Emergence of NDM-1- and CTX-M-3-Producing Raoultella ornithinolytica in Human Gut Microbiota

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Raoultella ornithinolytica is an opportunistic pathogen of the Enterobacteriaceae family and has been implicated in nosocomial infections in recent years. The aim of this study was to characterize a carbapenemase-producing R. ornithinolytica isolate and three extended-spectrum β-lactamase (ESBL)-producing R. ornithinolytica isolates from stool samples of adults in a rural area of Shandong Province, China. The species were identified using matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) and 16S rDNA sequence analysis. Antimicrobial susceptibility test showed that all four isolates were multidrug-resistant (MDR). The whole genome sequence (WGS) of these isolates was determined using an Illumina HiSeq platform, which revealed MDR-related genes. The S1 nuclease-pulsed-field gel electrophoresis (S1-PFGE) was used to characterize the plasmids carried by the R. ornithinolytica isolates. The blaNDM-1 and blaCTX-M-3 genes were probed using Southern blotting, which confirmed the location of both genes on the same plasmid with molecular weight of 336.5–398.4 kb. The transferability of blaNDM-1 and blaCTX-M was also confirmed by conjugation assays. Finally, BLAST analysis of both genes showed that mobile genetic elements were associated with the spread of drug resistance genes. Taken together, we report the presence of conjugative blaNDM-1 and blaCTX-M plasmids in R. ornithinolytica isolates from healthy humans, which indicate the possibility of interspecies transfer of drug resistance genes. To the best of our knowledge, this is the first study to isolate and characterize carbapenemase-producing R. ornithinolytica and ESBL-producing R. ornithinolytica isolates from healthy human hosts.

Keywords: Raoultella ornithinolytica, carbapenemase, extended-spectrum β-lactamase, blaNDM-1, blaCTX-M, whole-genome sequencing

INTRODUCTION

Raoultella is a genus of encapsulated Gram-negative aerobic bacilli of the Enterobacteriaceae family (Luo et al., 2017) that was initially part of the genus Klebsiella, but later reclassified based on the 16S rDNA sequence and the rpoB, gyrA, and gyrB genes (Drancourt et al., 2001). Raoultella ornithinolytica is one of the three species of the genus Raoultella (Singh et al., 2017).
and naturally exists in the soil, water, and plants (Ayoade et al., 2018). *R. ornithinolytica* can cause pneumonia, biliary or urinary tract infections and bacteremia and such cases are being increasingly reported (Sekowska et al., 2015; Van Cleve et al., 2018). Both organ-specific and systemic *R. ornithinolytica* infections can be life-threatening, especially in cancer patients after abdominal surgery (Hajjar et al., 2018). Therefore, it is vital to understand the pathogenic potential of *R. ornithinolytica* isolates in humans (De Petris and Ruffini, 2018). Cephalosporins, quinolones, and carbapenems are routinely used against this pathogen, and multi-drug resistant (MDR) *R. ornithinolytica* isolates have been reported in recent years (Wang et al., 2019).

Widespread use of third-generation cephalosporins and other β-lactam antibiotics in the past decades have led to the emergence of third-generation cephalosporin-resistant bacteria that produce extended spectrum β-lactamases (ESBLs) and AmpC β-lactamasas that are, respectively, encoded by plasmids and chromosomes (Kola et al., 2012; Dohmen et al., 2015). Consistent with this, infections caused by the ESBL-producing Gram-negative bacilli (GNB) have become increasingly prevalent worldwide, both in the healthcare and community settings, and pose significant therapeutic challenges (Wang et al., 2015). The class A serine CTX-M-type is the most common plasmid-encoded ESBLs that are produced by drug-resistant pathogens (He et al., 2016). The genetic elements encoding these CTX-M enzymes constantly evolve via random mutations and recombination between different resistance genes (Canton et al., 2012). A genetic epidemiological study on ESBLs found that *bla*_{CTX-M} has replaced *bla*_{TEM} and *bla*_{TEM} as the most common ESBL-encoding genes (Xia et al., 2014). While CTX-M-15 and CTX-M-14 are the most prevalent ESBLs worldwide (He et al., 2016), *bla*_{CTX-M-14} has been identified as the most prevalent ESBL gene in China, and epidemiological surveillance in Asia, Latin America, and Europe has revealed a dramatic increase in cephalosporin-resistant *Escherichia coli* and *Klebsiella* spp. strains due to spread of the CTX-M ESBLs (Zhang et al., 2016).

