Genetic characterization of two fully sequenced multi-drug resistant plasmids pP10164-2 and pP10164-3 from *Leclercia adecarboxylata*

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We previously reported the complete sequence of the resistance plasmid pP10164-NDM, harboring blaNDM (conferring carbapenem resistance) and bleMBL (conferring bleomycin resistance), which is recovered from a clinical *Leclercia adecarboxylata* isolate P10164 from China. This follow-up work disclosed that there were still two multidrug-resistant (MDR) plasmids pP10164-2 and pP10164-3 coexisting in this strain. pP10164-2 and pP10164-3 were completely sequenced and shown to carry a wealth of resistance genes, which encoded the resistance to at least 10 classes of antibiotics (β-lactams, macrolides, quinolones, aminoglycosides, tetracyclines, amphenicols, quaternary ammonium compounds, sulphonamides, trimethoprim, and rifampicin) and 7 kinds of heavy metal (mercury, silver, copper, nickel, chromate, arsenic, and tellurium). All of these antibiotic resistance genes are associated with mobile elements such as transposons, integrons, and insertion sequence-based transposable units, constituting a total of three novel MDR regions, two in pP10164-2 and the other one in pP10164-3. Coexistence of three resistance plasmids pP10164-NDM, pP10164-2 and pP10164-3 makes *L. adecarboxylata* P10164 tend to become extensively drug-resistant.

*Leclercia adecarboxylata*, which is ubiquitously distributed in nature, is a motile, aerobic member of Enterobacteriaceae, and it shows high degree of phenotypic similarity to *Escherichia coli*. *L. adecarboxylata* infections are rarely reported in humans, emphasizing the nature of this bacterium as an opportunistic pathogen¹³. In most cases, *L. adecarboxylata* is isolated as a pure culture from immunocompromised persons or patients with underlying medical conditions, and its can be occasionally found as a part of polymicrobial cultures in immunocompetent patients suggesting the dependence of this microorganism on co-flora to cause a disease¹³. In addition, only two cases of *L. adecarboxylata*-induced monomicrobial infections--without other coinciding pathogens--have also been reported in immunocompetent patients, indicating the relevant isolates may possess unique virulence factors not found in the other reported clinical isolates⁴. It has been postulated that *L. adecarboxylata* infections are underestimated and have been under-reported for a long time due to the fact of misidentification of this microorganism as *Escherichia coli*.⁶

*L. adecarboxylata* strains are naturally susceptible to all but two β-lactams (see below), azithromycin, tetracyclines, aminoglycosides, quinolones, and amphenicols, but resistant to penicillin G and oxacillin, erythromycin, roxithromycin and clarithromycin, fosfomycin, ketolides, lincosamides, glycopeptides, and rifampicin⁶. There are few reports describing the antimicrobial resistance of *L. adecarboxylata* due to acquisition of foreign resistance gene(s). The SHV-12-mediated cephalosporin resistance⁷, or the carbapenem resistance due to production of

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carbapenemase KPC-2 or VIM-19 has been observed in clinical \textit{L. adecarboxylata}. In addition, reported are two clinical isolates of multidrug-resistant (MDR) \textit{L. adecarboxylata}, one harboring \textit{bla}_{\text{TEM-1}}, \textit{bla}_{\text{CTX-M-3}}, and a class 1 integron cassette array \textit{dfrA12-orfF-aadA2}\textsuperscript{10}, and the other possessing \textit{bla}_{\text{SHV-12}}, \textit{bla}_{\text{DHA-1-ampR}} and a class 1 integron cassette array \textit{aacA4-cr-bla}_{\text{OXA-1-catB3-arr3}}\textsuperscript{11}.

We recently reported a fully sequenced resistance plasmid \textit{pP10164-NDM}, harboring a total of two resistance genes \textit{bla}_{\text{NDM}} (conferring carbapenem resistance) and \textit{ble}_{\text{MBL}} (conferring bleomycin resistance), from the clinical \textit{L. adecarboxylata} isolate P10164\textsuperscript{12}. Strain P10164 is resistant to β-lactams including carbapenems, quinolones, aminoglycosides, macrolides, fosfomycin, tetracyclines, amphenicols, and trimethoprim/sulfamethoxazole but remained susceptible to tigecycline and polymyxin E. This follow-up study provides the evidence for the presence of two additional resistance plasmids \textit{pP10164-2} and \textit{pP10164-3} in \textit{L. adecarboxylata} P10164. These two multidrug-resistant (MDR) plasmids were fully sequenced and shown to carry a large amount of antibiotic and heavy metal resistance genes.

