

# Drug resistance analysis of three types of avian-origin carbapenem-resistant Enterobacteriaceae in Shandong Province, China

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**ABSTRACT** Animal-derived Enterobacteriaceae bacteria such as *Escherichia coli* (*E. coli*), *Proteus mirabilis* (*P. mirabilis*), and *Klebsiella pneumoniae* (*K. pneumoniae*) are important food-borne zoonotic bacilli that exist widely in the broiler-breeding industry. Although carbapenem antibiotics are considered to be the last line of defense against multidrug-resistant bacteria, carbapenem-resistant Enterobacteriaceae (CRE) break through them. In our study, we therefore, examined the prevalence of CRE and characteristics of antimicrobial resistance in 6 conventional broiler-fattening farms in Shandong Province, China. Our study revealed isolation rates of 3.57% (6/168) for carbapenem-resistant *E. coli*, 10% (5/50) for carbapenem-resistant *P. mirabilis*, and 3.03% (1/33) for carbapenem-resistant *K. pneumoniae*. All 12 CRE bacterial strains showed varying degrees of

resistance to 27 antibiotics in 8 classes and were multidrug-resistant. The rate of the strains containing *bla*<sub>NDM</sub> genes, at 91.67% (11/12), was especially high. Among other results, the carrying rate of integrons in CRE bacteria was 91.67% (11/12), and 2 strains carried both class I and class II integrons, which accelerated the lateral transmission of resistant bacteria. Our first-ever finding of the 3 CRE bacteria *E. coli*, *P. mirabilis*, and *K. pneumoniae* on the same broiler farm suggests that poultry-derived CRE strains may pose a risk to humans. Moreover, our findings from surveillance can inform current understandings of the prevalence and characteristics of multidrug-resistant CRE in Shandong Province and, in turn, help to curb threats to food safety and public health and better prevent and control infectious zoonotic diseases.

**Key words:** *bla*<sub>NDM</sub>, carbapenem, drug resistance gene, multidrug resistance

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## INTRODUCTION

Bacterial resistance caused by the overuse and abuse of medical and veterinary antibiotics has become a major problem the world over. As a consequence, since the 1980s, multidrug-resistant (MDR) bacteria, including the likes of carbapenem-resistant Enterobacteriaceae (CRE), have continually been reported (Bi et al., 2011). CRE are superbacteria because of their strong resistance to frontline antibiotics, including treonam, ceftriaxone, and piperacillin, as well as ceftazidime, and

cefepime. Given those trends, curbing bacterial resistance demands sustained attention, or else antibiotics in general will no longer be effective (Sheu et al., 2019). At present, zoonotic Enterobacteriaceae already known to threaten the health of humans and livestock include *E. coli*, *P. mirabilis*, and *K. pneumoniae* (Liu et al., 2021a; Wang et al., 2021b; Li et al., 2022b; Li et al., 2022a). The emergence of such CRE means that the last line of defense against MDR bacteria is in jeopardy, a consequence of which is increasingly complicated clinical treatment (Woodford et al., 2014).

Since a strain of carbapenemase-producing *K. pneumoniae* was first reported in the U.S. state of North Carolina in 2001, CRE have been reported in many countries and regions (Yigit et al., 2001). Using data obtained from monitoring bacterial resistance in China in 2017, Hu et al. (2018) observed a resistance rate of Enterobacteriaceae to carbapenems approaching 10%. Therein, the resistance rate of *E. coli* to imipenem was

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1.9% and showed an upward trend compared with data from 2016, while the resistance rate of *K. pneumoniae* to carbapenems rose dramatically from 3.0% in 2005 to 20.9% in 2017. Although carbapenems have not been approved as antibiotics in animal breeding either in China or abroad, those circumstances have not prevented the transmission of CRE from livestock to humans (Feng et al., 2021). The trend suggests that the route of the livestock- and poultry-infecting bacteria may be direct contact or via the food chain from animals to humans. In either case, it poses a serious challenge for public health (Leverstein-van Hall et al., 2011). After all, in hens, *E. coli*, responsible for salpingitis, can be vertically transmitted to chicks and cause significant death in the first week of life, as well as induce peritonitis syndrome, which often leads to the acute death of poultry regardless of age (Landman and van Eck, 2015). Meanwhile, in humans, *P. mirabilis* can cause wounds, eye infections, and gastrointestinal and urinary tract infections (Sanchez et al., 2020), while *K. pneumoniae* can cause urinary, respiratory, and blood infections (Liu et al., 2017; Aires-de-Sousa et al., 2019). The latter can also cause mastitis in dairy cows and respiratory symptoms in broiler chickens (Yang et al., 2021). Species aside, because clinical treatment for CRE infection remains difficult, the mortality rate following infection continues to be high (Hamza et al., 2016).

The chief drug resistance mechanism of CRE is the production of carbapenemase, which can hydrolyze carbapenem antibiotics. According to the Ambler classification, carbapenems can be divided into 3 categories: A, B, and D. Carbapenemases in Enterobacteriaceae are primarily the class A enzymes KPC, SME, IMI, NMC, and GES; the class B enzymes IMP, VIM, and NDM; and the class D enzyme OXA (Cherak et al., 2021). The genes encoding carbapenemases can be captured by bacteria through the integron–gene cassette system, in a process mediated by chromosomes, plasmids, or transposons. After the carbapenemases hydrolyze imipenem, meropenem, or ertapenem, they can cause bacterial drug resistance, promote the spread of drug resistance genes among various bacterial genera, and ultimately increase the prevalence of carbapenem resistance (Sun et al., 2016; Li et al., 2019). Since the detection of *bla*<sub>NDM</sub> in *K. pneumoniae* isolated from a patient with urinary tract infection in Sweden was first reported in 2008, a total of 21 NDM enzyme variants, NDM-1 to NDM-21, have been identified globally (Tian et al., 2020). Among them, the NDM-1-producing enzyme is the most common in CRE (Iovleva and Doi, 2017). In 2010, eight carbapenem-insensitive strains were found on 8 chicken farms, on 6 duck farms, and in a pig slaughterhouse in China. One of those strains was identified as being positive for *bla*<sub>NDM-1</sub>, particularly on a 270 kb plasmid (Wang et al., 2012). Among the positive bacteria carrying the *bla*<sub>NDM-1</sub> gene reported in China from 2012 to 2016, most were reported in southwest China (Jia et al., 2018). More recently, in 2021, *P. mirabilis*, which produces *bla*<sub>NDM</sub> in broiler chickens, was first reported in Chengdu in Sichuan Province (Xie et al., 2021).

