Increased prevalence of *Escherichia coli* strains from food carrying *bla*\textsubscript{NDM} and *mcr-1*-bearing plasmids that structurally resemble those of clinical strains, China, 2015 to 2017

Xiaobo Liu\textsuperscript{1,4}, Shu Geng\textsuperscript{2}, Edward Wai-Chi Chan\textsuperscript{1,3}, Sheng Chen\textsuperscript{1,3}

1. Shenzhen Key Lab for Food Biological Safety Control, Food Safety and Technology Research Center, Hong Kong PolyU Shen Zhen Research Institute, Shenzhen, China
2. College of Food Science and Engineering, Northwest A&F University, Yangling, Shaanxi, China
3. The State Key Lab of Chirosciences, Department of Applied Biology and Chemical Technology, The Hong Kong Polytechnic University, Hung Hom, Kowloon, Hong Kong SAR

Correspondence: Sheng Chen (sheng.chen@polyu.edu.hk)

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Introduction: Emergence of resistance determinants of *bla*\textsubscript{NDM} and *mcr-1* has undermined the antimicrobial effectiveness of the last line drugs carbapenems and colistin. Aim: This work aimed to assess the prevalence of *bla*\textsubscript{NDM} and *mcr-1* in *E. coli* strains collected from food in Shenzhen, China, during the period 2015 to 2017. Methods: Multidrug-resistant *E. coli* strains were isolated from food samples. Plasmids encoding *mcr-1* or *bla*\textsubscript{NDM} genes were characterised and compared with plasmids found in clinical isolates. Results: Among 1,166 non-repeated cephalosporin-resistant *E. coli* strains isolated from 2,147 food samples, 390 and 42, respectively, were resistant to colistin and meropenem, with five strains being resistant to both agents. The rate of resistance to colistin increased significantly (p < 0.01) from 26% in 2015 to 46% in 2017, and that of meropenem resistance also increased sharply from 0.3% in 2015 to 17% in 2017 (p < 0.01). All meropenem-resistant strains carried a plasmid-borne *bla*\textsubscript{NDM} gene. Among the colistin-resistant strains, three types of *mcr-1*-bearing plasmids were determined. Plasmid sequencing indicated that these *mcr-1* and *bla*\textsubscript{NDM}-bearing plasmids were structurally similar to those commonly recovered from clinical isolates. Interestingly, both *mcr-1*-bearing and *bla*\textsubscript{NDM}-bearing plasmids were transferrable to *E. coli* strain J53 under selection by meropenem, yet only *mcr-1*-bearing plasmids were transferrable under colistin selection. Conclusion: These findings might suggest that mobile elements harbouring *mcr-1* and *bla*\textsubscript{NDM} have been acquired by animal strains and transmitted to our food products, highlighting a need to prevent a spike in the rate of drug resistant food-borne infections.
Several recent studies have reported the recovery of both the \textit{bla}\textsubscript{NDM} and \textit{mcr}-1 genes, including \textit{bla}\textsubscript{NDM}/\textit{bla}\textsubscript{NDM} and \textit{mcr}-1 in Enterobacteriaceae species, especially in \textit{E. coli} [17-19], as well as \textit{bla}\textsubscript{NDM} and \textit{mcr}-1 in \textit{Cronobacter sakazakii} [20]. These reports focused on the epidemiological features, evolutionary origin and dissemination routes of resistance genes in different countries. However, few of these studies involved surveillance of prevalence of the \textit{bla}\textsubscript{NDM} and \textit{mcr}-1 genes for a prolonged period. Here, we report the presence of \textit{bla}\textsubscript{NDM} and \textit{mcr}-1 in \textit{E. coli} isolated from food samples in Shenzhen, China, during the period 2015 to 2017 and assessed the prevalence of both genes in these \textit{E. coli} food isolates. We investigated the genomic structures of the mobile genetic elements that encoded \textit{bla}\textsubscript{NDM} and/or \textit{mcr}-1 in these strains to elucidate the transferability of these important resistance determinants.

