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Characterization of NDM-5-positive extensively resistant *Escherichia coli* isolates from dairy cows

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Highlights:

- Three NDM-5-positive *Escherichia coli* isolates were identified from mastitic cows.
- Co-existence of $bla_{\text{NDM-5}}$ and *mcr-1* was identified in one *E. coli* isolate.
- $bla_{\text{NDM-5}}$ gene is transferred via IncX3 pNDM-MGR194-like plasmid among *E. coli* isolates.
- Acquisition of $bla_{\text{NDM-5}}$ or *mcr-1*-bearing plasmid can incur host fitness cost.
- Host fitness cost did not cause loss of $bla_{\text{NDM-5}}$ or *mcr-1*-bearing plasmid.

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Abstract

The aim of this study was to investigate the prevalence of *bla*$_{NDM-5}$ gene in *Escherichia coli* isolates from dairy cows and to characterize the molecular traits of the *bla*$_{NDM-5}$-positive isolates. A total of 169 cows were sampled (169 feces and 169 raw milk samples) in three dairy farms in Jiangsu Province and 203 *E. coli* isolates were recovered. Among these strains, three isolates carried *bla*$_{NDM-5}$ gene, including one co-harboring *mcr-1*, which belonged to sequence type 446 and the other two belonged to ST2. Susceptibility testing revealed that the three *bla*$_{NDM-5}$-positive isolates showed extensively resistance to antimicrobials. The *bla*$_{NDM-5}$ gene was located on a ~46-kb IncX3 transferrable pNDM-MGR194-like plasmid in all three isolates, while *mcr-1* was located on a ~260-kb IncHI2 plasmid pXGE1*mcr*. Competition experiments revealed that acquisition of *bla*$_{NDM-5}$ or *mcr-1*-bearing plasmid can incur fitness cost of bacterial host, however, plasmid stability testing showed that both *bla*$_{NDM-5}$ and *mcr-1*-carrying plasmid maintained stable in the hosts after ten passages without antimicrobial selection. Whole genome sequencing revealed that the *mcr-1* gene coexisted with multiple resistance genes in pXGE1*mcr* and the backbone of this plasmid was similar to that of previously reported *mcr-1*-positive plasmid pHNSHP45-2. Moreover, pXGE1 could be conjugated into clinical NDM-5-positive *E. coli* isolates *in vitro*, thereby generating strains that approached pan-resistance. Active surveillance efforts are imperative to monitor the prevalence of *bla*$_{NDM-5}$ and *mcr-1* in carbapenem-resistant *Enterobacteriaceae* from dairy farms throughout China.

Keywords: *bla*$_{NDM-5}$, *mcr-1*, dairy cows, fitness cost, plasmid stability

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1. Introduction

NDM-5 belongs to NDM-type carbapenemase, which was a variant with increased carbapenemase activity in comparison with NDM-1 (Hornsey et al., 2011). *bla*$_{\text{NDM-5}}$ gene was first reported in an *Escherichia coli* strain (EC045) from a patient in the United Kingdom, and later has been identified in *E. coli* and *Klebsiella pneumoniae* from India, Algeria, Japan, Spain, the UK, USA, Dutch, Australia, Denmark, South Korea, Singapore and China (Bathoorn et al., 2015; Cho et al., 2015; Hammerum et al., 2015; Yousfi et al., 2015). The NDM-5-postive isolates reported were mainly recovered from clinical specimens from humans and there were sporadic cases of *bla*$_{\text{NDM-5}}$-carrying *E. coli* isolates from animals, including dog, cat and duck (Sun et al., 2016; Yang et al., 2016; Yousfi et al., 2015). The NDM-5-postive isolates showed multidrug resistance phenotype, which was caused by the carriage of multiple resistance genes in addition to *bla*$_{\text{NDM-5}}$. Moreover, *bla*$_{\text{NDM-5}}$ gene could coexist in the same isolate with the transferrable colistin resistance gene *mcr-1*, which represents the latest threat to public health, thus generating superbugs of extensively resistance or pan resistance (Du et al., 2016; Mediavilla et al., 2016). Recently, we investigated the typical dairy farms in China and found that there were ten *Klebsiella pneumoniae* isolates from cows carrying *bla*$_{\text{NDM-5}}$ gene and none of the *bla*$_{\text{NDM-5}}$-positive *K. pneumoniae* isolates carried *mcr-1*. The *bla*$_{\text{NDM-5}}$ gene was located on a ~46-kb IncX3 transferrable pNDM-MGR194-like plasmid in all *K. pneumoniae* isolates (He et al., 2017). However, the presence and characterization of *bla*$_{\text{NDM-5}}$ gene in *E. coli* from cows is unknown. Thus in this study, we aimed to investigate the prevalence of *bla*$_{\text{NDM-5}}$ gene in *E. coli* isolates from dairy cows, to characterize the molecular traits of *bla*$_{\text{NDM-5}}$-positive isolates, as well as the genetic contexts of the *bla*$_{\text{NDM-5}}$ and *mcr-1* gene.

