Characteristics and epidemiology of extended-spectrum β-lactamase-producing Multidrug-Resistant Klebsiella pneumoniae from Red Kangaroo,China

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Abstract

β-lactamase represents a serious challenge for treatment and public health, extended-spectrum β-lactamase (ESBL) producing Klebsiella pneumoniae clones have been increasingly reported worldwide.However, little is known about the prevalence and biological characteristics of drug-resistant strains in zoos.During a routine surveillance at in Zhengzhou zoo of China, we reported firstly that the Klebsiella pneumoniae isolate from healthy Red Kangaroos (Macropus Rufus) showed severe MDR, especially resistant to Cefuroxime Sodium (MIC, >64 μg/ml), Ceftriaxone (MIC, >8 μg/ml) and Cefepime (MIC, >64 μg/ml) and belonged to ST290. The whole genome sequencing showed that Chrome Chr-M297-1 harbored blaDHA-3, blaSHV-1, blaCTX-M-14, FosA5, dfrA3, sul3 etc., pM297-1.1(222,864bp, IncFIB(K)) carried 9 antimicrobial genes including blaCTX-M-14 , blaTEM-191, APH(3'')-Ib, APH(6)-Id and QnrS1 etc., and pM297-1.2 (225,763bp, IncFII(K)) carried 22 antimicrobial genes including blaTEM-1, blaCTX-M-3, APH(3')-Ia, AAC(3)-IIa, AAC(6')-Ib-cr, aadA16, QnrB2, QnrS1, QacEΔ1, mphA, sul1, dfrA27, etc. Traceability analysis revealed that these two plasmids are highly similar to those recovered from human clinical samples in some southern cities in Sichuan Province, China(> 99%), suggesting the spread and distribution of these plasmids in China. Furthermore, two plasmids harboring conjugal transfer genes facilitate the transmission of antimicrobial genes by conjugation with E.coli J53. Our research shows that the transmission and adaptation of Klebsiella pneumoniae producing ESBLs in zoo environment, suggesting that the zoo is gradually becoming an important potential reservoir of clinically important drug-resistant genes.Therefore, it is necessary to strengthen the monitoring of the emergence and spread of drug-resistant strains in zoo environment in captive wild animals.

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K. πνευμονιαε προδυςινγ β-λαςταμασε ιν ζοο

Summary

β-lactamase represents a serious challenge for treatment and public health, extended-spectrum β-lactamase (ESBL) producing *Klebsiella pneumoniae* clones have been increasingly reported worldwide.However, little is known about the prevalence and biological characteristics of drug-resistant strains in zoos.During a routine surveillance at in Zhengzhou zoo of China, we reported firstly that the *Klebsiella pneumoniae* isolate from healthy Red Kangaroos (*Macropus Rufus*) showed severe MDR, especially resistant to Cefuroxime Sodium (MIC, >64 μg/ml), Ceftriaxone (MIC, >8 μg/ml) and Cefepime (MIC, >64 μg/ml) and belonged to ST290. The whole genome sequencing showed that Chrome Chr-M297-1 harbored*bla* $_{\text{DHA-3}}$, *bla* $_{\text{SHV-1}}$,*bla* $_{\text{CTX-M-14}}$, FosA5, dfrA3, sul3 etc., pM297-1.1(222,864bp, IncFIB(K)) carried 9 antimicrobial genes including*bla* $_{\text{CTX-M-14}}$, *bla* $_{\text{TEM-191}}$, APH(3")-Ib, APH(6)-Id and QnrS1 etc., and pM297-1.2 (225,763bp, IncFII(K)) carried 22 antimicrobial genes including*bla* $_{\text{TEM-1}}$, *bla* $_{\text{CTX-M-3}}$, APH(3')-Ia, AAC(3)-IIa, AAC(6')-Ib-cr, aadA16, QnrB2, QnrS1, QacEΔ1, mphA, sul1, dfrA27, etc. Traceability analysis revealed that these two plasmids are highly similar to those recovered from human clinical samples in some southern cities in Sichuan Province, China(> 99%), suggesting the spread and distribution of these plasmids in China. Furthermore, two plasmids harboring conjugal transfer genes facilitate the transmission of antimicrobial genes by conjugation with *E.coli* J53. Our research shows that the transmission and adaptation of *Klebsiella pneumoniae* producing ESBLs in zoo environment, suggesting that the zoo is gradually becoming an important potential reservoir of clinically important drug-resistant genes.Therefore, it is necessary to strengthen the monitoring of the emergence and spread of drug-resistant strains in zoo environment in captive wild animals.

Key words: Multidrug-Resistance, Red Kangaroo, *Klebsiella pneumoniae* , Zoo, public health risk

1 INTRODUCTION

The emergence and dissemination of antimicrobial resistance (AMR) in the environment has become a global concern. Antimicrobial resistance (AMR) has been an area of focus during the past two decades and was recognizing AMR as a potential and serious threat for global public health among animals, environment and humans (Tacconelli et al., 2018). AMRs can be disseminated rapidly through various possible pathways, including foodborne pathogens, insects, wastewater, pet, food producing or wild animals, etc. (Q. E. Yang et al., 2019). In particular, more than 25 outbreaks of human infectious disease outbreaks were reported, 11 of which related to animals in farms, petting zoos and zoos during 1990 to 2000 (Bender & Shulman, 2004). Human may be infected by contacting wild animals directly or indirectly in some interactive activities of zoos, but this situation is easily ignored. The role of wild animals in zoos in the epidemiology of multidrug

resistance (MDR) is a little concerned. Some previous studies have shown that bacterial isolates from wild animal living in human activity areas show stronger drug resistance compared with wild animals living in remote areas (Allen et al., 2011; Cole et al., 2005; Kozak, Boerlin, Janecko, Reid-Smith, & Jardine, 2009; Rolland, Hausfater, Marshall, & Levy, 1985; Skurnik et al., 2006). As a part of human urban life, the captive wild animals in zoos are obviously more closely contacted with human. Therefore, the captive animals in zoos may become a potential natural reservoir for AMRs and antibiotic resistant bacteria.

It is now abundantly clear that bacteria are able to meet the evolutionary challenge of combating antimicrobial chemotherapy, often by acquiring preexisting resistance determinants from the bacterial gene pool. This is achieved through the concerted activities of mobile genetic elements able to move within or between DNA molecules, which include insertion sequences, transposons, and gene cassettes/integrons, and those that are able to transfer between bacterial cells, such as plasmids and integrative conjugative elements. It has been proved that MDR bacteria from captive wild animals can carry various mobile genetic elements, such as IncI1 harboring *bla* $_{\text{CTX-M-1}}$ and qnrS1 in *E.coli* from Czech zoo (Dobiasova et al., 2013), integron (class I and II) and plasmid carrying*bla* $_{\text{CMY-26}}$,*qnr* and *aac(6')-Ib-cr* in Gram-negative bacterial isolates from Japanese zoo(Ahmed et al., 2007), MDR *Salmonella Enterobacter* from captive wild animals and foreign animals in Ohio(Farias et al., 2015). It is suggested above that the emergence and dissemination of Multidrug Resistant pathogenic bacteria carried by wild animals in zoos may increase a serious public health risk between human, animal and environment.