The ability to investigate and monitor drug resistance in Enterobacteriaceae, especially carbapenem resistance, is important for assessing the economic and public health implications of such resistance. Given increasing forms of clinical CRE, it has become increasingly difficult to control bacterial infections. However, because no systematic analysis of CRE has been performed in China's Shandong Province, herein we report our study conducted on 6 large farms in the province, namely in the prefecture-level cities of Tai'an, Liaocheng, Weifang, Linyi, Heze, and Binzhou. We collected 707 samples on those farms and conducted basic studies on clinical CRE to determine the drug susceptibility of resistant bacteria and to characterize the drug resistance genes and drug resistance mechanisms of resistant strains. The data presented here deepen current understandings of characteristics of epidemics and the drug resistance of poultry-origin CRE strains in Shandong Province. Our findings thus provide a scientific basis for the clinical treatment and prevention of CRE infections in poultry in the surrounding region.

## MATERIALS AND METHODS

### Source of Strains

A total of 707 samples were collected on 6 large broiler farms in the cities of Tai'an, Liaocheng, Weifang, Linyi, Heze, and Binzhou in Shandong Province from May to July 2019 (Liu et al., 2021b). The samples were taken via anal swabs and environmental samples of broiler chickens collected during a breeding cycle, the latter of which came from walls, water, feces, and air. A total of 375 strains of *E. coli*, 50 strains of *P. mirabilis*, and 33 strains of *K. pneumoniae* were isolated from those samples. All of the *P. mirabilis* and *K. pneumoniae* isolates and 168 randomly selected strains, 24 to 30 per farm, were used in subsequent testing and analysis.

### Isolation and Identification of CRE

All *E. coli*, *P. mirabilis*, and *K. pneumoniae* isolates were again purified and confirmed using a microbial mass spectrometer (IVD MALDI Biotyper, Bruker, Bremen, Germany). In that experiment, we used imipenem (4 µg/mL) to verify whether the strains were resistant to carbapenems.

### Drug Susceptibility Testing of CRE

For the CRE, the minimum inhibitory concentrations (MICs) of 27 commonly used antibiotics in the following 8 categories were tested: 1) carbapenems in a class of antibiotics including imipenem (IPM), ertapenem (ETP), and meropenem (MEM); 2) β-lactam antibiotics including amoxicillin–clavulanic acid (AMC), ampicillin–sulbactam (SAM), piperacillin–tazobactam (TZP), cefazolin (CZ), cefepime (FEP), cefoperazone–sulbactam (SCP), ceftazidime (CAZ), ceftriaxone

(CRO), cefuroxime (CXM), ceftiofex (FOX), and aztreonam (ATM); 3) aminoglycoside antibiotics including gentamicin (GM), amikacin (AN), and tobramycin (NN); 4) tetracycline antibiotics including minocycline (MI), tetracycline (TE), and tigecycline (TGC); 5) quinolone antibiotics including moxifloxacin (MXF), ciprofloxacin (CIP), levofloxacin (LVX), and norfloxacin (NOR); 6) furan-class antibiotics including furan (FM); 7) peptide antibiotics including colistin (CL); and 8) chloramphenicol (C). The drug sensitivity results were assessed by reference to the American Institute of Clinical Laboratory Standardization (2022), and isolates resistant to more than three classes of antimicrobials were identified as MDR isolates. TGC and MXF were interpreted according to the standards recommended by the U.S. Food and Drug Administration (FDA).

### Integron Detection in CRE

Using integrase gene-specific primers, CRE carrying class I and class II integrons situation were detected by PCR. The relevant detection primer sequences and PCR conditions are shown in Table S1.

### Drug Resistance Genetic Screening for CRE

The DNA template was prepared by boiling extraction (Li et al., 2020). Six classes of 24 drug resistance genes were detected in CRE, including carbapenem ( $bla_{NDM}$ ,  $bla_{IMP}$ ,  $bla_{KPC}$ ,  $bla_{VIM}$ ,  $bla_{SPM}$ ,  $bla_{GIM}$ , and  $bla_{OXA}$ ) and other genes associated with resistance to  $\beta$ -lactams ( $bla_{TEM}$ ,  $bla_{SHV}$ , and  $bla_{CTX-M}$ ), aminoglycoside ( $aac(6')-Ib-cr$ ,  $aac1$ ,  $aac2$ , and  $aac3$ ), chloramphenicol ( $cmlA$  and  $stcM$ ), quinolone ( $qnrA$ ,  $qnrB$ ,  $qnrC$ ,  $qnrS$ , and  $oqxA$ ), and sulfonamide ( $sul1$ ,  $sul2$ , and  $sul3$ ) resistance genes. All primer-related information about the resistance genes is shown in Table S2.

## RESULTS

### Detection and Identification of CRE

As Figure 1 shows, 12 CRE were isolated in the samples collected across Shandong Province. CRE isolates were detected on broiler farms in 4 of the 6 cities: Tai'an, Liaocheng, Linyi, and Heze. Isolation rates were 3.57% (6/168) for carbapenem-resistant *E. coli*, 10% (5/50) for carbapenem-resistant *P. mirabilis*, and 3.03% (1/33) for carbapenem-resistant *K. pneumoniae*. Six strains of CRE were isolated in Linyi—3 strains of *E. coli* from feces, air, and anal swabs and 3 strains of *P. mirabilis* from anal swabs—for an overall isolation rate of 15.38%. Meanwhile, three strains of CRE were isolated in Heze—an *E. coli* strain, a *P. mirabilis* strain, and a *K. pneumoniae* strain—all from anal swabs, for an isolation rate of 9.68%. In Tai'an, 2 strains of CRE were isolated from feces and air, for an isolation rate of 3.64%, and, in Liaocheng, a strain of CRE was isolated from an anal swab of *P. mirabilis*, for an isolation rate of 2.17% (Table 1).

