**Complete genome sequence of *Enterobacter cloacae* R11
reveals multiple genes potentially associated with high-
level polymyxin E resistance**

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1 **Complete genome sequence of *Enterobacter cloacae* R11 reveals**
2 **multiple genes potentially associated with high-level polymyxin E**
3 **resistance**

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15

16 **Abstract**

17 *Enterobacter cloacae* strain R11 is a multidrug-resistant bacterium isolated from
18 sewage water near a swine feedlot in China. Strain R11 can survive in medium with
19 up to 192 µg/ml polymyxin E, indicating a tolerance for this antibiotic significantly
20 higher than that reported for other gram-negative bacteria. In this study, conjugation
21 experiments showed that partial polymyxin E resistance could be transferred from
22 strain R11 to *Escherichia coli* strain 25922, revealing that some genes related to

polymyxin E resistance are plasmid-based. The complete genome sequence of this strain was determined, yielding a total of 4,993,008 base pairs (G+C content, 53.15%) and 4,908 genes for the circular chromosome and four circular plasmids. Genome analysis revealed a total of 73 putative antibiotic resistance genes, including several polymyxin E resistance genes and genes potentially involved in multidrug resistance. These data provide insights into the genetic basis of the polymyxin E resistance and multidrug resistance of *E. cloacae*.

Keywords: *Enterobacter cloacae* R11, genome sequence, plasmids, polymyxin E, multidrug resistance.

Antibiotics are widely used to protect animal health by reducing infection and to increase yields by adding to livestock and poultry feed stuffs; however, broad-spectrum antibiotics are used increasingly more frequently even in cases where they are unnecessary (Threedeach et al. 2012). The overuse of antibiotics and the emergence of antibiotic-resistant microbes have caused global concern. Microbes resistant to almost all types of antibiotics have been reported in food animals (Alonso et al. 2017), and they have also been isolated from fresh milk and retail sausages (Giannino et al. 2009; Tew et al. 2016). Some of these microbes are opportunistic human pathogens (Hung et al. 2008). A member of the *Enterobacteriaceae* family, *Enterobacter cloacae* is primarily a commensal bacterium, but it can cause infections in the skin, wounds, urinary system and soft tissues (Shi et al. 2017). Additionally, the

development of multidrug resistance in *E. cloacae* causes greater concern about the pathogenic potential of this organism. Therefore, it is important to examine the genetic basis of the multidrug resistance of *E. cloacae*.

Strain R11 was isolated from sewage water near a swine feedlot in Jinan, China, and was identified as *E. cloacae* based on 16S rDNA sequence analysis. The isolated strain could survive in LB medium with up to 192 µg/ml polymyxin E, which is significantly higher than that of previously reported bacterial strains, and consequently, this strain may become a potential reservoir of polymyxin E-resistance genes (Nordqvist et al. 2016; Qureshi et al. 2015; Rhouma et al. 2016). Polymyxin E is considered the last line of defense against infection with antibiotic-resistant pathogens (Falagas et al. 2014; Roberts et al. 2015). However, polymyxin antibiotics, including polymyxin B and polymyxin E, have been widely used in livestock and the poultry breeding industry in order to prevent infection, and various polymyxin E-resistant bacteria have been isolated from different environments in the world, including clinical environments, causing public concern (van Duin and Paterson 2016).

To determine the minimal inhibitory concentrations (MICs) for polymyxin E and other antibiotics such as ampicillin and cefixime, the broth microdilution method was used, as described by the Clinical Laboratory Standard Institute (CLSI) guidelines (Behera et al. 2010). Results showed that strain R11 has multidrug resistance except for lincomycin as it could not grow in the presence of 1 µg/ml lincomycin; the MICs for the antibiotics assayed are shown in Table 1.

