

Characterization of Tn7-like transposons and its related antibiotic resistance among *Enterobacteriaceae* from livestock and poultry in China

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Abstract

Background: This study was aimed to investigate the prevalence and structure of Tn7-like in *Enterobacteriaceae* from livestock and poultry as well as their possible role as reservoir of antibiotic resistance genes (ARGs).

Methods: Polymerase chain reaction (PCR) and DNA sequencing analyses were used for the characterization of Tn7-like, associated integrons and ARGs. The antimicrobial resistance profile of the isolates was examined by using disc diffusion test.

Results: Three hundred and seventy-eight Tn7-like-positive strains of *Enterobacteriaceae* were isolated, and included *E. coli* (128), *Proteus*(150), *K. pneumonia*(17), *Salmonella*(13), *M. morgani*(21) and *A. baumannii*(1), wherein high resistance was observed for Trimethoprim/Sulfamethoxazole and Streptomycin, and fifty percent of the strains were multidrug-resistant. Integrons class 2 were detected in all of the isolates and there are high frequency mutation sites especially in 535, a stop mutation. Variable region of class 2 integrons carried same gene cassettes, namely *aadA1-sat2-dfrA1*. From the 378 isolated strains, we found a new type of Tn7-like on a plasmid, named Tn6765.

Conclusions: These findings proved that the Tn7-like can contribute to the horizontal transmission of antibiotic resistant genes in livestock and poultry. As potential vessels for antibiotic resistance genes (ARGs), Tn7-like could not be ignored due to their efficient transfer ability in environments.

Background

Enterobacteriaceae include a large group of gram-negative bacillus with similar biological characteristics, They are widely distributed in the natural environment and the intestinal tract of humans and animals. As an opportunistic pathogen, *Enterobacteriaceae* is associated with urinary tract infection (1), nosocomial infection (2), and food poisoning (3). The widespread and irrational use of antibiotics in humans and animals has given rise to numerous multidrug-resistant *Enterobacteriaceae* (MRE) via the dissemination of antimicrobial resistance genes (AMRg) (4). This multiresistance may be mediated by chromosomally located resistance determinants or mutations in a resident gene. however, it may also develop through the acquisition of resistance genes or an array of resistance genes by horizontal transfer. This latter phenomenon is currently thought to play an increasing role in the development of multidrug resistance in *Enterobacteriaceae* (5). These resistance determinants of *Enterobacteriaceae* are generally disseminated via mobile elements (integrons, transposons and plasmids) in bacterial populations from animals and humans (4). One significant finding over the past 10 years is the appreciation of the dissemination of Tn7, and related elements called Tn7-like elements that contain homologs of the Tn7 transposition proteins, in highly diverged bacteria adapted to a remarkable number of different environments (6–8).

Tn7 is typically large, often 14 kb in size (9). However, comparative analysis of some derivatives of the Tn7, Tn7-like, shows that these elements are very diverse in terms of genetic structure and have accumulated several accessory resistance regions (8, 10). Tn7-like carry the determinants for resistance not only to heavy metals such as mercuric ions, copper, silver ions, tellurite, arsenate and arsenite, but also to antibiotics such as β -lactams (including carbapenems), quinolones, aminoglycosides, tetracyclines, amphenicols, trimethoprim, sulfamethoxazole, streptomycin and fosfomycin (11). The Tn7-like, as an important mobile genetic element of transposons, transfers various resistance genes between bacteria through its transposase, promotes the horizontal spread of drug resistance in bacteria.

The sequences of the two ends of the Tn7 transposon are highly conserved, which are respectively transposition module and class 2 integron system. Transposition module encodes five proteins required for two transposition path-ways, *tnsA*, *tnsB*, *tnsC*, *tnsD*, and *tnsE* (*tnsABCDE*) (12). The basic transposition machinery of Tn7 and how it directs transposition into the attTn7 site is well understood and has been the subject of multiple reviews (13, 14). *tnsABC* are required for both Tn7 transposition pathways, but only allow transposition when they function with one of two target site selecting proteins, *tnsD* or *tnsE* (15, 16). Transposition with *tnsABC + tnsD* directs transposition into the single attTn7 site located downstream of *glmS* (15). Transposition with *tnsABC + tnsE* occurs preferentially into mobile plasmids through the ability of the *tnsE* protein to recognize features found enriched during DNA replication on the lagging-strand template (17, 18).

