

# Prevalence and antimicrobial susceptibility profiles of ESBL-producing *Klebsiella Pneumoniae* from broiler chicken farms in Shandong Province, China

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**ABSTRACT** *Klebsiella pneumoniae* (*K. pneumoniae*) is a conditionally pathogenic bacterium present in the intestinal or the respiratory tract of animals, and it is a common factor in acquired infections and a major threat to public health. Increased production of extended-spectrum  $\beta$ -lactamases (ESBLs) has become a serious issue in the treatment of *K. pneumoniae* infections. In this study, we examined the serotypes and antibiotic resistance profiles of *K. pneumoniae* isolated from broiler chickens on farms in Shandong Province, China. The *K. pneumoniae* isolation rate was 4.67% (33/707), and the serotype Capsular K54 (42.42%, 14/33) was the most prevalent serotype in broilers in Shandong. The antimicrobial susceptibility assay revealed that the 33 isolates were resistant to 28

antimicrobial drugs to varying degrees; among these, the highest resistance rate was observed for tetracyclines (90.91%), and the lowest rate of resistance was observed for moxifloxacin and fosfomycin (0%). The multidrug resistance (MDR) rate was 87.88% (29/33). The carrying rate of  $\beta$ -lactam-resistance genes was as high as 100%, with *bla*<sub>SHV</sub> having the highest rate (93.94%). It is worth noting that one carbapenem-resistant *K. pneumoniae* (CRKP) isolate carrying *bla*<sub>NDM-1</sub> and one colistin-resistant *K. pneumoniae* (COLR-KP) isolate carrying *mcr-3* were found in broiler chickens. This study indicates that ESBL-producing CRKP isolates and COLR-KP isolates have emerged on poultry farms in Shandong and could be a potential threat to food safety and public health.

**Key words:** *Klebsiella pneumoniae*, carbapenem-resistant, colistin-resistant, *bla*<sub>NDM-1</sub>, *mcr-3*

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## INTRODUCTION

As *K. pneumoniae* is a prominent zoonotic conditional pathogen in the Enterobacteriaceae family (Wang et al., 2020), its multidrug resistance (MDR) is worrying, given the risk of food-borne pathogens spreading to humans through the food chain and the emergence of super-resistant bacteria (Hartantyo et al., 2020). Human infection with this bacteria mainly causes urinary, respiratory, and bloodstream infections (Aires-de-Sousa et al., 2019). According to the 2021 bacterial resistance surveillance report of the China Bacterial

Resistance Surveillance Network (CARSS), the isolation rate of human *K. pneumoniae* ranked second, after *Escherichia coli* (*E. coli*), at 13.86% (<http://www.carss.cn/sys/account/login>). *K. pneumoniae* is also found in livestock and poultry farming; it mainly causes mastitis in cows (Yang et al., 2021) and respiratory symptoms in broiler chickens, and infection has a high mortality rate (Hamza et al., 2016). The general isolation rate of the bacteria is 9% to 35% (Hamza et al., 2016; Zhai et al., 2020; Yang et al., 2021).

The increasing resistance of bacteria to broad-spectrum  $\beta$ -lactam and peptide drugs has attracted public attention (Xiang et al., 2018; Song et al., 2020; Liu et al., 2021; Wang et al., 2021; Li et al., 2022).  $\beta$ -lactam drugs are the most commonly used drugs in clinical treatment of bacterial infections caused by Enterobacteriaceae, meaning that disease treatment has accelerated the process of bacterial resistance (Rubin and Pitout, 2014; Lee et al., 2021). In particular, the emergence of ESBL-producing bacteria has greatly restricted

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the use of such drugs. The global spread of ESBL-producing bacteria, especially ESBL-positive *E. coli* and *K. pneumoniae*, poses a challenge to treating MDR bacteria (Chong et al., 2018). The resistance of bacteria to colistin in peptide drugs has gradually gained attention as a result of greater attention to carbapenem-resistant *K. pneumoniae* (CRKP), and the emergence of the Mobile Colistin Resistance (*mcr*) gene poses no small threat to global public health (Wang et al., 2017a; Liu et al., 2021).

Environmental sources, food animals, and other factors have accelerated the spread of extended-spectrum  $\beta$ -lactamases (ESBL) in communities around the world (Chong et al., 2018)—especially the spread of carbapenem-resistant Enterobacteriaceae (CRE), which pose a major threat to global public health (Chen et al., 2014; Li et al., 2022). The first human-derived carbapenem-resistant gene *bla*<sub>KPC</sub> isolate of *K. pneumoniae* recorded in China was discovered in Zhejiang Province in 2004 (Wei et al., 2007). Since then, researchers have discovered *K. pneumoniae*-carrying *bla*<sub>NDM</sub> in livestock and poultry bred in China (He et al., 2017; Wang et al., 2017b; Xiang et al., 2018; Zhai et al., 2020). The emergence of CRE means that the last line of defense against MDR bacteria is threatened, making clinical treatment more challenging (Woodford et al., 2014; Hamza et al., 2016). It also means that this type of bacteria may spread from animals to humans through the food chain, endangering human health (Leverstein-van Hall et al., 2011).

However, *K. pneumoniae* in livestock, and especially in poultry, has not been systematically investigated in Shandong Province, China. This study aims to examine the epidemiological characteristics of MDR *K. pneumoniae* in six prefecture-level cities of Shandong, to assess the risk of animal-borne drug-resistant bacteria spreading to humans, and to provide epidemiological data for the emergence of MDR bacteria in the poultry industry. Here, we are the first to report one CRKP-carrying *bla*<sub>NDM-1</sub> isolate and one COLR-KP-carrying *mcr-3* isolate from 2 different poultry farms in Shandong. The data findings presented herein will develop our understanding of the prevalence and characteristics of *K. pneumoniae* strains in Shandong.

## MATERIALS AND METHODS

### Sample Collection and Identification of *K. pneumoniae*

The source of the sample is the same source in Liu et al. (2021). A total of 707 samples were collected from 6 large-scale broiler chicken farms—one each in the cities of Tai’an, Liaocheng, Weifang, Linyi, Heze, and Binzhou, all in Shandong—in May to July 2019 (Liu et al., 2021). The primary data were anal swabs and environmental samples of broiler chickens collected during a breeding cycle, with environmental samples coming from walls, water, feces, and air (Table 1). The water samples were centrifuged at 12,000 rpm for 60 s, after which most of the supernatant was discarded, and the remaining parts were cultured on MacConkey agar (Liu et al., 2021).

