Prevalence and characteristics of multidrug-resistant *Proteus mirabilis* from broiler farms in Shandong Province, China

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ABSTRACT Animal-derived *Proteus mirabilis* (***P. mirabilis***) is an important food-borne zoonotic bacillus and widely exists in the broiler-breeding industry. The present study was designed to explore the *P. mirabilis* prevalence and antimicrobial resistance characteristics in 6 conventional broiler-fattening farms in Shandong Province, China. The overall isolation rate of *P. mirabilis* was 7.07% (50/707). Antimicrobial resistance was very common in the *P. mirabilis* isolated from these farms and varied for different antibacterial drugs, with chloramphenicol, ciprofloxacin, and trimethoprim-sulfamethoxazole having the highest resistance rate (98%) and aztreonam the lowest (0%). Multidrug resistance was as high as 100%. The majority of the MDR isolates were resistant to between 9 and 12 of the antibiotics, with these accounting for 76% (38/50) of multidrug resistant strains. These *P. mirabilis* isolates carried 24 drug-resistance genes in 6 types, with *stcM* having the highest rate (96%) and *cmlA*, bla_{TEM}, and *qnrC* the lowest (2%). Superdrug resistance gene $bla_{NDM\text{-}1}$ was found in 10% (5/50) of isolates from poultry farms in Shandong. All the *P. mirabilis* isolates carried at least 6 virulence genes, with 100% detection rates of the *ireA* and *hpmA* genes. Our study revealed that the *P. mirabilis* strains isolated in the Shandong area all showed the MDR phenotype and the poultry-derived carbapenem-resistant MDR *P. mirabilis* strains may pose a potential risk to humans. Surveillance findings presented herein will be conducive to our understanding of the prevalence and characteristics of carbapenem-resistant *P. mirabilis* strains in Shandong, China.

Key words: *P. mirabilis*, multidrug-resistant, $bla_{NDM\text{-}1}$, virulence gene

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INTRODUCTION

Proteus mirabilis (***P. mirabilis***) is a gram-negative bacillus without spores, pili, or flagella and is an important zoonotic conditional pathogen that often causes human and animal infections. It can cause serious and persistent infections in humans, such as in the respiratory tract (Sun et al., 2020), wound infections, eye infections, and gastrointestinal and urinary tract infections (Sanches et al., 2020). Among the food-borne disease incidents reported in various countries, the proportion of food poisoning caused by *P. mirabilis* remains high (Gong et al., 2019; Wang et al., 2021). For example, from 1998 to 2013, it was reported that 294 people had food poisoning caused by *P. mirabilis* in 3 provinces of China (Gong et al., 2019). In recent years, the extensive use of antimicrobials has caused multidrug resistance in *P. mirabilis* and brought huge economic losses to the improvement of human life and to animal husbandry and has gradually developed into a worldwide problem (Cernohorská and Chvílová, 2011).

P. mirabilis is ubiquitous in the environment and has been frequently isolated from poultry (Yeh et al., 2018). Analyzed isolates from broiler chickens and human stool had high strain heterogeneity, and the same strains were not shared, but the antimicrobial resistance analysis was similar (Yu et al., 2021). The resistance of *P. mirabilis* is becoming more complicated and serious year by year, especially the manifestation of resistance to carbapenem drugs, making the prevention and control of the disease face greater challenges (van Duin and Doi, 2017). Some researchers have speculated that poultry products may act as a source of human infection, with a potential risk for public health (Yu et al., 2021).

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Animal-derived *P. mirabilis* is highly pathogenic and can cause infections of many human diseases (Sullivan et al., 2013; Armbruster et al., 2018). The pathogenicity of *P. mirabilis* mainly depends on its virulence factors to enhance invasiveness, including fimbriae, capsular polysaccharides to increase bacterial adhesion, immune evasion, and access to iron ions (Sanches et al., 2020; Sun et al., 2020). The virulence genes of *P. mirabilis* involve fimbriae coding genes *mrpA, pmfA, atfA*, and *ucaA*; protease coding genes *zapA* and *ptA*; hemolysin coding genes *hpmA* and *hlyA*; and the siderophore receptor gene *ireA* (Sanches et al., 2020).