The class 2 integron of Tn7 has an organization similar to that of the class I integron and carries three resistance gene cassettes—*aadA1*, *sat*, and *dfra1* (19–21)—close to an open reading frame, *IntI2**. *IntI2** has premature translation termination due to mutation of base 535 encoding integrase from C to T, and has no function. Although these gene cassettes are fixed in Tn7 due to mutations in the homologous recombinase, they can be rearranged in hosts expressing the relevant recombinase, resulting in other combinations of antibiotic resistance genes (22).

However, there is a paucity of data on the prevalence and role of Tn7-like transposons in antibiotic resistant *Enterobacteriaceae* in many countries such as China especially from livestock and poultry. The aim of this study was to determine the frequency of Tn7-like transposons and its associated integrons in *Enterobacteriaceae* which from swine and chicken, and to investigate the association between the presence of Tn7-like and antibiotic resistance patterns, so as to better understand the characteristics of Tn7-like and resistant *Enterobacteriaceae* in farming animals. Additionally, we plan to analyze the skeletal structure of our Tn7-like and compared it with other Tn7-like to obtain insight into the horizontal transfer of resistance genes and the diversity and evolution of Tn7-like.

Methods

Bacterial isolates

A total of 1482 consecutive and unduplicated clinical isolates of *Enterobacteriaceae* were collected from livestock and poultry in China from 2018 to January 2020. The isolates were from the intestines of dead animals, feces, cloacal swab and drinking water (Table 1). All the isolates were presumptively identified through phenotypic methods, including colony morphology on MacConkey Agar or Eosin-Methylene Blue Agar. And the identification of these isolates was later confirmed using 16S rRNA gene sequencing. All *Enterobacteriaceae* strains were stored at – 20 °C in 50% glycerol. The standard strain of *Escherichia coli* (ATCC25922) was used as a susceptibility control. Rifampin-resistant strain *E. coli*600 was used as the recipient strain in the conjugation experiments.

Table 1
Bacterial Strains Isolated from 2018 to 2020

Source	Species	No. of strains	Note
Pigs	<i>Escherichia coli</i>	413	Fecal sample, cloacal swab ,drinking
	<i>Proteus mirabilis</i>	208	water, or small intestine of dead
	<i>Providencia</i>	164	swine from 12 pig farms in 6
	<i>Morganella morganii</i>	146	different provinces of China.
	<i>Klebsiella pneumoniae</i>	141	
	<i>Salmonella enterica</i>	82	
	<i>Acinetobacter baumannii</i>	8	
Total		1162	
Chicken	<i>Escherichia coli</i>	121	Fecal sample, cloacal swab ,drinking
	<i>Proteus mirabilis</i>	53	water, or small intestine of dead
	<i>Providencia</i>	46	chicken from 10 poultry farms in 5
	<i>Klebsiella pneumoniae</i>	24	different provinces of China.
	<i>Salmonella enterica</i>	76	
Total		320	

Detection Of Tn7-like Transposons By Polymerase Chain Reaction (PCR)

Tn7-like transposons were tested in 1483 *Enterobacteriaceae* isolates using PCR targeting *tnsA*, a transposase-encoding gene. The isolates were grown overnight (18–24 h) in Brain Heart Infusion(BHI) broth (Oxoid, UK) at 37 °C with a rotation speed of 200 rpm, and the DNA template was prepared using the boiling method (23). The PCR mixture was prepared with a final volume of 20 µl, containing 1 µl of template DNA, 8 µl ddH₂O, 10 µl Taq PCR MasterMix and 0.5 µl each primer. The specific primers for detecting *tnsA* gene are shown in Table S1. The amplified PCR products were separated by electrophoresis in 1.2% agarose gels and visualised after staining with Goldview dye.