### Detection of *K. pneumoniae* Serotype

The test primarily screened for the 6 serotypes K1, K2, K5, K20, K54, and K57 (Wang et al., 2017b). The relevant detection primers are shown in the appended table S1. DNA templates were prepared using the boiling method of DNA extraction (Li et al., 2020). All the primers and annealing temperatures were slight modifications of those used in previously described procedures (Table S1).

### Antimicrobial Susceptibility Testing

The drug sensitivity tests were conducted using the BD Phoenix 100 NMIC/ID-4 composite board (Becton, Dickinson and Co., Franklin Lakes, NJ) (Li et al., 2020). Twenty-eight agents in 9 categories of commonly-used antibiotics were tested on 33 isolates. These agents included: Amikacin (AN), Amoxicillin-Clavulanate (AMC), Ampicillin-Sulbactam (SAM), Aztreonam (ATM), Cefazolin (CZ), Cefepime (FEP), Cefoperazone-Sulbactam (SCP), Cefoxitin (FOX), Ceftazidime (CAZ), Ceftriaxone (CRO), Cefuroxime (CXM), Chloramphenicol (C), Ciprofloxacin (CIP), Colistin (CL), Ertapenem (ETP), Fosfomycin (FF), Gentamicin (GM), Imipenem (IPM), Levofloxacin (LVX),

**Table 1.** Distribution of *K. pneumoniae*-positive samples collected from various locations on broiler chicken farms.

| Area       | % <i>K. pneumoniae</i> -positive samples (number of detections/total no.) for: |               |              |           |               |               |
|------------|--|---------------|--------------|-----------|---------------|---------------|
|            | Wall   | Water         | Feces        | Air       | Anal swab     | Total         |
| Tai’an     | 0 (0/12)   | 16.67 (2/12)  | 0 (0/18)     | 0 (0/26)  | 1.67 (1/60)   | 2.34 (3/128)  |
| He Ze      | 0 (0/6)  | 50.00 (3/6)   | 0 (0/12)     | 0 (0/12)  | 5.00 (3/60)   | 6.25 (6/96)   |
| Liao Cheng | 0 (0/12)   | 50.00 (7/14)  | 5.56 (1/18)  | 0 (0/22)  | 6.67 (4/60)   | 9.52 (12/126) |
| Lin Yi     | 0 (0/12)   | 0 (0/10)      | 0 (0/16)     | 0 (0/22)  | 0 (0/52)      | 0 (0/112)     |
| Wei Fang   | 0 (0/12)   | 8.33 (1/12)   | 0 (0/16)     | 0 (0/22)  | 6.67 (4/60)   | 4.10 (5/122)  |
| Bin Zhou   | 0 (0/12)   | 0 (0/12)      | 0 (0/20)     | 0 (0/18)  | 11.48 (7/61)  | 5.69 (7/123)  |
| Total      | 0 (0/66)   | 19.70 (13/66) | 1.00 (1/100) | 0 (0/122) | 4.25 (15/353) | 4.67 (33/707) |

Meropenem (MEM), Minocycline (MI), Moxifloxacin (MXF), Nitrofurantoin (FM), Norfloxacin (NOR), Piperacillin-Tazobactam (TZP), Tetracycline (TE), Tigecycline (TGC), and Tobramycin (NN). The results were interpreted in line with the Clinical and Laboratory Standards Institute guidelines (2019). MDR was defined as acquired nonsusceptibility to at least one agent in three or more antimicrobial categories.

### Screening for Antimicrobial-Resistance Genes

The  $\beta$ -lactam resistance-related genes (*bla*<sub>TEM</sub>, *bla*<sub>SHV</sub>, *bla*<sub>CTX-M1</sub>, *bla*<sub>CTX-M3</sub>, *bla*<sub>OXA23gp</sub>, *bla*<sub>OXA64gp</sub>, and *bla*<sub>DHA</sub>)—along with other genes associated with resistance to carbapenems (*bla*<sub>IMP-1</sub>, *bla*<sub>KPC-gp</sub>, *bla*<sub>NDM-1</sub>, *bla*<sub>OXA-48</sub>, and *bla*<sub>VIM</sub>), aminoglycosides (*aac* (6')-Ib-cr, *aac1*, *aac2*, and *aac3*), chloramphenicol (*cmlA* and *steM*), quinolones (*qnrA*, *qnrB*, *qnrC*, *qnrS*, and *oqxA*), peptides (*mcr-1* to *-10*), and tetracyclines (*tetB*)—were detected using Polymerase Chain Reaction (PCR). All the primers and annealing temperatures were slight modifications of those used in previously described procedures (Table S2).

## RESULTS

### Detection and Identification of *K. pneumoniae*

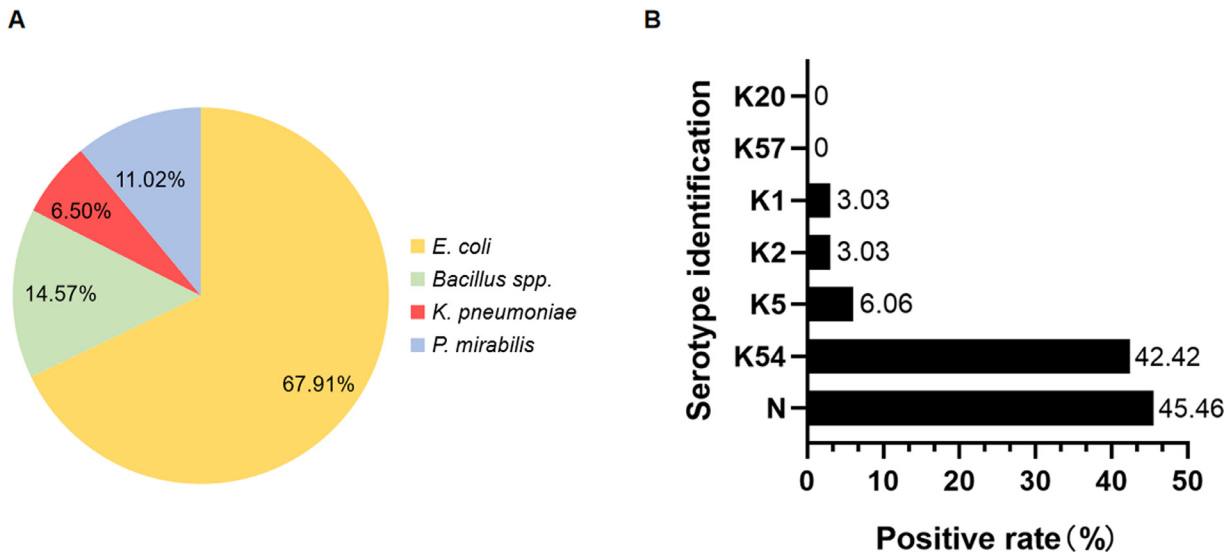
In this study, we examined the microbial diversity and antibiotic resistance profiles of bacteria isolated from broilers on 6 farms in Shandong. A total of 918 strains belonging to 128 species were isolated and identified from 707 samples; of these, *E. coli* (345/707, 53.04%) was the most frequently isolated, followed by *Proteus mirabilis* (56/707, 7.92%), *Bacillus cereus* (41/707,