### Methods

#### Bacterial isolation

\textit{E. coli} isolates were obtained from food samples, including pork, chicken, beef and shrimps, collected from wet markets and supermarkets in Shenzhen, Guangdong Province, China during the period from 10 August 2015 to 17 April 2017. Three isolation methods were employed to increase the yield. Details of these are described in the Supplement.

#### Antimicrobial susceptibility testing

All \textit{E. coli} isolates recovered from food samples were subjected to determination of their antimicrobial susceptibilities using the agar dilution method according to the Clinical and Laboratory Standards Institute (CLSI) except for colistin and tigecycline that was tested using the broth-microdilution method [21]. We used resistance breakpoints published by the CLSI [21]. \textit{E. coli} strain ATCC 25922 was tested as the quality control strain. Further \textit{E. coli} isolates from the same food sample and exhibiting identical MIC profile were considered as similar clones and eliminated from further analysis. Only non-duplicated \textit{E. coli} isolates from each sample were used for further analysis.

#### Characterisation of \textit{bla}\textsubscript{NDM} and \textit{mcr}-1 positive \textit{Escherichia coli} strains

All \textit{E. coli} strains were screened for the presence of the \textit{bla}\textsubscript{NDM} and \textit{mcr}-1 gene by PCR using primers as previously described [10,22]. PCR products were purified and subjected to Sanger sequencing to confirm the genetic identity.

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### Table 1

| Year | Number of food samples | Cefotaxime-resistant \textit{E. coli} isolates | \textit{mcr}-1-bearing \textit{E. coli} | \textit{bla}\textsubscript{NDM}-bearing \textit{E. coli}, with or without \textit{mcr}-1 |
|------|------------------------|---------------------------------------------|---------------------------------|--------------------------------------------------|
|      |                        | \textit{Total} Isolation rate (\%)<sup>a</sup> | \textit{Pork} | \textit{Beef} | \textit{Chicken} | \textit{Shrimp} | \textit{Total} \textit{mcr}-1 positivity rate (\%)<sup>a</sup> | \textit{Total} \textit{bla}\textsubscript{NDM} positivity rate (\%)<sup>a</sup> | Number of \textit{mcr}-1-positive strains | \textit{bla}\textsubscript{NDM}/\textit{mcr}-1 positivity rate (\%)<sup>a</sup> |
| 2015 | 747                    | 38.5 (A)                                          | 248 (73) | 37 (3) | 46 (30) | 97 (10) | 25 (10) | 0.3 (E) | 0 | 0 |
| 2016 | 1,039                  | 57.0 (A)                                          | 319 (52) | 61 (3) | 53 (6) | 168 (22) | 86 (22) | 12 | 0 | 0.2 |
| 2017 | 371                    | 57.2 (A)                                          | 143 (65) | 13 (3) | 56 (8) | 88 (1) | 5 (1) | 17 (F) | 5 | 2 |
| Total | 2,137                | 55 (A)                                              | 710 (60) | 107 (1) | 47 (31) | 375 (82) | 36 (10) | 402 (34) | 25 (42) | 4 (5) |

<sup>a</sup>Percentage was calculated by dividing the number of \textit{E. coli} isolates carrying the indicated resistance genes by the total number of cefotaxime-resistant \textit{E. coli} isolates obtained in each year; percentages labelled with the same letter in brackets indicated that no significant difference was observed between two variants; percentages labelled with different letters indicated that a significant difference was observed between two variants (p < 0.05); (B) vs (C): p = 0.000; (C) vs (D): p = 0.014; (B) vs (D): p = 0.000; (B) vs (C) vs (D): p = 0.000; (E) vs (F): p = 0.000.
Methodological details for the conjugation experiments, XbaI-PFGE, St-PFGE and Southern hybridisation are given in the Supplement.