**Results and Discussion**

**Overview of plasmids \textit{pP10164-2} and \textit{pP10164-3}**. The complete sequences of \textit{pP10164-2} and \textit{pP10164-3} were determined from the genomic DNA of strain P10164 by high-throughput shotgun sequencing (the mean sequencing coverages are 79 \times and 93 \times respectively) and PCR-based gap closing. These two plasmids have circularly closed DNA sequences, 313,395 bp and 80,460 bp in length with mean G + C contents of 47.3\% and 54.1\%, respectively, and they contain 356 and 91 predicted open reading frames (ORFs) in total, respectively (Fig. 1). The modular structure of each plasmid is discriminated as the backbone with insertion of multiple separate accessory modules.

The \textit{pP10164-2} backbone, 205 kb in length, is closely related (97\% query coverage and maximum 99\% nucleotide identity) to the prototype IncHI2 plasmid R478 from \textit{Serratia marcescens}\textsuperscript{13}, and almost identical (100\% coverage and 99\% identity) to another IncHI2 plasmid pKST313 from \textit{Salmonella enterica} serotype Typhimurium\textsuperscript{14}. Located in the \textit{pP10164-2} backbone are genes or gene clusters that encode the core IncHI2 plasmid determinants such as \textit{repHIA} and \textit{repHII2} (replication initiation), the \textit{tra1} and \textit{tra2} regions (conjugal transfer), \textit{parAB} and \textit{parM-parR} (partition) within \textit{tra2}, \textit{ter} (tellurium resistance), \textit{klaABC} (plasmid maintenance), and \textit{ars} (arsenic resistance). It has been proposed that the \textit{repHIA} replicon, the essential \textit{trh} (conjugal transfer), \textit{tra}, and \textit{oriT} (origin of transfer) sequences within \textit{tra1} and \textit{tra2}, and the \textit{parAB} partitioning module might represent the minimal IncHI2 determinants\textsuperscript{15}.

The \textit{pP10164-2} accessory regions, which are dramatically different from R478 and pKST313, are composed of the group IIb1 intron Kl.pn.I2, IS\textit{Kpn}26, two IS\textit{903D} elements, a novel insertion sequence (IS) of IS3-family designated IS\textit{Lad1}, a novel IS element of IS\textit{1202} group named IS\textit{Lad2}, and two novel MDR regions designated MDR-1 and MDR-2. The MDR-1 and MDR-2 regions, 61.3 kb and 40.3 kb in length respectively, are adjacent and isolated by a 1.9 kb backbone region composing of two ORFs \textit{orf381} and \textit{Aorj666}.  

**Figure 1.** Schematic maps of \textit{pP10164-2} (a) and \textit{pP10164-3} (b). Genes are denoted by arrows and colored based on gene function classification. The innermost circle presents GC-Skew [(G − C)/(G + C)] with a window size of 500 bp and a step size of 20 bp. The blue circle presents GC content. Shown also are backbone and accessory module regions.
The pP10164-3 backbone encodes the plasmid replication (repA) and maintenance (parFG and umuCD) functions as well as the residual conjugal transfer determinants (traA, mutated nikAB, and mobC), and overall it exhibits no significant sequence similarity to any known DNA sequences. The deduced replication initiator protein RepA belongs to the Rep_3 superfamily and cannot be assigned into any known incompatibility groups, and it matches various plasmid RepA proteins of unknown incompatibility groups from Leclercia, Cronobacter and Enterobacter with above 93% amino acid identity.

pP10164-3 is quite unusual because it has a relatively small (19 kb in length) backbone but carries much larger accessory contents including the 2.3 kb Klpn.12 intron and a 37.8 kb region composed of a MDR region and a carbohydrate utilization region. The carbohydrate utilization region is sequentially organized as a mixture of a novel ISI-family member (MIS1), a novel 14-gene locus probably accounting for galactan utilization, ISIF, a mutated sequence of a novel ISI-family member (MIS3), and a Tn2555 remnant. Both MIS1 and MIS3 cannot be discriminated as intact IS elements because their transposase genes insB and tnpA, respectively, becomes pseudogenes due to frameshift. The sucrose transposon Tn2555 from *E. coli* is an IS26-based composite transposon that carries the sucrose utilization gene cluster *scrKYABR*, two direct IS26 copies on its flanks and, sometimes, a third inverted IS26 copy inside the transposon15, while the Tn2555 remnant from pP10164-3 containing only *scrK* and *AscrY*.