In the present study, we conducted a comprehensive analysis to monitor the drug resistance and virulence gene-carrying status of *P. mirabilis* in broiler farms in some areas of Shandong. Our findings will provide a scientific basis for the clinical treatment and prevention of *P. mirabilis* infections in poultry and humans in the Shandong region. Meanwhile, the results of the virulence gene profile provide a basis for assessing the risk of poultry origin MDR *P. mirabilis* spreading to humans, which is a critical public health issue.

MATERIALS AND METHODS

Sample Collection and Identification of P. mirabilis

The sample source of this study is the same as that of Liu et al. (2021b). Two same-feeding period broiler houses on each farm were randomly selected for sampling. The cotton swabs and 1.5 mL centrifuge tubes used for sampling were sterilized. Every time we collected a new sample, we wore a new pair of PE gloves to avoid cross-contamination. A total of 707 samples were collected from 6 conventional broiler chicken farms located in 6 different prefecture-level cities in Shandong Province from May to July 2019. Apart from the 375 strains of *Escherichia coli* isolated on ordinary LB agar plates, 56 strains of *P. mirabilis* were also isolated. These strains show the characteristics of swarmlng growth phenomenon. In this work, these 56 isolates were recovered and inoculated on SS agar (Hope Bio-Technology Co., Qingdao, China) overnight at 37°C. The isolates formed round, flat, and translucent colonies on the SS plates. After three rounds of purification, putative *P. mirabilis* isolates were selected and confirmed using a microbial mass spectrometer (MALDI Biotyper, Bruker, Bremen, Germany).

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) and the paper method (test paper from Oxoid, Thermo Fisher, Basingstoke, England). The following antimicrobials were examined: amikacin (**AN**), amoxicillin-clavulanic acid (**AMZ**), ampicillin-sulbactam (**SAM**), aztreonam (**ATM**), cefazolin (**CZ**), cefepime (**FEP**), cefoperazone-sulbactam (**SCP**), cefoxitin (**FOX**), ceftazidime (**CAZ**), ceftriaxone (**CRO**), cefuroxime (**CXM**), chloramphenicol (**C**), ciprofloxacin (**CIP**), ertapenem (**ETP**), gentamicin (**GM**), levofloxacin (**LVX**), meropenem (**MEM**), imipenem (**IPM**), norfloxacin (**NOR**), piperacillin-tazobactam (**TZP**), tobramycin (**NN**), and trimethoprim-sulfamethoxazole (**SXT**). The results were interpreted based on the Clinical and Laboratory Standards Institute guidelines (2019). Multidrug-resistant (**MDR**) was defined as acquired nonsusceptibility to at least one agent in 3 or more antimicrobial categories.

Detection of Drug Resistance and Virulence Gene

DNA templates were prepared by the boiling method of DNA extraction (Li et al., 2020). The aminoglycosides resistance gene (*aaC1, aaC2, aaC3,* and *aac (6′) -Ib-cr*) and other genes associated with resistance to β-lactams ($bla_{CTX\text{-}M}$, bla_{SHV}, bla_{TEM}, bla_{PSE}, and bla_{OXA}); sulfonamides (*sul1, sul2,* and *sul3*); macrolides (*mefA* and *mrsD*); quinolones (*qnrA, qnrB, qnrC, qnrS,* and *oqxA*); chloramphenicol (*cmlA* and *stcM*); carbapenems-resistance genes (bla_{KPC}, bla_{IMP}, bla_{VIM}, and $bla_{NDM\text{-}1}$); and 10 other genes were screened by PCR (Poirel et al., 2011; Supplementary Table 2).