67 To verify if the polymyxin E resistance genes could be transferred from donor
68 strain R11 to other bacteria by horizontal gene transfer, the sodium azide-resistant
69 *Escherichia coli* 25922 strain, which has a polymyxin E MIC of 2 µg/ml, was used as
70 the recipient strain for examining conjugal transfer of resistance. After mixing the
71 donor and recipient, the mixture was spread on LB agar medium with X-Gluc
72 (5-bromo-4-chloro-3-indolyl-beta-D-glucuronic acid), sodium azide and polymyxin E
73 to screen the conjugants. The donor is inhibited by sodium azide, the recipient is
74 inhibited by polymyxin E, and only the conjugants can survive on the selective
75 medium and degrade X-Gluc to blue compounds. The conjugal transfer efficiency
76 between R11 and *E.coli* 25922 was about 4.12×10^{-4} /donor. Further analysis revealed
77 that the MIC of the conjugants for polymyxin E was 16 µg/ml, indicating that the
78 polymyxin E resistance of conjugants was much weaker than that of the donor strain
79 R11, which has a polymyxin E MIC of 192 µg/ml. One of these conjugants was
80 isolated and named 25922-T03. These data revealed that, although some genes related
81 to polymyxinE resistance might be located on one or more of the plasmids, other
82 genes contributing to polymyxinE resistance genes were potentially located on the
83 chromosome of strain R11, and therefore, the genome of strain R11 was sequenced to
84 find out the mechanism(s) of polymyxin E resistance.

85 The DNA sequencing was performed using an Illumina MiSeq platform and a
86 PacBio RS II platform at Personalbio Co., Ltd. (Shanghai, China). The sequences
87 were assembled *de novo* with Newbler version 2.8 and HGAP/Quiver and then
88 integrated with MUMmer software (Delcher et al. 2003). After the quality control

89 steps, approximately 0.91 Gb of data was obtained from the Illumina MiSeq platform,
90 and approximately 1.69 Gb of data was obtained from the PacBio RS II platform. A
91 total genome sequence of 4,993,008 bp with an average GC content of 53.15% was
92 assembled. The genome was annotated using the Prokaryotic Genome Annotation
93 Pipeline (PGAP) version 3.2 software by NCBI. Additional gene prediction was
94 performed by the RASTtk server (Brettin et al. 2015). The features of the complete
95 genome sequence are shown in Table 2. The genome was predicted to contain 4,908
96 genes, including 4,794 coding sequences, 25 rRNAs, 84 tRNAs, 102 ncRNAs, and
97 151 pseudogenes. Prophages in the genome were predicted using PHAST (Zhou et al.
98 2011), and two intact prophages in the chromosome and one incomplete prophage in
99 each of plasmids pASM1 and pASM2 were found. The Virulence Factors of
100 Pathogenic Bacteria Database (VFDB) was used to search for virulence factor genes
101 (Chen et al. 2012), and 10 virulence factor-encoding genes, including enterobactin
102 and aerobactin, were found in the chromosome. The Comprehensive Antibiotic
103 Resistance Database and RASTtk server were used for identification of
104 antibiotic-resistance genes (McArthur et al. 2013). Finally, a substantial number of
105 genes involved in multidrug resistance, including polymyxin E resistance, were
106 annotated, comprising 72 genes in the chromosome and one gene in plasmid
107 pASM1 (Supplementary Data Table S1). A list of genes predicted to be associated with
108 antibiotic resistance is shown in Table 3.

109 To investigate which plasmid and genes contributed to the polymyxin E
110 resistance of the conjugants, the total DNA of conjugant 25922-T03 was extracted,

111 and then PCR was performed with primers listed in Table S2 (Supplementary Data).
112 Total DNA from the recipient strain *E. coli* 25922 and the donor strain R11 were also
113 extracted and used as templates for PCR. Results showed that a specific fragment
114 from pASM1 could be amplified from strain R11 and conjugant 25922-T03, but not
115 from *E. coli* 25922, by PCR (Figure1). The identity of these PCR products was
116 confirmed by DNA sequencing. In contrast, specific targets from pASM2, pASM3
117 and pASM4 could not be amplified from conjugant 25922-T03, although they were
118 detectable in strain R11 (Figure1). These results indicated that only pASM1 was
119 acquired by the recipient strain *E. coli* 25922 and that one or more genes in pASM1
120 may contribute to polymyxin resistance in conjugant 25922-T03.