To further understand the routes of dissemination of AMRs harboring bacteria in zoo, in the present study, fresh animal feces samples were collected and *Enterobacteriaceae* isolates were isolated in the routine monitoring of bacterial diseases in Zhengzhou zoo, Henan province, China. An MDR *Klebsiella pneumoniae* isolate from Red Kangaroo was severe drug resistance, including cephalosporins, such as the second generation cephalosporins (*Cefuroxime Sodium*), the third generation cephalosporins (*Ceftriaxone*) and even the fourth generation cephalosporins (*Cefepime*). The whole genome sequencing (WGS) analysis was subjected to evaluate the relationship between its plasmid and drug-resistant gene related elements and human clinical isolates.

2 MATERIALS AND METHODS

2.1 Bacterial isolates

Enterobacteriaceae isolates were isolated from fresh fecal samples of animals at Zhengzhou Zoo, Henan province, China. Briefly, fecal samples were collocated into Eppendorf tube with 500μL sterile saline and shake gently and stood for 10 min. The supernatant was used to inoculate onto the MacConkey Agar (Beijing SanYao Science & Technology Development Co, Beijing, China) plate at 35°C for 18 hours. All the colonies with different morphologies and colors were stored and subjected to further analysis. Species identification was carried out using 16S rRNA sequence (TIANYI HUIYUAN,China).

2.2 DrugSusceptibility Testing

Drug susceptibility testing was performed by broth microdilution method (microbial susceptibility kit, BIOKONT, China) according to the CLSI guidelines (CLSI,2019). Fifteen antimicrobial drugs were used to screen MDR in this study, including *Ampicillin, Ampicillin/Sulbactam, Piperacillin/Tazobactam, Aztreonam, Cefuroxime sodium, Ceftriaxone, Cefepime, Ciprofloxacin, Levofloxacin, Meropenem, Colistin, Chloramphenicol* , *Trimethoprim/ Sulfamethoxazole, Nitrofurantoin*and *Amikacin* . The Drug susceptibility to *Tetracycline* and*Doxycycline* was tested by Kirby-Bauer disk diffusion method (OXOID, UK), and *E.coli* ATCC25922 was used as a quality-control strain.

2.3 Whole-genome sequencing and bioinformatics analysis

Based on drug susceptibility testing, a MDR *Klebsiella pneumoniae*isolate (named as M297-1) from Red Kangaroo was identified multidrug resistant (MDR) bacteria, and subjected to WGS using Oxford Nanopore Technologies (ONT) MinION platform (Biomarker Technologies, China) (Ashton et al., 2015; Loman, Quick, & Simpson, 2015). The sequencing was carried out according to the standard protocol provided by ONT,

and the high-quality genomic DNA, was extracted by Nanodrop, Qubit and 0.35% agarose gel electrophoresis for purity, concentration and integrity. Large fragments of DNA were recovered by BluePippin automatic nucleic acid recovery system. The library was constructed by ligation sequencing kit (SQK-LSK109 Ligation Sequencing Kit, Oxford Nanopore Technologies, UK), and the DNA damage repair and terminal repair, magnetic bead purification were used to connect, purify again, and the Qubit library was quantified and sequenced on the machine.

The phylogenetic tree was constructed based on 16s rRNAs*Klebsiella pneumoniae* isolate multilocus sequence typing (MLST) was conducted by using MLST 1.8 or PubMLST (http://pubmlst.org/)Plasmid replicon typing, plasmid multilocus sequence typing ,and identification of resistance genes were performed using Plasmid Finder (*https://cge.cbs.dtu.dk/services/PlasmidFinder/*) or pMLST 2.0, and Resfinder 2.0 or CARD, respectively. The comparison of the similarity between plasmids and known plasmids was conducted using PLSDB databases (*https://ccb-microbe.cs.uni-saarland.de/plsdb/*). Plasmid sequence was annotated with MiGAP (*https://www.migap.org/*), and the genomic structure was compared in EasyFig. The comparative map of plasmid genome was drawn by the Illustrator for Biological Sequences (IBS) software v1.0. (Liu et al., 2015) and modified manually. Transposons and insertion sequences were determined using ISfinder.

2.4 Conjugation Experiments and evaluation of plasmid stability

To investigate the transferability of plasmid in*Klebsiella pneumoniae* M297-l isolate, we performed conjugation assays with sodium-azide resistance*E.coli* J53 as a recipient strain. Briefly, overnight cultures of MDR*Klebsiella pneumoniae*M297-l as a donor and the recipient *E. coli* J53 strain were 1:10 mix and conducted on nitrocellulose membranes on a MacConkey Agar plate by incubation at 35°C for 16-20 hours. After incubation, we subsequently ten-fold serial diluted the mixed culture in sterile saline and aliquoted 100μl of diluted culture onto MacConkey Agar plates supplemented with 20mg/L of cefotaxime and 200mg/L of sodium azide. *E.coli* transconjugants were screened by drug susceptibility testing. To further evaluate the stability of the plasmid of *E.coli* transconjugants,*E.coli* transconjugants were passaged continuously in MHB without antibiotics and detected in McConkey Agar plate containing 20mg/L cefotaxime according to previous reports(Di Luca et al., 2017; Walsh, Weeks, Livermore, & Toleman, 2011; Wein, Hulter, Mizrahi, & Dagan, 2019).

Accession numbers: the sequence data and details of the sequenced samples, including the date and location of collection and source, were submitted to the GenBank. Accession numbers for Chr-M297-1, plasmid pM297-1.1and pM297-1.2 from *Klebsiella pneumoniae* M297-1, respectively. *Klebsiella pneumoniae* M297-1 assembly contigs are deposited under study accession number CP051490, CP051491, and CP051492.

3 RESULTS

3.1 MDR Klebsiella pneumoniae M297-1 isolates from Red Kangaroo

Overall, thirty-four isolates were isolated from animal fecal samples in Zhengzhou Zoo, including 33 isolates of *Escherichia coli* and 1 isolate of *Klebsiella pneumoniae* . The susceptibility profiles indicated that most of the isolates were susceptible to some of the common antimicrobial drugs in clinical use, while the antimicrobial resistances of the isolates from Red Kangaroo (n=4) were serious (Table 1). Among them, compared with other isolates, the isolate of*Klebsiella pneumoniae* (named as M297-1) had high resistance to major groups antimicrobial drugs including group A (*Ampicillin*), group B (*Cefuroxime, Cefepime, Ceftriaxone, Ciprofloxacin, Levofloxacin, Trimethoprim/ Sulfamethoxazole, Ampicillin/Sulbactam*), group C (*Aztreonam, Chloramphenicol*), group U (*Nitrofurantoin*). Furthermore, this isolate was subjected to whole genome sequencing analysis.