### Drug Resistance of CRE

For the 12 strains of CRE, eight classes of 27 antibiotics showed different degrees of resistance, with the rates of imipenem (IPM), ampicillin–sulbactam (SAM), ceftazolin (CZ), tetracycline (TE), ceftiofex (CRO), and cefuroxime (CXM) being as high as 100% (Figure 2A). The highest resistance rate was found for chloramphenicol (C), at 91.67%, followed by  $\beta$ -lactam at 85.61%, whereas the lowest resistance, 50%, was found for furan (FM). The drug resistance rates to most antibiotics exceeded 50% (Table S3), and all CRE strains had a multidrug resistance of 100% and were resistant to 15 to 25 antibiotics (Figure 2B). A heat map of the 9 CRE drug resistance profiles monitored on the 6 chicken farms appears in Figure 2C.

### Integron Carriage of CRE

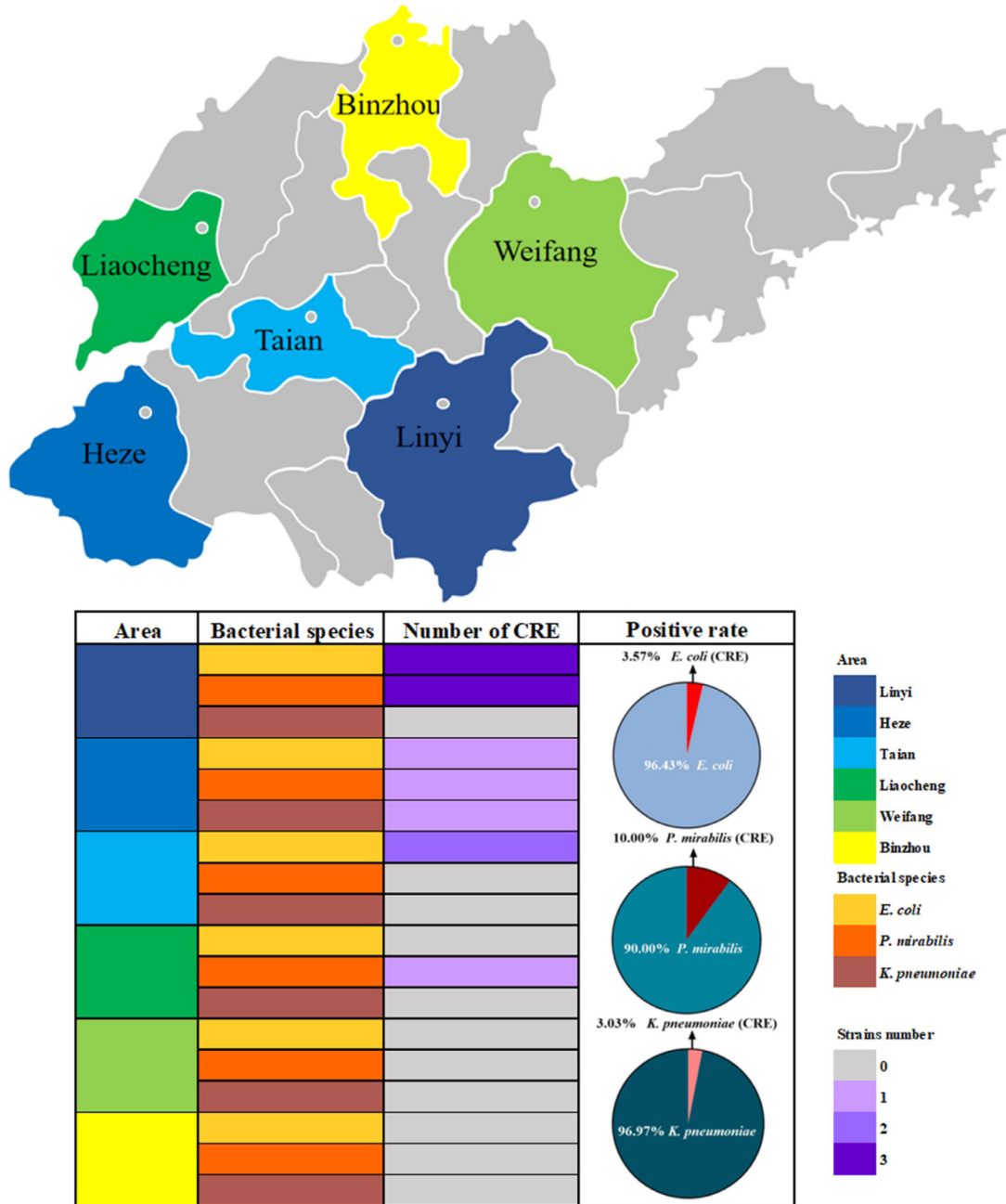
Of the 12 strains of CRE, 11 carried integrons in class I or II, whereas one carried neither. The integron carriage rate in those strains was 92%, and most carried class I integrons. All 6 strains of carbapenem-resistant *E. coli* carried class I integrons, including one strain that also carried class II integrons. Of the 5 strains of *P. mirabilis*, 3 carried class I integrons, 2 carried class II integrons, and one carried both. Beyond that, a carbapenem-resistant *K. pneumoniae* strain carried a class I integron (Table 2).

### Drug Resistance Genes of CRE

Six classes of 24 drug resistance genes were detected in the CRE, and, among the CRE isolates, the number of drug resistance genes detected varied (Figure 3A). One *E. coli* strain revealed the largest number of drug resistance genes (15), whereas one *P. mirabilis* strain revealed the fewest (4). Among the drug resistance genes,  $bla_{NDM}$  and  $stcM$  had the highest detection rate (91.67%), followed by  $bla_{CTX-M}$  (75%),  $sul1$  (75%),  $sul2$  (75%). The  $bla_{KPC}$ ,  $bla_{IMP}$ ,  $bla_{GIM}$ ,  $bla_{SPM}$ ,  $bla_{VIM}$ , and  $aac3$  genes were not detected (Table S4). Overall, 100% (12/12) of the CRE isolates contained at least one carbapenem resistance gene, 100% (12/12) contained at least one chloramphenicol resistance gene, 91.67% (11/12) contained at least one sulfonamide resistance gene, 83.33% (10/12) contained at least one aminoglycoside resistance gene, 75% (9/12) contained at least one  $\beta$ -lactam resistance gene, and 66.67% (8/12) contained at least one quinolone resistance gene (Figure 3B).