5.80%), and *K. pneumoniae* (33/707, 4.67%)—that is, important zoonotic pathogens (Figure 1A). As shown in Table 1, the number of *K. pneumoniae* isolated from different broiler farms varies: 3 strains were isolated in Tai'an, 12 strains in Liaocheng, 5 strains in Weifang, 7 strains in Binzhou, 6 strains in Heze, and zero in Linyi. In terms of the sources of sample separation and collection, we found that the separation rate of water samples were 19.70%, followed by anal swabs (4.25%). The positive rates of water samples in Liaocheng and Heze were both 50%.

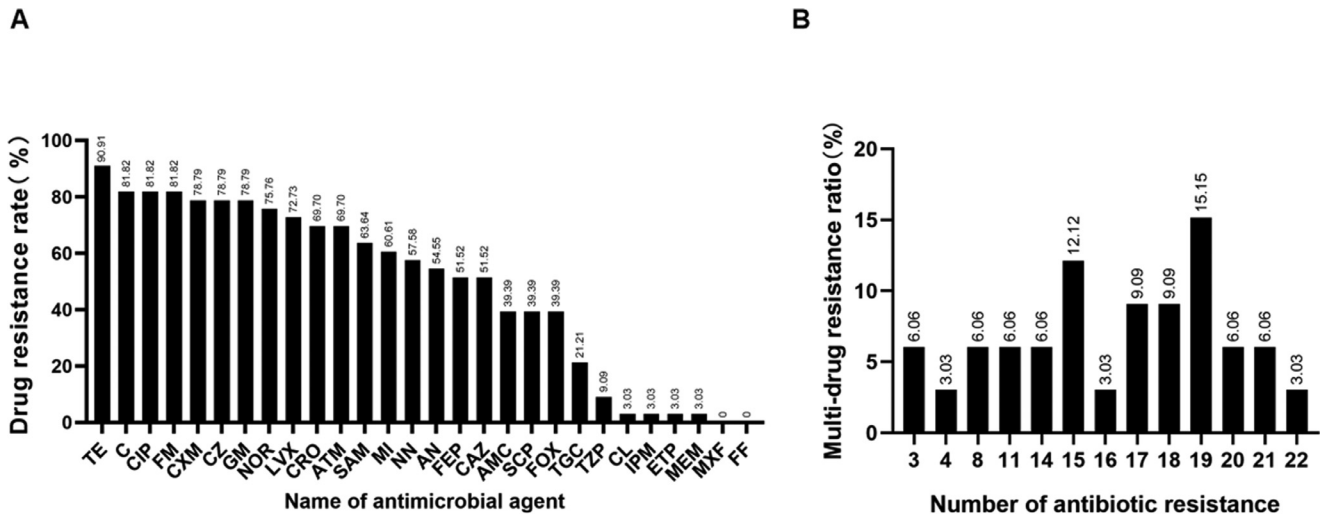
We used the method reported by Wang et al. (2017a) to perform serotyping analysis on *K. pneumoniae* isolates (Table S3). The analysis detected Capsular types K1 (3.03%), K2 (3.03%), K5 (6.06%), K54 (42.42%), and others (45.46%) in the sample. K20 and K57 were not detected (Figure 1B).

### Antimicrobial Resistance and MDR Profiles

As mentioned above, a total of 28 antibacterial agents in nine categories were screened in this study (Figure 2A). The drug susceptibility test of this experiment revealed different degrees of resistance to the 28 kinds of antibacterial drugs (Table S4). The resistance rate to tetracycline was the highest (90.91%), and the lowest resistance rate was to carbapenem drugs—that is, ETP, IPM, and MEM (3.03%); in addition, The *K. pneumoniae* isolates sensitive to both MXF and FF (0%). A high resistance rate of more than 50% was seen for 17 drugs, including FM and GM. In addition, for the first time, we discovered CRKP in poultry swabs from a poultry farm in Heze, Shandong, that was sensitive to FF, MXF, and TGC; the overall resistance rate of this farm was the highest among the six farms (63.69%) (Table S5). In terms of resistance to  $\beta$ -lactamase, *K. pneumoniae* showed a resistance rate of 50% to 80% for



**Figure 1.** The drug resistance, distribution map, and proportion of resistance genes and virulence genes of the *K. pneumoniae* strains in Shandong Province. (A) Proportion of strains in 707 samples. *E. coli* (345/707, 53.04%) was the most frequently isolated, followed by *Proteus mirabilis* (56/707, 7.92%), *Bacillus cereus* (41/707, 5.80%), and *K.pneumoniae* (33/707, 4.67%). (B) Serotype test results. The analysis detected Capsular types K1 (3.03%), K2 (3.03%), K5 (6.06%), K54 (42.42%), and others (45.46%) in the sample. K20 and K57 were not detected. N: others.



**Figure 2.** (A) A total of 28 antibacterial agents in nine categories were screened in this study. The resistance rate to tetracycline was the highest (90.90%), and the lowest resistance rate was to carbapenem drugs—that is, imipenem, ertapenem, and meropenem (3.03%). No strains were resistant to MXF and FF. A high resistance rate of more than 50% was seen for 17 drugs, including nitrofurantoin and gentamicin. In addition, for the first time, we discovered CRKP in poultry swabs from a farm in Heze that was only sensitive to tigecycline; the overall resistance rate of this farm was the highest of the 6 farms (63.69%). In terms of resistance to  $\beta$ -lactamase, *K. pneumoniae* showed a resistance rate of 50–80% for most cephalosporins and a resistance rate of 63.63% for ampicillin-sulbactam. (B) The antimicrobial susceptibility assay revealed that the MDR rate was 87.88% (29/33). Among the strains, the MDR number was concentrated in the range of 15–19 drugs, which accounted for 48.48% (16/33) of the MDR strains.

most cephalosporins, and a resistance rate of 63.64% for ampicillin-sulbactam.