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2. Materials and methods

2.1. Sample collection and bacterial strain identification

Samples in this study were collected from three dairy farms, located in the north, central and south, respectively, of Jiangsu Province in 2015. The three farms represent typical dairy production practices in each region and β-lactam agents were often used to treat bovine mastitis, such as penicillin, amoxicillin and ceftiofur. Raw milk and faecal samples were taken simultaneously from 169 individual cows (65 mastitic and 104 healthy), including 55, 60 and 54 cows from the three farms, respectively. A loop of fecal samples and 100 μl of raw milk were directly streaked on MacConkey agar (Luqiao, Beijing, China) and incubated at 37℃ for 16 h. The suspected clones with a red color were selected and boiled to extract the DNA, and then subjected to 16S rDNA sequencing, using previously described primers (Kim et al., 2010). The identified *E. coli* isolates were screened for the bla_{NDM} gene with the primers (NDM-up and NDM-dw) and the positive ones were sequenced (Zong and Zhang, 2013). The bla_{NDM}-positive *E. coli* isolates were further screened for the presence of *mcr-1* gene by PCR (Liu et al., 2016).

2.2. Antimicrobial susceptibility testing

For the bla_{NDM-5}-positive isolates and transconjugants/transformants, the minimum inhibitory concentrations (MICs) of β-lactams (ceftazidime, aztreonam and meropenem), gentamicin, florfenicol, tetracycline, tigecycline, ciprofloxacin, sulfamethoxazole/trimethoprim and colistin were determined using the broth microdilution method according to the recommendations of the CLSI document M100-S25 (CLSI, 2015). The *E. coli* isolate ATCC 25922 was used for quality control.

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2.3. Conjugation/transformation, S1-PFGE and hybridization

Conjugation by filter mating was performed between the *bla*$_{NDM-5}$-positive *E. coli* isolates and the azide-resistant *E. coli* J53, using a selection based on meropenem (2 μg/ml) and azide (100 μg/ml). For the donor isolate which contained the overlapping resistance profiles with the recipient *E. coli* strains (J53, azide^{R}; C600, streptomycin^{R} and rifampicin^{R}), electroporator was performed using *E. coli* DH5α as recipient, with meropenem (2 μg/ml) selection for the *bla*$_{NDM-5}$-containing transformants and colistin (2 μg/ml) selection for the *mcr-1*-containing transformants, respectively. Moreover, conjugation was conducted by using the two clinical *bla*$_{NDM-5}$-positive *E. coli* isolates in this study as receipt strains, with selection based on colistin (2 μg/ml) and ciprofloxacin (8μg/ml) for *mcr-1*-positive transcoujugants. Pulsed-field gel electrophoresis (PFGE) was used to determine the genetic relatedness of *mcr-1*-positive transcoujugants with the clinical receipt strains. S1 nuclease-PFGE and southern blotting were performed to determine the size of the *bla*$_{NDM-5}$-carrying plasmid and *mcr-1*-carrying plasmid in the donor strains and transconjugants, respectively.

2.4. Competition experiments to assess in vitro fitness

To assess the fitness impact of NDM or *mcr-1* carriage, pairwise competition assays were carried out using the *E. coli* transcoujugants/transformants carrying *bla*$_{NDM-5}$ or *mcr-1* gene competed with its plasmid-free counterparts. 24-hour competition experiments were performed as described previously (Lenski et al., 1994). Briefly, cultures were adjusted to a 1.0 McFarland standard, were diluted 1:10^{4} and

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then mixed at a volumetric ratio of 1:1 (time point zero). Colony counts were determined by plating serial dilutions of mixed culture on LB agar (LBA) with and without meropenem (2 μg/ml) or colistin (2 μg/ml) at 0 and 24 h. The number of colony forming unit (CFU) growing on antibiotic-supplemented LBA was subtracted from the number of CFU growing on antibiotic-free LBA to determine the number of suscepeible cells in the mixed population. All experiments were performed in triplicate and at least four replicates of each competition assay were performed. The relative fitness is calculated using the ratio of the growth rate (also defined as realized Malthusian parameters) of the resistant cells to that of the susceptible ones according to privious report (Gagneux et al., 2006). Mean values and standard deviations (SD) were calculated using Excel version 11.3.7 software. A relative fitness of 1 indicates that the transcoujugants/transformants undergo no fitness cost, whereas a ratio of greater than or less than 1 indicates increased or decreased fitness, respectively.

2.5. Plasmid stability testing

To estimate plasmid stability, a pure culture of *E. coli* transcoujugants/transformants carrying *bla*$_{\text{NDM-5}}$ or *mcr-1* was cultured in antibiotic-free LB broth. After 24h of growth, the cultures were diluted 1:10^4 in fresh LB medium and were further incubated in a duration of 10 passages (days). The ratio of colonies growing on antibiotic-supplemented LBA compared with antibiotic-free LBA was determined in triplicate for each passage. Newman-Keuls Multiple Comparison Test was used to evaluate differences between means, with a significant probability at a P value of ≤ 0.05. Presence of *bla*$_{\text{NDM-5}}$/*mcr-1* gene in the hosts after

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each passgage was verified by PCR, with colony grown on antibiotic-free/supplemented agar randomly selected (~20 colony per agar) as DNA template. For the transconjugants using clinical strains as receipts, both *bla*$_{\text{NDM-5}}$ and *mcr-1* genes were detected.