121 Several strategies have been employed by *Enterobacteriaceae* to enable them to
122 survive exposure to polymyxin antibiotics such as polymyxin B and polymyxin E.
123 The main strategy is a type of lipopolysaccharide (LPS) modification that can
124 decrease polymyxin binding to the bacterial outer membrane (Olaitan et al. 2014).
125 Recent studies revealed that the *mcr-1* gene related to this LPS modification is
126 plasmid-based, making this the first known plasmid-mediated resistance mechanism
127 to polymyxin E (Gao et al. 2016; Liu et al. 2016). The *mcr-1* gene encodes a
128 phosphoethanolamine transferase, and in strain R11, we found two separate and
129 different chromosomal genes encoding phosphoethanolamine transferases, and one
130 chromosomal gene cluster involved in the lipid A-Ara4N pathway (polymyxin B
131 resistance), whereas no *mcr-1* gene was detected on the plasmids; notably, none of
132 these genes were detected in conjugant 25922-T03 by PCR. The *arn* and *pmr* operons

133 and the PhoP/PhoQ two-component regulatory system have also been shown to be
134 involved in polymyxin E hetero-resistance in the *E. cloacae* complex (Guerin et al.
135 2016; Olaitan et al. 2014), and homologues to these genes were found in the
136 chromosome of R11. In addition, it has been reported that efflux pumps can also
137 contribute to polymyxin resistance (Olaitan et al. 2014), and our analyses identified
138 17 genes in the chromosome and one gene in pASM1 encoding efflux pump proteins
139 (Table S1). Considering that there were no other genes related to antibiotic resistance
140 in the plasmids, this plasmid-based efflux pump may contribute to the resistance of
141 the conjugants to polymyxin E in the above conjugation experiment.

142 In summary, this study presents the complete genome of *E. cloacae* R11, which
143 is a highly polymyxin-resistant bacterium. Analysis of the genome shows that it
144 contains a large number of antibiotic-resistance genes, virulence factor genes, and
145 four intact or incomplete prophages. We hope that these findings will facilitate a
146 deeper understanding of polymyxin E resistance mechanisms and the role of
147 horizontal transfer in resistance.

148 **Nucleotide sequence accession number**

149 The complete genome sequence of *Enterobacter cloacae* R11 has been deposited
150 in GenBank under the accession numbers CP019839-CP01984.

151 **Conflicts of interest**

152 The authors declare no conflicts of interest.

153 **Acknowledgements**

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231 Figure and table legends

232

233 **Fig.1. Detection of R11 plasmids in conjugant 25922-T03 by PCR.** Genes of
234 plasmids pASM1, pASM2, pASM3 and pASM4 were amplified by PCR using the
235 following templates for each panel: lane 1, the total DNA of conjugant 25922-T03;
236 lane 2, the total DNA of strain R11; lane 3, the total DNA of strain 25922.

237

238 **Table1** The antibiotic-resistance characteristics of strain R11

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240 **Table 2** Genomic features of strain R11

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242 **Table3** List of putative antibiotic resistance genes in strain R11

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Fig.1. Detection of R11 plasmids in conjugant 25922-T03 by PCR. Genes of plasmids pASM1, pASM2, pASM3 and pASM4 were amplified by PCR using the following templates for each panel: lane 1, the total DNA of conjugant 25922-T03; lane 2, the total DNA of strain R11; lane 3, the total DNA of strain 25922.

Table1 The antibiotic-resistance characteristics of strain R11

Antibiotic	MIC ($\mu\text{g}\cdot\text{ml}^{-1}$)	Susceptibility ^a
Ampicillin	40	Resistant
Cefixime	20	Resistant
Meropenem	16	Resistant
Florfenicol	60	Resistant
Amikacin	10	Resistant
Tetracycline	10	Resistant
Lincomycin	<1	Susceptible
Sulfamethoxazole	32	Resistant
Ciprofloxacin	10	Resistant
Polymyxin E	192	Resistant

^a “Resistant” indicates that R11 grew in the presence of $>2\ \mu\text{g}\cdot\text{ml}^{-1}$ of the antibiotic.