3.2 Genomic structure of the Chr-M297-1 harboring $bla_{\text{DHA-3}}$,$bla_{\text{SHV-1}}$ and $bla_{\text{CTX-M-14}}$

The chromosomal DNA of M297-1 (named as Chr-M297-1) is 5,750,384 bp in size, and exhibits 100% identity with a query coverage of 100% to the *K.pneumoniae* by Ribosomal Multilocus Sequence Typing (rMLST). Isolate M297-1 belongs to ST 290)(*https://pubmlst.org/rmlst/*) , has the highest homology with *Klebsiella pneumoniae* isolate (99.93%) from human clinical samples based on phylogenetic tree of 16s rRNA (Figure

S1). For ST290, only five isolates were recorded in the database, of which one strain was from dairy cow and the other four strains were from human clinical samples. Chr-M297-1 possesses 9 gene islands and 1 prophage, 169 antimicrobial genes most importantly carrying 3 β-lactamase genes (*bla* $_{\text{DHA-3}}$, *bla* $_{\text{SHV-1}}$, and*bla* $_{\text{CTX-M-14}}$), FosA5, dfrA3, sul3 *etc.*(Table 2). In addition, the resistance pump was mainly composed of ABC family genes, as well as MFS, SMR and MATE family genes.

3.3 Genomic structure of plasmid carrying important antimicrobial genes

K.pneumoniae isolate M297-1 contains two plasmids pM297-1.1(222,864bp) and pM297-1.2(225,763bp), which belong to IncFIB(K) and IncFII(K),respectively. pM297-1.1 contains five Genomic_islands, two β-lactamase genes (*bla* $_{\text{CTX-M-14}}$and *bla* $_{\text{TEM-191}}$), two aminoglycoside resistant genes (APH(3")-Ib and APH(6)-Id), quinolone resistance gene QnrS1 and others. According to PLSDB database, only three plasmids were similar to pM297-1.1 focusing on multidrug resistant region, which is TnpA-other-$bla_{\textbf{CTX-M-14}}$-TnpA/Tn903-$bla_{\textbf{TEM-191}}$ – TnpA/IS2-other- QnrS1 -tnpR/Tn552-ISKpn19- tnpR/Tn4653 (Figure 1). Specially, conjugal transfer protein genes (*traABCDEGHIKLMNPQTUVX- trbBCEFI- FinO*), Lactose permease and two aminoglycoside resistant genes (transposase- *APH(3")-Ib* and *APH(6)-Id*) co-located in the same larger Genomic_island 11 (42,044bp-101,359bp).

pM297-1.2 contains 7 Genomic_islands and 1 prophage. Comparing to pM297-1.1, pM297-1.2 harbors much more antimicrobial genes including 2 extended-spectrum β-lactamase (ESBLs) gene (*bla* $_{\text{CTX-M-3}}$and *bla* $_{\text{TEM-1}}$), 5 aminoglycoside resistance genes (*APH(6)-I,APH(3')-Ia, AAC(3)-IIa,aadA16, AAC(6')-Ib-cr*) , fluoroquinolones resistant genes (*QnrB2* and *QnrS1*), *mphA, sul1,* QacEdelta1 multidrug exporter, *etc* . Notably, pM297-1.2 carries chloramphenicol resistant gene (floR), tetracycline resistant gene (tetG), sulfonamide resistance gene (sul1,sul2) (Table 2), but pM297-1.1 does not contain these genes.

Furthermore, the genomic region of pM297-1.1 and pM297-1.2 were divided into different Multidrug Resistance regions according to composition of antimicrobial genes and related antimicrobial genes. The pM297-1.1possessed one Multidrug resistance gene region (TnpA-other-*bla* $_{\text{CTX-M-14}}$-InsC/Tn903-*bla* $_{\text{TEM-191}}$-insD-other-insC21-*QnrS1* - tnpR/Tn552 -other-tnpR/Tn4653) which was entirely conserved in p911021-tetA, p1_020098, pLAP2_020009, NZ_CP040176.1 (similarity,¿99.86%). This genomic region was flanked by InsC/Tn903 and insC21 sequences and co-harbored other genes encoding extended-spectrum β-lactamases and one gene conferring resistance to fluoroquinolones.

The multidrug resistance region of pM297-1.2 appeared to consists of three parts, which are named MDR1, MDR2 and MDR3. MDR1 was bracketed by derivatives of Tn903, Tn3 and Tn1721(Tn21 subfamily) and harbored resistance genes against aminoglycoside, sulfonamide and chloramphenicol. MDR2 carried one class I integron (intl1) and co-harbored other genes resistance to aminoglycoside, sulfonamide, rifampicin, and fluoroquinolones. Notably, MDR3 was bracketed by derivatives of Tn3 harboring two β-lactamases genes*bla* $_{\text{CTX-M-3}}$ and *bla* $_{\text{TEM-1}}$and Tn4653 gene carrying one gene resistance to fluoroquinolones. Some plasmid, including pKp21774-135, pKPNH54.1, pKF3-94, pL22-5, p2, pR210-2-CTX, pCTXM15_020019, pSCM96-1, pNH25.2, were found to possess the MDR3 by a database search. Obviously, these two plasmids evolved from other plasmids by inserting, deleting or replacing.

3.4 Traceability analysis of these two plasmids

In PLSDB database, five plasmids from *Klebsiella pneumoniae* in human clinical samples were high similarity to plasmid pM297-1.1. pM297-1.2 was high similarity to thirteen plasmids, of which 9 were derived from *Klebsiella pneumoniae* , and 1 from *Klebsiella variicola* , *Klebsiella quasipneumoniae* , *Klebsiella sp.* and *Escherichia coli* in human clinical samples from different regions (Table S1).