The genes *mrpA, pmfA, atfA,* and *ucaA* (fimbriae), *zapA* and *ptA* (proteases), *hpmA* and *hlyA* (hemolysins), and *ireA* (siderophore receptors), which are associated with the virulence of *P. mirabilis* (Sanches et al., 2020), were detected using PCR. All the primers and annealing temperatures were slight modifications of those used in previously described procedures (Supplementary Table 3).

RESULTS

Detection and Identification of P. mirabilis

Fifty-six strains of *P. mirabilis* were initially identified in the laboratory, and 50 strains were finally reidentified in this study (Supplementary Table 1). The overall isolation rate of *P. mirabilis* was 7.07% (50/707). Twenty-two strains were from Tai'an, 13 from Weifang, 9 from Linyi, 4 from Liaocheng, and 1 each from Heze and Binzhou (Figure 1D). In this study, *P. mirabilis* was isolated from all broiler farms, with the highest proportion from Tai'an chicken farms, accounting for 50% of the total (25/50), and the lowest proportion from Binzhou and Heze, with only one strain isolated from each. Among these isolates, the air sample had the highest separation rate, which was 13.75% (22/160), and the water sample had the lowest separation rate, which was 1.37% (1/73) (Table 1).

Antimicrobial Resistance and MDR Profiles

A total of 22 antibacterial agents in 9 categories were screened in this study (Figure 1B). The multidrug