Antibiotic Sensitivity Test

The antibiotic resistance profile of all Tn7-like positive isolates was determined using the disc diffusion method, as per the Clinical and Laboratory Standards Institute standards (56). Briefly, Mueller-Hinton agar was used as the test medium for each of the following antimicrobials (all discs from Oxoid, Basingstoke, UK): Ampicillin (AMP, 10 µg), Imipenem(IPM, 10 µg), Cefoxitin (FOX, 30 µg), Ciprofloxacin (CIP, 5 µg), Streptomycin(S, 10 µg), Gentamicin(CN,10 µg), Florfenicol(FFC, 30 µg), Enrofloxacin(ENR, 5 µg), Tetracycline(TGC, 30 µg), Aztreonam(ATM, 30 µg), Ceftazidime(CAZ, 30 µg) and Trimethoprim/Sulfamethoxazole (SXT,1.25/23.75 µg). *E. coli* ATCC 25922 was used as a quality control strain.

Characterization Of The Integrons And Associated ARGs

The isolated Tn7-like *Enterobacteriaceae* strains were checked for the presence of *intl2* integrase genes by PCR, using primers(Table S1) and methodology as previously described by (24, 25), and these mutation site sequence of *intl2* were visualized with Weblogo (<http://weblogo.berkeley.edu/logo.cgi>). Subsequently, strains harboring *intl2* genes were elaborated for the amplification of its three resistance gene cassettes (*aadA1*, *sat2* and *dfrA1*). For this purpose, primer pairs which are listed in Table S1, were used to amplify the gene cassette by targeting *aadA1*, *sat2* and *dfrA1*, respectively.

WGS And Analysis

We also analysed genetic environment of Tn7-like transposons among strains exhibiting unique resistance phenotypes. The whole genome of the Tn7-like-positive strain was sequenced using Illumina MiSeq with a 200-fold sequencing depth and the nanopore platform with a 100-fold sequencing depth. Genome assembly was carried out by de novo assembly in SMRT portal version 3.2.0, and the sequence was annotated using the NCBI Prokaryotic Genome Annotation Pipeline v4.2. Mobile elements, resistance genes, and other features were annotated by INTEGRALL (26), ISfinder (27), ResFinder (28), PlasmidFinder (29), and the Tn Number Registry (30) online databases, and the analysis was conducted using the BLAST program (<http://blast.ncbi.nlm.nih.gov/Blast.cgi>).

Horizontal Transfer Of Tn7-like Transposon

Conjugation was performed using rifampin-resistant *E. coli* EC600 as the recipient and the *P. mirabilis* SCBX1.1 isolate as the donor with selection on Salmonella Shigella agar plates containing 20 mg/liter rifampin and 8 mg/liter florfenicol. Successful horizontal transfer of plasmid p1.1.2 containing Tn7-like transposon was confirmed using antibiotic sensitivity test and PCR as mentioned above, then the conjugation frequency was calculated as transconjugants divided by number of donors (31). The stability of p1.1.2 in the positive transconjugants (designated EC-600-Pp1.1.2) was determined by passage in Brain Heart Infusion(BHI) broth lacking antibiotics as described previously (32).

Statistical analysis

Variables are expressed as percentages (%). Comparison of incidence of Tn7-like in *Enterobacteriaceae* isolates versus Tn7-like lacking isolates was assessed using the Chi square test. Statistical analysis was conducted with the GraphPad Prism 8 software.

P value of < 0.05 was considered as significant.

Genbank Accession Number

The complete sequences of p1.1.1 (CP047113), p1.1.2 (CP047114), and all Tn7-like; Tn6763 (MN641830), Tn6764 (MN628641), and Tn6765 (CP047113), identified in the present study were submitted to NCBI GenBank.

Results

Prevalence of Tn7-like transposon in Enterobacteriaceae

A total of 1482 strains of *Enterobacteriaceae* were isolated during the period 2018.6-2020.1 through analysis of samples collected from Sichuan, Hainan, Chongqing, Shandong, Hebei and Anhui provinces. Of the 1482 *Enterobacteriaceae* strains examined, 378 (25.5%) contained Tn7-like transposon. They included 128(8.6%) *Escherichia coli*, 150(10.1%) *Proteus*, 48(3.2%) *Providencia*, 21(1.4%) *Morganella morganii*, 17(1.1%) *Klebsiella pneumoniae*, 13(0.9%) *Salmonella enterica* and 1 (0.06%) *Acinetobacter baumannii* (Fig. 1). The positive rate of Tn7-like transposon in *proteus* was significantly higher than that of other strains ($p < 0.0001$).