**The MDR-1 region of pP10164-2.** The pP10164-2 MDR-1 region (Fig. 2a) is organized sequentially as a novel Tn3-family unit transposon designated Tn6317, the Tn-3-IS26-bla_12, a Tn5396-associated region, IS26, In27pP10164-2, the IS26-attA(A)-tetR(C)-unit, IS26, and a Tn5396-like transposon remnant.

Tn6317 is generated from the insertion of Tn5058 into a backbone remnant of Tn6256, a Tn3-family TnPa38-related transposon from clinical *Citrobacter freundii* from Italy16. Each of the two 39 bp terminal inverted repeats (IRL: inverted repeat left; IRR: inverted repeat right) of Tn6317 is disrupted by IS4321R into two separate parts (IR-5′ plus IR-3′), which is also observed in Tn6256. It seems that the Tn5058 insertion is accompanied by not only the truncation of IS4321R but also the loss of downstream IRL-3′ and the core transposition module tnpA (transposase) at the 5′ region of Tn6317 relative to Tn6256 (Fig. 2b). Tn5058 is composed of a Tn5053-family core transposition module tniA (transposase)-tnib (ATP-binding protein)-tniQ (transposition auxiliary protein)-res (resolution site)-tnir (serine resolvase) and two mercury resistance gene clusters named mer1 and mer2, which is delimited by terminal 25 bp IRL and IRR. Tn5058 differs from the prototype Tn5058 (accession number Y17897) from *Pseudomonas* sp. ED23-33 by the insertion of IS075 into each of the two internal inverted repeats IRR_mer1 and IRR_mer2. The IS1111-family IS4321 and its close derivative IS5075 are known to be targeted by the terminal inverted repeats of the Tn21-subgroup transposons of Tn3 family17.

The Tn-3-IS26-bla_12, a unit likely derived from a precursor Tn3 [IRL-tnpA-res-tnpR (resolvase)-bla_12-IRR]-like element, is the IS26 unit (which is known to be transposable among plasmids17) upstream of IS4321R, leading to the truncation of IS4321R, the IRL-3′-tnpA-res of Tn3, and the truncation of tnpR of Tn3. The connection of Tn3-IS26-bla_12, together with IS4321R, orientated in opposite directions likely results in the loss of the IRR of Tn3, making Tn3-IS26-bla_12, which cannot be discriminated as a transposon due to the absence of one of the paired IRL/IRR routinely bracketing at both ends. Both bla_12 and IS4321R encode class A β-lactamases, whose activity can be inhibited by clavulanic acid. TEM-1 is able to hydrolyze penicillins but not extended-spectrum cephalosporins; by contrast, SFO-1 exhibits significant hydrolytic activity against both penicillins and extended-spectrum cephalosporins, but it has no detectable activity against carbapenems and cephapains.16. The *bla_12*, expression is inducible, which is regulated by the transcriptional regulatory activity encoded by umuCD that is in IS26. Tn5058 is an IS26-based composite transposon from the *Citrobacter freundii* plasmid pCTX-M-3 and displays a modular structure IS26-In27-iscr1-AISEc28-arsA1-ISEc29- msr(E)-mph(E)-orf543-IS2611,22. Notably, Tn5618 lacks the paired short direct repeats (DRs), which represent the target site duplication signals routinely bracketing at both ends of a composite transposon. Tn5418 and various Tn5418-associated elements (with insertion of different class 1 integrons or integron-like sequences between IS26 and *iscr1*) are thought to promote the dissemination of the aminoglycoside resistance gene *armA*, the macrolide resistance operon *msr(E)-mph(E)*, and other classes of antibiotic resistance genes within the inserted integrons23. The Tn5418-associated region from pP10164-2 differs from Tn5618 by the replacement of In27 by a novel class 1 integron named In1262, and the deletion of orf543-IS26 originally at the 3′ region of Tn5618 (Fig. 2d). The connection of immediately upstream IS26 and immediately downstream *iscr1* with In1262 leads to the loss of two terminal 25 bp inverted repeats (IRs: inverted repeat initial; IRT: inverted repeat terminal) and the truncation of *intI* (integration) occurs for this integron. In1262 carries two gene cassettes *gcu167* and *aucA3* (aminoglycoside resistance); *attC*_attA, The novel gene cassette *gcu167* of unknown function contains two consecutive ORFs *gcu167a* (putative nucid hydrolase) and *gcu167b* (putative nucleotidase), followed by a single *attC*_attA site.