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Table 2 Genomic features of strain R11

Features	Chromosome	pASM1	pASM2	pASM3	pASM4
Genome size (bp)	4812230	85262	79101	11748	4667
G + C content (%)	55.92	52.63	51.00	54.98	51.23
Total genes	4699	105	109	22	7
Protein coding genes	4488	105	109	22	7
RNA genes	211	0	0	0	0
Prophage number	2	1	1	0	0
Virulence factor genes	10	0	0	0	0
Antibiotic resistance genes	72	1	0	0	0
GenBank accession no.	CP019839	CP019840	CP019841	CP019842	CP019843

Table3 List of putative antibiotic resistance genes in strain R11

Antibiotic type	Gene number	Location ^a
Polymyxin	9	Chromosome
Tetracycline	2	Chromosome
Methicillin	6	Chromosome
Beta-lactam	2	Chromosome
Bicyclomycin	1	Chromosome
Streptothricin	1	Chromosome
Fosfomycin	2	Chromosome
RND ^b efflux system	17	Chromosome
Multidrug resistance system	32	Chromosome
RND efflux system	1	pASM1

^a the specific genes are listed in Table S1

^b RND, resistance-nodulation-division family

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Table S1 Supplementary Data

type	location	contig_id	start-stop	strand	function	aliases	evidence_codes
Methicillin	chr	fig 6666666.2370.77.peg.156	chr_182097_183200	+	Undecaprenyl-phosphate N-acetylglucosaminyl 1-phosphate transferase (EC 2.7.8.-)	FIG00662416	isu;Methicillin_resistance_in_Staphylococci
Polymyxin	chr	fig 6666666.2370.77.peg.256	chr_305287_306426	+	UDP-4-amino-4-deoxy-L-arabinose--oxoglutarate aminotransferase (EC 2.6.1.-)	FIG00046703	icw(2);Lipid_A-Ara4N_pathway_(Polymyxin_resistance_)
Polymyxin	chr	fig 6666666.2370.77.peg.257	chr_306428_307411	+	Polymyxin resistance protein ArnC, glycosyl transferase (EC 2.4.-.-)	FIG00022019	icw(3);Lipid_A-Ara4N_pathway_(Polymyxin_resistance_)
Polymyxin	chr	fig 6666666.2370.77.peg.258	chr_307408_309390	+	UDP-glucuronic acid oxidase (UDP-4-keto-hexauronic acid decarboxylating) (EC 1.1.1.305) / UDP-4-amino-4-deoxy-L-arabinose formyltransferase (EC 2.1.2.13)	FIG00002423	icw(2);Lipid_A-Ara4N_pathway_(Polymyxin_resistance_) icw(3);Lipid_A-Ara4N_pathway_(Polymyxin_resistance_)
Polymyxin	chr	fig 6666666.2370.77.peg.259	chr_309387_310289	+	Polymyxin resistance protein PmrJ, predicted deacetylase	FIG00002427	icw(1);Lipid_A-Ara4N_pathway_(Polymyxin_resistance_)
Polymyxin	chr	fig 6666666.2370.77.peg.260	chr_310286_311941	+	Polymyxin resistance protein ArnT, undecaprenyl phosphate-alpha-L-Ara4N transferase; Melittin resistance protein PqaB	FIG00001379	icw(4);Lipid_A-Ara4N_pathway_(Polymyxin_resistance_)
Polymyxin	chr	fig 6666666.2370.77.peg.261	chr_311941_312276	+	Polymyxin resistance protein PmrL, sucrose-6 phosphate hydrolase	FIG00002456	isu;Lipid_A-Ara4N_pathway_(Polymyxin_resistance_)
Polymyxin	chr	fig 6666666.2370	chr_312273_	+	Polymyxin resistance protein PmrM	FIG000	icw(4);Lipid_A-Ara4N_pathway_(Polymyxin_resistance_)