3.5 Conjugative transfer and stability of plasmids harboring MDR regions

Transconjugants were obtained from mating experiments with*Klebsiella pneumoniae* M297-1 donors, in *E. coli* J53 recipients, at rates of 10^{-5} to 10^{-6}transconjugants per recipient cell. All 13 putative transconjugants tested were found to be the recipient background species and to be β-lactamase positive by antimicrobial

susceptibility testing. Notably, 8 of the 13 transconjugants were resistance to *sulfamethoxazole, chloramphenicol* and *tetracycline* , which was suggested to be transconjugants carrying with pM297-1.2 (Table 3). The another 4 strains were not resistant to the *sulfamethoxazole, chloramphenicol* and*tetracycline* (Table 3),which was considered to be transconjugants carried pM297-1.1. All positive transconjugants colonies tested, resistance to *cefotaxime* , remained detectable throughout the 6-day passage experiment, even in the absence of antibiotic selection. Therefore, it was confirmed that the two plasmids carried by*Klebsiella pneumoniae* M297-1 had stronger transferability and stability in new host bacteria.

4 DISCUSSION

In this study, we reported firstly the multidrug-resistant*Klebsiella pneumoniae* M297-1 carried by healthy Red Kangaroo, which is suggested that asymptomatic animal hosts were more likely to be ignored and may become an important reservoir for drug-resistant pathogens, such as *Salmonella entericus* (Perron, Quessy, & Bell, 2008). *Salmonella* isolated from captive wild animals in Ibadan, western Nigeria, has been observed to be resistant to sulfadiazine and penicillin (Falade & Durojaiye, 1976). Among the 232 isolates of Gram-negative bacteria isolated from mammals, reptiles and birds raised in Asakusa Zoo, Hiroshima Prefecture, Japan, 21.1% of Gram-negative bacteria carry at least one drug-resistant gene and have multiple drug-resistant phenotypes (Ahmed et al., 2007). ESBLs and*fluoroquinolone* resistance genes detected in Czech zoos were associated with the transmission of specific *E.coil* clones and plasmids of specific incompatible groups between different animal species (Dobiasova et al., 2013). *S.aureus* from a zoo and wildlife in Germany from 2008 to 2016, two isolates from juvenile red squirrels showed multiple drug resistance phenotypes (Fessler et al., 2018). The frequency of AMRs detected in animals living in human settlements is significantly higher than that in animals living in the wild environment (Grall et al., 2015; Kock et al., 2018). The isolates from wild animals show a similar pattern of drug resistance to*E.coil* from human clinical sources in their study areas (Jobbins & Alexander, 2015). another some studies, drug-resistant Salmonella isolates identified from zoo environments, animals and their keepers have been confirmed to be clone-related (Farias et al., 2015; Milton et al., 2018). Therefore, wild animals may be important hosts and storage hosts for the spread of drug-resistant bacteria, and human activities significantly affect the microbial community of captive wild animals in zoo environments that are easy to be ignored.

In the present study, the ST290 sequence types *K.pneumoniae*M297-1 from Red Kangaroo is closely related to that of human clinical isolates, but only 5 cases of this sequence type have been reported worldwide, reported by the United States, Australia and China (Figure 2). The plasmid of isolates carrying *bla* $_{\text{KPC-2}}$,*bla* $_{\text{IMP-8}}$, *bla* $_{\text{TEM-1}}$ and*bla* $_{\text{CTX-M-15}}$ genes transmission have been found in neonatal infections (Jin et al., 2017; Kong et al., 2020). The first case of nosocomial epidemics infection caused by NDM-5 metallo-β-lactamase ST290 isolates was reported in China (Wang et al., 2019). Therefore, our study further showed that zoo-derived MDR bacteria are closely related to human-derived MDR.

bGWAS provided comprehensive information about isolate M297-1. The whole genome analysis of M297-1 confirmed that it's plasmids carried clinically related ESBLs genes *bla* $_{\text{TEM-1}}$, *bla* $_{\text{TEM-191}}$, *bla* $_{\text{CTX-M-3}}$ and*bla* $_{\text{CTX-M-14}}$ (Table 2 and Figure 1). Since the late 1990s, the multidrug-resistant *Enterobacteriaceae* that produces ESBLs has become an important cause of urinary tracts and bloodstream infections in the community (Pitout & Laupland, 2008; Wyres et al., 2020). This is a rapidly evolving class of β-lactamases, usually from *bla* $_{\text{TEM-1}}$, *bla* $_{\text{TEM-2}}$ or*bla* $_{\text{SHV-1}}$ genes, which have the ability to hydrolyze third-generation *cephalosporins* and *aztreonam*and can be inhibited by clavulanic acid (Paterson & Bonomo, 2005). Although new members of the ESBLs family are often found, the earlier*bla* $_{\text{CTX-M-14}}$ and*bla* $_{\text{CTX-M-15}}$ enzymes are prevalent in world (Bush & Bradford, 2020; Bush & Fisher, 2011), while*bla* $_{\text{CTX-M-3}}$ enzymes are prevalent mainly in Europe (Canton et al., 2008). Resistance to extended-spectrum cephalosporins such as *ceftazidime, cefotaxime* and *cefepime* was often observed when ESBLs appeared in *Klebsiella sp.* (Babic, Hujer, & Bonomo, 2006). ESBLs, was also detected on M297-1 chromosome, but it also stably carried ESBLs positive plasmids pM297-1.1 and pM297-1.2, indicating that drug resistance itself are not the only selection criterion for maintaining ESBLs cod plasmids. In addition, in this study, other drugs resistance genes of *sulfonamides, fluoroquinolones, aminoglycosides* and *tetracyclines* on chromosomes and plasmids suggest that it may have

evolved under the pressure of many antibiotics.

Mobile genetic elements, such as insertion sequence, transposon, integron and prophage, can mobilize antibiotic resistance genes. The detection of *bla* $_{\text{TEM}}$ and*bla* $_{\text{CTX-M}}$ type β-lactamases genes in a variety of genetic backgrounds suggests that their mobilization may involve multiple mechanisms. In this study, for plasmid pM297-1.1, IS1380 is located at upstream of *bla* $_{\text{CTX-M-14}}$, and downstream detected IS903 is also located at upstream of*bla* $_{\text{TEM-191}}$. *bla* $_{\text{TEM-191}}$ may also be related to its downstream truncated IS2 gene, and similar structures also exist in similar plasmids retrieved (p911021-tetA,MG288679.1;p1_020098,NZ_CP036307.1; pLAP2_020009, CP038004.1) (Figure 1). This seems to imply that the β-lactamase gene carried by pM297-1.1 may form a tandem structure of drug resistance gene after multiple homologous recombination. For MDR 3 region of plasmid pM297-1.2, the resistance gene box of β-lactamase genes in Tn3-like transposon was*tnpA-tnpR-bla* $_{\text{TEM-1}}$*-other-bla* $_{\text{CTX-M-3}}$, and also located on the prophage. We found that the similar plasmid (pKF3-94, pL22-5, p2, pCTXM15_ 020019, pSCM96-1) also had a complete or truncated similar structure (Figure 1). There are usually two IS431mec insertion sequence genes from *Staphylococcus aureus* at upstream and downstream of *aminoglycosides, fluoroquinolones, sulfonamides*and *tetracyclines* in the pM297-1.2 MDR region. The structure of the intI1- gene cassette carried by the plasmid pM297-1.2 supports the concept of mobile elements to transfer antimicrobial genes between different bacteria. The drug resistance gene cassette (*aac(6')-1b-cr-arr-3-dfrA27-aadA16)* is derived from the plasmid (ACC_NUCCORE : EU675686) carried by the multi-drug resistance*E.coil* isolated from the urine of patients in Huashan Hospital (Wei et al., 2009). This structure is completely preserved in pM297-1.2 from *Klebsiella pneumoniae* M297-1 (Figure 1) and is located on the Genomic Island. The drug resistance gene cassette mainly encodes β-lactamases, acetyltransferases and nucleoside transferases, which do not to require significant cell interaction and integrated into the metabolic network. Therefore, the interference from the existing genome is minimal, and it is the best example of a single gene corresponding to a single phenotype (Ghaly, Geoghegan, Tetu, & Gillings, 2020), but in any case, this genetic factor will help bacteria evolve multiple determinants of antibiotic resistance in different habitats (Edge & Hill, 2005).