In27_pP10164-2 resembles a complex class 1 integron, whose modular structure can be generally divided sequentially into 5′-conserved segment [3′CS: *intI*-attI], variable region 1 (VR1), the first copy of 3′-conserved segment [3′CS: *gcuE*A1 (quaternary ammonium compound resistance)-sulf (sulfonamide resistance)], common region *iscr1*, VR2, and the second copy of 3′CS (3′CS2), bordered by terminal 25 bp IRI and IRR24. In27_pP10164-2 comprises Δ5′CS (ΔintI-attI), VR1 [three sequentially arranged gene cassettes: *dfrA12* (trimethoprim resistance-attC*_attA_, gcuF (unknown function)-attC*_attA_, and *aadA2* (aminoglycoside resistance)); *attC*_attA, 3′CS1, VR2 [orf639 (putative β-lactamase)-IS1 × 4], an ISCR-like element, 3′CS2, orf5, and *dfrA6*: Δ5′CS and *dfrA6* are in truncated formats and IRI and IRR are absent, which is likely resulted from the connection of IS26 at both ends of In27_pP10164-2. The common region *iscr1*, which is commonly located between 3′CS1 and VR2 of a typical complex class 1 integron, is not found in In27_pP10164-2, but a 1.4 kb element (which encodes a putative protein with 80%
amino acid sequence similarity to the ISCR20 transposase but lacks the oriIS element characteristic of ISCRs) is found between VR2 and 3′CS2 of In27\textsubscript{pP10164-2}.

Figure 2. The pP10164-2 MDR-1 region and comparison to related regions. Genes are denoted by arrows and colored based on gene function classification. Shading regions denote regions of homology (>95% nucleotide similarity).
IS26-tetA(C)-tetR(C) represents a putative mobile unit carrying a tetracycline resistance module tetA(C) (tetracycline efflux protein)-tetR(C) (transcriptional repressor of tetA); moreover, similar genetic elements are found in various plasmids such as the IncN1 plasmid N330 and originally found in the MDR plasmid R100 (accession number AP000342) from Enterobacter cloacae while the IRL shows the insertion of IS26-1R elements but lacks the right terminal IS26, and IS26-yrB-tetA(C)-orf378-tetR(C)-orf477 -IS26. Each of them contains two terminally flanking IS26 elements but cannot be annotated as a composite transposon, because the paired DR sequences are not identified. The common component IS26 would act as an adaptor to mediate massive recombination and transposition events, facilitating the assembly of the MDR-1 region with a very complex mosaic structure.

The MDR-2 region of p10164-2. The p10164-2 MDR-2 region (Fig. 3) is mainly composed of IS26, A\n705, A\n706, a novel Tn3-family unit transposon designated Tn3262, ISKpn19, IS\nPpu12 lacking IRL, sil, IS1R, IS903D, Δcop and ren in order of their priority. A\n705 contains Δ5′CS (ΔintI-attI, truncated by connection of IS26 upstream of A\n705) and a single gene cassette aadA1ai (aminoglycoside resistance)attC\nadA1ai. The aadA1ai gene is a derivative of the prototype aadA1 gene (accession number X12870), displaying the Val5Met amino acid substitution. Tn2670 is an IS1-based composite transposon, which is composed of a backbone region with Tn21 inserted within it and originally found in the MDR plasmid R100 (accession number AP000342) from Shigella flexneri. The IS26-270 backbone consists of two IS1 elements flanking a 1.5 kb central region that harbors the amphenicol resistance gene catA1 and the ybjA gene encoding putative acetyl transferase. A\n706 from the p10164-2 MDR-2 region resembles the Tn2670 backbone but lacks the right terminal IS1 and, notably, similar structures are found in other IncH2 plasmids such as pRH-R27 and in the chromosomally located resistance island AbaR1 and its derivatives from Acinetobacter baumannii.

Tn6322 is composed of the Tn21 core transposition module mpap-res together with a novel mercury resistance gene cluster designated mer3, and the mer3 region differs dramatically (92% coverage and maximum 86% nucleotide identity) from the mer locus from Tn21, indicating the capture of mer3 by the Tn21 core transposition module during the genesis of Tn6322. The mer3 region is mostly similar (100% coverage and maximum 96% nucleotide identity) to the counterpart of the Enterobacter cloacae transposon Tn6005 belonging to the Tn5036/Tn3926 subgroup of Tn3 family. Tn6322 is flanked by 38bp IRL/IRR resembling those of Tn21: the IRR is intact, while the IRL shows the insertion of IS3075.