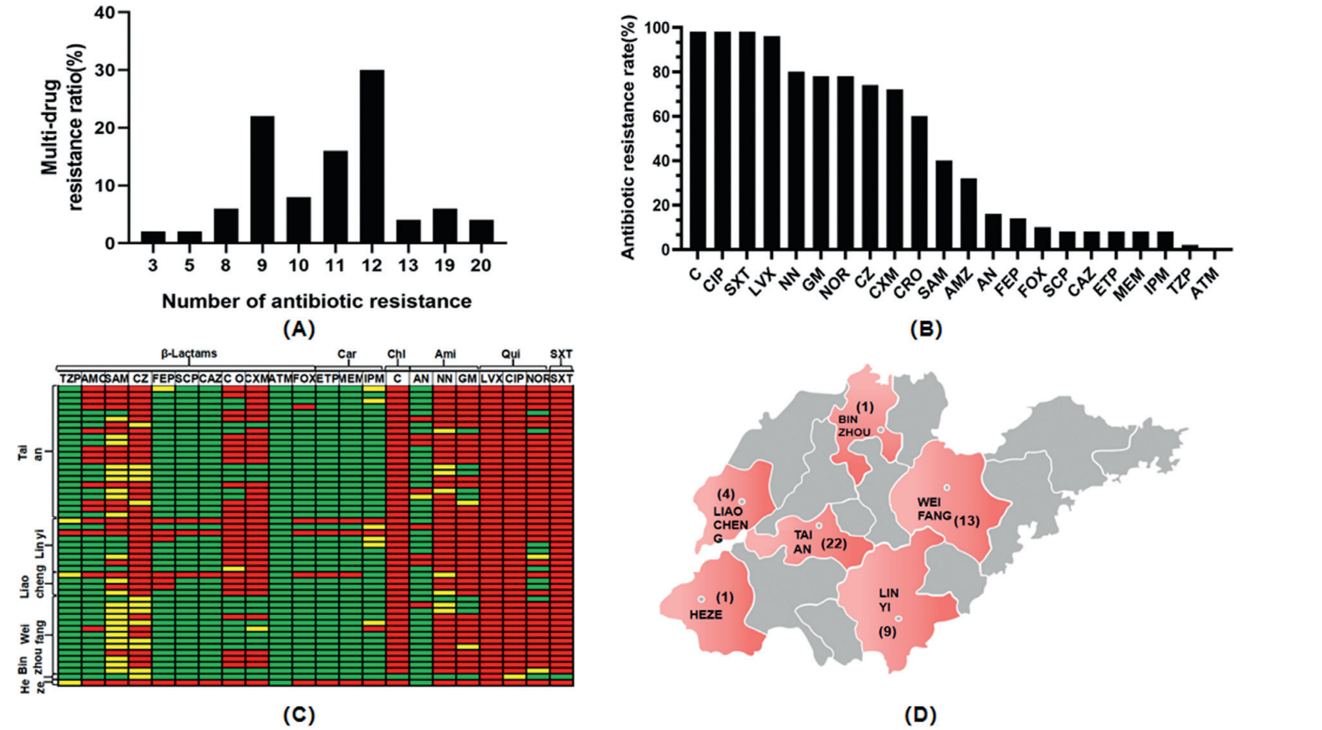

Figure 1. The drug resistance of P. mirabilis and the distribution map of the strains in Shandong Province. (A) The multidrug resistance of 50 strains of *P. mirabilis*, of which the strains resistant to 12 drugs are the most (30%), the strains resistant to 3 and 5 drugs are the least (2%), and 50 strains are resistant to up to 20 drugs. (B) The susceptibility results of 22 kinds of antibacterial drugs are presented in the resistance rate (%). P. mirabilis showed varying degrees of resistance to 22 antibacterial drugs, with the highest resistance to chloramphenicol, ciprofloxacin, and trimethoprim-sulfamethoxazole (98%), and the highest sensitivity to aztreonam (0%). (C) P. mirabilis Heat Resistance Spectrum. Among them, red represents drug resistance, green represents sensitivity, and yellow represents moderate sensitivity. (D) Distribution map of 50 strains of *P. mirabilis* in Shandong Province. Twenty-two strains were isolated from Tai'an, 13 from Weifang, 9 from Linyi, 4 from Liaocheng, 1 from Heze, and 1 from Binzhou. Abbreviations: A, aminoglycosides; B, β-lactams; C, chloramphenicol; Q, quinolones, S, sulfonamides.

resistance of *P. mirabilis* sampled in this sample is relatively common. The multidrug resistance rate was 100% (50/50), and the proportion of resistance to 12 drugs was the largest, which was 30% (15/50). The majority of the MDR isolates were resistant to between 9 and 12 of the antibiotics, accounting for 76% (38/50) of the multidrug-resistant strains (Figure 1A). Two strains (4%) with the highest 20 drug-resistant strains were detected, which came from Weifang farm. A total of 24 drug-resistance profiles were monitored in the 6 chicken farms in Shandong Province. The heat map of the drug-resistance profiles is shown in Figure 1C. Among them were 6 kinds of main-drug resistance spectra (number of drug-resistant strains ≥3), which contained 4 species of the dominant drug-resistance spectrum (number of drug-resistant strains ≥5). Fifty-eight percent (29/50) of the tested *P. mirabilis* drug-resistance spectrum represented the main drug-resistance spectrum, and 46% (23/50) of the strains were the dominant drug-resistance spectrum (Table 2). Carbapenem-resistant strains were mainly from Linyi farm, followed by Heze and Liaocheng, and the detection rate of carbapenem drugs was 10%.

Prevalence of Antimicrobial Resistance Genes and Virulence Genes

Twenty-five antimicrobial resistance genes were identified among the 50 *P. mirabilis* isolates, including aminoglycosides resistance genes (*aaC1, aaC2, aaC3*, and *aac (6′) -Ib-cr*); β-lactams resistance genes ($bla_{CTX\text{-}M}$,