### Plasmid sequencing and bioinformatics analyses

Plasmid sequencing was performed as previously described using the Illumina and PacBio RS II platforms [14]. All plasmid sequences were submitted to the RAST tool for annotations and modified manually by BLAST [23]. The Easyfig software was used in comparative plasmid analysis [24].

### Statistical analysis

The statistical analysis was performed by Minitab16 software (Minitab, version 16.2.3, Minitab Inc., State College, United States). The resistance rates of *E. coli* to colistin and meropenem in 2015, 2016 and 2017 were determined by the chi-squared test. The threshold for significant difference was $p < 0.05$.

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**Figure 1**

XbaI-PFGE pattern of *Escherichia coli* isolates resistant to meropenem, Shenzhen, 2015–2017 (n = 42)

| Strain number | Isolation sources | Isolation date (d/m/y) | Resistance gene | bla NDM-1 plasmid (kb) | mcr-1 plasmid (kb) |
|---------------|-------------------|------------------------|----------------|------------------------|------------------|
| 975 pork      | 09/01/17          | **bla**<sub>NDM</sub>46 (IncX3) |               | 46 (IncX3)            |                  |
| 1140 pork     | 10/04/17          | **bla**<sub>NDM</sub>46 (IncX3) |               | 46 (IncX3)            |                  |
| 1108 chicken  | 27/03/17          | **bla**<sub>NDM</sub>, mc<sub>r</sub>-1 97(IncX1-N) |               |                  | 60 (IncI2)       |
| 972 chicken   | 09/01/17          | **bla**<sub>NDM</sub>46 (IncX3) |               | 46 (IncX3)            |                  |
| 973 chicken   | 09/01/17          | **bla**<sub>NDM</sub>46 (IncX3) |               | 46 (IncX3)            |                  |
| 1138 chicken  | 10/04/17          | **bla**<sub>NDM</sub>46 (IncX3) |               | 46 (IncX3)            |                  |
| 1079 chicken  | 20/03/17          | **bla**<sub>NDM</sub>46 (IncX3) |               | 46 (IncX3)            |                  |
| 1083 chicken  | 20/03/17          | **bla**<sub>NDM</sub>46 (IncX3) |               | 46 (IncX3)            |                  |
| 1089 pork     | 20/03/17          | **bla**<sub>NDM</sub>46 (IncX3) |               | 46 (IncX3)            |                  |
| 1144 chicken  | 10/04/17          | **bla**<sub>NDM</sub>46 (IncX3) |               | 46 (IncX3)            |                  |
| 1143 pork     | 10/04/17          | **bla**<sub>NDM</sub>46 (IncX3) |               | 46 (IncX3)            |                  |
| 1087 pork     | 20/03/17          | **bla**<sub>NDM</sub>46 (IncX3) |               | 46 (IncX3)            |                  |
| 1088 beef     | 20/03/18          | **bla**<sub>NDM</sub>46 (IncX3) |               | 46 (IncX3)            |                  |
| 1043 beef     | 06/03/17          | **bla**<sub>NDM</sub>46 (IncX3) |               | 46 (IncX3)            |                  |
| 1045 pork     | 06/03/17          | **bla**<sub>NDM</sub>46 (IncX3) |               | 46 (IncX3)            |                  |
| 1081 pork     | 20/03/17          | **bla**<sub>NDM</sub>46 (IncX3) |               | 46 (IncX3)            |                  |
| 1005 pork     | 20/02/17          | **bla**<sub>NDM</sub>46 (IncX3) |               | 46 (IncX3)            |                  |
| 974 pork      | 09/01/17          | **bla**<sub>NDM</sub>, mc<sub>r</sub>-1 74 (IncFII) 60 (IncI2) |               |                  | 60 (IncI2)       |
| 787 pork      | 05/09/17          | **bla**<sub>NDM</sub>, mc<sub>r</sub>-1 46 (IncX3) 33 (IncX4) |               |                  | 33 (IncX4)       |