## DISCUSSION

Carbapenems are  $\beta$ -lactam antibiotics, which are generally suitable for severe Gram-negative bacterial infections, drug-resistant bacterial infections, and mixed infections due to their broad spectrum and strong



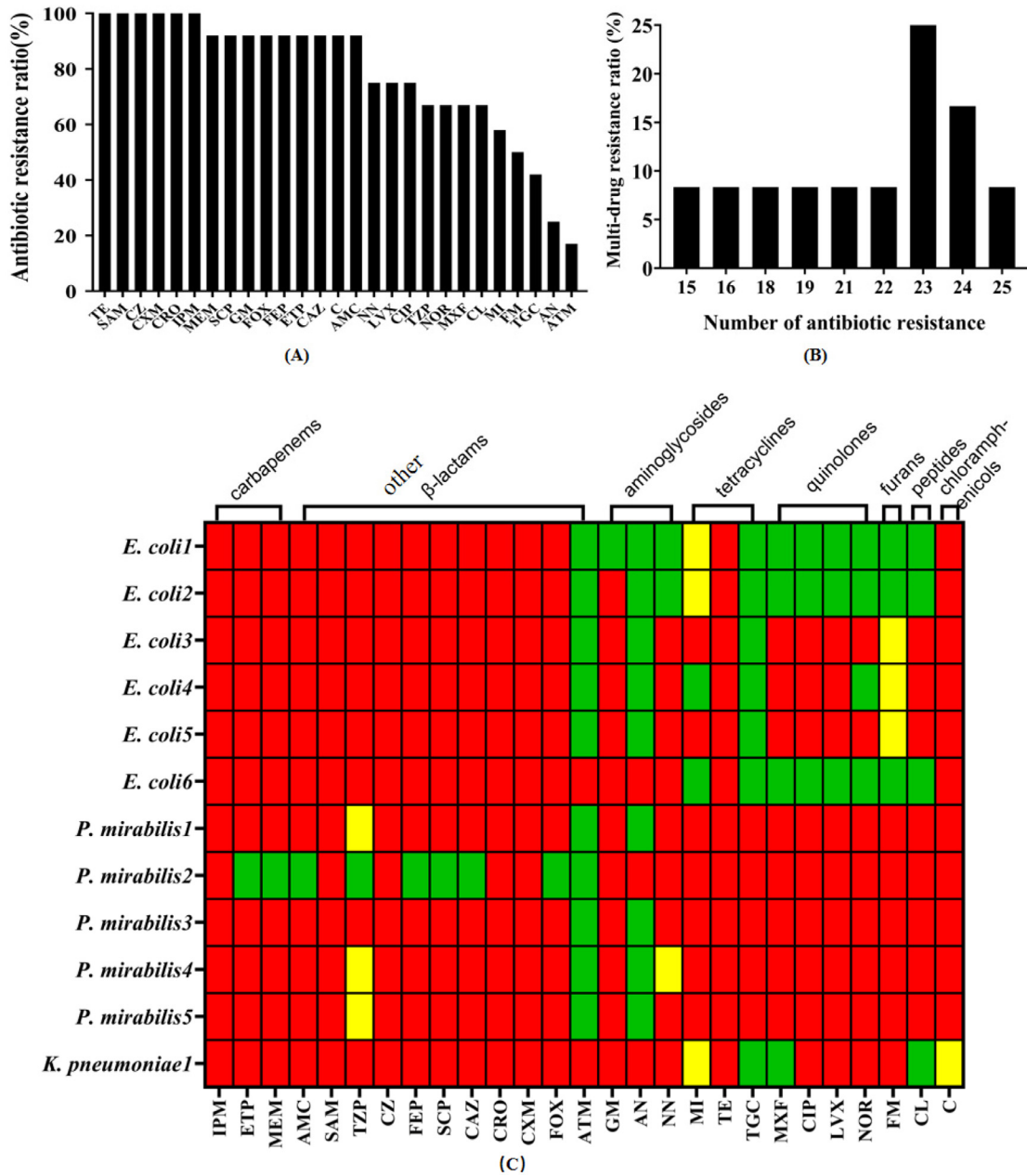
**Figure 1.** Geographic distribution of the sampling sites and CRE isolates across different prefecture-level cities in Shandong, China. A total of 12 CRE bacteria samples were collected from 6 broiler fattening farms in the Tai'an, Linyi, Liaocheng, Weifang, Binzhou, and Heze areas. Among them, Weifang and Binzhou have no CRE bacteria.

effects. Moreover, they are regarded as the last line of defense against MDR bacterial infections (Cheng et al., 2019). More recently, however, the emergence of CRE

able to resist such defenses increasingly poses a serious threat to the health of humans as well as livestock, especially poultry.

**Table 1.** Source status and isolation rate of CRE bacteria from six broiler farms.

Area	<i>E. coli</i>					<i>P. mirabilis</i>					<i>K. pneumoniae</i>					Isolate rate
	Feces	Air	Swab	Walls	Water	Feces	Air	Swab	Walls	Water	Feces	Air	Swab	Walls	Water	
Taian	1/7	1/1	0/19	0/3	0/0	0/0	0/11	0/10	0/1	0/0	0/0	0/0	0/1	0/0	0/2	3.64%
Heze	0/7	0/0	1/15	0/0	0/2	0/0	0/0	1/1	0/0	0/0	0/0	0/0	1/3	0/0	0/3	9.68%
Linyi	1/10	1/1	1/17	0/2	0/0	0/0	0/3	3/5	0/0	0/1	0/0	0/0	0/0	0/0	0/0	15.38%
Liaocheng	0/12	0/3	1/14	0/0	0/1	0/0	0/3	0/1	0/0	0/0	0/1	0/0	0/4	0/0	0/7	2.17%
Weifang	0/9	0/1	0/18	0/0	0/2	0/0	0/4	0/9	0/0	0/0	0/0	0/0	0/4	0/0	0/1	0%
Binzhou	0/5	0/4	0/15	0/0	0/0	0/0	0/1	0/0	0/0	0/0	0/0	0/0	0/7	0/0	0/0	0%