2.6. Molecular analysis of bla$_{NDM-5}$-positive isolates

The genetic relatedness of *bla*$_{\text{NDM-5}}$-positive isolates was investigated by multilocus sequence typing (MLST) using primers as described in the website (http://bigsdb.pasteur.fr/ecoli/primers_used.html), and also PFGE according to the PulseNet protocol for *E. coli* (http://www.cdc.gov/pulsenet/PDF/ecoli-shigella-salmonella-pfge-protocol-508c.pdf). To obtain comprehensive understanding of the genetic basis of the antibiotic resistance phenotypes in the three *bla*$_{\text{NDM-5}}$-positive *E. coli* isolates, genome DNA of these strains were subjected to whole genome sequencing (WGS) by constructing a shotgun library using Illumina Hiseq 2000, which produced 100 bp paired-end reads (Berry Genomics Company, Beijing, China). A draft assembly of the sequences was conducted using CLC Genomics Workbench 5 (CLC Bio, Aarhus, Denmark) and the resulting contigs were reassembled with those of PacBio contigs generated by HGAP3.0. All contigs were searched for potential antimicrobial resistance genes using Resfinder 2.1 (https://cge.cbs.dtu.dk/services/ResFinder/). Sequence analysis was conducted using the ORF Finder (http://www.ncbi.nlm.nih.gov/gorf/gorf.html) and BLAST functions (http://blast.ncbi.nlm.nih.gov/Blast.cgi). Alignment of similar *mcr-1*-positive plasmids was created by BRIG tools

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(http://sourceforge.net/projects/brig/). The complete nucleotide sequences of pXGE1*mcr* were submitted to GenBank with the accession number KY990887.

3. Results and discussion

3.1. Identification of bla$_{NDM-5}$-positive isolates

In this study, 203 *E. coli* isolates were recovered from 169 cows, including 169 isolates from feces and 34 isolates from mastitic milk. These isolates were screened for *bla*$_{NDM}$ gene and only three were positive, including one isolates (XG-E1) from farm 1, two isolates (TQ-E1, and TQ-E2) from farm 2. All the *E. coli* isolates were recovered from fecal samples from three mastitic cows and harbored *bla*$_{NDM-5}$ allele, which exhibited 100% nucleotide identity to that from *E. coli* strain EC405 (GenBank accession no. JN104597) (Hornsey et al., 2011). To be noted, ten *bla*$_{NDM-5}$-positive *K. pneumoniae* isolates were also identified from these samples, as described in our previous study (He et al., 2017). The three *E. coli* isolates were further screened for the presence of *mcr-1*, and only one isolate (XG-E1) contained the gene. This discovery was concerning, as polymyxin has never been approved for use in dairy cows for therapeutic or preventive purposes, although it is extensively used in swines and chickens in China. Coexistence of *bla*$_{NDM-5}$ and *mcr-1* has been reported in *E. coli* isolates from chicken, swines and a Muscovy Duck in China, and also one *E. coli* isolate from a patient in the United States (Kong et al., 2017; Mediavilla et al., 2016; Wang et al., 2017; Yang et al., 2016). As far as I know, this was the first time that the *E. coli* co-harboring *bla*$_{NDM-5}$ and *mcr-1* was identified from cows.

3.2. Antimicrobial resistance patterns of bla$_{NDM-5}$-positive isolates

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Susceptibility testing revealed that all three isolates were resistant to most of the antimicrobials tested, including β-lactams (ceftazidime, MICs > 256 μg/ml; aztreonam, MICs = 64 μg/ml; meropenem, MICs = 128 μg/ml), gentamycin (MICs > 256 μg/ml), florfenicol (MICs ≥ 128 μg/ml), tetracycline (MICs ≥ 128 μg/ml), and sulfamethoxazole/trimethoprim (MICs ≥ 152/8 μg/ml). *E. coli* XG-E1 was also resistant to colistin (MICs = 8 μg/ml), while TQ-E1 and TQ-E2 were resistant to ciprofloxacin (MICs = 64 μg/ml). However, all isolates remained susceptible to tigecycline (MICs < 0.5 μg/ml). Our data showed that all the $bla_{\text{NDM-5}}$-carrying *E. coli* isolates were identified as extensively resistant isolates (resistant to most classes of antimicrobial agents), which will further limit clinical therapeutic options for these cows. Drug usage record revealed that only β-lactam agents were used to treat bovine mastitis in these dairy farms and the extensively resistance phenotype of these $bla_{\text{NDM-5}}$-positive isolates may be due to the using of none β-lactams to treat the bovine infectious diseases other than bovine mastitis.