| 908 pork      | 31/10/16          | **bla**<sub>NDM</sub>46 (IncX3) |               | 46 (IncX3)            |                  |
| 847 pork      | 26/09/16          | **bla**<sub>NDM</sub>46 (IncX3) |               | 46 (IncX3)            |                  |
| 1142 chicken  | 10/04/17          | **bla**<sub>NDM</sub>46 (IncX3) |               | 46 (IncX3)            |                  |
| 976 chicken   | 90/01/17          | **bla**<sub>NDM</sub>46 (IncX3) |               | 46 (IncX3)            |                  |
| 1107 chicken  | 27/03/17          | **bla**<sub>NDM</sub>, mc<sub>r</sub>-1 110 (IncB) 220 (IncHI2) |               |                  | 220 (IncHI2)     |
| 1141 pork     | 10/04/17          | **bla**<sub>NDM</sub>46 (IncX3) |               | 46 (IncX3)            |                  |
| 714 pork      | 15/08/16          | **bla**<sub>NDM</sub>46 (IncX3) |               | 46 (IncX3)            |                  |
| 1166 chicken  | 17/04/17          | **bla**<sub>NDM</sub>46 (IncX3) |               | 46 (IncX3)            |                  |
| 1106 chicken  | 27/03/17          | **bla**<sub>NDM</sub>, mc<sub>r</sub>-1 110 (IncB) 60 (IncI2) |               |                  | 60 (IncI2)       |
| 1082 pork     | 20/03/17          | **bla**<sub>NDM</sub>46 (IncX3) |               | 46 (IncX3)            |                  |
| 1084 pork     | 20/03/17          | **bla**<sub>NDM</sub>46 (IncX3) |               | 46 (IncX3)            |                  |
| 1085 beef     | 20/03/17          | **bla**<sub>NDM</sub>46 (IncX3) |               | 46 (IncX3)            |                  |
| 948 shrimp    | 05/12/16          | **bla**<sub>NDM</sub>46 (IncX3) |               | 54 (IncX3)            |                  |
| 40 pork       | 17/08/15          | **bla**<sub>NDM</sub>46 (IncX3) |               | 46 (IncX3)            |                  |
| 977 pork      | 09/01/17          | **bla**<sub>NDM</sub>46 (IncX3) |               | 46 (IncX3)            |                  |
| 1004 pork     | 06/03/17          | **bla**<sub>NDM</sub>46 (IncX3) |               | 46 (IncX3)            |                  |
| 1165 chicken  | 17/04/17          | **bla**<sub>NDM</sub>46 (IncX3) |               | 46 (IncX3)            |                  |
| 1006 chicken  | 20/02/17          | **bla**<sub>NDM</sub>46 (IncX3) |               | 46 (IncX3)            |                  |
| 1044 pork     | 06/03/17          | **bla**<sub>NDM</sub>46 (IncX3) |               | 46 (IncX3)            |                  |
| 1078 pork     | 20/03/17          | **bla**<sub>NDM</sub>46 (IncX3) |               | 46 (IncX3)            |                  |
| 1139 pork     | 10/04/17          | **bla**<sub>NDM</sub>46 (IncX3) |               | 46 (IncX3)            |                  |
| 1080 pork     | 20/03/17          | **bla**<sub>NDM</sub>46 (IncX3) |               | 46 (IncX3)            |                  |
| 1086 pork     | 20/03/17          | **bla**<sub>NDM</sub>46 (IncX3) |               | 46 (IncX3)            |                  |

All these plasmids were conjugative plasmids.
Grey shading indicates homologies between the corresponding genetic loci on each plasmid. Arrows indicate coding sequences, with arrowheads indicating the direction of transcription. Red: antibiotic resistance-encoding genes; blue: mobile elements; yellow: replicon proteins; grey: maintenance/stability functioning genes or hypothetical proteins.
Prevalence of cephalosporin-resistant *Escherichia coli* in food products