Silver and copper compounds are used as antimicrobial agents in hospitals, and the relevant resistance determinants could serve as hygienic fitness factors and thus improve bacterial survival in hospital environments. In R478, the silver and copper resistance gene clusters, called sil and cop respectively, are located adjacent and associated with an upstream Tn7-like core transposition module tnsABCD. Similar tnsABCD-sil-cop structures

Figure 3. The p10164-2 MDR-2 region and comparison to related region. Genes are denoted by arrows and colored based on gene function classification. Shading regions denote regions of homology (>95% nucleotide similarity).
are widely found in IncHI2 plasmids such as pMRVIM0813 (accession number KP975077), pSTm-A54650 (LK056646), pKST3134 and pRH-R2735, although considerable variations in both genetic content and nucleotide sequence are observed among different plasmids. Similarly, a multi-heavy metal resistance region ISKpn19-ΔISPpu12-sil-IS1R-orf1623-Δcop-rcn is found in the pP10164-2 MDR-2 region: compared with the prototype tnsABCD-sil-cop structure, ISKpn19-ΔISPpu12 replaces tnsABCD, the insertion of IS1R-orf1623 (putative metal-dependent hydrolase)-IS903D between sil and cop marked the truncation of cop into ΔcopS-copE, and a rcn locus (encoding the RcnA efflux pump responsible for nickel/cobalt detoxification and the rcnA repressor RcnR) is added immediately downstream of ΔcopS-copE. Notably, the IncHI2 plasmid pRH-R2735 carries a very similar structure from ΔISPpu12 to rcn with further insertion of a fragment composed of three hypothetical ORFs between IS1R and orf1623. The MDR-2 region of pP10164-2 and the corresponding MDR region of pRH-R2735 are genetically related and might share a much more recent ancestor, although they contain dramatically different sets of resistance genes upstream of the ΔISPpu12 to rcn region.

The MDR region of pP10164-3. The pP10164-3 MDR region (Fig. 4) is 35.5 kb in length and can be divided into two components, namely a 4.9 kb qnrS1 (quinolone resistance) region and a novel Tn3-family unit transposon designated Tn6308. The qnrS1 genetic platform ΔISEcl2-qnrS1-ΔtnpR (truncated Tn3-family resistance)–ISKpn19 is widely found in resistance plasmids from Enterobacteriaceae species57. Replacement of the 5’ terminal ΔISEcl2 by ΔISKpn19 generates a novel qnrS1 region ΔISKpn19-qnrS1-ΔtnpR–ISKpn19 as observed in the pP10164-3 MDR region.

The Tn6308 backbone is a hybrid of the core transposition module tnpAR-res of Tn1696 and the mer region of Tn21, and it is bordered by the intact 39 bp IRL and the IS5075-disrupted IRR at both ends in the absence of DRs. Tn1696 and Tn21 are both the members of the Tn21 subgroup of Tn3 family, but they have independent histories and origins with limited nucleotide sequence similarity (79 to 96%) between corresponding backbone genes60. The res site, originally 120 bp in length, is truncated into an 83 bp remnant in Tn6308 due to the insertion of a class 1 integron In37b. Notably, all the three novel Tn3-family transposons Tn6317, Tn6322, and Tn6308 identified in this work have undergone at least two evolutionary events after their initial transposition into pP10164-2 or pP10164-3: i) the disruption of one or both terminal IR sequences by insertion of IS5075 or IS4321R, making them deficient in further mobilization; and ii) the removal of target site duplication signals, making them lack of terminal DR sequences.