Antimicrobial Resistance Phenotypes

A total of 378 Tn7-like positive isolates were examined for their resistance against 10 antibiotics (Table 2). Two hundred and fourteen (56.61%) isolates showed resistance to three or more antibiotic classes and were considered as multidrug-resistance (MDR). Except for *Acinetobacter baumannii*, *Proteus* and *Salmonella* have the highest multidrug resistance rates of 65.69% and 63.90%, respectively. Notably, high resistance rates were observed for Streptomycin (88.7%) and Trimethoprim/Sulfamethoxazole (74.6%), followed by Ampicillin (63.23%). And low resistance rates to Ceftazidime (5.82%), Cefoxitin (5.82%) and Aztreonam (4.23%) were detected. Most of the *Enterobacteriaceae* isolates were highly susceptible to Imipenem.

Table 2
Rates of Resistance to Antimicrobial Agents of Tn7-like Positive Isolates.

Antimicrobial agent	E.coli (n = 128), %	Proteus (n = 150), %	Providencia (n = 48), %	M.morganii (n = 21), %	K.pneumoniae (n = 17), %	S.enterica (n = 13), %	A.baumannii (n = 1), %	Total (n = 378), %
CN (10 µg)	24.22	30.67	12.50	9.52	11.76	38.46	0.00	24.34
S (10 µg)	84.38	90.00	95.83	90.48	70.59	84.62	100.00	87.83
TGC (30 µg)	56.25	32.67	16.67	4.76	47.06	69.23	100.00	39.15
CAZ (30 µg)	6.25	3.33	8.33	0.00	0.00	38.46	0.00	5.82
IPM (10 µg)	1.56	0.00	0.00	0.00	0.00	0.00	0.00	0.53
FOX (30 µg)	7.03	6.00	2.08	0.00	11.76	7.69	0.00	5.82
SXT(1.25/23.75 µg)	67.97	85.33	75.00	52.38	41.18	76.92	100.00	74.07
ATM (30 µg)	3.91	1.33	4.17	0.00	5.88	46.15	0.00	4.23
CIP (5 µg)	22.66	28.00	16.67	4.76	0.00	23.08	0.00	21.96
AMP (10 µg)	60.94	70.67	58.33	52.38	35.29	69.23	100.0	63.23
MDR	55.22	65.96	52.25	29.93	22.83	63.90	100.0	56.61

Prevalence And Cassette Gene Of Tn7-like Integrons

The positive rate of class 2 integrons in 378 *Enterobacteriaceae* strains was 100%. Through sequencing and sequence comparison, the results showed that *intI2* usually had mutation sites, and most of them were at 349,372,379,535,617,767 and 774 (Fig. 2). On amplification, 347 isolates were found to carry the resistance gene cassette (*aadA1*, *sat2* and *dfrA1*) related to *intI2*, whereas 31 strains had different *aadA1*, *sat2* and *dfrA1* gene deletions, respectively. 24 isolates were missing one gene cassette, and 7 isolates were missing two gene cassette. A single *S.enterica* only harbored *dfrA1* (Table 3).

Table 3
Cassette Gene of Tn7-like Integrons.

No. of isolates	Cassette gene
121(<i>E.coli</i>),142(<i>Proteus</i>),41(<i>Providencia</i>),19(<i>M.morganii</i>)15(<i>K.pneumoniae</i>),8 (<i>S.enterica</i>),1(<i>A.baumannii</i>)	<i>aadA1,sat2,dfrA1</i>
2(<i>E.coli</i>), 3(<i>Proteus</i>), 1(<i>Providencia</i>),2(<i>S.enterica</i>)	<i>aadA1,sat2</i>
2(<i>E.coli</i>), 5(<i>Proteus</i>), 1(<i>Providencia</i>),2(<i>M.morganii</i>),2 (<i>K.pneumoniae</i>)	<i>aadA1,dfrA1</i>
1(<i>E.coli</i>),1(<i>Providencia</i>), 2(<i>S.enterica</i>)	<i>sat2,dfrA1</i>
1(<i>E.coli</i>),1(<i>Providencia</i>),	<i>aadA1</i>
1(<i>E.coli</i>),3(<i>Providencia</i>),	<i>sat2</i>
1(<i>S.enterica</i>)	<i>dfrA1</i>