A total of 1,166 (55%) non-repeated cephalosporin-resistant *E. coli* isolates were recovered from 2,137 food samples purchased during the period from 2015 to 2017 in Shenzhen, China, for the Food-borne Pathogen Surveillance Programme conducted by the Shenzhen Key Laboratory for Food Biological Food Safety Control. No food sample was found to contain more than one type of *E. coli* isolate. Among the 2,137 food samples, 1,368 were purchased from wet markets, and 769 samples were purchased from supermarkets of different regions in Shenzhen. These food samples included 1,182 pork, 230 beef, 383 chicken and 342 shrimp samples (Supplementary Table S1). Among the 1,166 ceftaxidime-resistant *E. coli* isolates, 385 (52%) *E. coli* were isolated from 747 food samples in 2015, 570 (56%) from 1,019 food samples in 2016, and 211 (57) from 371 food samples in 2017 (Table 1). Among these *E. coli* isolates, 710 were isolated from the 1,182 pork samples (60%), 107 were recovered from the 230 beef samples (47%), 315 from the 383 chicken samples (82%) and 34 from the 342 shrimp samples (10%). The detailed sampling and isolation information is provided in Supplementary Table S1.

Antimicrobial susceptibility of *Escherichia coli* food isolates

We determined the minimal inhibitory concentrations (MIC) of various antibiotics for all 1,166 cephalosporin-resistant *E. coli* isolates. These strains were found to be resistant to most of the antibiotics tested, with a resistance rate of 100% to ampicillin, cefotaxime and ceftriaxone, and resistance to the other agents as follows: 95% to sulfamethoxazole/trimethoprim, 95% to tetacycline, 86% to chloramphenicol, 82% to nalidixic acid, 64% to kanamycin and 59% to ciprofloxacin. The rate of resistance to colistin was ca 33%. On the other hand, these strains were susceptible to tigecycline (100%), amikacin (97%), ceftazidime/avibactam (96%) and meropenem (96%). For the 390 *E. coli* strains that were resistant to colistin, the rate of resistance to different antibiotics was slightly higher than the resistance rate of all *E. coli* isolates, with the of exception of tigecycline (0%), ceftazidime/avibactam (2%) and meropenem (2%) (Supplementary Table S2). Forty-two *E. coli* strains that were resistant to meropenem were also resistant to most of other antibiotics. Five of these 42 strains were also resistant to colistin, but were mostly susceptible to amikacin (98%) (Supplementary Table S2). Among the 390 colistin-resistant *E. coli* strains, the rate of resistance to colistin increased significantly over the years, with 26% in 2015, 36% in 2016 and 46% in 2017 (p<0.05). Among the 42 strains that were resistant to meropenem, the rate of meropenem resistance increased significantly from 0.3% in 2015 to 1% in 2016 and 17% in 2017 (p<0.001) (Table 1). Interestingly, five meropenem-resistant strains were also resistant to colistin, accounting for 0.4% of the total number of *E. coli* strains isolated from food products in this study (Table 1).

Prevalence of mcr-1 and bla<sub>NDM</sub> in *Escherichia coli* isolates from food

All 390 colistin-resistant *E. coli* strains were subjected to screening for the presence of the *mcr-1*, *mcr-2*, *mcr-3* and *mcr-4* genes; all of them carried the *mcr-1* gene but not the other *mcr* genes. Screening for carbapenemase genes among the 42 meropenem-resistant *E. coli* strains showed that all of them harboured the *bla<sub>NDM</sub>* gene except for strains 787, 974, 977, 1079, 1106 and 1107, which were found to harbour the *bla<sub>NDM</sub>* gene. No other carbapenemase genes, such as the *bla<sub>KPC</sub>, *bla<sub>VIM</sub>, *bla<sub>IMP</sub>, *bla<sub>OXA-4</sub>*-like genes, could be detected in these strains. Consistently, the five *E. coli* strains that were resistant to both meropenem and colistin carried both *mcr-1* and *bla<sub>NDM</sub>* genes. Genetic characterisation by PFGE of the 42 *bla<sub>NDM</sub>*-carrying *E. coli* strains showed that the majority were genetically unrelated, suggesting that the spread of these strains was mainly due to acquisition of *bla<sub>NDM</sub>* by different *E. coli* strains, rather than clonal dissemination of strains that had acquired the *bla<sub>NDM</sub>* gene (Figure 1). Nevertheless, evidence of clonal transmission was also observed; examples include strains 1080/1086 and 972/973, which were isolated from different pork or chicken samples purchased on the same date, as well as strains 1043/1045/1081, 1082/1084/1085, 1006/1044 and 977/1004, which were isolated from different meat products on different dates.