Carbapenem-resistant Enterobacteriaceae (CRE) is a serious public health concern worldwide because of its rapid spread and limited therapeutic drugs (Zheng et al., 2018). Metallo-β-lactamasas (MBLs) are produced by many species of Gram-negative bacteria and confer resistance to carbapenems, cephalosporins, and penicillins except monobactams (Tada et al., 2019). New Delhi Metallo-beta-Lactamase 1 (NDM-1) is a plasmid-associated Ambler class B β-lactamase/carbapenemase that was first reported in clinical *E. coli* and *Klebsiella pneumoniae* isolates from an Indian patient in Sweden in 2008 (Yong et al., 2009). Subsequent cases of carbapenemase-producing isolates have since been reported in Britain, Australia, India, Russia, etc. (Yong et al., 2009; Kumarasamy et al., 2010; Bocanegra-Ibarias et al., 2017), and clinical isolates of *R. ornithinolytica* from urethral effluent, fester, and rectum samples have recently been found to produce this enzyme (Li et al., 2012; Khajuria et al., 2013; Zhou et al., 2013; Zheng et al., 2015; Paskova et al., 2018).

*R. ornithinolytica* infections are largely nosocomial and have rarely been reported in a healthy community (Seng et al., 2016). Nevertheless, the high rates of antimicrobial resistance in *R. ornithinolytica* isolates should be characterized in order to provide a basis for treating infections. To this end, we conducted a cross-sectoral study as part of the Sino-Swedish Integrated Multisectoral Partnership for Antibiotic Resistance Containment (IMPACT) in the Shandong Province in China using a One Health approach. The aim of this project is to study the relationship between the development of drug resistance in human (symbiotic and clinical), zoonotic, food, and environmental isolates of *R. ornithinolytica*. We identified NDM-1 and ESBL-producing *R. ornithinolytica* strains from healthy subjects and analyzed the drug resistance phenotypes and underlying mechanisms. To the best of our knowledge, this is the first report detailing the presence of an NDM-producing *R. ornithinolytica* strain in the human gut microbiota.

**METHODS**

**Bacterial Isolation and Identification**

A total of 1,380 fecal samples were collected from healthy people in rural communities in July 2017 according to a previously described sampling procedure (Sun et al., 2018). Briefly, the samples were collected into ESwab tubes (Copan, Brescia, Italy) and stored at −80°C until cultivation. After thawing, the fecal samples were cultured on ChromID CARBA agar and ChromID ESBL agar plates (bioMérieux, Marcy l’Etoile, France) for 18 h at 37°C to respectively screen for the carbapenemase- and ESBL-producing *R. ornithinolytica* strains. The suspected *R. ornithinolytica* colonies identified based on color and morphology were picked and sub-cultured on CHROMagar Orientation agar (CHROMagar Company, Paris, France) overnight at 37°C. The resulting isolates were identified using MALDI-TOF MS and then confirmed by 16S rDNA sequence analysis against the bacterial 16S rDNA gene sequence in GenBank. The genomic average nucleotide identity (ANI) was calculated as described previously (Jiang et al., 2018).

**DNA Extraction and PCR**

DNA was extracted from pure cultures of *R. ornithinolytica* using a Gentra Puregene Yeast/Bact. Kit (QIAGEN, Hilden, Germany), and the 16S rDNA sequences were amplified by PCR with primers designed using the Primer 5.0 software (3′-AGAGTTTGATCCTGCTCAG-5′/3′-GGTTACCTTGTTAAGACTT-5′). The optimized cycling conditions were: initial denaturation at 94°C for 5 min followed by 30 cycles of amplification each with 94°C for 1 min, 52°C for 1 min, and 72°C for 1 min and final extension at 72°C for 5 min. The PCR product was detected by capillary electrophoresis as described previously (Zhang et al., 2019). The *bla*_{NDM-1}, *bla*_{CTX-M}, and *bla*_{CTX-M-14} genes were amplified by PCR using primers published previously (Shimizu et al., 2017; Chetri et al., 2019), and the products were confirmed by capillary electrophoresis followed by Sanger sequencing.

**Antimicrobial Resistance Test**

To verify carbapenemase production by the isolates, resistance against imipenem, ertapenem, and/or meropenem were determined.
using the modified Hodge test. ESBL-production was verified using the double disk diffusion method with cefotaxime, ceftazidime, and/or clavulanic acid according to Clinical and Laboratory Standards Institute (CLSI, 2017).