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

Area	% *P. mirabilis*-positive samples (number of detections/total no.) for:					
	Environment				Animal	
	Wall	Water	Feces	Air	Anal swab	Total
Tai'an	8.33(1/12)	0(0/12)	0(0/18)	42.31(11/26)	16.67(10/60)	17.19(22/128)
Heze	0(0/6)	0(0/6)	0(0/12)	0(0/12)	1.67(1/60)	1.04(1/96)
Liao Cheng	0(0/12)	0(0/14)	0(0/18)	13.64(3/22)	1.67(1/60)	3.17(4/126)
Lin Yi	0(0/12)	10.00(1/10)	0(0/16)	13.64(3/22)	9.62(5/52)	8.04(9/112)
Wei Fang	0(0/12)	0(0/12)	0(0/16)	18.18(4/22)	15.00(9/60)	10.66(13/122)
Bin Zhou	0(0/12)	0(0/12)	0(0/20)	5.56(1/18)	0(0/61)	0.81(1/123)
Total	1.52(1/66)	1.52(1/66)	0(0/100)	18.03(22/122)	7.37(26/353)	7.07(50/707)

Table 2. Dominant resistance spectrum and main resistance spectrum.

Strain number	Quantity	Resistance spectrum
30,31,32,40,43,44	6	AMC+SAM+CZ+CRO+CXM+C +NN+GM+LVX+CIP+NOR +SXT
10,11,12,26,39,45	6	AMC+SAM+CZ+CRO+CXM+C +NN+GM+LVX+CIP+NOR +SXT
7,16,17,19,36,48	6	SAM+CZ+CRO+CXM+C+NN +GM+LVX+CIP+NOR+SXT
18,25,28,29,46	5	SAM+CZ+CRO+CXM+C+AN+NN +GM+LVX+CIP+NOR+SXT
15,24,47	3	CZ+CRO+CXM+C+NN+GM +LVX+CIP+SXT
6,37,38	3	SAM+CZ+C+NN+LVX+CIP +NOR+SXT

bla_{SHV}, bla_{TEM}, bla_{PSE}, and bla_{OXA}); carbapenems resistance genes (bla_{KPC}, bla_{IMP}, bla_{VIM}, and $bla_{NDM\text{-}1}$); sulfonamides resistance genes (*sul1, sul2,* and *sul3*); macrolides resistance genes (*mefA* and *mrsD*); quinolones resistance genes (*qnrA, qnrB, qnrC, qnrS,* and *oqxA*); and chloramphenicol resistance genes (*cmlA* and *stcM*). The highest detection rate was for *stcM* (96%), and the lowest for *aaC3*, bla_{PSE}, bla_{KPC}, bla_{IMP}, and bla_{VIM} (0%). A total of 28 drug resistance gene profiles were found. Five carbapenem-resistant *P. mirabilis* isolates carrying $bla_{NDM\text{-}1}$ were isolated (Table 3).

The 50 isolates of pathogenic *P. mirabilis* were tested by PCR for 9 virulence genes. The results showed that all strains carried the *ireA* and *hpmA* virulence genes. The 50 isolates of *P. mirabilis* carried a maximum of 8 kinds of 6 virulence genes, and a minimum of 6 kinds of virulence genes. The detection rates of *ucaA, mrpA,*

Table 3. Detection of resistance genes.

Types of resistance genes	Drug resistance gene name	Detection rate % (number of positives/total number of samples)
Aminoglycosides	*aaC1*	4% (2/50)
	aaC2	76% (38/50)
	aaC3	0
	aac (6') -Ib-cr	92% (46/50)
β-lactams	$bla_{CTX\text{-}M}$	52% (26/50)
	bla_{SHV}	4% (2/50)
	bla_{TEM}	2% (1/50)
	bla_{PSE}	0
	bla_{OXA}	86% (43/50)
Carbapenems	bla_{KPC}	0
	bla_{IMP}	0
	$bla_{NDM\text{-}1}$	10%(5/50)
	bla_{VIM}	0
Sulfonamides	*sul1*	94% (47/50)
	sul2	94% (47/50)
	sul3	18% (9/50)
Macrolides	*mefA*	4% (2/50)
	mrsD	20% (10/50)
Quinolones	*qnrA*	26% (13/50)
	qnrB	18% (9/50)
	qnrC	2% (1/50)
	qnrS	16% (8/50)
	oqxA	16% (8/50)
Chloramphenicol	*cmlA*	2% (1/50)
	stcM	96% (48/50)

atfA, zapA, and *ptA* were 96%−98%, the detection rate of *pmfA* was the lowest at 94%, and the *hlyA* gene was not detected (Figure 2B).