In37b from pP10164-3, In37c from the C. freundii plasmid p112298-KPC57 and In37d from the Aeromonas plasmid pPG1 are all derivatives of the typical complex class 1 integron In37 from Escherichia coli58 (Fig. 5). In37 is sequentially organized as 5′CS, VR1 [aacA4cr (quinolone and aminoglycoside resistance):attCaacA4cr], blaOXA-1 (β-lactam resistance):attCblaOXA-1, catB3 (aminophenol resistance):attCcatB3, and arr3 (rifampicin resistance):attCat3, 3′CS1, ISCR1, VR2 [qnrA1 (quinolone resistance) and ampR (LysR-family regulator)], 3′CS2, and orf5-orf6-IS6100, which is bracketed by 25 bp IRi/IRr and associated 5 bp DRs59.
Compared to In37, In37b has undergone the insertion of Tn6309 into intI1, the loss of ISCR1-VR2-3’CS2, the truncation of orf5-orf6 into Δorf5 due to the insertion of the chromate resistance unit IRLchrA-chrA-orf98\textsuperscript{40} (the 38 bp IRL\textsubscript{chrA} is further disrupted by IS\textsubscript{5075}), and the replacement of IS\textsubscript{6100} by the macrolide resistance unit IS\textsubscript{26}-mph(A)-mrx-mphR(A)-IS\textsubscript{6100}\textsuperscript{40} followed by ΔtniA Tn\textsubscript{21} (Fig. 5). Tn6309 is an IS\textsubscript{26}-based composite transposon containing the tetracycline resistance module tetA(C)-tetR(C); although just being named in this work, Tn6309 has been found in the genomic island Sm1-MDRGI from Stenotrophomonas maltophilia\textsuperscript{41} and also in the three sequenced plasmids pB3 from Pseudomonas sp. GFP142, pKAZ3 from an uncultured bacterium\textsuperscript{43} and pNDM-116-1 (accession number LN831184) from Vibrio cholerae. The absence of IRi and ΔtniA\textsubscript{Tn21} and the truncation of intI1 are observed but there is no insertion of Tn6309 in In37c compared to In37b (Fig. 5). Insertion of Tn6309 and IRL\textsubscript{chrA-chrA-orf98} are not found in In37d, leaving intI1 and orf5-orf6 intact, but ΔtniA\textsubscript{Tn21}-IRt is absent from In37d compared to In37b (Fig. 5). The above observations indicate that extensive recombination and transposition events have occurred during derivation of In37, In37b, In37c and In37d from an In37-like precursor, making them to integrate different sets of additional resistance genes, but the core resistance gene cassette array aacA4cr-bla\textsubscript{OXA-1}-catB3-arr3 is shared by these integrons.

Figure 5. **Comparison of In37b with its derivatives.** Genes are denoted by arrows and colored based on gene function classification. Shading regions denote regions of homology (>95% nucleotide similarity).

**Concluding remarks.** This is the first report of detection of MDR plasmids and determination of their complete sequences in *L. adecarboxylata*. Coexistence of three resistance plasmids pP10164-NDM, pP10164-2 and pP10164-3 makes *L. adecarboxylata* P10164 tend to become extensively drug-resistant. This bacterial species may serve as a potential reservoir of antimicrobial resistance genes in clinical settings. Data presented here would promote us to gain deeper understanding of plasmid-mediated mechanisms of drug resistance in *L. adecarboxylata*. Prevalence of the resistance plasmids pP10164-NDM, pP10164-2 and pP10164-3 in *L. adecarboxylata* and other bacterial species from the clinical settings cultures especially those from immunocompromised patients needs to be elucidated.

**Methods**

Bacterial genomic DNA were isolated by classical phenol/chloroform method followed by diethyl ether removal of polysaccharides that contaminate genomic DNA\textsuperscript{44}, and then sequenced with a paired-end library with an average insert size of 500 bp and a mate-pair library with average insert size of 5,000 bp, using HiSeq 2500 sequencer (Illumina, CA, USA). In order to get complete plasmid sequences, the contigs were assembled with Velvet, and the gaps were filled through combinatorial PCR and Sanger sequencing on ABI 3730 Sequencer (LifeTechnologies, CA, USA).

The open reading frames and pseudogenes were predicted with GeneMark\textsuperscript{S}\textsuperscript{TM} (http://topaz.gatech.edu/GeneMark), RAST (http://rast.nmpdr.org/), and Prodigal (http://compbio.ornl.gov/Prodigal), and further annotated by BLASTP and BLASTN against UniProtKB/Swiss-Prot (http://web.expasy.org/docs/swiss-prot_guideline.html) and NCBI NR databases.

Annotation of resistance genes, mobile elements and other gene futures was based on the relevant databases including CARD (http://arpcard.mcmaster.ca), BacMet (http://bacmet.biomedicine.gu.se/), β-lactamases...
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Author Contributions
D.S.Z., P.X. and Y.F. conceived the study and designed experimental procedures. F.S., Q.S., W.L., Q.W. and W.F. performed the experiments. F.S., D.S.Z., Q.S. and D.F.Z. analyzed the data. Y.T., W.C. and Y.F. contributed reagents and materials. D.S.Z., F.S., P.X. and Y.F. wrote this manuscript.

Additional Information
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