3.3. Conjugation/transformation and plasmid size estimation

The $bla_{\text{NDM-5}}$-carrying plasmid was successfully conjugated into azide-resistant *E. coli* strain J53 from donor isolates TQ-E1 and TQ-E2 with a conjugation rate of ~10^{-3} per receipt strain. For isolate XG-E1 which was resistant to azide and streptomycin /rifampicin, the $bla_{\text{NDM-5}}$-carrying plasmid and *mcr-1*-carrying plasmid in this strain were transformed into *E. coli* DH5α, respectively. Moreover, the *mcr-1*-containing plasmid from XG-E1 was successfully conjugated into ciprofloxacin-resistant clinical isolates of *E. coli* TQ-E1 and TQ-E2, at a conjugation rate of ~10^{-5} and ~10^{-6} per

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receipt strain, respectively. PFGE revealed that the transconjugants were clonal related with the clinical receipt strains (Data not shown). S1-PFGE and and southern blotting showed that the *bla*$_{\text{NDM-5}}$ was located on a ~46-kb plasmid in all three *E. coli* isolates, while *mcr-1* was located on a ~260-kb plasmid in XG-E1 and the corresponding transconjugants (Fig. 1). The *bla*$_{\text{NDM-5}}$-positive transconjugants/transformants only showed resistance to ceftazidime (MICs > 128 μg/ml) and meropenem (MICs ≥ 32 μg/ml), while the *mcr-1*-positive transconjugants showed a pan-drug resistance phenotype, with the exception of tigecycline (data not shown).

3.4. Fitness cost and plasmid stability

The transconjugants carrying *bla*$_{\text{NDM-5}}$-positive plasmid originated from *E. coli* TQ-E1 and TQ-E2 showed a relative fitness of 0.88±0.07 and 0.87±0.05 at 95% confidence intervals (CI), respectively, whereas the relative fitness for *bla*$_{\text{NDM-5}}$-positive and *mcr-1*-positive transformants originated from *E. coli* XG-E1 at 95% CI was 0.85±0.06 and 0.75±0.05, respectively (Fig. 2). The *mcr-1*-positive transconjugants using *E. coli* TQ-E1 and TQ-E2 as receipt showed a relative fitness of 0.78±0.06 and 0.72±0.08 at 95% CI, respectively (Fig. 2). These results have shown that the acquisition of *bla*$_{\text{NDM-5}}$ or *mcr-1*-bearing plasmid can place an energy burden on the bacterial host and incur fitness cost, which is in consistance with the previous report that carriage of pNDM-1 plasmid resulted in a loss of fitness for *E. coli* J53 receipt (Gottig et al., 2016). The difference in the fitness reduction between *bla*$_{\text{NDM-5}}$ and *mcr-1* may be due to the fact that the additional resistance genes carried on the

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mcr-1-positive plasmid might have led to a lot of energy demands on the host, whereas *bla*$_{NDM-5}$-positive plasmid carries no additional resistance genes as verified in the following plasmid content analysis. Quantification of *bla*$_{NDM-5}$ or *mcr-1*-carrying plasmid loss was performed by serial passages of transconjugants/transformants in antibiotic-free medium. The ratio of CFU growing on antibiotic-supplemented LBA to CFU on antibiotic-free LBA was insignificant different ($P>0.05$) after each passage (Table S1). PCR also verified that all the selected colony harbored the corresponding resistance gene, and for the *mcr-1*-carrying transconjugants, both *bla*$_{NDM-5}$ and *mcr-1* genes were identified after series passages. These results indicated that the plasmid harboring *bla*$_{NDM-5}$ or *mcr-1* gene maintains stable in the hosts and bacterial fitness cost could not cause plasmid loss. Previous reports also identified that *bla*$_{NDM-1}$-carrying plasmid was stabe in the receipt without antibiotics after four passages (Gottig et al., 2016), which may be attributed to the compensatory mechanisms employed by the bacterial hosts, and further raises doubts over the strategy that containing the spread of resistance would be to suspend the use of a particular antibiotic until resistant genotypes had declined to low frequency (Lenski, 1998).

3.5. Molecular analysis of bla$_{NDM-5}$-positive isolates

MLST revealed that XG-E1 from farm 1 belonged to sequence type (ST) 446 (PFGE pattern A), whereas TQ-E1 and TQ-E2 from farm 2 belonged to ST2 (PFGE patterns B and C, respectively). *bla*$_{NDM-1}$ has recently been identified in ST2 *E. coli* isolates from humans in China (Du et al., 2017), but neither ST2 nor ST446 *E. coli*

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strains have previously been shown to harbor *bla*$_{NDM-5}$, indicating independent acquisition of NDM genes by *E. coli* from dairy farms. Whole genome sequencing was used to analyze the genetic context of *bla*$_{NDM-5}$ and *mcr-1*, and to examine the coexistence of resistance genes in the original isolates. All isolates harbored the ~46-kb *bla*$_{NDM-5}$-positive plasmid (46161bp, 46253bp and 46253bp, respectively), which is almost identical (>99% coverage and >99% nucleotide identity) to the IncX3 plasmid pNDM-MGR194 (GenBank accession no. KF220657) from a human *K. pneumoniae* isolate reported in India (Krishnaraju et al., 2015), and also to the *bla*$_{NDM-5}$-carrying plasmid found in the *K. pneumoniae* strains previously isolated from the samples used in the current study (He et al., 2017). Among these reports, the IncX3 pNDM-MGR194-like plasmids were self-transmissible without other resistance determinants, which is in agreement with the resistance phenotype of the *bla*$_{NDM-5}$-positive transconjugants/transformants. Moreover, findings in our study strongly indicate plasmid-mediated horizontal transfer of the *bla*$_{NDM-5}$ gene between *E. coli* and *K. pneumoniae* strains from the same dairy farm (farms 1 and 2).