DISCUSSION

In this study, a total of 707 samples from broiler farms in Shandong Province, including Tai'an, Liaocheng,

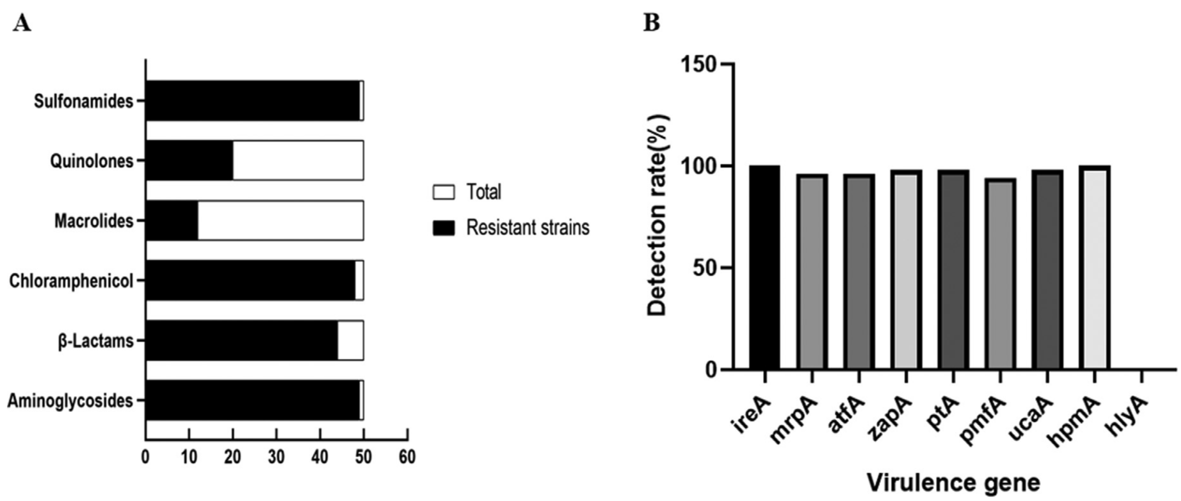

Figure 2. Proportion of resistance genes and virulence genes. (A) Overall, it was found that 98% (49/50) of *P. mirabilis* strains carry aminoglycoside resistance genes, 88% (44/50) of strains carry β-lactam resistance genes, 29% (14/50) of strains carry tetracycline resistance genes, 98% (49/50) of strains carry sulfa resistance genes, 8% (4/50) of strains carry polymyxin resistance genes, 24% (12/50) of strains carry macrolide resistance genes, 40% (20/50) of strains carry quinolone resistance genes, and 96% (48/50) of strains carry chloramphenicol resistance genes. (B) The 50 isolates of pathogenic *P. mirabilis* were tested by PCR for nine virulence genes. The results showed that the virulence genes ireA and hpmA had the highest detection rates, both of which were 100%; the detection rates of zapA, ptA, ucaA mrpA, and atfA were 98, 98, 98, 96, and 96%, respectively; the detection rate of pmfA was the lowest at 94%, and the hlyA gene was not detected.

Heze, Weifang, Linyi, and Binzhou, were collected (Liu et al., 2021b). This experiment reidentified 50 strains of *P. mirabilis*, with a positive rate of 7.07%. Some studies have found that *P. mirabilis* can be separated from chicken manure together with other intestinal bacteria, indicating that it is part of the normal intestinal flora (Drzewiecka, 2016), which will lead to the transfer of these bacteria to the slaughter line during the slaughter process. Neutralization may cause cross-contamination (Projahn et al., 2018). In this study, we found that the air sample separation rate reached 18.03%, followed by chicken anal swabs 7.37%, which also confirmed the above view. Therefore, the rational handling of excreta such as livestock and poultry manure is a public health issue that farms need to pay attention to. In addition, the Weifang broiler factory had the highest isolation rate of 18.06%. At the same time, the air sample isolation rate of the factory was as high as 57.12%, indicating that the environmental sanitation may not be up to standard.