Asia is one of the centers of antibiotic resistance, there is a surprising number of drug-resistant strains, including*K.pneumoniae* , a large number of acquired gram-negative MDR strains had been found (Jean & Hsueh, 2011). The prevalent STs in Asia include ST15, ST23, ST14 and ST231. Among them, ST15 is very common, ST23 is significantly related to Southeast Asia, while ST14 and ST231 are significantly related to South Asia (Wyres et al., 2020). In this study, most of the plasmids retrieved from PLSDB database were carried by *K.pneumoniae* . Among the *K.pneumoniae* isolates carrying highly similar plasmids to pM297-1.2, only two isolates of ST15 were isolated from Thailand and China, and one strain of ST23 and one strain of ST14 from China. The isolates carrying highly similar plasmids to pM297-1.1 did not detect the above four sequence types (Table S1). As a result, the two plasmids in our study belong to IncF, a narrow host spectrum plasmid widely in *Enterobacteriaceae* , and can carry a variety of AMR genes and play a major role in the spread of specific antimicrobial genes (Carattoli, 2011; Rozwandowicz et al., 2018). Some studies showed that IncFIB and IncFII plasmids are effective vectors of β-lactamases. These plasmids can promote the transfer of antimicrobial genes when exposed to antibiotics (Rooney et al., 2019).*bla* $_{\text{NDM}}$*, bla* $_{\text{OXA}}$ and other genes spread in the *Enterobacteriaceae* flora of medical facilities by relying on these plasmids (Simner et al., 2018; Strydom et al., 2020; Wu et al., 2019), while *bla* $_{\text{CTX-M}}$ can become the dominant gene carried by IncFII plasmids in France and China (Dahmen, Haenni, Chatre, & Madec, 2013; Du et al., 2012), but it seems that *bla* $_{\text{CMY-42}}$ is replacing*bla* $_{\text{CTX-M-15}}$ in some areas (Paul, Babenko, & Toleman, 2020). In particular, some IncFIB can not only express a high level of drug resistance, but also enhance the virulence of*K.pneumoniae* after conjugation with *K.pneumoniae* (Yang, Wai-Chi Chan, Zhang, & Chen, 2019). To sum up, our results suggested that the co-prevalence of plasmid IncFIB and IncFII in the same isolates may lead to serious public health problems.

Similar plasmids were found by Sweden, Thailand and China respectively. Except for one isolate in Sweden and two isolates in Thailand, the other isolates carrying similar plasmids mainly distributed among the southern cities of Sichuan province, China (Figure 3, Table S1). From 2010 to 2019, it is suggested that the epidemic distribution of this drug-resistant plasmid is gradually spreading in China, and the difference in

carrying drug-resistant genes indicates that it is evolving. However, the phylogenetic analysis of the related isolates showed that M297-1 located on different branch with other*K.pneumoniae* isolates carrying similar plasmids (Figure 4). The possible interpretation may be that it comes from samples in Red Kangaroo, other reference isolates from clinical samples in human.

In conclusion, our study demonstrates the high resistance of 13 drugs including *Ceftriaxone* and *Cefepime*on MDR *K. pneumoniae*isolate from healthy red kangaroos in Zhengzhou zoo, China. Most importantly, this is the first report on a *K. pneumoniae* M297-1 (ST290) from wild animal carrying *bla* $_{\text{DHA-3}}$,*bla* $_{\text{SHV-1}}$, *bla* $_{\text{CTX-M-14}}$,*bla* $_{\text{TEM-191}}$, *bla* $_{\text{TEM-1}}$, and*bla* $_{\text{CTX-M-3}}$ in China and two conjugal transferable plasmids co-harboring other antimicrobial genes *AΠH(3′)-Ia, AΠH(3')-Iβ, AΠH(6)-Iδ, AA"(3)-IIa, AA"(6′)-Iβ-ςρ, aaδA16, XνρB2, XνρΣ1, XaςEΔ1, μπηA, συλ1* and *dfrA27* etc. our research confirmed that there is a close relationship between drug-resistant strains carried by wild animals in zoos and human clinical isolates.it is suggested that the zoo may be becoming an important reservoir of clinically important MDR isolate, which pose a potential public health risk that can be ignored.

Data Availability Statement

All data generated or used during the study appeared in the submitted article.

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CONFLICT OF INTERESTS

We declare that we and our co-authors do not have any financial conflicts of interest and the funder(s).

Ethical Statement

Ethical Statement is not applicable.

REFERENCES

Ahmed, A. M., Motoi, Y., Sato, M., Maruyama, A., Watanabe, H., Fukumoto, Y., & Shimamoto, T. (2007). Zoo animals as reservoirs of gram-negative bacteria harboring integrons and antimicrobial resistance genes.*Appl Environ Microbiol* , 73(20), 6686-6690. doi:10.1128/aem.01054-07

Allen, S. E., Boerlin, P., Janecko, N., Lumsden, J. S., Barker, I. K., Pearl, D. L., . . . Jardine, C. (2011). Antimicrobial resistance in generic *Escherichia coli* isolates from wild small mammals living in swine farm, residential, landfill, and natural environments in southern Ontario, Canada. *Appl Environ Microbiol* , 77(3), 882-888. doi:10.1128/aem.01111-10

Ashton, P. M., Nair, S., Dallman, T., Rubino, S., Rabsch, W., Mwaigwisya, S., . . . O'Grady, J. (2015). MinION nanopore sequencing identifies the position and structure of a bacterial antibiotic resistance island. *Nat Biotechnol* , 33(3), 296-300. doi:10.1038/nbt.3103