Structural Analysis Of Tn7-like

Among the Tn7-like-carrying strains, three multi-drug resistant bacteria were randomly selected for whole-genome sequencing, and yielded three complete Tn7-like structures. According to the transposon nomenclature (<http://transposon.lstmed.ac.uk/>), we designated them Tn6763, Tn6764, and Tn6765, respectively. Tn6763 (GenBank accession number MN641830) and Tn6764 (GenBank accession number MN628641) were located on the chromosomes of *Proteus* and *E. coli*, respectively. They are typical Tn7 transposons comprising the transposition module (*tnsA*, *tnsB*, *tnsC*, *tnsD* and *tnsE*), three gene cassettes (*aadA1*, *sat2* and *dfrA1*), and an inactive class 2 integrase gene. Besides, both of them inserted an *insB-1* next to the *aadA1* gene (Fig. 3).

Tn6765 is located on a plasmid of *Proteus mirabilis* SCBX1.1, named p1.1.2 (GenBank accession number CP047114). Sequence analysis showed that 19 resistance genes, except for *cfp*, were carried by the Tn7-like transposon (Fig. 3). The novel MDR transposon harboring 18 different resistance genes. including *blaDHA-1* (cephalosporin resistance), *qnrA1* (fluoroquinolone), *aac(6)-Ib-cr*. (fluoroquinolone and aminoglycosides), *floR* (chloramphenicol/florfenicol), *mphE* and *msrE* (macrolide), and *lunF* (lincosamide) genes. While the *cfp* gene is carried by another 12795 bp plasmid p1.1.1 (GenBank accession number CP047113), which exists in the same strain as p1.1.2. Tn6765 is 64752 bp (corresponding to bases 47309 to 112060 in GenBank accession number CP047114) with an average GC content of 52.94% that differs from that of the rest of the *P. mirabilis* plasmid (GC content, 44.35%). It has partial characteristics of the Tn7 transposon (Fig. 3), which contained transposase genes *tnsA*, *tnsB* and *tnsC*. But its transposition module lost *tnsE* gene and the *tnsD* was truncated by insertion of the inverted repeat (IR mcp) of Tn1721.

Tn6765 was highly homologous with Tn6450 located on the chromosome detected by Chen (33). The similarity of nucleic acid sequence was more than 99%, and the coverage was 96%. The differences between the two are mainly in the following four aspects: (1) Tn6765 has a hypothetical protein gene before *blaDHA-1*; (2) The *ampR* gene of Tn6450 was replaced by *lysR* and *HypA* in Tn6765; (3) An incomplete *aac(3)-IV* was inserted between ISEc59 and *aph(4)-Ia*; (4) Tn6450 is inserted downstream of the *glmS* gene, encoding glucosamine 6-phosphate synthase and surrounded by 5-bp direct repeats (CCAAT), Whereas Tn6765 is located on the plasmid, there is no *glmS* gene and repeated sequences at both ends (Figs. 3 and 4). The plasmid carrying Tn6765 was 138818 bp in size and had a GC content of 44.35%, which was named as p1.1.2. Blastn results showed that, except for the Tn6765 Tn7-like MDR region, other regions of plasmid p1.1.2. showed 99.98% similarity and 91% coverage with nucleic acid

sequence of *P.mirabilis* plasmid pPm60 (GenBank accession number MG516911) that also carried the Tn7-like transposon published in NCBI database (Fig. 4).

Horizontal Transfer Of Tn7-like

Double-antibiotics Salmonella Shigella agar plates (300ug / ml rifampicin + 10.24ug / ml flufenicol) were used to screen the transconjugant. The results of drug sensitivity, electrophoresis, and sequencing showed that the plasmid p1.1.2 could be successfully transferred to *E. coli* EC600 (Table 4). A novel Tn7-like (Tn6765) transconjugant was obtained through the conjugative transfer test, and at a frequency of 1.5×10^{-1} transconjugants per donor (average of three independent determinations). The stability of Tn6765 was determined by continuous passage propagation lasting 10 days (20 passages) in the absence of antimicrobial pressure. One hundred clones in the 21st passage were picked to detect the presence of Tn6765. The results of all PCRs were positive. Showed that Tn6765 can be stably inherited in the recipient bacteria.