Antimicrobial Susceptibility Test
A total of 17 antibiotics belonging to 13 antimicrobial classes were tested, including cephalosporins (ceftaxime and ceftazidime), cephapencils (cefoxitin), β-lactam/β-lactamase inhibitor complexes (amoxicillin-clavulanate and piperacillin-tazobactam), carbapenems (imipenem and meropenem), penicillins (ampicillin), aminoglycosides (gentamicin and amikacin), fluoroquinolones (ciprofloxacin), folate metabolic pathway inhibitors (trimethoprim-sulfamethoxazole), tetracycline, chloramphenicol (chloramphenicol), colistin, furantoin, and tigecycline. The minimal inhibitory concentrations (MICs) of colistin and tigecycline were determined by the broth microdilution method and other antibiotics using the agar dilution method. The results were interpreted according to CLSI (2017) and European Committee on Antimicrobial Susceptibility Testing (EUCAST) (version 8.1, 2018) guidelines. E. coli ATCC25922 was used as the quality control strain. Since clinical breakpoints of florfenicol are not available for Enterobacteriaceae in EUCAST or CLSI, the resistance breakpoint of >16 mg/L was selected based on the epidemiological cut-off values for the closely related E. coli and Salmonella spp. (Chi et al., 2019).

Whole-Genome Sequencing
Whole-genome sequencing was performed on the extracted DNA by Sangon Biotech (Shanghai, China) using the Illumina HiSeq sequencing platform. The quality of the high-throughput sequence data was assessed by FastQC1. SPAdes 3.11.0 was used for assembling raw sequences (Hu et al., 2011). All draft genomes were deposited in the NCBI database under accession numbers VJYE00000000-VJYH00000000. Acquired antimicrobial resistance genes and plasmid replicons were identified using ResFinder 2.1 and PlasmidFinder 1.3, respectively. The gene sequences surrounding \( \text{bla}_{\text{NDM-1}}, \text{bla}_{\text{CTX-M-3}}, \) and \( \text{bla}_{\text{CTX-M-14}} \) were annotated using RAST2 and Easyfig 2.2.3 (Chi et al., 2019). To find the core genes of the \( R. \ ornithinolytica \) genomes, Roary was used for SNPs analysis (Page et al., 2015). Maximum likelihood-based phylogenetic reconstruction was performed with RAxML version 8.2.10 (Stamatakis, 2014) and visualized with FastTree (Price et al., 2009).

S1 Nuclease-Pulsed-Field Gel Electrophoresis and Southern Blotting
The location of \( \text{bla}_{\text{NDM-1}}, \text{bla}_{\text{CTX-M-3}}, \) and \( \text{bla}_{\text{CTX-M-14}} \) on the plasmids was validated by S1-PFGE and southern blotting. Briefly, the isolates were embedded in 10 g/L Seakem Gold gel, digested with endonuclease S1 nuclease (TakaRa, Dalian, China), and subjected to pulsed-field gel electrophoresis (Parameters: 14°C, voltage 6 V/cm, electric field angle 120°, conversion time 2.16–63.8 s, and electrophoresis 16 h). The DNA fragments were transferred horizontally to a nylon membrane (Millipore, USA), and hybridized with three digoxin-labeled probes obtained by PCR amplification (Yu et al., 2002) and the Dig High Prime DNA Labeling and Detection Starter Kit (Roche Diagnostics). The genomic DNA of Salmonella enterica serovar Braenderup H9812 strain cut with XbaI was used as the DNA marker.

RESULTS

Multidrug-Resistant Raoultella ornithinolytica Strains Were Isolated From the Human Fecal Samples
One carbapenemase-producing (ROF058) and three ESBL-producing (ROE007, ROE058, and ROI014) \( R. \ ornithinolytica \) strains were isolated from 1,380 fecal samples (Table 1) and confirmed by MALDI-TOF MS, 16S rDNA sequencing, and ANI analysis (Figure 1A). Phylogenetic analysis revealed that isolates ROE007 and ROE058 are clonally related although they were recovered from different villagers in the same natural village (Figure 1B). The \( \text{bla}_{\text{NDM-1}}, \text{bla}_{\text{CTX-M-3}}, \) and \( \text{bla}_{\text{CTX-M-14}} \) genes were subsequently identified in all the strains, and multidrug resistance was confirmed by antimicrobial susceptibility tests. ROF058 was resistant to 12 antibiotics other than carbapenem, but sensitive to amikacin, tigecycline, furantoin, colistin, and trimethoprim-sulfamethoxazole. The resistance profiles of the three ESBL-producing isolates were similar, and all were resistant to gentamicin, tetracycline, cefotaxime, trimethoprim-sulfamethoxazole, and ampicillin. Furthermore, all trans-conjugants exhibited MDR phenotypes similar to the donor strain (Table 1).