Babic, M., Hujer, A. M., & Bonomo, R. A. (2006). What's new in antibiotic resistance? Focus on beta-lactamases. *Drug Resist Updat* , 9(3), 142-156. doi:10.1016/j.drup.2006.05.005

Bender, J. B., & Shulman, S. A. (2004). Reports of zoonotic disease outbreaks associated with animal exhibits and availability of recommendations for preventing zoonotic disease transmission from animals to people in such settings. *J Am Vet Med Assoc* , 224(7), 1105-1109. doi:10.2460/javma.2004.224.1105

Bush, K., & Bradford, P. A. (2020). Epidemiology of beta-Lactamase-Producing Pathogens. *Clin Microbiol Rev* , 33(2). doi:10.1128/cmr.00047-19

Bush, K., & Fisher, J. F. (2011). Epidemiological expansion, structural studies, and clinical challenges of new beta-lactamases from gram-negative bacteria. *Annu Rev Microbiol* , 65, 455-478. doi:10.1146/annurev-micro-090110-102911

Canton, R., Novais, A., Valverde, A., Machado, E., Peixe, L., Baquero, F., & Coque, T. M. (2008). Prevalence and spread of extended-spectrum beta-lactamase-producing *Enterobacteriaceae* in Europe. *Clin Microbiol Infect* , 14 Suppl 1, 144-153. doi:10.1111/j.1469-0691.2007.01850.x

Carattoli, A. (2011). Plasmids in Gram negatives: molecular typing of resistance plasmids. *Int J Med Microbiol* , 301(8), 654-658. doi:10.1016/j.ijmm.2011.09.003

Cole, D., Drum, D. J., Stalknecht, D. E., White, D. G., Lee, M. D., Ayers, S., . . . Maurer, J. J. (2005). Free-living Canada geese and antimicrobial resistance. *Emerg Infect Dis* , 11(6), 935-938. doi:10.3201/eid1106.040717

Dahmen, S., Haenni, M., Chatre, P., & Madec, J. Y. (2013). Characterization of blaCTX-M IncFII plasmids and clones of*Escherichia coli* from pets in France. *J Antimicrob Chemother* , 68(12), 2797-2801. doi:10.1093/jac/dkt291

Di Luca, M. C., Sorum, V., Starikova, I., Kloos, J., Hulter, N., Naseer, U., . . . Samuelsen, O. (2017). Low biological cost of carbapenemase-encoding plasmids following transfer from *Klebsiella pneumoniae* to *Escherichia coli* . *J Antimicrob Chemother* , 72(1), 85-89. doi:10.1093/jac/dkw350

Dobiasova, H., Dolejska, M., Jamborova, I., Brhelova, E., Blazkova, L., Papousek, I., . . . Literak, I. (2013). Extended spectrum beta-lactamase and fluoroquinolone resistance genes and plasmids among*Escherichia coli* isolates from zoo animals, *Czech Republic* . FEMS Microbiol Ecol, 85(3), 604-611. doi:10.1111/1574-6941.12149

Du, X. D., Li, D. X., Hu, G. Z., Wang, Y., Shang, Y. H., Wu, C. M., . . . Li, X. S. (2012). Tn1548-associated armA is co-located with qnrB2, aac(6')-Ib-cr and blaCTX-M-3 on an IncFII plasmid in a *Salmonella enterica* subsp. enterica serovar Paratyphi B strain isolated from chickens in China. *J Antimicrob Chemother* , 67(1), 246-248. doi:10.1093/jac/dkr407

Edge, T. A., & Hill, S. (2005). Occurrence of antibiotic resistance in*Escherichia coli* from surface waters and fecal pollution sources near Hamilton, Ontario. *Can J Microbiol* , 51(6), 501-505. doi:10.1139/w05-028

Falade, S., & Durojaiye, O. A. (1976). Salmonellae isolated from captive animals in Ibadan, Western State of Nigeria. *J Wildl Dis* , 12(3), 464-467. doi:10.7589/0090-3558-12.3.464

Farias, L. F., Oliveira, C. J., Medardus, J. J., Molla, B. Z., Wolfe, B. A., & Gebreyes, W. A. (2015). Phenotypic and Genotypic Characterization of *Salmonella enterica* in Captive Wildlife and Exotic Animal Species in Ohio, USA. *Zoonoses Public Health* , 62(6), 438-444. doi:10.1111/zph.12170

Fessler, A. T., Thomas, P., Muhldorfer, K., Grobbel, M., Brombach, J., Eichhorn, I., . . . Schwarz, S. (2018). Phenotypic and genotypic characteristics of *Staphylococcus aureus* isolates from zoo and wild animals. *Vet Microbiol* , 218, 98-103. doi:10.1016/j.vetmic.2018.03.020

Ghaly, T. M., Geoghegan, J. L., Tetu, S. G., & Gillings, M. R. (2020). The Peril and Promise of Integrons: Beyond Antibiotic Resistance. *Trends Microbiol* . doi:10.1016/j.tim.2019.12.002

Grall, N., Barraud, O., Wieder, I., Hua, A., Perrier, M., Babosan, A., . . . Andremont, A. (2015). Lack of dissemination of acquired resistance to beta-lactams in small wild mammals around an isolated village in the Amazonian forest. *Environ Microbiol Rep* , 7(5), 698-708. doi:10.1111/1758-2229.12289

Jean, S. S., & Hsueh, P. R. (2011). High burden of antimicrobial resistance in Asia. *Int J Antimicrob Agents* , 37(4), 291-295. doi:10.1016/j.ijantimicag.2011.01.009

Jin, Y., Song, X., Liu, Y., Wang, Y., Zhang, B., Fan, H., & Shao, C. (2017). Characteristics of carbapenemase-producing *Klebsiella pneumoniae* as a cause of neonatal infection in Shandong, China.*Exp*

Ther Med , 13(3), 1117-1126. doi:10.3892/etm.2017.4070

Jobbins, S. E., & Alexander, K. A. (2015). FROM WHENCE THEY CAME–ANTIBIOTIC-RESISTANT *ESCHERICHIA COLI* IN AFRICAN WILDLIFE. *J Wildl Dis* , 51(4), 811-820. doi:10.7589/2014-11-257

Kock, R., Daniels-Haardt, I., Becker, K., Mellmann, A., Friedrich, A. W., Mevius, D., . . . Jurke, A. (2018). Carbapenem-resistant*Enterobacteriaceae* in wildlife, food-producing, and companion animals: a systematic review. *Clin Microbiol Infect* , 24(12), 1241-1250. doi:10.1016/j.cmi.2018.04.004