Table 4
Antimicrobial resistance phenotype and inhibitory zone diameter of *Proteus mirabilis* SCBX1.1 isolate, *Escherichia coli* EC600 and EC600 with plasmid P1.1.2

Antibiotics	Inhibitory zone diameter(mm)			Antimicrobial resistance phenotype		
	SCBX1.1	EC600	EC600 +P1.1.2	SCBX1.1	EC600	EC600 +P1.1.2
CN(10 µg)	9	24	8	R	S	R
S (10 µg)	0	27	0	R	S	R
FFC(30 µg)	0	25	0	R	S	R
CAZ(30 µg)	13	30	11	R	S	R
ENR(5 µg)	0	28	9	R	S	R
FOX(30 µg)	13	30	0	R	S	R
SXT(1.25/23.75 µg)	0	31	0	R	S	R
CIP(5 µg)	11	27	0	R	S	R
AMP (10 µg)	0	22	0	R	S	R
R, resistant; S, susceptible; I, intermediate.						

Discussion

Laboratory experiments have shown that Tn7 and its derivatives are capable of transposition in many different hosts, but few naturally occurring examples have been isolated (7) In this study, we examined the occurrence and prevalence of Tn7-like in *Enterobacteriaceae* isolates obtained from several farms of chicken and swine raised for meat purpose. Of 7 *Enterobacteriaceae* species in the collection, the most prevalent species included *Proteus* (150 isolates, 39.7%) and *Escherichia coli* (128 isolates, 33.9%). One hundred isolates (26.5%) were assigned to other *Enterobacteriaceae* species. Statistical analysis showed that the presence of Tn7-like was different among different bacteria genera ($P < 0.0001$) (Fig. 1). The result indicated *Proteus malleable* genomes contribute to facilitate high-frequency insertion of transposons like Tn7-like, which was similar to the findings of other studies (31, 34, 35). Also, *Escherichia coli* is the representative strain of the genus *Escherichia*, which is the abundant type of bacteria in the intestine of humans and animals (36), so its proportion in Tn7-like positive *Enterobacteriaceae* is also large. Our results provide evidence of the pigs and chickens as a possible reservoir of Tn7-like carrying strains with the antibiotic resistance gene. *Enterobacteriaceae*, as a widespread intestinal bacterium, its carriage Tn7-like are likely to have a serious impact on human life through animals such as pigs and chickens, and is a risk that deserves our attention.

In multidrug-resistant *Enterobacteriaceae* bacteria, Tn7-like transposons play a very vital role due to their high capability for transferring antimicrobial resistance genes (37). Through drug susceptibility tests, we found that the multi-drug resistance of *Enterobacteriaceae* carrying Tn7-like is widespread in various strains. Except for *M.morganii* and *K.pneumoniae*, the multi-drug resistance rates of other strains are above 50%. Among them, *Proteus* (65.69%) and *Salmonella* (63.90%) are more prominent (Table 2). *Proteus* is an important drug-resistant storage bacteria because of its highly efficient genetic structure. It is usually considered commensal in the gut, but it can also cause food poisoning and is most commonly recognized clinically as a cause of urinary tract infections (38, 39). *Salmonella*, one of the most common and widely distributed foodborne pathogenic microorganisms, is a major cause of food-borne salmonellosis (40, 41). And the food-borne salmonellosis is typically acquired through consumption of contaminated poultry meat and eggs (42). Obviously, for both *Proteus* and *Salmonella*, carriage of Tn7-like, each containing a set of resistance genes, may increase the chances of horizontal transfer of multiple resistance determinants to susceptible strains, and may in turn confer unique advantages to the host and enable them survive a strong antimicrobial selection pressure especially in poultry and livestock farm settings.