The Raoultella ornithinolytica Strains Harbor Multidrug-Resistant Genes
MDR was defined as acquired non-susceptibility to at least one agent in three or more antimicrobial categories, and XDR

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1. http://www.bioinformatics.babraham.ac.uk/projects/fastqc/
2. https://cge.cbs.dtu.dk
3. http://rast.nmpdr.org/rast.cgi
was defined as non-susceptibility to at least one agent in all but two or fewer antimicrobial categories (Magiorakos et al., 2012). Whole genome sequencing showed that ROF058 is an extensively drug-resistant (XDR) strain, while ROE007, ROE058, and ROI014 are MDR strains (Table 1). In addition to the carbapenemase-encoding \( \text{bla}_{\text{NDM-1}} \), ROF058 also carries genes encoding for other ESBLs (\( \text{bla}_{\text{CTX-M-3}}, \text{bla}_{\text{OXA-1}}, \text{bla}_{\text{TEM-1B}}, \) and \( \text{bla}_{\text{DHA-1}} \)), as well as resistance factors against, aminoglycosides [\( \text{aac}(3)\text{-IId} \)], rifampicins (\( \text{arr-3} \)), chloramphenicols (\( \text{catB3} \)), tetracyclines [\( \text{tet}(D) \)], quinolones [\( \text{qnrB4 and aac(6')-Ib-cr} \)], fosfomycin (\( \text{fosA} \)), and sulfonamides (\( \text{sul1} \)) (Figure 2). The remaining isolates harbored genes encoding the ESBLs (\( \text{bla}_{\text{CTX-M-14}} \)), quinolones (\( \text{qnrS1} \)), aminoglycosides [\( \text{aac(3)-IId} \)], sulfonamides (\( \text{sul1} \)), tetracyclines [\( \text{tet}(A) \)], sulfanilamides (\( \text{dfrA1} \)), and fosfomycins (\( \text{fosA} \)) resistance genes.

**The Multidrug-Resistant Genes Are Located on Mobile Genetic Elements**

Whole genome sequencing of strain ROF058 generated 5,747,150 clean reads, which were then assembled to 209 contigs with a GC content of 51.38%; WGS of strain ROE007 generated 5,214,213 clean reads, which were then assembled to 203 contigs with a GC content of 49.36%; WGS of strain ROE058 generated 5,235,762 clean reads, which were then assembled to 199 contigs.
with a GC content of 50.28%; WGS of strain ROI014 generated 5,510,178 clean reads, which were then assembled to 215 contigs with a GC content of 54.66%. The plasmids in the different isolates were identified using the Center for Genomic Epidemiology program (Luo et al., 2017), which showed that ROFO58 harbored the IncFIB(K)-type plasmid, ROE007 and ROE058 contained IncFIC(K) and IncFII-type plasmids, and ROI014 carried IncFIB(K) and IncFII plasmids. In addition, S1-PFGE and southern blot hybridization revealed three different plasmids in the *R. ornithinolytica* isolates ranging from 104.5 to 398.4 kb (Figure 3). ROFO58 contained the larger plasmid (336.5–398.4 kb) harboring the *bla*<sub>NDM-1</sub> and *bla*<sub>CTX-M-3</sub> genes. ROE007 and ROE058 had identical plasmid profiles, and the *bla*<sub>CTX-M-14</sub> gene was located on a 138.9 kb plasmid in both isolates. The *bla*<sub>CTX-M-14</sub> gene in ROI014 was located on a 216.9–244.4 kb plasmid. Furthermore, the *bla*<sub>NDM-1</sub> and *bla*<sub>CTX-M-3</sub> genes could be transferred from each of the isolates into recipient *E. coli* strains via conjugation (Table 1), which was confirmed by PCR (data not shown).