The *mcr-1*-carrying plasmid designated pXGE1*mcr* is 254048 bp in length and belongs to the IncHI2 group. As shown in Fig. 3, the backbone of pXGE1*mcr* is almost identical (95% coverage and 99% nucleotide identity) to that of plasmid pHNSHP45-2 (GenBank accession no. KU341381) in the strain SHP45 from which *mcr-1* was first discovered (Liu et al., 2016). In both plasmids, IS*Apl1* is in the upstream of *mcr-1*. However, IS*Apl1-mcr-1* segment is in a different location in pXGE1*mcr*, which showed 99% nucleotide identity with the corresponding region

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(10,214–104,078 bp) of the recently reported *E. coli* plasmid pMR0516*mcr* from a patient in the USA (GenBank accession no. KX276657) (McGann et al., 2016). pXGE1*mcr* also carried additional resistance genes (Table 1), including *bla*$_{\text{CTX-M-14}}$ (β-lactam resistance), *aph(3')-Ia*, *aac(3)-IVa*, *aadA1* and *aadA2* (aminoglycoside resistance), *mph(A)* (macrolide resistance), *floR* and *cmlA1* (phenicol resistance), *fosA3* (fosfomycin resistance), *dfrA12* (trimethoprim resistance), and *sul1*, *sul2* and *sul3* (sulfanilamide resistance). In addition, donor strain XG-E1 also harbored *bla*$_{\text{TEM-1}}$, *bla*$_{\text{OXA-10}}$, *aph(4)-Ia*, *arr-2*, *tet*(A), *tet*(M) and *dfrA14*, which mediate resistance to β-lactams, aminoglycosides, rifampicin, tetracycline and trimethoprim, respectively.

E. coli isolates TQ-E1 and TQ-E2 also carried multiple resistance genes, making them extensively resistant to a range of antibiotics (Table 1). Of note, TQ-E1 also harbored carbapenemase gene *bla*$_{\text{VIM-2}}$ in addition to the plasmid-located *bla*$_{\text{NDM-5}}$ gene. The *bla*$_{\text{VIM-2}}$-flanking region shared 6,861-bp homologous sequence (91% identity) with a resistance plasmid from *Pseudomonas putida* strain PPV2-2, in which *bla*$_{\text{VIM-2}}$ and *aacA4* were located within a class 1 integron construct (GenBank accession no. GQ227991) (Juan et al., 2010). The co-existence of *bla*$_{\text{VIM-2}}$ and *bla*$_{\text{NDM-1}}$ in clinical *P. aeruginosa* isolates from India has been reported (Paul et al., 2016); however, this is the first time that these two carbapenemase genes have been found to coexist in *E. coli* from dairy cows.

4. Conclusions

In summary, we isolated and identified three NDM-5-producing *E. coli* isolates

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from dairy cows, including one co-producing MCR-1 and another co-harboring VIM-2. The *bla*$_{\text{NDM-5}}$-carrying and *mcr-1*-harboring plasmids reduced fitness of bacterial hosts but maintained stable in the receipt strain. The *mcr-1*-carrying plasmid could be conjugated into the NDM-5-positive *E. coli* isolates *in vitro*, thereby generating strains that approached pan-resistance. Active surveillance efforts are imperative to monitor the prevalence of *bla*$_{NDM-5}$ *and mcr-1* genes in carbapenem-resistant *Enterobacteriaceae* from dairy farms throughout China.

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Conflicts of interest

None.

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Figure Legends

Fig. 1. Identification of *bla*$_{NDM-5}$ and *mcr-1* in three *Escherichia coli* isolates and their transformants/transconjugants. (a) Plasmid size determination by S1 nuclease pulsed-field gel electrophoresis. (b) and (c) Southern blotting hybridization with the *bla*$_{NDM-5}$-specific and *mcr-1*-specific probes, respectively. Lane M: *Salmonella enterica* serotype *Braenderup* strain H9812 marker. Lanes 1, 3, and 5: *E. coli* isolates XG-E1, TQ-E1, and TQ-E2, respectively. Lane 2: *mcr-1*-carrying transformants from *E. coli* XG-E1. Lane 4: *bla*$_{NDM-5}$- and *mcr-1*-positive transconjugants (donor: *E. coli* XG-E1; recipient: TQ-E1). Lane 6: *bla*$_{NDM-5}$- and *mcr-1*-positive transconjugants (donor: *E. coli* XG-E1; recipient: TQ-E2).

Fig. 2. Relative fitness of transformants/transconjugants carrying *bla*$_{NDM-5}$ or *mcr-1*. A relative fitness of 1 indicates that the transcoujugants/transformants undergo no fitness cost and all transformants/transconjugants in this study had a fitness cost (error bars indicate 95% confidence intervals).