Tn7-like isolates are particularly resistant to Trimethoprim/Sulfamethoxazole and Streptomycin because of the *intl2*-associated resistance gene cassette (*aadA1*, *sat2* and *dfrA1*) carried by Tn7-like (43, 44). The gene cassettes of *intl2* contained the aminoglycoside adenylyltransferase (*aadA1* and *aadA2*), dihydrofolate reductase (*dfrA1*), and streptothricin acetyltransferase (*sat2*) encoding genes, which are responsible for streptomycin-spectinomycin, trimethoprim, and streptothricin resistance, respectively (45). In this study, we used the PCR method to determine the occurrence of class 2 integrons and associated gene cassettes (*aadA1*, *sat2* and *dfrA1*) in Tn7-like-positive isolates. Similar gene cassettes (*aadA1*, *sat2*, and *dfrA1*) have been obtained in the most of animal-derived *Enterobacteriaceae* isolates (46, 47). The presence of the gene cassette and its corresponding phenotypic resistance was matched (Tables 2 and 3). This explains why Tn7-like bacteria are highly resistant to Trimethoprim/Sulfamethoxazole and Streptomycin, and may pose the risk of widespread dissemination of large, single assemblage repositories of resistance genes and threatening the emergence of a post-antibiotic era (48). The high prevalence of the Tn7-like containing antibiotic resistance genes detected in the current study could be attributed to extensive use of antibiotics for disease prevention, treatment and growth enhancement in farm animals in the studied. Specifically, spectinomycin is widely used against the gonorrhea and respiratory/enteric infections in humans and animals (25). Tn7-like have been so successful at spreading into diverse relevant taxa that they could be used as a proxy for anthropogenic pollution (49, 50).

The *intl2* downstream of Tn7-like transposons mediate the deletion of gene cassettes (*aadA1*, *sat2*, and *dfrA1*). Through sequencing, we found that there were multiple mutated sites in *intl2*, among which 535 base mutations (C mutated to T) were terminating mutations (Fig. 2), which led to premature termination of integrase gene translation, making its gene cassette sequence show a high degree of stability, usually *aadA1*, *sat2* and *dfrA1* (51). Karin Hansson et al. changed the stop codon 179 of the *intl2* gene into the codon encoding glutamic acid (E), and found that *intl2**179E promoted the specific excision of each gene cassettes in Tn7 at different frequencies (22). Some of the gene cassettes deletions investigated in this study are precisely because the 535 base mutation produces a functional integrase gene *intl2* (Fig. 2 and Table 3). We show here experimentally that the second class of integrase and associated gene cassettes in Tn7 can promote recombination between resistant gene present in the host, but only after changing the termination codon to a sense codon (22). This makes Tn7-like a better reservoir of antibiotic resistance.

The structure of the Tn7-like transposon is changeable and interrelated. We have obtained a new transposon Tn6765. By comparison analysis, we know that Tn6765 and Tn6450 have a very high similarity. Both are Tn7-like transposons, which are generated by inserting sequences and transposons carrying flanking drug resistance genes integrated into the Tn7 transposon variable region. Their transposable module was interrupted by the transposon Tn1721 at the transposase gene *tnsD*, and Tn1721 was interrupted by the downstream IS26 (33). The structures of Tn6765 and Tn6450 are complex and highly homologous, suggesting that they may have undergone similar evolutionary processes. Tn6765 carries five IS26, two ISCR1, one IS1353, one IS1006, one ISCR2, one ISAb1, one ISEC59, and 2 defective transposons Tn1721 and Tn21, indicating that this transposon is generated by multiple mobile genetic elements carrying lateral resistance genes integrated into the variable region. There are a few genetic differences between Tn6450 and Tn6765 (Fig. 3). Noteworthy, Tn6450 is located on the chromosome of *Proteus mirabilis* but Tn6765 is located on the plasmid (Fig. 4), which confirmed that Tn7-like can be transmitted alternately on the chromosome and plasmid by cutting and inserting. Through the conjugative transfer test, Tn6765 was successfully transferred to EC600 with the plasmid. 41 Ominously, like its distant relative Tn7, Tn6765 may exhibit the potential to jump bacterial species readily. To make

matters worse, Tn6450 comes from a chicken source (33) and Tn6765 from a pig source, suggesting that Tn7-like can be transmitted from one animal to another with bacterial hosts.