in		77.peg.262	312653			02758	
Multiple antibioti c	chr	fig 6666666.2370 77.peg.287	chr_333787_ 333194	-	Multiple antibiotic resistance protein marC	FIG010 67880	ff
Tetracyc line	chr	fig 6666666.2370 77.peg.371	chr_423067_ 425181	+	Translation elongation factor G	FIG019 69619	idu(1);Tetracycline_resistance,_ribosome_protection_type icw(1);Mycobacterium_virulence_operon_involved_in_prot ein_synthesis_(SSU_ribosomal_proteins) idu(1);Tetracycline_resistance,_ribosome_protection_type ,_too idu(1);Universal_GTPases idu(1);Translation_elongation_factor_G_family idu(1);Translation_elongation_factors_bacterial
RND	chr	fig 6666666.2370 77.peg.422	chr_466315_ 463202	-	RND efflux system, inner membrane transporter CmeB	FIG000 34851	idu(2);Multidrug_Resistance_Efflux_Pumps idu(2);Multidrug_efflux_pump_in_Campylobacter_jejuni_(CmeABC_operon)
RND	chr	fig 6666666.2370 77.peg.423	chr_467466_ 466327	-	Membrane fusion protein of RND family multidrug efflux pump	FIG002 07095	icw(1);Multidrug_Resistance_Efflux_Pumps
RND	chr	fig 6666666.2370 77.peg.449	chr_496052_ 496984	+	Membrane fusion component of tripartite multidrug resistance system	FIG000 01080	idu(4);Multidrug_Resistance,_Tripartite_Systems_Found_i n_Gram_Negative_Bacteria
RND	chr	fig 6666666.2370 77.peg.640	chr_701241_ 699763	-	Type I secretion outer membrane protein, TolC precursor	FIG000 28132	isu;Multidrug_Resistance_Efflux_Pumps isu;Ton_and_Tol_transport_systems
multidru g	chr	fig 6666666.2370 77.peg.951	chr_1044191_ _1042644	-	Multidrug resistance protein B	FIG013 56213	ff
multidru g	chr	fig 6666666.2370 77.peg.952	chr_1045380_ _1044208	-	Multidrug resistance protein A	FIG013 03929	ff
multidru g	chr	fig 6666666.2370 77.peg.953	chr_1046037_ _1045507	-	Transcription repressor of tripartite multidrug resistance system	FIG000 02687	ff

RND	chr	fig 6666666.2370.77.peg.1167	chr_1292458_1289345	-	RND efflux system, inner membrane transporter CmeB	FIG00034851	idu(2);Multidrug_Resistance_Efflux_Pumps idu(2);Multidrug_efflux_pump_in_Campylobacter_jejuni_(CmeABC_operon)
bicyclo mycin	chr	fig 6666666.2370.77.peg.1351	chr_1506898_1508094	+	MFS family multidrug transport protein, bicyclomycin resistance protein	FIG01296751	ff
RND	chr	fig 6666666.2370.77.peg.1395	chr_1557670_1559088	+	RND efflux system, outer membrane lipoprotein, NodT family	FIG00005510	idu(1);Multidrug_Resistance_Efflux_Pumps
multidru g	chr	fig 6666666.2370.77.peg.1440	chr_1606005_1605283	-	Response regulator BaeR	FIG00229357	icw(1);The_mdtABCD_multidrug_resistance_cluster
multidru g	chr	fig 6666666.2370.77.peg.1441	chr_1607405_1606002	-	Sensory histidine kinase BaeS	FIG01303885	icw(2);The_mdtABCD_multidrug_resistance_cluster
multidru g	chr	fig 6666666.2370.77.peg.1442	chr_1608817_1607402	-	Multidrug transporter MdtD	FIG00003550	icw(3);The_mdtABCD_multidrug_resistance_cluster
multidru g	chr	fig 6666666.2370.77.peg.1443	chr_1611895_1608818	-	Multidrug transporter MdtC	FIG00147841	isu;The_mdtABCD_multidrug_resistance_cluster
multidru g	chr	fig 6666666.2370.77.peg.1444	chr_1615018_1611896	-	Multidrug transporter MdtB	FIG00137528	icw(1);The_mdtABCD_multidrug_resistance_cluster
RND	chr	fig 6666666.2370.77.peg.1445	chr_1616220_1615018	-	Probable RND efflux membrane fusion protein	FIG00229283	icw(1);The_mdtABCD_multidrug_resistance_cluster
multidru g	chr	fig 6666666.2370.77.peg.1548	chr_1735237_1736649	+	Outer membrane component of tripartite multidrug resistance system	FIG00004305	idu(2);Multidrug_Resistance,_Tripartite_Systems_Found_in_Gram_Negative_Bacteria
multidru g	chr	fig 6666666.2370.77.peg.1549	chr_1736649_1737848	+	Membrane fusion component of tripartite multidrug resistance system	FIG00001080	icw(1);Multidrug_Resistance,_Tripartite_Systems_Found_in_Gram_Negative_Bacteria
multidru g	chr	fig 6666666.2370.77.peg.1550	chr_1737852_1739384	+	Inner membrane component of tripartite multidrug resistance system	FIG00005204	icw(2);Multidrug_Resistance,_Tripartite_Systems_Found_in_Gram_Negative_Bacteria
Beta-lac	chr	fig 6666666.2370	chr_1762285	-	Beta-lactamase class C and other penicillin	FIG000	isu;Beta-lactamase