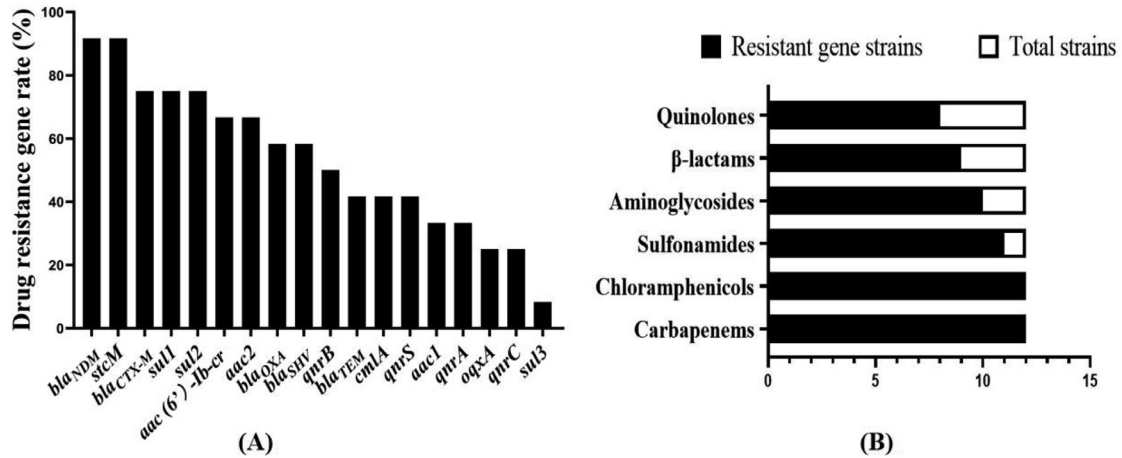


**Figure 2.** Drug resistance status of CRE bacteria. (A) Resistance rate of CRE bacteria to some antimicrobial agents. (B) The multidrug resistance of CRE bacteria. (C) CRE bacteria resistance spectrum, red indicates resistance, green indicates sensitivity, yellow indicates moderate sensitivity.

**Table 2.** The integron gene detection results.

Bacterial species	Strain number	Area	Class I	Class II
<i>E. coli</i>	1	Taian	+	-
	2	Taian	+	+
	3	Linyi	+	-
	4	Linyi	+	-
	5	Linyi	+	-
<i>P. mirabilis</i>	6	Heze	+	-
	1	Linyi	+	-
	2	Linyi	-	-
	3	Linyi	+	-
	4	Liaocheng	-	+
<i>K. pneumoniae</i>	5	Heze	+	+
	1	Heze	+	-

An important factor of bacterial resistance to antibiotics is bacteria’s tendency to carry resistance-related genes (Song et al., 2020; Liu et al., 2021b; Wang et al., 2021a; Liu et al., 2023). In past studies, the *bla<sub>NDM</sub>* gene has often been observed in poultry and poultry environments (Hamza et al., 2016; Zhu et al., 2021), while other resistance-related genes, including *bla<sub>KPC</sub>*, *bla<sub>IMP</sub>*, and *bla<sub>VIM-2</sub>*, have also appeared in carbapenem-resistant Gram-negative bacteria (Bocharova et al., 2020; Algammal et al., 2021; Elmonir et al., 2021). In our study, all CRE strains carried genes encoding carbapenemases, and we observed detection rates of 91.7% (11/12) for *bla<sub>NDM</sub>* and 58.3% (7/12) for *bla<sub>OXA</sub>*. However, no other genes involved in carbapenem-resistance were



**Figure 3.** Drug resistance genes of CRE. (A) The detection rates of antibiotic resistance genes in CRE isolates. (B) Detection rates of diverse classes of ARGs in CRE isolates.

found (Table S4), which suggests that among the characteristics of encoding class B and D enzymes (Cherak et al., 2021), the presence of *bla<sub>NDM</sub>* and *bla<sub>OXA</sub>* might be the main cause of resistance to carbapenem agents.

Most genes related to carbapenem resistance exist in various mobile genetic structures that collectively bolster the genes' transmission (Nordmann et al., 2012). Integrons in bacteria can take and spread resistance genes, which is an important means for the distribution of antibiotic resistance genes through horizontal transfer. In particular, of class I and class II integrons—the 2 most common types in clinical isolates—class I integrons play a major role in the transmission of antibiotic resistance genes (Koratzanis et al., 2011; Sabbagh et al., 2021). In our study, integrons were found in 11 CRE strains—90.9% (10/11) carried class I integrons, while 27.3% (3/11) carried class II integrons—and thus might be the chief factor in the risk of transmission of CRE resistance genes. Surprisingly, the resistance of CRE strains to chloramphenicol was as high as 91.7% (11/12), as shown in Figure 2; however, chloramphenicol has been banned for veterinary use for many years. Interestingly, all CRE strains carried chloramphenicol resistance genes, and *cmlA* was also encoded by class I integron (Ma et al., 2019). The characteristics of integrons that can carry multiple resistance genes (Bocharova et al., 2020) may be responsible for the emergence of resistance-related genes, thereby leading to a tolerance to chloramphenicol in CRE isolates. Moreover, we found that carbapenem resistance genes often appeared together with other drug resistance genes such as sulfonamide, aminoglycoside, and quinolone-related resistance genes (Figure 3B), which also indicates the possibility that various resistance genes coexist on the same integron. Therefore, the in-depth study of mobile elements encoding resistance genes is critical to understanding the mechanism of antibiotic resistance and will thus be the next focus of our research.

CRE strains present significant trouble when it comes to treating bacterial infections. In our study, CRE

strains exhibited MDR, with resistance rates to TE, SAM, CZ, and other drugs reaching 100% (Figure 2A). Furthermore, the isolates' resistance to 23 or 24 kinds of antibiotics was most common in CRE strains (Figure 2B), which showcases the feature of pan-resistance. Meanwhile, antibiotic resistance spectrum analysis revealed that none of the commonly used antibiotics could effectively inhibit 12 CRE isolates. Frustratingly, 41.7% (5/12) and 66.7% (8/12) of CRE strains were resistant to TGC and CL (Figures 2A and 2C), respectively, which were the last lines of defense against carbapenem-resistant bacteria (Chang et al., 2020; Yaghoubi et al., 2022). ATM and AN showed partial effects in fighting CRE in having the lowest resistance rate of CRE strains (Figures 2A and 2C). Beyond that, combining ATM, AN, CL, and TGC shows promise as an antagonist for CRE isolates. Therefore, the use of carbapenems should be carefully to effectively prevent public health problems.