Fig. 3. Alignment of conjugative pXGE1*mcr* identified in this study with the *E. coli* plasmid pHNSHP45-2 and pMR0516*mcr*. The circular map was created by BRIG tools. Plasmid pXGE1*mcr*，pMR0516*mcr* labeled with different colors were aligned to the reference plasmid pHNSHP45-2. Genes in plasmid pHNSHP45-2 and the *mcr-1* gene in the other two plasmids are labeled. The gaps in the plasmid represent the missing sequences when compared to the reference plasmid.

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Table 1 Characterization of three NDM-5-positive *Escherichia coli* isolates from cows and their transformants/transconjugants.

				Transformants or transconjugants[a]			
	XG-E1	TQ-E1	TQ-E2	XG-E1-TF*mcr*	XG-E1-TF_{NDM}/ TQ-E1-TC_{NDM}/ TQ-E2-TC_{NDM}	TQ-E1-TC*mcr*	TQ-E2-TC*mcr*
Location	Farm 1	Farm 2	Farm 2				
MLST type	ST446	ST2	ST2				
PFGE pattern	A	B	C				
Plasmid replicon type (~kb)				IncHI2 (260kb)	IncX3 (46kb)		
Resistance genes	*bla*$_{NDM-5}$, *bla*$_{CTX-M-14}$, *bla*$_{TEM-1}$, *bla*$_{OXA-10}$, *mcr-1*, *aac(3)-IVa*, *aph(3')-Ia*, *aph(4)-Ia*, *aadA1*, *aadA2*, *fosA3*, *mph(A)*, *floR*, *cmlA1*, *arr-2*, *tet(A)*, *tet(M)*, *sul1*, *sul2*, *sul3*, *dfrA12*, *dfrA14*	*bla*$_{NDM-5}$, *bla*$_{VIM-2}$, *bla*$_{TEM-199}$, *bla*$_{CTX-M-55}$, *aph(3')-VIa*, *aacA4*, *strA*, *strB*, *rmtB*, *aac(6')-Ib-cr*, *fosA3*, *erm(B)*, *mph(A)*, *floR*, *catA3*, *sul2*, *tet(A)*	*bla*$_{NDM-5}$, *bla*$_{TEM-199}$, *bla*$_{CTX-M-55}$, *aac(3)-IVa*, *aph(3')-Ia*, *aph(4)-Ia*, *armA*, *aadA16*, *aacA4*, *strA*, *strB*, *rmtB*, *aac(6')-Ib-cr*, *fosA3*, *erm(B)*, *mph(A)*, *msr(E)*, *floR*, *arr-6*, *sul1*, *sul2*, *dfrA27*	*mcr-1*, *bla*$_{CTX-M-14}$, *aac(3)-IVa*, *aph(3')-Ia*, *aadA1*, *aadA2*, *mph(A)*, *floR*, *cmlA1*, *fosA3*, *sul1*, *sul2*, *sul3*, *dfrA12*	*bla*$_{NDM-5}$	*bla*$_{NDM-5}$, *bla*$_{VIM-2}$, *bla*$_{TEM-199}$, *bla*$_{CTX-M-14}$, *bla*$_{CTX-M-55}$, *aph(3')-VIa*, *aacA4*, *strA*, *strB*, *rmtB*, *aac(3)-IVa*, *aph(3')-Ia*, *aadA1*, *aadA2*, *aac(6')-Ib-cr*, *fosA3*, *erm(B)*, *mph(A)*, *floR*, *catA3*, *cmlA1*, *sul1*, *sul2*, *sul3*, *tet(A)*, *dfrA12*, *mcr-1*	*bla*$_{NDM-5}$, *bla*$_{TEM-199}$, *bla*$_{CTX-M-14}$, *bla*$_{CTX-M-55}$, *aac(3)-IVa*, *aph(3')-Ia*, *aph(4)-Ia*, *armA*, *aadA1*, *aadA2*, *aadA16*, *aacA4*, *strA*, *strB*, *rmtB*, *aac(6')-Ib-cr*, *fosA3*, *erm(B)*, *mph(A)*, *msr(E)*, *floR*, *cmlA1*, *arr-6*, *sul1*, *sul2*, *sul3*, *dfrA12*, *dfrA27*, *mcr-1*

[a] TF indicates transformants and TC indicates transconjugants. XG-E1-TF*mcr* was obtained by colistin (2 μg/ml) selection. XG-E1-TF_{NDM}, TQ-E1-TC_{NDM} and TQ-E2-TC_{NDM} were obtained by meropenem (2 μg/ml) selection.