Kong, N., Li, G., Yang, C., Wang, X., Cao, M., Zhang, L., . . . Wei, Q. (2020). Prevalence of blaKPC-2-harbouring *Klebsiella pneumoniae*ST290 in a tertiary hospital in China. *J Glob Antimicrob Resist* , 20, 344-345. doi:10.1016/j.jgar.2020.01.025

Kozak, G. K., Boerlin, P., Janecko, N., Reid-Smith, R. J., & Jardine, C. (2009). Antimicrobial resistance in *Escherichia coli* isolates from swine and wild small mammals in the proximity of swine farms and in natural environments in Ontario, Canada. *Appl Environ Microbiol* , 75(3), 559-566. doi:10.1128/aem.01821-08

Liu, W., Xie, Y., Ma, J., Luo, X., Nie, P., Zuo, Z., . . . Ren, J. (2015). IBS: an illustrator for the presentation and visualization of biological sequences. *Bioinformatics* , 31(20), 3359-3361. doi:10.1093/bioinformatics/btv362

Loman, N. J., Quick, J., & Simpson, J. T. (2015). A complete bacterial genome assembled de novo using only nanopore sequencing data. *Nat Methods* , 12(8), 733-735. doi:10.1038/nmeth.3444

Milton, A. A. P., Agarwal, R. K., Priya, G. B., Athira, C. K., Saminathan, M., Reddy, A., . . . Kumar, A. (2018). Occurrence, antimicrobial susceptibility patterns and genotypic relatedness of*Salmonella spp* . isolates from captive wildlife, their caretakers, feed and water in India. *Epidemiol Infect* , 146(12), 1543-1549. doi:10.1017/s0950268818001553

Paterson, D. L., & Bonomo, R. A. (2005). Extended-spectrum beta-lactamases: a clinical update. *Clin Microbiol Rev* , 18(4), 657-686. doi:10.1128/cmr.18.4.657-686.2005

Paul, D., Babenko, D., & Toleman, M. A. (2020). Human carriage of cefotaxime-resistant *Escherichia coli* in North-East India: an analysis of STs and associated resistance mechanisms. *J Antimicrob Chemother* , 75(1), 72-76. doi:10.1093/jac/dkz416

Perron, G. G., Quessy, S., & Bell, G. (2008). A reservoir of drug-resistant pathogenic bacteria in asymptomatic hosts. *PLoS One* , 3(11), e3749. doi:10.1371/journal.pone.0003749

Pitout, J. D., & Laupland, K. B. (2008). Extended-spectrum beta-lactamase-producing *Enterobacteriaceae* : an emerging public-health concern. *Lancet Infect Dis* , 8(3), 159-166. doi:10.1016/s1473-3099(08)70041-0

Rolland, R. M., Hausfater, G., Marshall, B., & Levy, S. B. (1985). Antibiotic-resistant bacteria in wild primates: increased prevalence in baboons feeding on human refuse. *Appl Environ Microbiol* , 49(4), 791-794.

Rooney, C. M., Sheppard, A. E., Clark, E., Davies, K., Hubbard, A. T. M., Sebra, R., . . . Chilton, C. H. (2019). Dissemination of multiple carbapenem resistance genes in an in vitro gut model simulating the human colon. *J Antimicrob Chemother* , 74(7), 1876-1883. doi:10.1093/jac/dkz106

Rozwandowicz, M., Brouwer, M. S. M., Fischer, J., Wagenaar, J. A., Gonzalez-Zorn, B., Guerra, B., . . . Hordijk, J. (2018). Plasmids carrying antimicrobial resistance genes in *Enterobacteriaceae* .*J Antimicrob Chemother* , 73(5), 1121-1137. doi:10.1093/jac/dkx488

Simner, P. J., Antar, A. A. R., Hao, S., Gurtowski, J., Tamma, P. D., Rock, C., . . . Timp, W. (2018). Antibiotic pressure on the acquisition and loss of antibiotic resistance genes in Klebsiella pneumoniae.*J Antimicrob Chemother* , 73(7), 1796-1803. doi:10.1093/jac/dky121

Skurnik, D., Ruimy, R., Andremont, A., Amorin, C., Rouquet, P., Picard, B., & Denamur, E. (2006). Effect of human vicinity on antimicrobial resistance and integrons in animal faecal *Escherichia coli* .*J Antimicrob Chemother* , 57(6), 1215-1219. doi:10.1093/jac/dkl122

Strydom, K. A., Chen, L., Kock, M. M., Stoltz, A. C., Peirano, G., Nobrega, D. B., . . . Pitout, J. D. D. (2020). Klebsiella pneumoniae ST307 with OXA-181: threat of a high-risk clone and promiscuous plasmid in a resource-constrained healthcare setting. *J Antimicrob Chemother* , 75(4), 896-902. doi:10.1093/jac/dkz550

Tacconelli, E., Carrara, E., Savoldi, A., Harbarth, S., Mendelson, M., Monnet, D. L., . . . Magrini, N. (2018). Discovery, research, and development of new antibiotics: the WHO priority list of antibiotic-resistant bacteria and tuberculosis. *Lancet Infect Dis* , 18(3), 318-327. doi:10.1016/s1473-3099(17)30753-3

Walsh, T. R., Weeks, J., Livermore, D. M., & Toleman, M. A. (2011). Dissemination of NDM-1 positive bacteria in the New Delhi environment and its implications for human health: an environmental point prevalence study. *Lancet Infect Dis* , 11(5), 355-362. doi:10.1016/s1473-3099(11)70059-7

Wang, Z., Li, M., Shen, X., Wang, L., Liu, L., Hao, Z., . . . Yu, F. (2019). Outbreak of blaNDM-5-Harboring *Klebsiella pneumoniae*ST290 in a Tertiary Hospital in China. *Microb Drug Resist* , 25(10), 1443-1448. doi:10.1089/mdr.2019.0046

Wei, Q., Jiang, X., Yang, Z., Chen, N., Chen, X., Li, G., & Lu, Y. (2009). dfrA27, a new integron-associated trimethoprim resistance gene from *Escherichia coli* . *J Antimicrob Chemother* , 63(2), 405-406. doi:10.1093/jac/dkn474

Wein, T., Hulter, N. F., Mizrahi, I., & Dagan, T. (2019). Emergence of plasmid stability under non-selective conditions maintains antibiotic resistance. *Nat Commun* , 10(1), 2595. doi:10.1038/s41467-019-10600-7

Wu, W., Feng, Y., Tang, G., Qiao, F., McNally, A., & Zong, Z. (2019). NDM Metallo-beta-Lactamases and Their Bacterial Producers in Health Care Settings. *Clin Microbiol Rev* , 32(2). doi:10.1128/cmr.00115-18