In this study, MDR *K. pneumoniae* was found to be a serious phenomenon, as the bacteria demonstrate a MDR rate of 87.88%; most MDR strains are resistant to 15 to 19 kinds of drugs (48.48%, or 16/33), with the largest rates of resistance to 19 drugs, at 15.15% (5/33), while the highest resistance was to 22 kinds of drugs. The MDR rate of *K. pneumoniae* sampled in Heze is relatively common (63.69%) (Figure 2B). In addition, a total of 29 drug-resistance profiles were found in 5 of the chicken farms in Shandong (Table S5). The heat map of the drug-resistance profiles is shown in Figure 3. Among these profiles, there is no dominant drug resistance spectrum (number of drug-resistant strains  $\geq 5$ ) nor a main drug resistance spectrum (number of drug-resistant strains  $\geq 3$ ).

### Antimicrobial Resistance Gene Profiles

In this study, 7 kinds of 33 drug-resistance genes were detected against peptides, carbapenems, tetracyclines, aminoglycosides, chloramphenicols, quinolones, and  $\beta$ -lactams (Figure 4A). These *K. pneumoniae* isolates carried seven types and 16 drug-resistance genes, harboring 32 drug-resistance gene profiles (Figure 4B). The positive rate of drug-resistance genes ranged from 3.03% to 100%, with the highest positive rate being that of  $\beta$ -lactams (100%), and the lowest positive rate being that of carbapenems and peptides (3.03%).

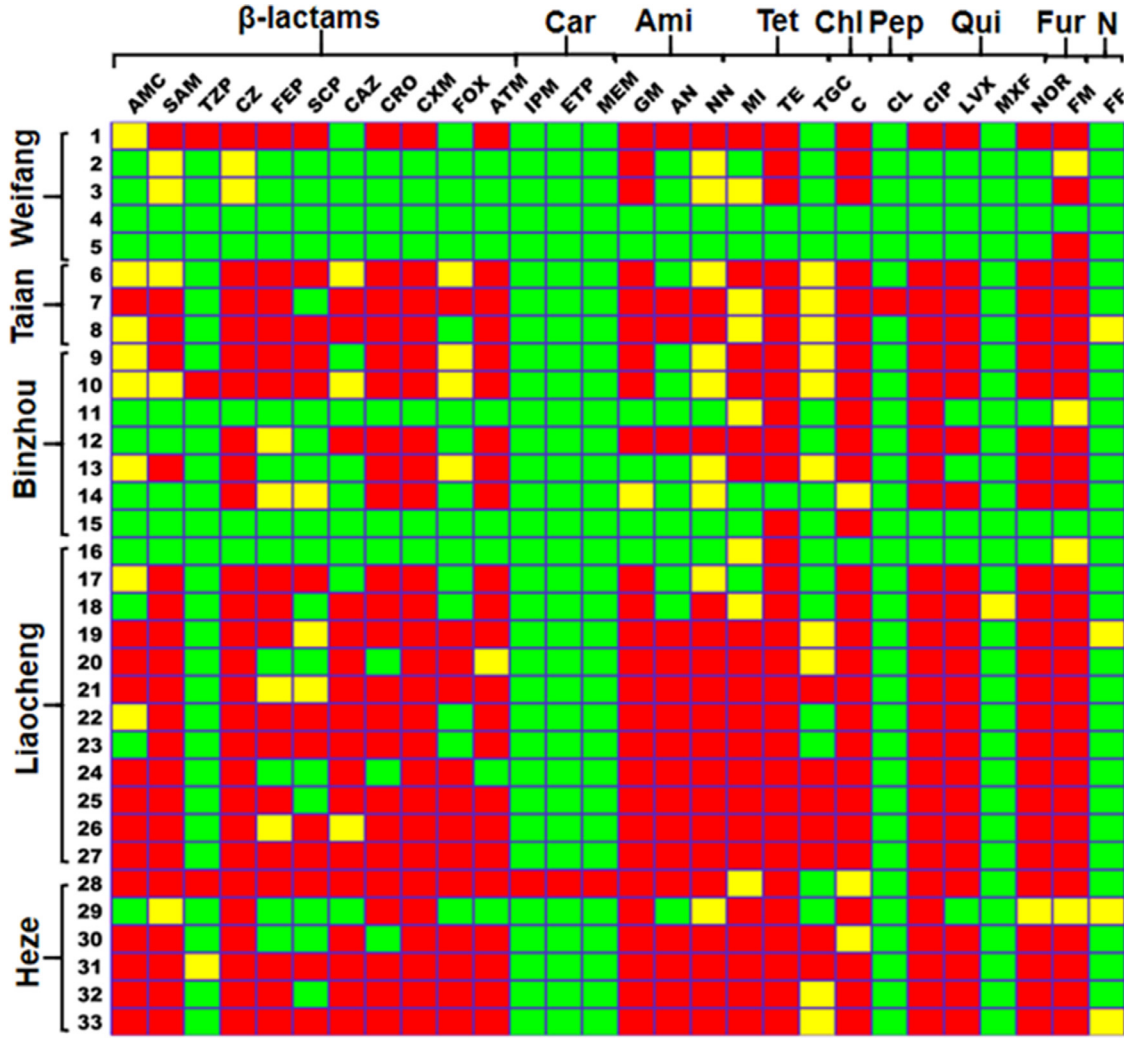
## DISCUSSION

The isolation rate of *K. pneumoniae* in this study was 33/707, or 4.67%. For these isolates, the total isolation