In our study, CRE were detected on farms in Taian, Heze, Linyi, and Liaocheng but not in Binzhou and Weifang. Studies have shown that good biosafety measures and breeding environments are essential for broiler production (de Castro Burbarelli et al., 2017; Van Limbergen et al., 2018). The farm in Heze, built in 2008, has old breeding equipment and thus frequently confronts fecal accumulation that has led to the gathering of drug-resistant bacteria. Meanwhile, on the farm in Taian, built in 2010, ventilation equipment is poor, and ammonia is heavy in the air. Both of those farms have adopted traditional modes of raising chickens—that is, with the poultry standing on netting such that their feces is not cleaned up, and disinfection is not timely—which has resulted in a high rate of drug-resistant strains. In our study, 83.3% of CRE were isolated in swab or fecal samples (Table 1). Because animal feces is an important host for drug-resistant bacteria and antibiotic resistance genes (Furtula et al., 2010; Chuppava et al., 2019; Luiken et al., 2019, 2020) and because antibiotic-resistant bacteria can transmit antibiotic resistance to the environment through feces, the

disposal of feces is critical to curbing the emergence of resistant bacteria. No CRE were detected on the farms in Binzhou and Weifang, however, primarily because they were both built in 2019, meaning a short operation time coupled with advanced breeding equipment. Therefore, our experimental results also verified that the breeding environment and duration of a farm's operation may have a certain impact on the prevalence of CRE (Table S5). Terrible environments and the long-term use of antibiotics lead to the accumulation of CRE strains, which only increases the prevalence of resistant microorganisms on older farms. Therefore, regular environmental disinfection and hygienic cleaning should be performed on farms, and poultry feces should be disposed of regularly to prevent the accumulation of any resistance of CRE strains.

## CONCLUSIONS

Our findings clarify the characteristics of the current epidemic of CRE bacteria and their drug resistance characteristics on poultry farms in Shandong Province, China. The isolation rates of carbapenem-resistant *E. coli*, *P. mirabilis*, and *K. pneumoniae* were 3.57% (6/168), 10% (5/50), and 3.03% (1/33), respectively. All 12 CRE strains had MDR, and the detection rate of the drug resistance gene *bla*<sub>NDM</sub> and *stcM* were the highest (91.67%). The carriage rate of integrons in CRE was 91.67%, and 2 strains carried both class I and class II integrons. We also found, for the first time ever, *E. coli*, *P. mirabilis*, and *K. pneumoniae*—that is, three CRE bacteria—on the same broiler farm. Altogether, our results suggests that to prevent a sudden, severe epidemic of such resistant strains, researchers should strengthen epidemiological investigations and research on resistance mechanisms of resistant bacteria, as well as actively monitor drug-resistant genes in order to better prevent and control zoonotic diseases.

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Ethical approval: The authors confirm that the ethical policies of the journal, as noted on the journal's author guidelines page, have been adhered to and the appropriate ethical review committee approval has been received.

Data availability statement: The data that support the findings of this study are openly available in the article, supplementary material. The raw data supporting the conclusions of this manuscript will be made available by the authors, without undue reservation, to any qualified researcher.

## DISCLOSURES

The authors declare no conflict of interest.

## SUPPLEMENTARY MATERIALS

Supplementary material associated with this article can be found in the online version at [doi:10.1016/j.psj.2023.102483](https://doi.org/10.1016/j.psj.2023.102483).

## REFERENCES

- Aires-de-Sousa, M., J. M. Ortiz de la Rosa, M. L. Gonçalves, A. L. Pereira, P. Nordmann, and L. Poirel. 2019. Epidemiology of carbapenemase-producing *Klebsiella pneumoniae* in a hospital, Portugal. *Emerg. Infect. Dis.* 25:1632–1638.
- Algammal, A. M., H. R. Hashem, K. J. Alffi, H. F. Hetta, N. S. Sheraba, H. Ramadan, and R. M. El-Tarabili. 2021. atpD gene sequencing, multidrug resistance traits, virulence-determinants, and antimicrobial resistance genes of emerging XDR and MDR-*Proteus mirabilis*. *Sci. Rep.* 11:9476.
- Bi, S., H. Yan, M. Chen, Z. Zhang, L. Shi, and H. Wang. 2011. New variant *Salmonella* genomic island 1-U in *Proteus mirabilis* clinical and food isolates from South China. *J. Antimicrob. Chemother.* 66:1178–1179.
- Bocharova, Y., T. Savinova, A. Lazareva, S. Polikarpova, N. Gordinskaya, N. Mayanskiy, and I. Chebotar. 2020. Genotypes, carbapenemase carriage, integron diversity and oprD alterations among carbapenem-resistant *Pseudomonas aeruginosa* from Russia. *Int. J. Antimicrob. Agents* 55:105899.
- Chang, J., B. Tang, Y. Chen, X. Xia, M. Qian, and H. Yang. 2020. Two IncHI2 plasmid-mediated colistin-resistant *Escherichia coli* strains from the broiler chicken supply Chain in Zhejiang province, China. *J. Food Prot.* 83:1402–1410.
- Cheng, P., F. Li, R. Liu, Y. Yang, T. Xiao, M. Ishfaq, G. Xu, and X. Zhang. 2019. Prevalence and molecular epidemiology characteristics of carbapenem-resistant *Escherichia coli* in Heilongjiang Province, China. *Infect. Drug Resist.* 12:2505–2518.
- Cherak, Z., L. Loucif, A. Moussi, and J. M Rolain. 2021. Carbapenemase-producing gram-negative bacteria in aquatic environments: a review. *J. Glob. Antimicrob. Resist.* 25:287–309.
- Chuppava, B., B. Keller, A. Abd El-Wahab, C. Sürie, and C. Visscher. 2019. Resistance reservoirs and multi-drug resistance of commensal *Escherichia coli* from excreta and manure isolated in broiler houses with different flooring designs. *Front. Microbiol.* 10:2633.
- Clinical and Laboratory Standards Institute (CLSI). 2022. Performance Standards for Antimicrobial Susceptibility Testing. 32nd ed. CLSI, Wayne, PA Volume M100.
- de Castro Burbarelli, M. F., G. do Valle Polycarpo, K. Deliberali Lelis, C. A. Granghelli, A. C. Carão de Pinho, S. Ribeiro Almeida Queiroz, A. M. Fernandes, R. L. Moro de Souza, M. E. Gaglianone Moro, R. de Andrade Bordin, and R. de Albuquerque. 2017. Cleaning and disinfection programs against *Campylobacter jejuni* for broiler chickens: productive performance, microbiological assessment and characterization. *Poult. Sci.* 96:3188–3198.
- Elmonir, W., N. K. Abd El-Aziz, Y. H. Tartor, S. M. Moustafa, E. M. Abo Remela, R. Eissa, H. A. Saad, and A. A Tawab. 2021. Emergence of colistin and carbapenem resistance in extended-spectrum beta-lactamase producing *Klebsiella pneumoniae* isolated from chickens and humans in Egypt. *Biology (Basel)* 10:743468, doi:10.3389/fmicb.2021.743468.