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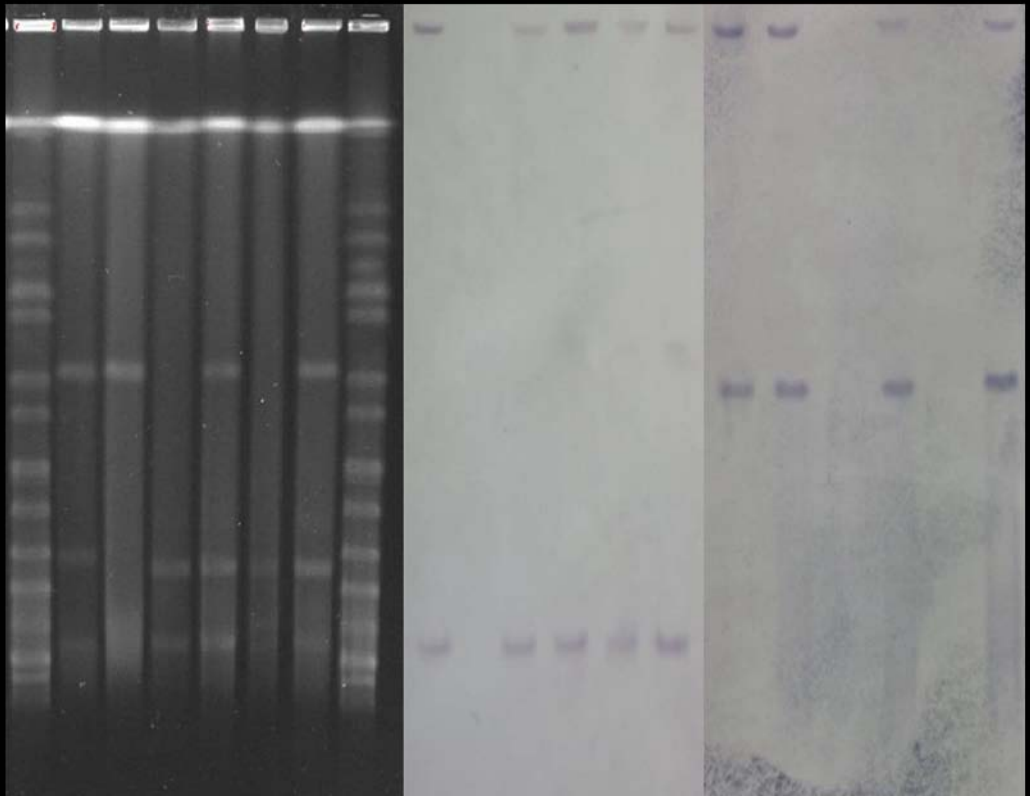
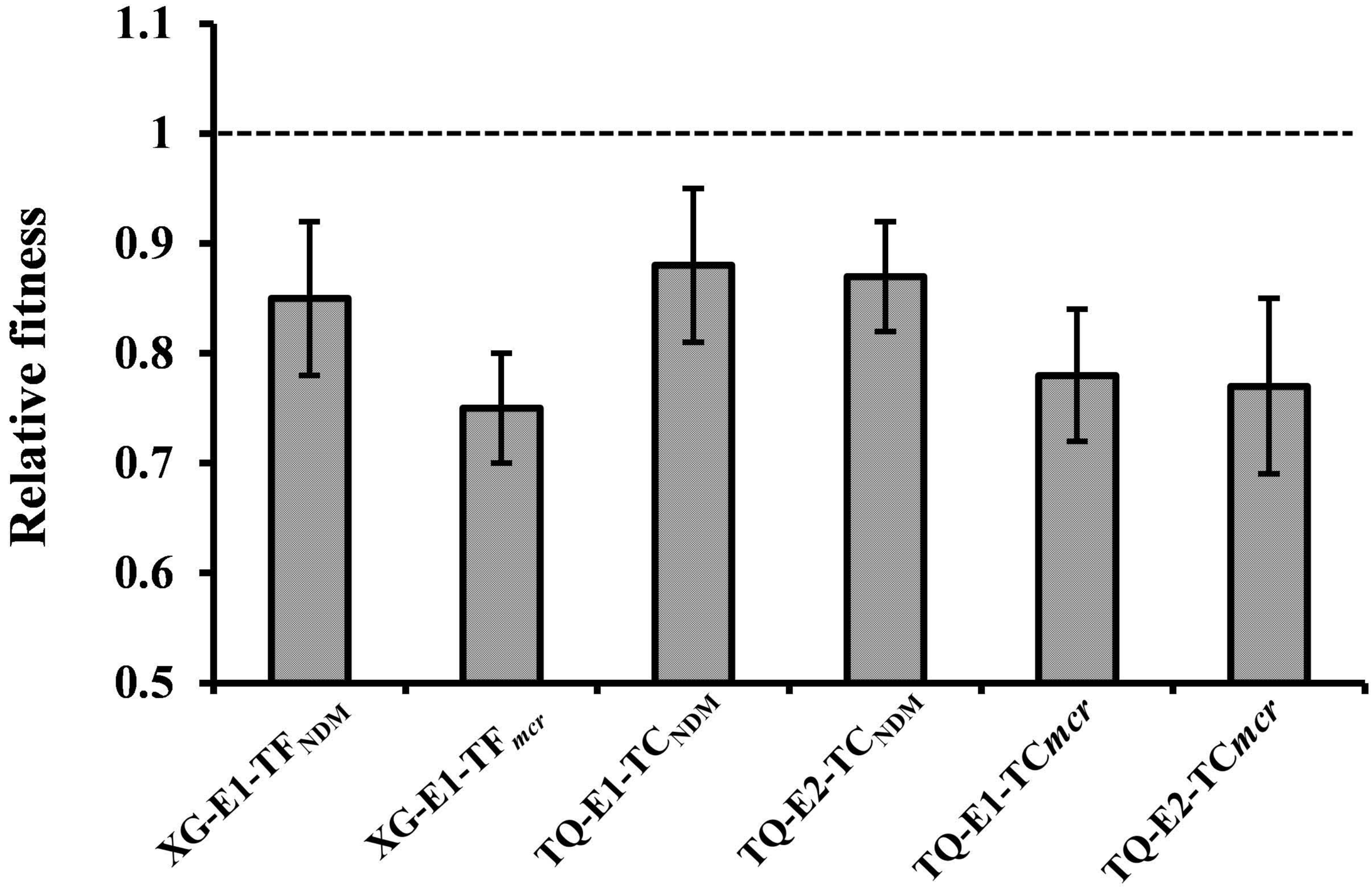
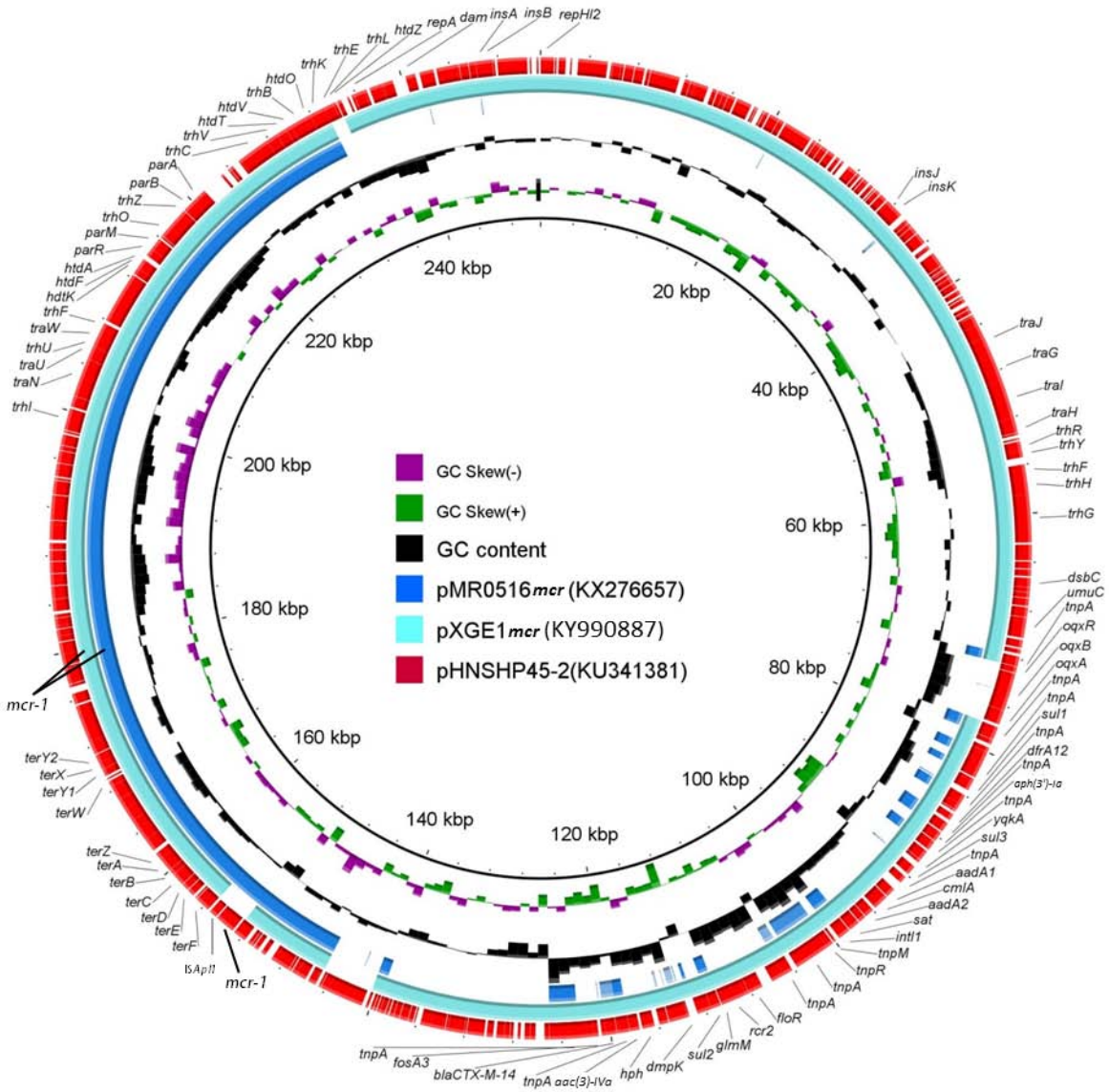
TQ-E1-TC*mcr* and TQ-E2-TC*mcr* were obtained by colistin (2 μg/ml) and ciprofloxacin (8 μg/ml) selection.




Relative fitness
1.1
1
0.9
0.8
0.7
0.6
0.5
XG-E1-TF$_{NDM}$
XG-E1-TF$_{mcr}$
TQ-E1-TC$_{NDM}$
TQ-E2-TC$_{NDM}$
TQ-E1-TC*mcr*
TQ-E2-TC*mcr*
Transformants/transconjugants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