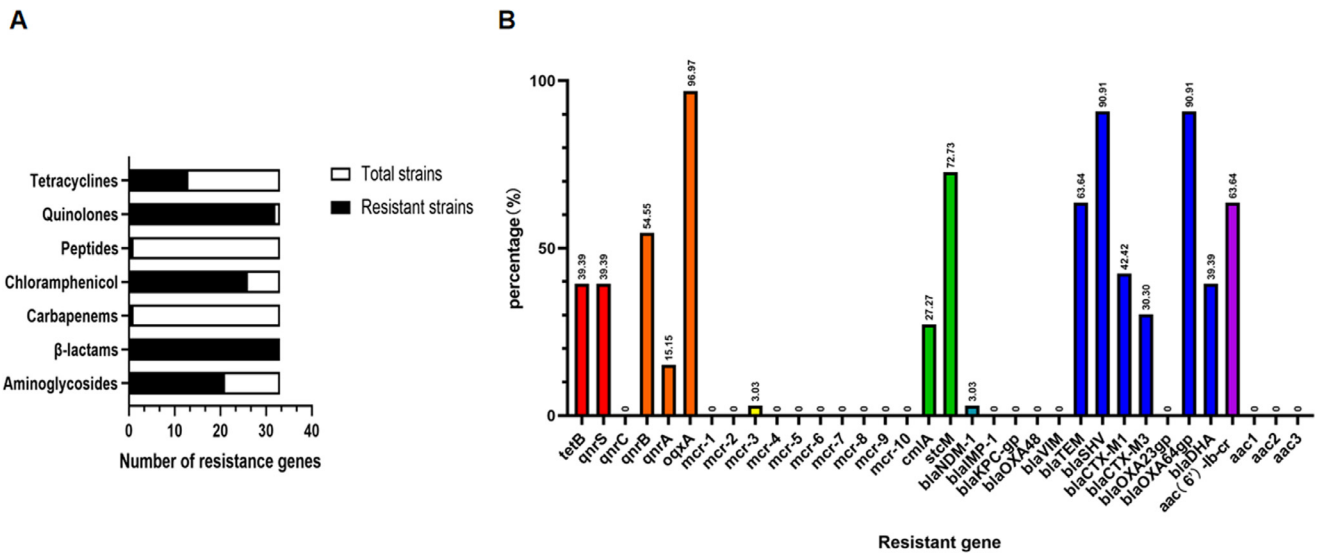
rate of *K. pneumoniae* was slightly lower than the 7.80% separation rate found on a poultry farm in India (Bhardwaj et al., 2021), which in turn was lower than the detection rate of 25.80% found in broiler cecum samples by the Norwegian veterinary department in 2018 (Franklin-Alming et al., 2021). The proportion is higher than that of bacteria isolated in Shandong dairy farms (1/233, 0.43%), but lower than the detection rate in Jiangsu dairy farms (7.83%) (Yang et al., 2021). In terms of the sources of sample separation and collection, we found that the separation rate of water samples reached 19.70%. It is well known that improper treatment of wastewater from livestock and poultry farms may cause the spread of MDR bacteria (Gomi et al., 2018); in addition, the use of contaminated irrigation water is another key reason behind this phenomenon (Furlan and Stehling, 2021). Therefore, it is necessary to pay more attention to the proper discharge of wastewater and sewage.

Capsule is an important virulence factor that protects *K. pneumoniae* from lethal serum factors and phagocytosis (Hsu et al., 2011). However, in some bacteria (such as *Streptococcus pneumoniae*), due to the frequent horizontal transfer of the capsular polysaccharide (CPS) operon that is responsible for the synthesis of capsular polysaccharides, the capsule (K) type may be distributed across many unrelated individuals (Wang et al., 2017a). Of the 77 K types described in the serotyping protocol, the serotypes K1, K2, K5, and K57 are most closely related to aggressiveness and high pathogenicity (Wang et al., 2017a; Turton et al., 2010; Wei et al., 2021). Of these types, K1 and K2 are the most important pathogenic serotypes, and the toxicity of K1 and K2 pneumonia has been verified in mice; the 2 types dominate human infections and are frequently reported in community-acquired pneumonia (Hsu et al., 2011).





**Figure 3.** The heat map of the drug-resistance profiles: red represents drug resistance, green represents sensitivity, and yellow represents moderate sensitivity. Car: Carbapenems, Ami: Aminoglycosides, Tet: Tetracyclines, Chl: Chloramphenicol, Pep: Peptides, Qui: Quinolones, Fur: Furans, N: Other types of antibiotics.



**Figure 4.** (A) A total of 7 kinds of 33 drug-resistance genes were detected against peptides, carbapenems, tetracyclines, aminoglycosides, chloramphenicol, quinolones, and  $\beta$ -lactams. The positive rate of drug-resistance genes ranged from 3.03% to 100%. The positive rate of  $\beta$ -lactams is the highest (100%), and the positive rate of carbapenems and peptides is the lowest (3.03%). (B) These *K. pneumoniae* isolates carried seven types and 16 drug-resistance genes, harboring 32 drug-resistance gene profiles. The positive rate of drug-resistance genes ranged from 3.03% to 100%. Red: tetracyclines drug-resistant genes, orange: quinolones drug-resistant genes, yellow: peptides drug-resistant genes, green: chloramphenicol drug-resistant genes, cyan-blue: carbapenems drug-resistant genes, blue:  $\beta$ -lactams drug-resistant genes, purple: aminoglycosides drug-resistant genes.

Aggressive diseases, such as liver abscess and endogenous endophthalmitis, are associated with the envelope serotypes K1 and K2 (Chew et al., 2017). In the present study, we found that the serotype Capsular K54 (42.42%, 14/33) was the most prevalent serotype in broilers in Shandong. There are almost no reports on serotypes in poultry, but there was a report on food-borne *K. pneumoniae* in Singapore: in this report, 8% (11 of 147) of isolates carried at least one type (K1, K2, or K54) (Hartantyo et al., 2020).

The potential risk of zoonotic bacteria spreading from animals to humans and the emergence of MDR bacteria and super bacteria are of great concern (Wang et al., 2021). Due to the lack of effective vaccines and other green prevention and control technologies, food-borne zoonotic bacterial diseases are usually treated with antibiotics. However, excessive and/or improper use of antibiotics in livestock and poultry has led to an increase in antimicrobial-resistant and MDR bacteria worldwide, becoming a global concern for human and animal health (Li et al., 2020; Wang et al., 2021; Yu et al., 2022). In this study, MDR *K. pneumoniae* was a serious phenomenon, as the bacteria showed a MDR rate of 87.88%—higher than the 22% MDR prevalence rate in Hong Kong, China, similar to the 85% rate in India (Poudel et al., 2019), and lower than the 96.67% rate in a commercial broiler slaughter factory in Shandong (Wu et al., 2016). These numbers fully reflect the MDR of *K. pneumoniae* (Wyres et al., 2020).

One of the reasons for bacterial resistance is the widespread existence of resistance genes. This feature is related to the production of plasmid-encoded antibiotic resistance genes (ARG), mainly observed in China, South Asia, and Southeast Asia (Qiao et al., 2018; Wyres et al., 2020). With the frequent use of antibiotics, *K. pneumoniae* can continuously accumulate ARG and form “super-resistant bacteria” (Navon-Venezia et al., 2017; Wang et al., 2020).