ta		77.peg.1568	_1760987		binding proteins	00681	
RND	chr	fig 6666666.2370 77.peg.1945	chr_2169428 _2166333	-	RND multidrug efflux transporter; Acriflavin resistance protein	FIG001 32942	idu(2);Multidrug_Resistance_Efflux_Pumps
Tetracycline	chr	fig 6666666.2370 77.peg.2048	chr_2278595 _2280694	+	Translation elongation factor G	FIG019 69619	idu(1);Tetracycline_resistance,_ribosome_protection_type idu(1);Mycobacterium_virulence_operon_involved_in_protein_synthesis_(SSU_ribosomal_proteins) idu(1);Tetracycline_resistance,_ribosome_protection_type,_too idu(1);Universal_GTPases idu(1);Translation_elongation_factor_G_family idu(1);Translation_elongation_factors_bacterial
Streptothricin	chr	fig 6666666.2370 77.peg.2145	chr_2388272 _2387745	-	Streptothricin acetyltransferase, Streptomyces lavendulae type	FIG000 03590	isu;Streptothricin_resistance
multidrug	chr	fig 6666666.2370 77.peg.2208	chr_2454329 _2453664	-	Multiple antibiotic resistance protein MarC	FIG000 04188	icw(3);Multiple_Antibiotic_Resistance_MAR_locus
multidrug	chr	fig 6666666.2370 77.peg.2209	chr_2454585 _2455019	+	Multiple antibiotic resistance protein MarR	FIG000 04042	isu;Multiple_Antibiotic_Resistance_MAR_locus
multidrug	chr	fig 6666666.2370 77.peg.2210	chr_2455041 _2455421	+	Multiple antibiotic resistance protein MarA	FIG000 04085	isu;Commensurate_regulon_activation icw(2);Multiple_Antibiotic_Resistance_MAR_locus
multidrug	chr	fig 6666666.2370 77.peg.2211	chr_2455455 _2455673	+	Multiple antibiotic resistance protein MarB	FIG000 06555	icw(1);Multiple_Antibiotic_Resistance_MAR_locus
RND	chr	fig 6666666.2370 77.peg.2282	chr_2546341 _2547720	+	RND efflux system, outer membrane lipoprotein, NodT family	FIG000 05510	idu(1);Multidrug_Resistance_Efflux_Pumps
RND	chr	fig 6666666.2370 77.peg.2396	chr_2664659 _2661504	-	RND multidrug efflux transporter; Acriflavin resistance protein	FIG001 32942	icw(1);Multidrug_Resistance_Efflux_Pumps
RND	chr	fig 6666666.2370 77.peg.2397	chr_2665910 _2664684	-	RND efflux system, membrane fusion protein CmeA	FIG019 60366	isu;Multidrug_Resistance_Efflux_Pumps isu;Multidrug_efflux_pump_in_Campylobacter_jejuni_(Cm