Genetic characterisation of *bla<sub>NDM</sub>*-bearing *Escherichia coli* strains

To investigate the genetic characteristics of *E. coli* strains carrying *bla<sub>NDM</sub>* conjugation experiments were performed. The meropenem resistance phenotype of all 42 *bla<sub>NDM</sub>*-bearing strains tested was transferrable to *E. coli* J53 and EC600, with only data on J53 being shown here. S1-PFGE and Southern hybridisation performed on the parental and transconjugant of all 42 strains showed that *bla<sub>NDM</sub>* was located on plasmids of six different sizes, with the ca44 kb IncX3-type plasmid being the most dominant, accounting for 36 of the 42 conjugative plasmids. Two strains were found to harbour an IncB plasmid of ca110 kb, and one strain each carried a plasmid of 54 kb (IncX3), 74 kb (IncFII), 97 kb (IncX1-IncN) and 130 kb (IncFIB) in size (Figure 1). The ca44 kb and ca54 kb IncX3-type *bla<sub>NDM</sub>*-bearing plasmids have been commonly reported in clinical isolates, while they were rarely reported in carbapenem-resistant Enterobacteriaceae strains isolated from non-clinical settings. To confirm that these plasmids, recovered from *E. coli* of food origin, were genetically similar to those in human clinical isolates, one ca44 kb plasmid and one ca54 kb plasmid were recovered from the transconjugants and subjected to complete plasmid sequencing using both Illumina and Nanopore sequencing platforms.
The complete map of the \textit{bla} \textit{NDM} \textsuperscript{5}\text{-}bearing plasmid from transconjugant \textit{E. coli} MTC787, designated as pMTC787, was 46,161 bp in size with a GC content of 46.7%; it was confirmed to belong to an IncX3 replicon type. BLASTN analysis of this plasmid revealed that p787-NDM displayed 100% query coverage and 99% nucleotide identity with a plasmid pCRCB-101\textsubscript{1} (CP024820) carried by a \textit{Citrobacter freundii} strain CRCB-101 isolated from open pus of a person in South Korea, and with plasmid pNDM-EC36 (MG591703) carried by an \textit{E. coli} strain EC36 isolated in Henan, China (Figure 2A). Moreover, p787-NDM showed high homology (98% in both identity and coverage) to other similar IncX3, \textit{bla} \textit{NDM} \textsuperscript{5}\text{-}bearing plasmids reported in GenBank, such as plasmid pNDM\textsubscript{K1} from \textit{K. pneumoniae} (NC_022740.1), plasmid pP855-NDM\textsubscript{5} (MF547508.1) and plasmid pP888A-NDM\textsubscript{5} (MF547507.1) from \textit{clinical E. coli} strains and plasmid pNDM\textsubscript{5}SSH006 (MTKV01000083.1) from \textit{Salmonella Typhimurium} strain SHO06.

