Isolates collection**

263 A total of 316 samples of swine wastewater were collected from 29 swine farm in
264 Shandong, China. The samples were cultured by inoculation onto MacConkey plates
265 containing 2.0 mg/L meropenem. A red colony was selected from each plate for identification.
266 Cultures were identified using MALDI-TOF MS Axima^{TM} (Shimadzu-Biotech Corp., Kyoto,
267 Japan) and 16S rRNA sequencing. For the carbapenem-resistant isolates, five major
268 carbapenemase resistance genes (*bla*$_{KPC}$, *bla*$_{NDM}$, *bla*$_{IMP}$, *bla*$_{OXA-48}$-like and *bla*$_{VIM}$) were

269 detected for in carbapenem-resistant isolates by PCR using previously described primers (27).

270 **Antimicrobial susceptibility testing**

271 The MICs of 14 antibiotics (cefotaxime, ceftazidime, aztreonam, amikacin, gentamicin,
272 ciprofloxacin, tetracycline, tigecycline, fosfomycin, sulfamethoxazole/trimethoprim,
273 imipenem, meropenem, colistin, and florfenicol) for all recovery isolates were determined by
274 agar dilution and interpreted according to the CLSI guidelines (CLSI, 2024). The breakpoints
275 of colistin and tigecycline for *Enterobacteriaceae* were interpreted according to the EUCAST
276 criteria ("European Committee on Antimicrobial Susceptibility Testing. Breakpoint tables for
277 interpretation of MICs and zone diameters, 2024"). *E. coli* ATCC 25922 served as a quality
278 control strain for susceptibility testing.

279 **Conjugation assay testing**

280 To determine the transferability of the resistance genes, streptomycin resistant *E. coli* strain
281 C600 was used as the recipient and the conjugation assay was performed using a filter mating
282 method. Transconjugants were selected using MacConkey agar plates containing both 1.0 mg/L
283 meropenem and 1,500 mg/L streptomycin. The identification of the transconjugants used PCR
284 (28).

285 **WGS and phylogenetic analysis**

286 Clonal relatedness of all of the *Enterobacteriaceae* was analyzed by ERIC-PCR as
287 described previously (29). The genomic DNA of all Non-clonal bla_{NDM}-positive
288 *Enterobacteriaceae* was subjected to 250 bp paired-end WGS using the Illumina MiSeq system
289 (30), and the paired-end Illumina reads were assembled by SPAdes v3.6.2.18 MLST (31),
290 antibiotic resistance genes (ARGs), plasmid replicon types was analysed using the CGE server
291 (https:// cge.cbs.dtu.dk/services/).

292 The hosts and countries of 196 *E. coli* isolates were retrieved from NCBI
293 (https://www.ncbi.nlm.nih.gov/pathogens) and the assembly genomes of the 196 isolates
294 downloaded from the NCBI database (as of Sep. 2024). All assembly genomes were used for
295 core-genome alignments to produce a phylogenetic tree using the Parsnp software of the
296 Harvest suite (32). In this pipeline, bases that have likely undergone recombination are

removed using PhiPack and only columns passing a set of filters based on these criteria are considered reliable core-genome SNPs (33). The final set of core-genome SNPs was submitted to FastTree 2 for reconstructing a maximum likelihood phylogenetic tree using default parameters (34). The VCF file of all variants identified by Parsnp was then used to determine pair-wise single nucleotide variant distances between the core genomes of all strains. For the phylogenetic tree, a reference genome was randomly selected using the '-r!' switch. The lineages of the phylogenetic tree were defined using rhierbaps version 6.0 (35). The heat map was generated using R 3.3.2 (R Foundation for Statistical Computing) and was used to construct the tree that was visualized using FigTree v1.4.2 and iTOL v4 (36). Generation of plasmid maps were performed with BRIG.

DECLARATION OF COMPETING INTEREST

All authors approved the manuscript and gave their consent for submission and publication.

Ying Chu: Writing – original draft, Writing-review & editing. **Lan Yang:** Writing-original draft. **Yujie Miao:** Data curation, Software. **Liya Huang:** Validation, Visualization. **Rong Wang:** Methodology, Visualization. **Fengting Liao:** Visualization. **Xiang Luo:** Validation. **Shuancheng Bai:** Conceptualization, Formal analysis, Project administration, Supervision.**Yubao Li:** Conceptualization, Resources

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DATA AVAILABILITY

All WGS data have been deposited in the NCBI (Sequence Read Archive (SRA) submission: SUB15732648).

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