						eABC_operon)
RND	chr	fig 6666666.2370_77.peg.2525	chr_2793812_2795185	+	Multi antimicrobial extrusion protein (Na(+)/drug antiporter), MATE family of MDR efflux pumps	FIG00000402 isu;Riboflavin_FMN_and_FAD_metabolism_in_plants isu;Multidrug_Resistance_Efflux_Pumps
multidrug	chr	fig 6666666.2370_77.peg.2835	chr_3091970_3093187	+	Multidrug-efflux transporter, major facilitator superfamily (MFS) (TC 2.A.1)	FIG00087875 idu(1);Multidrug_Resistance_Efflux_Pumps
Methicillin	chr	fig 6666666.2370_77.peg.2911	chr_3164777_3165934	+	Poly(glycerophosphate chain) D-alanine transfer protein DltD	FIG00001546 isu;D-Alanyl_Lipoteichoic_Acid_Biosynthesis isu;Methicillin_resistance_in_Staphylococci
Methicillin	chr	fig 6666666.2370_77.peg.2913	chr_3166078_3167181	+	D-alanyl transfer protein DltB	FIG00001531 icw(1);D-Alanyl_Lipoteichoic_Acid_Biosynthesis icw(1);Methicillin_resistance_in_Staphylococci
Methicillin	chr	fig 6666666.2370_77.peg.2914	chr_3167178_3168611	+	D-alanine--poly(phosphoribitol) ligase subunit 1 (EC 6.1.1.13)	FIG00001579 icw(2);D-Alanyl_Lipoteichoic_Acid_Biosynthesis icw(2);Methicillin_resistance_in_Staphylococci
multidrug	chr	fig 6666666.2370_77.peg.3011	chr_3279751_3277811	-	Macrolide export ATP-binding/permease protein MacB (EC 3.6.3.-)	FIG00001667 isu;Multidrug_Resistance_Efflux_Pumps
multidrug	chr	fig 6666666.2370_77.peg.3012	chr_3280890_3279748	-	Macrolide-specific efflux protein MacA	FIG00001179 icw(1);Multidrug_Resistance_Efflux_Pumps
multidrug	chr	fig 6666666.2370_77.peg.3073	chr_3345377_3344145	-	Multidrug translocase MdfA	FIG00053455 ff
multidrug	chr	fig 6666666.2370_77.peg.3105	chr_3383666_3382143	-	Outer membrane component of tripartite multidrug resistance system	FIG00004305 idu(2);Multidrug_Resistance,_Tripartite_Systems_Found_in_Gram_Negative_Bacteria
multidrug	chr	fig 6666666.2370_77.peg.3106	chr_3385237_3383666	-	Inner membrane component of tripartite multidrug resistance system	FIG00005204 icw(2);Multidrug_Resistance,_Tripartite_Systems_Found_in_Gram_Negative_Bacteria
multidrug	chr	fig 6666666.2370_77.peg.3107	chr_3386328_3385234	-	Membrane fusion component of tripartite multidrug resistance system	FIG00001080 icw(1);Multidrug_Resistance,_Tripartite_Systems_Found_in_Gram_Negative_Bacteria
RND	chr	fig 6666666.2370_77.peg.3343	chr_3642165_3639010	-	RND multidrug efflux transporter; Acriflavin resistance protein	FIG000132942 idu(2);Multidrug_Resistance_Efflux_Pumps

RND	chr	fig 6666666.2370.77.peg.3344	chr_3643236 _3642175	-	Probable RND efflux membrane fusion protein	FIG002 29283	idu(1);The_mdtABCD_multidrug_resistance_cluster
Fosmid omycin	chr	fig 6666666.2370.77.peg.3405	chr_3707760 _3708968	+	Fosmidomycin resistance protein	FIG008 01557	ff
RND	chr	fig 6666666.2370.77.peg.3420	chr_3725225 _3724572	-	Transcription repressor of multidrug efflux pump acrAB operon, TetR (AcrR) family	FIG000 07172	isu;Multidrug_Resistance_Efflux_Pumps
RND	chr	fig 6666666.2370.77.peg.3421	chr_3725366 _3726559	+	Membrane fusion protein of RND family multidrug efflux pump	FIG002 07095	icw(2);Multidrug_Resistance_Efflux_Pumps
RND	chr	fig 6666666.2370.77.peg.3422	chr_3726582 _3729728	+	RND efflux system, inner membrane transporter CmeB	FIG000 34851	icw(1);Multidrug_Resistance_Efflux_Pumps idu(2);Multidrug_efflux_pump_in_Campylobacter_jejuni_(CmeABC_operon)
multidru g	chr	fig 6666666.2370.77.peg.3448	chr_3753645 _3751864	-	Multidrug resistance-like ATP-binding protein mdIB	FIG007 31543	ff
multidru g	chr	fig 6666666.2370.77.peg.3449	chr_3755410 _3753638	-	ATP-binding component of a transport system	FIG010 47725	ff
multidru g	chr	fig 6666666.2370.77.peg.3518	chr_3827972 _3826758	-	Multidrug-efflux transporter, major facilitator superfamily (MFS) (TC 2.A.1)	FIG000 87875	idu(1);Multidrug_Resistance_Efflux_Pumps
multidru g	chr	fig 6666666.2370.77.peg.3542	chr_3853653 _3855059	+	Outer membrane component of tripartite multidrug resistance system	FIG000 04305	idu(2);Multidrug_Resistance,_Tripartite_Systems_Found_i n_Gram_Negative_Bacteria
multidru g	chr	fig 6666666.2370.77.peg.3543	chr_3855109 _3856251	+	Membrane fusion component of tripartite multidrug resistance system	FIG000 01080	icw(1);Multidrug_Resistance,_Tripartite_Systems_Found_i n_Gram_Negative_Bacteria
multidru g	chr	fig 6666666.2370.77.peg.3544	chr_3856343 _3857785	+	Inner membrane component of tripartite multidrug resistance system	FIG000 05204	icw(2);Multidrug_Resistance,_Tripartite_Systems_Found_i n_Gram_Negative_Bacteria
Methicill in	chr	fig 6666666.2370.77.peg.3706	chr_4048017 _4046659	-	UDP-N-acetylmuramoylalanyl-D-glutamyl-2,6-di aminopimelate--D-alanyl-D-alanine ligase (EC 6.3.2.10)	FIG000 24401	icw(4);Peptidoglycan_Biosynthesis icw(2);Peptidoglycan_biosynthesis--gjo icw(1);Methicillin_resistance_in_Staphylococci