In recent years, the drug resistance of *P. mirabilis* has become increasingly serious (Magali Decôme et al., 2020). The drug susceptibility test of this experiment showed different degrees of drug resistance to 22 antimicrobials. The highest resistance rate was to chloramphenicol, ciprofloxacin, and trimethoprim-sulfamethoxazole (98%), and the lowest was to aztreonam (0%). It maintained a high resistance rate to 10 drugs, such as cefazolin and tobramycin, and the resistance rate exceeded 50%. In addition, we found 2 strain resistance to 20 drugs in Linyi, which was only sensitive to aztreonam and amikacin, and the overall drug resistance rate in this farm was also high, which may be caused by frequent use of antimicrobials due to environmental sanitation. Binzhou had the lowest drug resistance rate, which may be because the factory was a new chicken farm. From the perspective of β-lactamase resistance, the resistance rate of *P. mirabilis* to most cephalosporins was 60 to 75%, the resistance rate to ampicillin-sulbactam was 40%, and the resistance rate to carbapenems was 10%, and it was only very sensitive to aztreonam. In this study, *P. mirabilis* MDR (Poudel et al., 2019) was a serious phenomenon, with the largest proportion of resistance to 12 drugs. The majority of the MDR isolates, accounting for 76%, were resistant to between 9 and 12 of the antibiotics. It was resistant to 20 drugs at most, and the multidrug resistance rate, which reached 100%, was higher than the 76.7% reported in Northeast China (Sun et al., 2020) and the 78.13% reported in Brazil (Sanches et al., 2019), which fully reflects the multidrug resistance of *P. mirabilis*.

An important factor in bacterial resistance to antimicrobials is that they carry related resistance genes (Song et al., 2020; Liu et al., 2021a). There was a strong correlation between the phenotypic resistance pattern to the antibiotics and the presence of resistant genes (Table 2). It can be seen from the statistical results that the highest detection rate was the chloramphenicol resistance gene *stcM* (96%), followed by the sulfonamides *sul*1 and *sul*2 (94%), the aminoglycosides *aac (6′) -Ib-cr* (92%), and β-lactams bla_{OXA} (86%), while the lowest were *cmlA*, bla_{TEM}, and *qnrC* (2%). Not detected were *aacC3*, bla_{PSE}, bla_{VIM}, bla_{KPC}, and bla_{IMP}. In addition, in the recent 25 yr of reports on the drug resistance of *P. mirabilis*, the most resistant has been the β-lactamase-producing *P. mirabilis* (Bontron et al., 2019), which is distributed all over the world and has many types, including broad-spectrum β-lactamase (**ESBLs**) (Sohn et al., 2011), cephalosporinase (**AmpC**) (Jacoby, 2009), and carbapenemase, among which ESBLs are mainly the TEM and CTX-M types (Nakano et al., 2012). In this study, the detection rate of β-lactamase resistance genes was as high as 88% (44/50). This result is mainly based on the bla_{OXA} gene and is very similar to the result of 88.10% (89/101) isolated from a study in India (Poudel et al., 2019). Carbapenems are the treatment of choice for severe infections caused by ESBL-producing microorganisms (Rawat and Nair, 2010). However, the five carbapenem-resistant isolates in this experiment brought greater challenges to clinical treatment. Coproduction of ESBL and carbapenemase was observed in 6% (3/50) of isolates from Linyi and Liaocheng and may act as a source of human infection, with the potential risk to public health (Yu et al., 2021).