Wyres, K. L., Nguyen, T. N. T., Lam, M. M. C., Judd, L. M., van Vinh Chau, N., Dance, D. A. B., . . . Holt, K. E. (2020). Genomic surveillance for hypervirulence and multi-drug resistance in invasive*Klebsiella pneumoniae* from South and Southeast Asia. *Genome Med* , 12(1), 11. doi:10.1186/s13073-019-0706-y

Yang, Q. E., Tansawai, U., Andrey, D. O., Wang, S., Wang, Y., Sands, K., . . . Niumsup, P. R. (2019). Environmental dissemination of mcr-1 positive *Enterobacteriaceae* by *Chrysomya spp.* (common blowfly): An increasing public health risk. *Environ Int* , 122, 281-290. doi:10.1016/j.envint.2018.11.021

Yang, X., Wai-Chi Chan, E., Zhang, R., & Chen, S. (2019). A conjugative plasmid that augments virulence in *Klebsiella pneumoniae* .*Nat Microbiol* , 4(12), 2039-2043. doi:10.1038/s41564-019-0566-7

Figure legends

FIGURE 1. Antimicrobial genes, insertion sequences and composite transposons in plasmids from MDR *K. pneumoniae* isolate M297-1.

A. pM297-1.1 and its similar linear property particles characteristics and comparison; B. pM297-1.2 and its similar linear property particles characteristics and comparison. Dark green and golden shadows represent shared areas with high similarity (¿90%), showing the relative positions of genes identified in the complete nucleotide sequences of these bacteria. These genes are marked with arrows, which represent the coding sequence and the associated transcription direction, and the size of the arrow is proportional to the length of the gene. The red shadow represents the antibiotic resistance gene, the blue shadow indicates the insertion sequence, the yellow shadow indicates the transposase gene, the green shadow indicates the integrase gene, and the orange shadow indicates the serine recombinase family protein gene. the blank indicates that it has nothing to do with drug resistance or unknown functional protein genes. Turquoise shadow, gene island; purple shadow, prophage.

FIGURE 2. The distribution of the ST290 of *Klebsiella pneumoniae* isolates in the world, among which three isolates were in China, one in USA and one in Australia.

FIGURE 3. The distribution of different isolates carrying similar plasmids in the world. The distribution of different isolates carrying similar plasmids that are highly similar to plasmids pM297-1.1(A) and pM297-1.2 (B), while C and D indicate the distribution in China.The reporting date, region and ST typing of the isolates are shown in the box.

FIGURE 4. Phylogenetic relationship between reference*K.pneumoniae* isolates carrying similar plasmids and M297-1 based on 16s rRNA sequences. The circle indicates the strain carrying highly similar plasmids to pM297-1.1, the triangle indicates the strain carrying highly similar plasmids to pM297-1.2, and the red arrow points to M297-l. Orange, China; light green, Sweden; dark blue, Thailand; gray, missing information.

FIGURE S1. Phylogenetic relationship between *Klebsiella pneumoniae* isolate M297-1 and reference strain from human clinical samples based on the 16s rRNA

Table legends

TABLE 1. The drug susceptibility profiles of*Enterobacteriaceae* isolated from Red Kangaroo

Bacteria species
Escherichia coli
Escherichia coli
Escherichia coli
Klebsiella variicola
Citrobacter freundii
Morganella morganii
Klebsiella pneumoniae
+AMP,Ampicillin;SAM,Ampicillin/Sulbactam;ATM,Aztreonam;CXM,Cefuroxime;CRO,Ceftriaxone;FEP,Cefepime;CL,Colis

TABLE 2. Genomic information of *Klebsiella pneumoniae*isolate M297-1 from Red Kangaroo in Zhengzhou zoo, Henan province

Chromosome	Chr- M29
Plasmids	pM297-1.1
	pM297-1.2
+N/A, No Prophage.	+N/A, N
++β-lactamase genes are shown in bold. Number in parentheses indicates the number of drug resistance genes.	++β-lacta

TABLE 3. Drug Susceptibility Testing of *E.coli*transconjugants from *Klebsiella pneumoniae* M297-1 as donor and*E.coli* J53 as a recipient strain.

Agent+
Ampicillin
Ampicillin/Sulbactam
Piperacillin/Tazobactam
Aztreonam
Cefazolin
Cefotaxime

Agent+
Ciprofloxacin
Meropenem
Chloramphenicol
Trimethoprim/Sulfamethoxazole
Tetracycline
Doxycycline
+Chloramphenicol, Trimethoprim/Sulfamethoxazole, Tetracycline and Doxycycline are shown in bold. S,susceptible; I,inter

TABLE S1.Comparison between reference plasmid and pM297-1.1 and pM297-1.2 from *Klebsiella pneumoniae* M297-1 from Red Kangaroo in Zhengzhou zoo, Henan province.

Similar plasmid+	ACC_NUCCORE	Plasmid name	CreateDate	Location	Host
pM297-1.1	MG288679.1	p911021-tetA	2018/4/16	N/A	N/A
	NZ_CP025577.1	p08EU827_1	2019/1/12	Sweden:Stockholm	*Homo sapiens*
	NZ_CP036307.1	p1_020098	2019/2/20	China:Chengdu,Sichuan	*Homo sapiens*
	CP038004.1	pLAP2_020009	2019/3/15	China:Bazhong,Sichuan	*Homo sapiens*
	NZ_CP040176.1	unnamed1	2019/5/20	China:Chongqing	*Homo sapiens*
pM297-1.2	NC_013950.1	pKF3-94	2010/3/19	N/A	N/A
	NZ_CP011334.1	unnamed	2015/5/6	N/A	N/A
	NZ_CP014005.1	unnamed1	2016/1/24	China:Jiangxi	*Homo sapiens*
	NZ_CP025966.2	pQnrB_LL34	2018/2/1	China:Chengdu,Sichuan	*Homo sapiens*
	NZ_CP026588.1	p2	2018/3/1	China:Nanchang,Jiangxi	*Homo sapiens*
	NZ_CP028553.2	pCTXM15_020019	2018/4/5	China:Meishan,Sichuan	*Homo sapiens*
	NZ_CP024917.1	pKPNH54.1	2018/4/11	Thailand	*Homo sapiens*
	NZ_CP024876.1	pNH25.2	2018/4/11	Thailand	*Homo sapiens*
	NZ_CP028717.1	pSCM96-1	2018/4/25	China	N/A
	NZ_CP031262.1	pL22-5	2018/9/8	China	*Homo sapiens*
	NZ_CP034085.1	pR210-2-CTX	2018/12/4	China	*Homo sapiens*
	NZ_CP022442.1	unnamed1	2019/1/12	N/A	N/A
	MG878868.1	pKp21774-135	2019/2/28	N/A	N/A
+N/A,Unknown	+N/A,Unknown	+N/A,Unknown	+N/A,Unknown	+N/A,Unknown	+N/A,Unknown