Methicillin	chr	fig 6666666.2370.77.peg.3707	chr_4049429_4048014	-	UDP-N-acetylmuramoylalanyl-D-glutamate--2,6-diaminopimelate ligase (EC 6.3.2.13)	FIG00000243	isu;Peptidoglycan_Biosynthesis icw(1);Peptidoglycan_biosynthesis--gjo isu;Methicillin_resistance_in_Staphylococci
Fosfomycin	chr	fig 6666666.2370.77.peg.3879	chr_4239118_4238699	-	Fosfomycin resistance protein FosA	FIG00040288	isu;Fosfomycin_resistance
Beta-lactamase	chr	fig 6666666.2370.77.peg.4015	chr_4397975_4399120	+	Beta-lactamase (EC 3.5.2.6)	FIG00000747	idu(1);Beta-lactamase idu(1);Tn552
Multidrug	chr	fig 6666666.2370.77.peg.4261	chr_4669922_4668969	-	Membrane fusion component of tripartite multidrug resistance system	FIG00001080	idu(4);Multidrug_Resistance,_Tripartite_Systems_Found_in_Gram_Negative_Bacteria
Multidrug	chr	fig 6666666.2370.77.peg.4263	chr_4671305_4670940	-	Multidrug resistance efflux pump		
Multidrug	chr	fig 6666666.2370.77.peg.4364	chr_4789107_4790264	+	Multidrug resistance protein D	FIG00053847	ff
Polymyxin	chr	fig 6666666.2370.77.peg.2510	chr_2779522_2781147	+	Phosphoethanolamine transferase EptA specific for the 1 phosphate group of core-lipid A	FIG00002204	isu;Lipid_A_modifications
Polymyxin	chr	fig 6666666.2370.77.peg.4210	chr_4614182_4612491	-	Phosphoethanolamine transferase specific for the outer Kdo residue of lipopolysaccharide	FIG00007854	isu;Lipid_A_modifications
RND	plasmid1	fig 6666666.2370.78.peg.11	plasmid1_10087_10380	+	RND efflux system, inner membrane transporter CmeB		isu;Multidrug_efflux_pump_in_Campylobacter_jejuni_(CmeABC_operon)

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Table S2 Supplementary Data

Primers	Sequence of primers (5'→3')	Gene function
P1-73For	agagaaatgcacttccatccagaat	hypothetical protein
P1-73Rev	atctaaagcccctgtgttatagtgggt	
P2-94For	gttcattctcctggctagaaagccaatccc	hypothetical protein
P2-94Rev	cgaaccagcgtggcgtgcatcatgttc	
P3-10For	acgctggcgaaggacttagagcagcaa	hypothetical protein
P3-10Rev	ctactcctgtttcaggcggatccatgtcg	
P4-2For	tgacgacccgaacgcaccggaga	hypothetical protein
P4-2Rev	ctcgccgcaggctcgagagctgcacgcc	
Chr-2566For	gcctctcgcgggcgaactactccctg	Phosphoethanolamine transferase
Chr-2566Rev	tagtccggtgacagccagaacataaacg	
Chr-4362For	tcgctgtggcgagtgtggcaacgttt	Phosphoethanolamine transferase
Chr-4362Rev	gtatcacaggaatagccacgataggcaa	