- Feng J., Q. Xiang, J. Ma, P. Zhang, K. Li, K. Wu, M. Su, R. Li, D. Hurley, L. Bai, J. Wang, and Z. Yang. 2021. Characterization of Carbapenem-Resistant Enterobacteriaceae Cultured From Retail Meat Products, Patients, and Porcine Excrement in China. *Front. Microbiol.* 12:743468.
- Furtula, V., E. G. Farrell, F. Diarrassouba, H. Rempel, J. Pritchard, and M. S. Diarra. 2010. Veterinary pharmaceuticals and antibiotic resistance of *Escherichia coli* isolates in poultry litter from commercial farms and controlled feeding trials. *Poult. Sci.* 89:180–188.
- Hamza, E., S. M. Dorgham, and D. A. Hamza. 2016. Carbapenemase-producing *Klebsiella pneumoniae* in broiler poultry farming in Egypt. *J. Glob. Antimicrob. Resist.* 7:8–10.
- Hu, F., D. Zhu, F. Wang, and M. Wang. 2018. Current status and trends of antibacterial resistance in China. *Clin. Infect. Dis.* 67: S128–S134.
- Iovleva, A., and Y. Doi. 2017. Carbapenem-resistant Enterobacteriaceae. *Clin. Lab. Med.* 37:303–315.
- Jia, X., W. Dai, W. Ma, J. Yan, J. He, S. Li, C. Li, S. Yang, X. Xu, S. Sun, J. Shi, and L. Zhang. 2018. Corrigendum: carbapenem-resistant *E. cloacae* in Southwest China: molecular analysis of resistance and risk factors for infections caused by *NDM-1*-producers. *Front. Microbiol.* 9:2694.
- Koratzanis, E., M. Souli, I. Galani, Z. Chryssouli, A. Armaganidis, and H. Giamarellou. 2011. Epidemiology and molecular characterisation of metallo-beta-lactamase-producing Enterobacteriaceae in a university hospital intensive care unit in Greece. *Int. J. Antimicrob. Agents* 38:390–397.
- Landman, W. J., and J. H. van Eck. 2015. The incidence and economic impact of the *Escherichia coli* peritonitis syndrome in Dutch poultry farming. *Avian Pathol.* 44:370–378.
- Leverstein-van Hall, M. A., C. M. Dierikx, J. Cohen Stuart, G. M. Voets, M. P. van den Munckhof, A. van Essen-Zandbergen, T. Platteel, A. C. Fluit, N. van de Sande-Bruinsma, J. Scharinga, M. J. Bonten, and D. J. Mevius. 2011. Dutch patients, retail chicken meat and poultry share the same ESBL genes, plasmids and strains. *Clin. Microbiol. Infect.* 17:873–880.
- Li, S., F. Z. Guo, X. J. Zhao, Q. Wang, H. Wang, Y. Z. An, and F. X. Zhu. 2019. Impact of individualized active surveillance of carbapenem-resistant Enterobacteriaceae on the infection rate in intensive care units: a 3-year retrospective study in a teaching hospital of People's Republic of China. *Infect. Drug Resistance* 12:1407–1414.
- Li, X., L. Li, L. Yu, S. Liu, L. Liu, X. Wei, Y. Song, C. Liu, M. Jiang, and F. Wang. 2020. Prevalence of avian-origin *mcr-1*-positive *Escherichia coli* with a potential risk to humans in Tai'an, China. *Poult. Sci.* 99:5118–5126.
- Li Z., C. Peng, G. Zhang, Y. Shen, Y. Zhang, C. Liu, M. Liu, and F. Wang. 2022. Prevalence and characteristics of multidrug-resistant *Proteus mirabilis* from broiler farms in Shandong Province, China. *Poult. Sci.* 101:101710.
- Li Z., L. Xin, C. Peng, C. Liu, P. Wang, L. Yu, M. Liu, and F. Wang. 2022. Prevalence and antimicrobial susceptibility profiles of ESBL-producing *Klebsiella pneumoniae* from broiler chicken farms in Shandong Province, China. *Poult. Sci.* 101:102002.
- Liu C., P. Wang, Y. Dai, Y. Liu, Y. Song, L. Yu, C. Feng, M. Liu, Z. Xie, Y. Shang, S. Sun, and F. Wang. 2021. Longitudinal monitoring of multidrug resistance in *Escherichia coli* on broiler chicken fattening farms in Shandong, China. *Poult. Sci.* 100:100887.
- Liu C., C. Feng, Y. Duan, P. Wang, C. Peng, Z. Li, L. Yu, M. Liu, and F. Wang. 2023. Ecological risk under the dual threat of heavy metals and antibiotic resistant *Escherichia coli* in swine-farming wastewater in Shandong Province, China. *Environ. Pollut.* 319:120998.
- Liu, B. T., F. J. Song, M. Zou, Q. D. Zhang, and H. Shan. 2017. High incidence of *Escherichia coli* strains coharboring *mcr-1* and *bla<sub>NDM</sub>* from chickens. *Antimicrob. Agents Chemother.* 61:e02347–16.
- Liu, C., Y. Liu, C. Feng, P. Wang, L. Yu, D. Liu, S. Sun, and F. Wang. 2021. Distribution characteristics and potential risks of heavy metals and antimicrobial resistant *Escherichia coli* in dairy farm wastewater in Tai'an, China. *Chemosphere* 262:127768.
- Luiken, R. E. C., L. Van Gompel, A. Bossers, P. Munk, P. Joosten, R. B. Hansen, B. E. Knudsen, S. García-Cobos, J. Dewulf, F. M. Aarestrup, J. A. Wagenaar, L. A. M. Smit, D. J. Mevius, D. J. J. Heederik, and H. Schmitt. 2020. Farm dust resistomes and bacterial microbiomes in European poultry and pig farms. *Environ. Int.* 143:105971.