*K. pneumoniae* is an important zoonotic pathogen, and the prevalence of ESBL-producing *K. pneumoniae* makes it one of the MDR pathogens of particular public health significance. A study in the Netherlands showed that antibiotic-resistance genes may be transmitted from poultry through the food chain to humans who are in close contact with them, as similar ESBL-producing isolates have been found in poultry meat and humans that are in close contact (Leverstein-van Hall et al., 2011). In this study, the overall prevalence of ESBL genes in *K. pneumoniae* isolates was 100% ( $n = 33$ ), a proportion that is higher than: that of a Shandong broiler slaughterhouse, where 96.67% (87/90) were ESBL-producing strains (Wu et al., 2016); the ESBL production rate of a healthy meat and poultry farm in India (1/20, 5%) (Bhardwaj et al., 2021); that of broiler liver from Algeria, where the proportion of ESBL isolates was 3.70% (Chenouf et al., 2021); and Germany’s separation rate of chicken meat, which was 17.50% (35/200) (Eibach et al., 2018). The most common ESBL genes were  $bla_{SHV}$  ( $n = 31/33$ , 93.94%),  $bla_{OXA64gp}$  ( $n = 30/33$ , 90.91%), and  $bla_{TEM}$  ( $n = 21/33$ , 63.64%).

CRKP has attracted public attention because in the past, carbapenems were used to treat diseases caused by  $\beta$ -lactamase-resistant strains (Nicolau, 2008). Currently, as a result of the abuse of antibiotics, Gram-positive bacteria in many areas has begun to show resistance to carbapenem drugs (Tumbarello et al., 2012). Carbapenem-resistance genes primarily include  $bla_{KPC}$ ,  $bla_{IMP}$ ,  $bla_{VIM}$ ,  $bla_{OXA48}$ , and  $bla_{NDM}$  (Candan and Aksöz, 2015; Liu et al., 2016). In this paper, we detected only one strain of *K. pneumoniae* carrying  $bla_{NDM-1}$  in Shandong poultry farms; the other four carbapenem resistance-related genes— $bla_{KPC}$ ,  $bla_{IMP}$ ,  $bla_{VIM}$ , and  $bla_{OXA48}$ —were not detected. As of this writing, in livestock and poultry breeding, a handful of articles on CRKP carrying  $bla_{KPC}$ ,  $bla_{IMP}$ ,  $bla_{VIM}$ ,  $bla_{OXA48}$ , and  $bla_{NDM}$  have been reported in China and other countries (Candan and Aksöz, 2015; Hamza et al., 2016; Köck et al., 2018; Zhai et al., 2020; Chaalal et al., 2021; Ejikeugwu et al., 2021; Elmonir et al., 2021). For poultry isolates studied in Egypt, 15% (15/100) were resistant to carbapenems: all of them were positive for  $bla_{NDM}$  (Hamza et al., 2016). A study starting in 2017 identified 91 strains of CRKP and 72 strains of carbapenem-resistant *E. coli* in a broiler house in Hebei Province, China (Zhai et al., 2020). In a study of chicken in Algeria, a CRKP rate of 16.02% (29/181) was detected, which is a higher rate than this study (Chaalal et al., 2021); in addition, a rate of 10.53% of  $bla_{NDM}$  was detected in broilers in Egypt (2/19) (Elmonir et al., 2021). Research in Nigeria showed that of the 370 isolates collected from slaughterhouses, cows’ anuses, and poultry cloacas, 83.30% were found to be encoded by Metallo- $\beta$ -lactamase genes (especially  $bla_{IMP-1}$  and  $bla_{IMP-2}$ ) of *K. pneumoniae* (Ejikeugwu et al., 2021).  $bla_{OXA48}$  was first discovered in Turkey, and the co-infection of NDM-1 and OXA-48 was found in the country 3 years later, with a detection rate of 8% (4/50) (Candan and Aksöz, 2015). However, the co-infection of carbapenem resistance-related genes was not found in our tests in this study.

In recent years, the rate of resistance to colistin in *Enterobacteriaceae* has increased every year (Liu et al., 2016; Liu et al., 2021), especially in *K. pneumoniae*, which has led researchers from all over the world to conduct extensive research on this issue. A study in eastern China found a total of 53 strains of *K. pneumoniae* (4.5%, 53/1,171) that were confirmed to be COLR-KP, of which 8 strains carried the mobile colistin resistance (*mcr*) gene (Liu et al., 2021).

In this study, we make the first discovery of *K. pneumoniae* (in 33 isolates collected from 5 broiler chicken farms in Shandong) carrying these types of drug-resistance genes. In this study, 2 new drug-resistance genes were identified in poultry in Shandong. We found one strain (Capsular types K54) carrying  $bla_{NDM-1}$  genes in a Heze broiler factory and one strain carrying *mcr-3* in a Tai’an broiler factory (Table S5). It can be understood that the spread of carbapenem-resistant and colistin-resistant genes

brings no small challenges to treating livestock and poultry farms. Our discovery provides valuable data for monitoring the spread of *bla*<sub>NDM-1</sub>-producing and *mcr-3*-positive *K. pneumoniae* in poultry farms in Shandong and for monitoring its potential spread from broilers to humans. However, we did not detect important carbapenem-resistance genes and colistin-resistance genes other than *bla*<sub>NDM-1</sub> and *mcr-3*. Therefore, in the follow-up monitoring of MDR *K. pneumoniae*, the sampling range and quantity need to be further expanded.

## CONCLUSIONS

The *K. pneumoniae* isolation rate was 4.67% (33/707), and the serotype Capsular K54 (42.42%, 14/33) was the most prevalent serotype in broiler chickens in Shandong. The antimicrobial susceptibility assay revealed that the MDR rate was 87.88% (29/33). Among the strains, the MDR number was concentrated in the range of 15 to 19 drugs, which accounted for 48.48% (16/33) of the MDR strains. The carrying rate of  $\beta$ -lactam-resistance genes reached as high as 100%, with the highest rate for *bla*<sub>SHV</sub> (93.94%). It is worth noting that one CRKP isolate (serotype Capsular K54) carrying *bla*<sub>NDM-1</sub> genes was found in an anal swab sample in Heze. In addition, one COLR-KP isolate carrying *mcr-3* was found in water samples in Tai'an. This study indicates that ESBL-producing CRKP isolates and COLR-KP isolates emerging on poultry farms in Shandong could be a potential threat to food safety and public health.

## ETHICS STATEMENT

The study protocol and the poultry examinations were approved by the Animal Care and Use Committee of Shandong Agricultural University, Tai'an, China.

## AUTHOR CONTRIBUTIONS

FW and LX participated in the study design. ZL, CP, and LX carried out the study and drafted the manuscript. CL, PW, LY, and ML collected the important background information. All authors read and approved the final manuscript.

## ACKNOWLEDGMENTS

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## DISCLOSURES

No conflict of interest exists in the submission of this manuscript, and the manuscript has been approved for publication by all authors.

## SUPPLEMENTARY MATERIALS

Supplementary material associated with this article can be found in the online version at [doi:10.1016/j.psj.2022.102002](https://doi.org/10.1016/j.psj.2022.102002).