Carbapenem-resistant Enterobacter is actually a group of enteric bacteria, which contains more than 70 kinds of bacteria. This type of bacteria is called “super bacteria” (Yong et al., 2009) because it is resistant to many new antimicrobials, and the death rate of infected persons is very high. The emergence of Enterobacter carbapenemase strains has gradually attracted public attention, especially New Delhi metal β-lactamase (**NDM-1**). This enzyme makes almost all β-lactam drugs ineffective, and only a few antimicrobials can be treated, such as colistin and tigecycline. NDM-1 is encoded by the $bla_{NDM\text{-}1}$ gene located in the plasmid. It can spread among a variety of bacteria mediated by the plasmid and may cause urinary tract infections, lung infections, peritonitis, sepsis (Day et al., 2013), and other complex and diverse diseases. *P. mirabilis* carrying this gene is resistant to carbapenem drugs and is a “super-resistant bacterium,” sensitive to only one or two drugs, and thus posing huge challenges to clinical treatment. NDM mainly occurs in the Indian subcontinent (van Duin and Doi, 2017), the Balkans and the Middle East. Carbapenemase-producing *Klebsiella pneumoniae* was first reported in Zhejiang, China, in 2007 (Shen et al., 2010) and gradually spread to the whole country. Among the positive bacteria carrying $bla_{NDM\text{-}1}$ gene reported in China from 2012 to 2016, southwest China reported the most (Jia et al., 2018). In 2021, the first report of NDM-1 producing *P. mirabilis* in broiler chickens was in Chengdu, Sichuan, China (Xie et al., 2021). In this study, we isolated the NDM-1 resistance gene for the first time in Shandong, China. The drug resistance gene screening revealed that 10% (5/50) carried the $bla_{NDM\text{-}1}$ gene, from the broiler farms in Linyi, Liaocheng, and Heze. It is worthy of warning that we found that 3 of them carried ESBLs resistance genes

bla_{OXA} or $bla_{CTX\text{-}M}$. This finding provides valuable information for monitoring the spread of NDM-1-producing *P. mirabilis* in poultry farms of Shandong Province and tracing the potential transmission from broiler chickens to humans.

Animal-derived *P. mirabilis* carries multiple virulence genes and is an important zoonotic pathogen (Sullivan et al., 2013; Armbruster et al., 2018). The isolates in this study carry virulence genes, such as the *ireA, mrpA, ucaA, pmfA, atfA, ptA, zapA*, and *hpmA* genes (Sanches et al., 2019). Among them, *ireA* and *hpmA* had the highest detection rate of 100%, and the lowest was *pmfA at* 94%. The *hlyA* gene was not detected, which is consistent with the results reported in Brazil (Sanches et al., 2019). The results of this study also showed that almost all isolates have the *zapA* gene, which can provide protection for mucosal immune response and is thus consistent with other studies (Mirzaei et al., 2019).

CONCLUSIONS

In this study, the MDR of *P. mirabilis*-isolated strains from chickens in Shandong Province is serious, as high as 100%, most of which are resistant to between 9 and 12 drugs, accounting for 76% (38/50). *P. mirabilis* isolated 8 types of drug-resistance genes and 22 drug-resistance genes, of which *stcM* had the highest detection rate (96%). Twenty-eight drug-resistance gene profiles were tested and 4 dominant drug-resistance gene profiles. In addition, we first detected the superdrug resistance gene $bla_{NDM\text{-}1}$ (5/50, 10%) in poultry farms in Shandong. These results provide valuable information for monitoring the spread of NDM-1-producing ESBLs-*P. mirabilis* in poultry farms of Shandong Province and tracing the potential transmission from chickens to humans.

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

Author contributions: FW participated in the study design. ZL and CP carried out the study and drafted the manuscript. GZ, YZ, CL, ML, and YS collected the important background information. All authors read and approved the final manuscript.

DISCLOSURES

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

SUPPLEMENTARY MATERIALS

Supplementary material associated with this article can be found in the online version at doi:10.1016/j.psj.2022.101710.

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