- Luiken, R. E. C., L. Van Gompel, P. Munk, S. Sarrazin, P. Joosten, A. Dorado-García, R. Borup Hansen, B. E. Knudsen, A. Bossers, J. A. Wagenaar, F. M. Aarestrup, J. Dewulf, D. J. Mevius, D. J. J. Heederik, L. A. M. Smit, and H. Schmitt. 2019. Associations between antimicrobial use and the faecal resistome on broiler farms from nine European countries. *J. Antimicrob. Chemother.* 74:2596–2604.
- Ma, X., N. Guo, S. Ren, S. Wang, and Y. Wang. 2019. Response of antibiotic resistance to the co-existence of chloramphenicol and copper during bio-electrochemical treatment of antibiotic-containing wastewater. *Environ. Int.* 126:127–133.
- Nordmann, P., L. Dortet, and L. Poirel. 2012. Carbapenem resistance in Enterobacteriaceae: here is the storm!. *Trends Mol. Med.* 18:263–272.
- Sabbagh, P., M. Rajabnia, A. Maali, and E. Ferdosi-Shahandashti. 2021. Integron and its role in antimicrobial resistance: a literature review on some bacterial pathogens. *Iran J. Basic Med. Sci.* 24:136–142.
- Sanches, M. S., A. A. S. Baptista, M. de Souza, M. F. Menck-Costa, L. Justino, E. K. Nishio, A. Oba, A. Bracarense, and S. P. D. Rocha. 2020. *Proteus mirabilis* causing cellulitis in broiler chickens. *Braz. J. Microbiol.* 51:1353–1362.
- Sheu, C. C., Y. T. Chang, S. Y. Lin, Y. H. Chen, and P. R. Hsueh. 2019. Infections caused by carbapenem-resistant Enterobacteriaceae: an update on therapeutic options. *Front. Microbiol.* 10:80.
- Song Y., L. Yu, Y. Zhang, Y. Dai, P. Wang, C. Feng, M. Liu, S. Sun, Z. Xie, and F. Wang. 2020. Prevalence and characteristics of multidrug-resistant *mcr-1*-positive *Escherichia coli* isolates from broiler chickens in Tai'an, China. *Poult. Sci.* 99:1117–1123.
- Sun, Y., M. Li, L. Chen, H. Chen, X. Yu, J. Ye, Y. Zhang, C. Ma, and T. Zhou. 2016. Prevalence and molecular characterization of carbapenemase-producing gram-negative bacteria from a university hospital in China. *Infect. Dis. (Lond)* 48:138–146.
- Tian, D., B. Wang, H. Zhang, F. Pan, C. Wang, Y. Shi, and Y. Sun. 2020. Dissemination of the *bla<sub>NDM-5</sub>* gene via IncX3-type plasmid among Enterobacteriaceae in children. *mSphere* 5:e00699–19.
- Van Limbergen, T., J. Dewulf, M. Klinkenberg, R. Ducatelle, P. Gelaude, J. Méndez, K. Heinola, S. Papisolomontos, P. Szeleszczuk, and D. Maes. 2018. Scoring biosecurity in European conventional broiler production. *Poult. Sci.* 97:74–83.
- Wang, F., W. Zhang, and D. Niu. 2021. Editorial: foodborne Enterobacteriaceae of animal origin. *Front. Cell Infect. Microbiol.* 11:772359.
- Wang, Y., C. Wu, Q. Zhang, J. Qi, H. Liu, Y. Wang, T. He, L. Ma, J. Lai, Z. Shen, Y. Liu, and J. Shen. 2012. Identification of New Delhi metallo- $\beta$ -lactamase 1 in *Acinetobacter lwoffii* of food animal origin. *PLoS One* 7:e37152.
- Wang W., L. Yu, W. Hao, F. Zhang, M. Jiang, S. Zhao, and F. Wang. 2021. Multi-Locus Sequence Typing and Drug Resistance Analysis of Swine Origin *Escherichia coli* in Shandong of China and Its Potential Risk on Public Health. *Front. Public Health.* 9:780700.
- Woodford, N., D. W. Wareham, B. Guerra, and C. Teale. 2014. Carbapenemase-producing Enterobacteriaceae and non-Enterobacteriaceae from animals and the environment: an emerging public health risk of our own making? *J. Antimicrob. Chemother.* 69:287–291.
- Xie, X., J. Zhang, H. N. Wang, and C. W. Lei. 2021. Whole genome sequence of a New Delhi metallo- $\beta$ -lactamase 1-producing *Proteus mirabilis* isolate SNYG35 from broiler chicken in China. *J. Glob. Antimicrob. Resist.* 24:266–269.
- Yaghoubi, S., A. O. Zekiy, M. Krutova, M. Gholami, E. Kouhsari, M. Sholeh, Z. Ghafouri, and F. Maleki. 2022. Tigecycline antibacterial activity, clinical effectiveness, and mechanisms and epidemiology of resistance: narrative review. *Eur. J. Clin. Microbiol. Infect. Dis.* 41:1003–1022.
- Yang, Y., Y. Peng, J. Jiang, Z. Gong, H. Zhu, K. Wang, Q. Zhou, Y. Tian, A. Qin, Z. Yang, and S. Shang. 2021. Isolation and characterization of multidrug-resistant *Klebsiella pneumoniae* from



- raw cow milk in Jiangsu and Shandong provinces, China. *Transbound. Emerg. Dis* 68:1033–1039.
- Yigit, H., A. M. Queenan, G. J. Anderson, A. Domenech-Sanchez, J. W. Biddle, C. D. Steward, S. Alberti, K. Bush, and F. C. Tenover. 2001. Novel carbapenem-hydrolyzing beta-lactamase, *KPC-1*, from a carbapenem-resistant strain of *Klebsiella pneumoniae*. *Antimicrob. Agents Chemother.* 45:1151–1161.
- Zhu, X., Y. Zhang, Z. Shen, L. Xia, J. Wang, L. Zhao, K. Wang, W. Wang, Z. Hao, and Z. Liu. 2021, 2443. Characterization of *NDM-1*-producing carbapenemase in *proteus mirabilis* among broilers in China. *Microorganisms* 9.