## REFERENCES

- Aires-de-Sousa, M., J. M. Ortiz de la Rosa, M. L. Goncalves, 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.
- Bhardwaj, K., M. S. Shenoy, S. B. U. Baliga, B. S. Baliga, and V. K. Shetty. 2021. Research Note: Characterization of antibiotic resistant phenotypes and linked genes of *Escherichia coli* and *Klebsiella pneumoniae* from healthy broiler chickens, Karnataka, India. *Poult. Sci.* 100:101094.
- Candan, E. D., and N. Aksöz. 2015. *Klebsiella pneumoniae*: characteristics of carbapenem resistance and virulence factors. *Acta Biochim. Pol.* 62:867–874.
- Chaalal, N., A. Touati, S. Bakour, M. A. Aissa, A. Sotto, J. P. Lavigne, and A. Pantel. 2021. Spread of OXA-48 and NDM-1-Producing *Klebsiella pneumoniae* ST48 and ST101 in Chicken Meat in Western Algeria. *Microb. Drug Resist.* 27:492–500.
- Chen, L., B. Mathema, K. D. Chavda, F. R. DeLeo, R. A. Bonomo, and B. N. Kreiswirth. 2014. Carbapenemase-producing *Klebsiella pneumoniae*: molecular and genetic decoding. *Trends Microbiol.* 22:686–696.
- Chenouf, N. S., I. Carvalho, C. R. Messai, L. Ruiz-Ripa, O. M. Mama, Y. Titouche, A. Zitouni, A. Hakem, and C. Torres. 2021. Extended spectrum  $\beta$ -lactamase-producing *Escherichia coli* and *Klebsiella pneumoniae* from Broiler Liver in the Center of Algeria, with detection of CTX-M-55 and B2/ST131-CTX-M-15 in *Escherichia coli*. *Microb. Drug Resist.* 27:268–276.
- Chew, K. L., R. T. P. Lin, and J. W. P. Teo. 2017. *Klebsiella pneumoniae* in Singapore: hypervirulent infections and the carbapenemase threat. *Front. Cell Infect. Microbiol.* 7:515.
- Chong, Y., S. Shimoda, and N. Shimono. 2018. Current epidemiology, genetic evolution and clinical impact of extended-spectrum  $\beta$ -lactamase-producing *Escherichia coli* and *Klebsiella pneumoniae*. *Infect. Genet. Evol.* 61:185–188.
- Eibach, D., D. Dekker, K. Gyau Boahen, C. Wiafe Akenten, N. Sarpong, C. Belmar Campos, L. Berneking, M. Aepfelbacher, R. Krumkamp, E. Owusu-Dabo, and J. May. 2018. Extended-spectrum beta-lactamase-producing *Escherichia coli* and *Klebsiella pneumoniae* in local and imported poultry meat in Ghana. *Vet Microbiol* 217:7–12.
- Ejikegwu, C., O. Nworie, M. Saki, H. O. M. Al-Dahmoshi, N. S. K. Al-Khafaji, C. Ezeador, E. Nwakaeze, P. Eze, E. Oni, C. Obi, I. Iroha, C. Esimone, and M. U. Adikwu. 2021. Metallo- $\beta$ -lactamase and AmpC genes in *Escherichia coli*, *Klebsiella pneumoniae*, and *Pseudomonas aeruginosa* isolates from abattoir and poultry origin in Nigeria. *BMC Microbiol* 21:124.
- 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)*. 10:373.
- Franklin-Alming, F. V., H. Kaspersen, M. A. K. Hetland, R. J. Bakksjø, L. L. Nesse, T. Leangapichart, I. H. Löhr, A. A. Telke, and M. Sunde. 2021. Exploring *Klebsiella pneumoniae* in healthy poultry reveals high genetic diversity, good biofilm-forming abilities and higher prevalence in Turkeys than broilers. *Front. Microbiol.* 12:725414.
- Furlan, J. P. R., and E. G. Stehling. 2021. Multiple sequence types, virulence determinants and antimicrobial resistance genes in multidrug- and colistin-resistant *Escherichia coli* from agricultural and non-agricultural soils. *Environ. Pollut.* 288:117804.
- Gomi, R., T. Matsuda, M. Yamamoto, P. H. Chou, M. Tanaka, S. Ichiyama, M. Yoneda, and Y. Matsumura. 2018. Characteristics of carbapenemase-producing *Enterobacteriaceae* in wastewater revealed by genomic analysis. *Antimicrob. Agents Chemother.* 62:1128.