**DISCUSSION**

Intermittent but lethal nosocomial infections by *R. ornithinolytica* have been increasingly reported in the past decade (De Petris and Ruffini, 2018), and the advent of techniques like MALDI-TOF MS has enabled identification of new isolates (De Jong et al., 2013; Seng et al., 2016). Notable reports of *R. ornithinolytica* infections include sepsis in a patient with mitochondrial disease in Spain (Sanchez-Codez et al., 2019), urinary tract infection in a pediatric patient in Turkey (Buyukcam et al., 2019), and ventilator associated pneumonia in two immunocompetent trauma patients in USA (Van Cleve et al., 2018). *R. ornithinolytica* has also been implicated in joint infections and appendicitis in the clinical setting (Beye et al., 2018; Hajjar et al., 2018). In addition, MDR strains of this pathogen have emerged in recent years, primarily due to the spread of the resistance genes. The first NDM-1-producing *R. ornithinolytica* strain was isolated from the pus of hospitalized patients in India in 2013 (Khajuria et al., 2013) and was followed by reports of resistant *R. ornithinolytica* in USA, China, India, Spain, Korea, Bangladesh, and Brazil. In addition, some resistant strains have also been isolated from animals, vegetables, wastewater, and river water. The major drug resistance genes identified in these isolates include *bla*<sub>NDM-1</sub>, *bla*<sub>KPC-2</sub>, *mcr-1*, *bla*<sub>CTX-M-3</sub>, *bla*<sub>IMP-4</sub>, and *bla*<sub>OXA-1</sub> (Zhou et al., 2014; Hasan et al., 2015; Zheng et al., 2015; Hernandez-Garcia et al., 2018; Miao et al., 2018; Yoon et al., 2018; Carramachi et al., 2019; Wang et al., 2019). However, little is known regarding the carbapenemase- and ESBL-producing *R. ornithinolytica* strains. To the best of our knowledge, this is the first report on resistant *R. ornithinolytica* isolated from human gut microbiota.

Studies show that bacterial strains producing NDM-1 are resistant to most of the antibiotics used clinically and are only sensitive to a few such as colistin and tigecycline (Khan et al., 2017). However, Wang et al. (2018) described a strain of *K. pneumoniae* isolated from both humans and animals.
that was positive for the NDM-1 gene and showed resistance to polymyxin and colistin (Wang et al., 2018). Consistent with this, our drug susceptibility test showed that ROFO58 was resistant to cefotaxime, ceftazidime, gentamicin, tetracycline, and ciprofloxacin. *R. ornithinolytica* is naturally resistant to aminopenicillin due to the presence of the chromosomal class A β-lactamase gene (Vasaikar et al., 2017), and all isolates did exhibit resistance to ampicillin. The presence of drug resistance genes strongly correlated with resistant phenotypes. Therefore, the WGS approach can rapidly detect antibiotic resistance genes that have been annotated and predict drug-resistance in a particular isolate. Although all trans-conjugants showed an MDR phenotype similar to that of the donor strain in our study, the NDM-1-producing isolate had lower resistance to gentamicin and were sensitive to imipenem, ciprofloxacin, and amoxicillin-clavulanic acid. This could be due to factors other than the resistance genes. For example, studies have shown that the same resistance gene confers different susceptibility patterns when under the control of different promoters (Zhou et al., 2019).

A previous report showed that the *bla*<sub>NDM-1</sub> gene in Enterobacteriaceae is located on a rapidly transferable 50–200 kb plasmid belonging to several incompatibility groups such as IncL/M, IncH1, IncFII, IncF, or untypable (Ahmad et al., 2018). In this study, the plasmid carrying *bla*<sub>NDM-1</sub> was larger than 300 kb and of type IncFIB. Furthermore, this plasmid could be transferred from R0F058 to *E. coli* EC600 but not to *E. coli* J53. We surmised therefore that the plasmid was rare and selectively transferred. Epidemiological and genetic studies have shown that plasmid ligation and transposition of mobile genetic elements play an important role in the horizontal transmission of *bla*<sub>NDM-1</sub> (Khajuria et al., 2013). BLAST analysis showed the presence...
of the aminoglycoside acetyltransferase gene \( [\text{aac(3)}] \) and bleomycin resistance gene \( \text{bla}_{\text{NDM-1}} \), respectively. There were two insertion sequences (\( \Delta IS91 \)) flanking both resistance genes, indicating its important role in the spread of \( \text{bla}_{\text{NDM-1}} \) and other resistant genes. In addition, a remnant of \( \Delta ISaba125 \) was located upstream of \( \text{bla}_{\text{NDM-1}} \) (Figure 2). A previous study has shown that partial \( \Delta ISaba125 \) is a promoter of the \( \text{bla}_{\text{NDM-1}} \) gene (Poirel et al., 2011). The \( \text{bla}_{\text{NDM-1}} \) and \( \text{bla}_{\text{CTX-M-3}} \) genes were detected in the ROF058 trans-conjugant, indicating that \( \text{bla}_{\text{NDM-1}} \) can be co-transferred with \( \text{bla}_{\text{CTX-M-3}} \) and across species, and thereby disseminate drug resistance. Although clinically important bacteria such as \( \text{Enterobacter cloacae} \) and \( \text{Klebsiella pneumoniae} \) produce both \( \text{NDM-1} \) and \( \text{CTX-M} \), and other resistant genes. In this study, we have shown that the co-existence of \( \text{bla}_{\text{NDM-1}} \) and \( \text{bla}_{\text{CTX-M-3}} \) in one plasmid for the first time.