The complete sequence \textit{bla} \textit{NDM} \textsuperscript{5}\text{-}bearing plasmid from the transconjugant of \textit{E. coli} strain 948, MT9498 was obtained and designated as pMT9498. It was confirmed as an IncX3 type with a size of 53,770 bp. Sequence analysis showed that it was highly homologous to plasmid pNDM\textsubscript{K1}020049 which is carried by a \textit{Klebsiella pneumoniae} strain SCKP020049 isolated from a person in Sichuan, China and to plasmid p2ZDC33 which is carried by an \textit{E. coli} strain ZHDC33 isolated in Zhejiang, China. Plasmid pMT9498 aligned well with plasmid p787-NDM, with only an additional mobile element carrying IS26-bla \textit{SHV}-\textsubscript{12} (Figure 2B). This plasmid also displayed high homology (> 98% in both identity and coverage) with other IncX3, \textit{bla} \textit{NDM} \textsuperscript{5}\text{-}bearing plasmids reported in GenBank, such as plasmid pNDM-HN380 (NC_019162.1) from \textit{K. pneumoniae}, plasmid pNDM-EcHK001 (NZ_CMO08823.1) from \textit{Enterobacter cloacae} strain EcHK001 and plasmid pYQ13500-NDM (KR059865.1) from \textit{En. cloacae} strain EC-YQ13500.

Among the 42 \textit{E. coli} strains carrying \textit{bla} \textit{NDM} five also carried \textit{mcr-1} (Figure 1). Conjugation experiments were performed to assess the transferability of the colistin resistance phenotype; it could be successfully transferred to \textit{E. coli} J53 from all strains except strain 1107, where the \textit{mcr-1} bearing plasmid was not conjugative and could not be transferred by transformation. Si-PFGE and Southern hybridisation were performed on strain 1107 and showed that the \textit{mcr-1} gene was located on a ca 220 kb plasmid belonging to the InH12 type. Two types of conjugative \textit{mcr-1} bearing plasmids could be recovered from strains 787, 1106 and 1108 with three strains carrying a ca 60 kb IncI2 plasmid and one harbouring a ca 33 kb, IncX4 plasmid (Figure 1). Interestingly, during conjugation, both the \textit{mcr-1} bearing and the \textit{bla} \textit{NDM} \textsuperscript{5}\text{-}bearing plasmids in strains 787, 974 and 1108 could be simultaneously transferred to \textit{E. coli} J53 under selection of meropenem, yet only \textit{mcr-1} bearing plasmids could be transferred to J53 under selection of colistin. The data suggested that even though the \textit{mcr-1} and \textit{bla} \textit{NDM} \textsuperscript{5} genes were located on different plasmids, each of these two plasmids could be transferred to other bacteria under meropenem selection pressure (Figure 3).

One ca 60 kb (IncI2) and one ca 33 kb (IncX4) \textit{mcr-1} bearing plasmid were recovered from the transconjugants and subjected to complete plasmid sequencing using both Illumina and Nanopore sequencing platforms. The complete map of the ca 30 kb \textit{mcr-1} bearing plasmid recovered from transconjugant \textit{E. coli} CTC787, designated as pCTC787, was shown to belong to IncX4 type with a size of 33,301 bp. It aligned well (>99% in both identity and coverage) with several previously reported plasmids such as p14EC007a (CP024132), which was carried by an \textit{E. coli} strain 14EC007 isolated from clinical patients in China, and pGM17–001\textsubscript{2} (CP028174) which was carried by a \textit{Salmonella enterica} strain CFSA064033 isolated from a turkey sample in Germany (Figure 2C). The complete sequence of a ca 60 kb \textit{mcr-1} bearing conjugative plasmids, recovered from the transconjugant of \textit{E. coli} CTC1106 strain was shown to belong to the IncI2 type with a size of 60,960 bp and a GC content of 42.3%. It was designated as p1106-IncI2. BLASTN analysis revealed that...
it exhibited the highest homology with pP111, carried by a Salmonella enterica (KY120365) strain P111 isolated in Taiwan, and with pColR644SK1, carried by an E. coli strain ColR644SK1 (MF175188) isolated in Switzerland. It also exhibited high homology (>99% in both identity and coverage) with pHNSHP45 (KP147127) and other plasmids reported previously [25,26]. Similar to other IncI2 type plasmids, p1106-IncI2 contained genes and proteins responsible for replication (repB), plasmid stability (mok), partitioning (parA), plasmid transferability (traL), plasmid-borne site-specific recombinase (rci) and pilus-encoding loci (pilV) (Figure 2D).