- Hamza, E., S. M. Dorgham, and D. A. Hamza. 2016. Carbapenemase-producing *Klebsiella pneumoniae* in broiler poultry farming in Egypt. *J. Glob. Antimicrob. Res.* 7:8–10.
- Hartantyo, S. H. P., M. L. Chau, T. H. Koh, M. Yap, T. Yi, D. Y. H. Cao, R. A. GutiÉrrez, and L. C. Ng. 2020. Foodborne *Klebsiella pneumoniae*: virulence potential, antibiotic resistance, and risks to food safety. *J. Food Prot.* 83:1096–1103.
- He, T., Y. Wang, L. Sun, M. Pang, L. Zhang, and R. Wang. 2017. Occurrence and characterization of *bla*<sub>NDM-5</sub>-positive *Klebsiella pneumoniae* isolates from dairy cows in Jiangsu, China. *J. Antimicrob. Chemother.* 72:90–94.
- Hsu, C. R., T. L. Lin, Y. C. Chen, H. C. Chou, and J. T. Wang. 2011. The role of *Klebsiella pneumoniae rmpA* in capsular polysaccharide synthesis and virulence revisited. *Microbiology (Reading)* 157:3446–3457.
- Köck, R., I. Daniels-Haardt, K. Becker, A. Mellmann, A. W. Friedrich, D. Mevius, S. Schwarz, and A. Jurke. 2018. Carbapenem-resistant *Enterobacteriaceae* in wildlife, food-producing, and companion animals: a systematic review. *Clin. Microbiol. Infect.* 24:1241–1250.
- Lee, D., J. Y. Oh, S. Sum, and H. M. Park. 2021. Prevalence and antimicrobial resistance of *Klebsiella* species isolated from clinically ill companion animals. *J. Vet. Sci.* 22:e17.
- 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, 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.
- 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.
- 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.
- Liu, C., C. Feng, Y. Liu, P. Wang, L. Yu, M. 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.
- Liu, Y., Y. Lin, Z. Wang, N. Hu, Q. Liu, W. Zhou, X. Li, L. Hu, J. Guo, X. Huang, and L. Zeng. 2021. Molecular mechanisms of colistin resistance in *Klebsiella pneumoniae* in a tertiary care teaching hospital. *Front. Cell Infect. Microbiol.* 11:673503.
- Liu, Y., Y. Wang, T. R. Walsh, L. Yi, R. Zhang, J. Spencer, Y. Doi, G. Tian, B. Dong, X. Huang, L. Yu, D. Gu, H. Ren, X. Chen, L. Lv, D. He, H. Zhou, Z. Liang, J. Liu, and J. Shen. 2016. Emergence of plasmid-mediated colistin resistance mechanism MCR-1 in animals and human beings in China: a microbiological and molecular biological study. *Lancet Infect. Dis.* 16:161–168.
- Navon-Venezia, S., K. Kondratyeva, and A. Carattoli. 2017. *Klebsiella pneumoniae*: a major worldwide source and shuttle for antibiotic resistance. *FEMS Microbiol. Rev.* 41:252–275.
- Nicolau, D. P. 2008. Carbapenems: a potent class of antibiotics. *Expert Opin. Pharmacol.* 9:23–37.
- Poudel, A., T. Hathcock, P. Butaye, Y. Kang, S. Price, K. Macklin, P. Walz, R. Cattle, A. Kalalah, F. Adekanmbi, and C. Wang. 2019. Multidrug-resistant *Escherichia coli*, *Klebsiella pneumoniae* and *Staphylococcus spp.* in houseflies and blowflies from farms and their environmental settings. *Int. J. Environ. Res. Public Health* 16:3390.
- Qiao, M., G. G. Ying, A. C. Singer, and Y. G. Zhu. 2018. Review of antibiotic resistance in China and its environment. *Environ. Int.* 110:160–172.
- Rubin, J. E., and J. D. Pitout. 2014. Extended-spectrum beta-lactamase, carbapenemase and AmpC producing *Enterobacteriaceae* in companion animals. *Vet. Microbiol.* 170:10–18.
- 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.
- Tumbarello, M., P. Viale, C. Viscoli, E. M. Trecarichi, F. Tumietto, A. Marchese, T. Spanu, S. Ambretti, F. Ginocchio, F. Cristini, A. R. Losito, S. Tedeschi, R. Cauda, and M. Bassetti. 2012. Predictors of mortality in bloodstream infections caused by *Klebsiella pneumoniae* carbapenemase-producing *K. pneumoniae*: importance of combination therapy. *Clin. Infect. Dis.* 55:943–950.
- Turton, J. F., C. Perry, S. Elgohari, and C. V. Hampton. 2010. PCR characterization and typing of *Klebsiella pneumoniae* using capsular type-specific, variable number tandem repeat and virulence gene targets. *J. Med. Microbiol.* 59:541–547.
- Wang, Y., R. Zhang, J. Li, Z. Wu, W. Yin, S. Schwarz, J. M. Tyrrell, Y. Zheng, S. Wang, Z. Shen, Z. Liu, J. Liu, L. Lei, M. Li, Q. Zhang, C. Wu, Q. Zhang, Y. Wu, T. R. Walsh, and J. Shen. 2017a. Comprehensive resistome analysis reveals the prevalence of *NDM* and *MCR-1* in Chinese poultry production. *Nat. Microbiol.* 2:16260.
- Wang, F., W. Zhang, and D. Niu. 2021. Editorial: Foodborne Enterobacteriaceae of Animal. *Front Cell Infect Microbiol.* 11:772359.
- Wang, G., G. Zhao, X. Chao, L. Xie, and H. Wang. 2020. The characteristic of virulence, biofilm and antibiotic resistance of *Klebsiella pneumoniae*. *Int. J. Environ. Res. Public Health* 17:3390.
- Wang, J., Y. Shang, S. Guo, F. Diao, Y. Yu, X. Wei, Z. Feng, S. Jiang, and Z. Xie. 2017b. Serotype and virulence genes of *Klebsiella pneumoniae* isolated from mink and its pathogenesis in mice and mink. *Sci Rep.* 7:17291, doi:10.1038/s41598-017-17681-8.
- 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.
- Wei, D., X. Xiong, Y. Mei, F. Du, L. Wan, and Y. Liu. 2021. Microbiological and clinical characteristics of *Klebsiella pneumoniae* Isolates of K57 capsular serotype in China. *Microb. Drug Resist.* 27:391–400.
- Wei, Z., X. Du, Y. Yu, P. Shen, Y. Chen, and L. Li. 2007. Plasmid-mediated *KPC-2* in a *Klebsiella pneumoniae* isolate from China. *Antimicrob. Agents Chemother.* 51:763–765.
- 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.
- Wu, H., M. Wang, Y. Liu, X. Wang, Y. Wang, J. Lu, and H. Xu. 2016. Characterization of antimicrobial resistance in *Klebsiella* species isolated from chicken broilers. *Int. J. Food Microbiol.* 232:95–102.
- Wyres, K. L., T. N. T. Nguyen, M. M. C. Lam, L. M. Judd, N. van Vinh Chau, D. A. B. Dance, M. Ip, A. Karkey, C. L. Ling, T. Miliya, P. N. Newton, N. P. H. Lan, A. Sengduangphachanh, P. Turner, B. Veeraraghavan, P. V. Vinh, M. Vongsouvath, N. R. Thomson, S. Baker, and K. E. Holt. 2020. Genomic surveillance for hypervirulence and multi-drug resistance in invasive *Klebsiella pneumoniae* from South and Southeast Asia. *Genome Med.* 12:11.
- Xiang, R., X. Ye, H. Tuo, X. Zhang, A. Zhang, C. Lei, Y. Yang, and H. Wang. 2018. Co-occurrence of *mcr-3* and *bla*<sub>(NDM-5)</sub> genes in multidrug-resistant *Klebsiella pneumoniae* ST709 from a commercial chicken farm in China. *Int. J. Antimicrob. Agents* 52:519–520.
- 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.
- Yu, L., Y. Liu, M. Liu, Z. Li, L. Li, and F. Wang. 2022. Research Note: Molecular characterization of antimicrobial resistance and virulence gene analysis of *Enterococcus faecalis* in poultry in Tai'an, China. *Poult. Sci.* 101:101763.
- Zhai, R., B. Fu, X. Shi, C. Sun, Z. Liu, S. Wang, Z. Shen, T. R. Walsh, C. Cai, Y. Wang, and C. Wu. 2020. Contaminated in-house environment contributes to the persistence and transmission of *NDM*-producing bacteria in a Chinese poultry farm. *Environ. Int.* 139:105715.