The isolates in our study carried the \( \text{bla}_{\text{CTX-M-3}} \) and \( \text{bla}_{\text{CTX-M-14}} \) genes, which is similar to the \( \text{CTX-M} \) types of ESBL-producing \( \text{K. pneumoniae} \) isolated from environmental samples (Chi et al., 2019). This likely indicates spread of resistant bacteria between human hosts and the environment. The \( \text{bla}_{\text{CTX-M}} \) genes are usually present on the \( \text{IncFIC} \), \( \text{IncHI2} \), \( \text{IncL/M} \), and \( \text{IncK} \) plasmid types (Zhang et al., 2016). WGS analysis showed that three ESBL-producing \( R. ornithinolytica \) isolates in our study carry the \( \text{IncFIC} \) plasmid, along with a \( \Delta ISEcp1 \) sequence upstream of \( \text{bla}_{\text{CTX-M-3}} \) in ROF058, and \( \text{bla}_{\text{CTX-M-14}} \) in ROE007 and ROE058. A study showed that 97.6% of the \( \text{bla}_{\text{CTX-M}} \) genes have an upstream \( \Delta ISEcp1 \) sequence (Adamski et al., 2015), which plays an important role in the transposition of \( \text{bla}_{\text{CTX-M}} \) and other ESBL genes (Decano et al., 2019). ROE007 and ROE058 isolated from the same village showed an identical sequence flanking \( \text{bla}_{\text{CTX-M-14}} \), i.e., \( \Delta ISEcp1\text{-bla}_{\text{CTX-M-14}}\text{-\( \Delta IS903 \)) \), which could be the result of either the clonal expansion of the original host bacteria or lateral transfer of the genetic elements. A linear \( \text{fecE-fecD} \)-\( \text{fecC-fecB-fecA} \)-\( \text{fecR-fecI} \) ferric citrate genes were detected upstream of \( \text{bla}_{\text{CTX-M-14}} \) in ROI014, which is very similar to that reported in an environmental \( \text{K. pneumoniae} \) isolate (accession no. KF914891) from the same area (Chi et al., 2019). These findings strongly indicate the spread of drug-resistance genes between \( K. pneumoniae \) and \( R. ornithinolytica \).

To summarize, we characterized \( R. ornithinolytica \) isolates from healthy human feces in terms of their antibiotic susceptibility, drug resistance genes, and the transfer mechanism of the mobile genetic elements. The strains co-producing \( \text{NDM-1} \) and \( \text{CTX-M} \) can confer higher levels of resistance to multiple antibiotics and can transfer the genes to other strains via plasmids by conjugation. Therefore, the patterns of antibiotic resistance and transmission should be closely monitored for \( R. ornithinolytica \), especially in healthy individuals.

**DATA AVAILABILITY STATEMENT**

The datasets generated for this study can be found in the National Center for Biotechnology Information, VJJYE00000000-VVYH000000000, https://submit.ncbi.nlm.nih.gov/.

**ETHICS STATEMENT**

The studies involving human participants were reviewed and approved by Ethics Committee for Shandong Center for Disease Control and Prevention; Shandong Center for Disease Control and Prevention. The patients/participants provided their written informed consent to participate in this study.

**AUTHOR CONTRIBUTIONS**

SW and LX contributed to perform the experiments, data analysis, and manuscript writing. XC and YL contributed to perform the experiments. XC, ZK, PH, HX, ZB, and BZ contributed to the sample collection and data analysis. BZ, ZB, and HX contributed to conceive and design the experiments and reviewed the article.

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