Discussion
Mobile carbapenemase genes have emerged in clinical isolates but are rare in isolates from animals. In China, the first blaNDM-1 gene was reported in an E. coli strain (HK-01) in 2011 [8] and has since disseminated extensively in clinical settings [27-29]. The blaNDM-1 gene has mainly been reported on a ca 46kb and 54kb IncX3-type conjugative plasmid in China [9,29,30]. Although such a gene has since been reported in non-clinical bacterial isolates of different origins, such as animals and the environment, it was often carried by plasmids of various types mainly through insertion of the blaNDM-1-bearing mobile element into the backbone of different plasmids in bacterial strains of the same origin. These data appear to imply that bacterial strains, in particular E. coli, that commonly reside in animals and the farm environment might not be adaptable to the IncX3 type plasmids. Recently, Wang Y et al. reported the prevalence of NDM and MCR-1-producing Enterobacteriaceae strains in poultry and the farm environment, and that the prevalence of blaNDM-1 in these strains has increased sharply [39]. E. coli isolates carrying an IncX3 blaNDM-1-bearing plasmid were found to be prevalent in Enterobacteriaceae strains isolated from the poultry production chain [19]. Wang R et al. also reported the co-existence of mcr-1 and different blaNDM-1 variants in E. coli and K. pneumoniae isolates originating from broiler, swine and cattle samples [31]. These findings suggest that co-transmission of blaNDM-1 and the already prevalent mcr-1 gene has occurred among Enterobacteriaceae in animals, leading to selection of strains carrying both blaNDM-1 and mcr-1 which are a risk to human health. This risk will become imminent if Enterobacteriaceae strains carrying both resistance genes become prevalent in our food products. However, isolation of E. coli carrying both the blaNDM-1 and mcr-1 gene, in particular carriage of blaNDM-1 by the IncX3-type plasmid in E. coli strains of food origin, has not been reported previously.

We have been conducting comprehensive surveillance of multidrug-resistant E. coli in food products in China in the past few years. E. coli strains of food origin were shown in our study to carry mcr-1 at an increasing rate, whereas the blaNDM-1 gene was rarely detected. In this study, we have shown that the blaNDM-1-positive isolates were increasingly detectable over time, with a prevalence rate of 0.3% and 1% in 2015 and 2016, respectively, rising sharply to 17% in 2017. This sharp increase in the prevalence of blaNDM-1-bearing E. coli strains in food products was mainly due to transmission of a ca 446kb IncX3 plasmid commonly harboured by clinical Enterobacteriaceae isolates. In addition, the increasing prevalence of E. coli strains carrying blaNDM-1 implies emergence of E. coli strains that carry both blaNDM-1 and mcr-1. In this work, all three commonly reported mcr-1-bearing plasmids were able to co-exist in a single E. coli strain. In addition to the highly prevalent IncX3 blaNDM-1-bearing plasmid, another five plasmid types were also reported in E. coli of food origin. Furthermore, all blaNDM-1-bearing plasmids reported in our study were conjugative, implying that they were transmissible to other E. coli strains of food origin. The increasing prevalence of Enterobacteriaceae strains carrying blaNDM-1 and mcr-1 in food products will lead to increased colonisation of the human gastrointestinal tract with these Enterobacteriaceae strains, a phenomenon that has been associated with the high prevalence of drug-resistant infections in clinical settings [32]. Importantly, E. coli carrying blaNDM-1 or mcr-1 could be detected in pork, beef and even shrimp, suggesting that these strains have already widely disseminated to different food products. An increasing rate of food-borne infections caused by such strains would be a serious concern. Further surveillance of blaNDM-1 and mcr-1 in other food products is warranted.

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Conflict of interest
None declared.

Authors’ contributions
XBL, designed and performed the research and drafted the manuscript; GX participated in the supervision of the research; EWCC, participated in data analysis and manuscript writing; SC supervised the whole project, analysed the data and wrote the manuscript.

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