Proteus SCBX1.1 carrying Tn6765 also contains another *cfp* plasmid P1.1.1 (Fig. 6). The *cfp*-containing segment (corresponding to bases 8925 to 12726 to 4624 in GenBank accession number CP047113) harbours a genetic structure (Fig. 5), showing homology to the *cfp* segment characterized in *P. cibarius* G11 (Genebank accession number CP047287), which is partially different from the Chen et al. reported the existence of IS26-*cfp*-Dn554 tnpB-Dn3 family tnpA-IS26 section in another Tn7-like Tn6451 (52) (Fig. 5). Although the *cfp* section of Tn6765 and Tn6451 is slightly different, this also suggests an evolutionary direction of the Tn6765 carrying strain. By comparing the genetic structure of different Tn7-like transposons, we can speculate that the multidrug-resistant Tn7-like transposons have a certain evolutionary relationship, which confirmed that Tn7-like plays a vital role in the field of storing resistance genes.

Unlike the DNA blueprints of higher organisms, *Enterobacteriaceae* bacterial genomes exhibit an extraordinary degree of plasticity and intraspecies diversity (53). A key feature of *Enterobacteriaceae* is the constant mobility of various segments of the genome both within a single cell and between cells. This mobility plays a significant role in the evolution of bacteria through tuning of genetic architecture and the lateral acquisition of new genes that may provide a survival advantage, thus further adapting bacteria to new environments (54). Transposons as the major contributors to the acquisition and dissemination of antibiotic resistance genes (ARGs), and Tn7 are their central agents (55). One Tn7 relative may operate as a “founder element” that can locate and safely transpose into the bacterial chromosome, bringing with it other mobile elements and possibly attachment sites for bacteriophages, integrons, or other transposons. After multiple Tn7-like transposition events accumulate in the attachment site, recombination between these elements may enhance evolution by reassortment (7).

Conclusions

To the best of our knowledge, this study is the first report showing the prevalence and baseline characteristics of Tn7-like in *Enterobacteriaceae* isolates from livestock and poultry in China. Our results showed that Tn7-like transposon has a great effect on bacterial resistance, and they can continuously accumulate ARGs in the evolutionary process to consolidate the bacteria's defense ability, which makes bacterial treatment more difficult. Animals are possible reservoirs and a source for the dissemination of Tn7-like transposon associated ARGs in the environment. Multiple antibiotic resistance gene determinants in Tn7-like, especially when found in producing chickens and swine is highly worrisome and may become a serious threat by spreading associated resistance genes in other nearby animals and the humans connected therewith. Therefore, strict preventive measures should be taken to stop the spread and emergence of mobile genetic resistance determinants in food producing poultry and livestock in China and the world, and our study provides an important reference for this.

Abbreviations

PCR: polymerase chain reaction

MDR: multidrug resistance;

MRE: multidrug-resistant *Enterobacteriaceae*;

ARGs: antibiotic resistance genes;

AMRg: antimicrobial resistance genes ;

AMP: Ampicillin;

IPM: Imipenem;

FOX: Cefoxitin;

CIP: Ciprofloxacin;

S: Streptomycin;

CN: Gentamicin;

FFC: Florfenicol;

ENR: Enrofloxacin;

TGC: Tetracycline;

ATM: Aztreonam;

CAZ: Ceftazidime;

SXT: Trimethoprim/Sulfamethoxazole;

E.coli: Escherichia coli;

M. morganii: Morganella morganii;

K.pneumoniae: Klebsiella pneumoniae;

S.enterica: Salmonella enterica;

A.baumannii: Acinetobacter baumannii

Declarations

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Availability of data and materials

Please contact author for data requests.

Authors' Contributions

All authors listed, have made substantial, direct and intellectual contribution to the work, and approved it for publication. Juan He conceived the study and drafted the manuscript. Juan He and Cui Li collected samples and conducted data statistics. Juan He, Cui Li and Pengfei Cui performed experiments. Hongning Wang supervised the research.

Ethics approval and consent to participate

Not applicable in view of the nature of the study.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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Figures

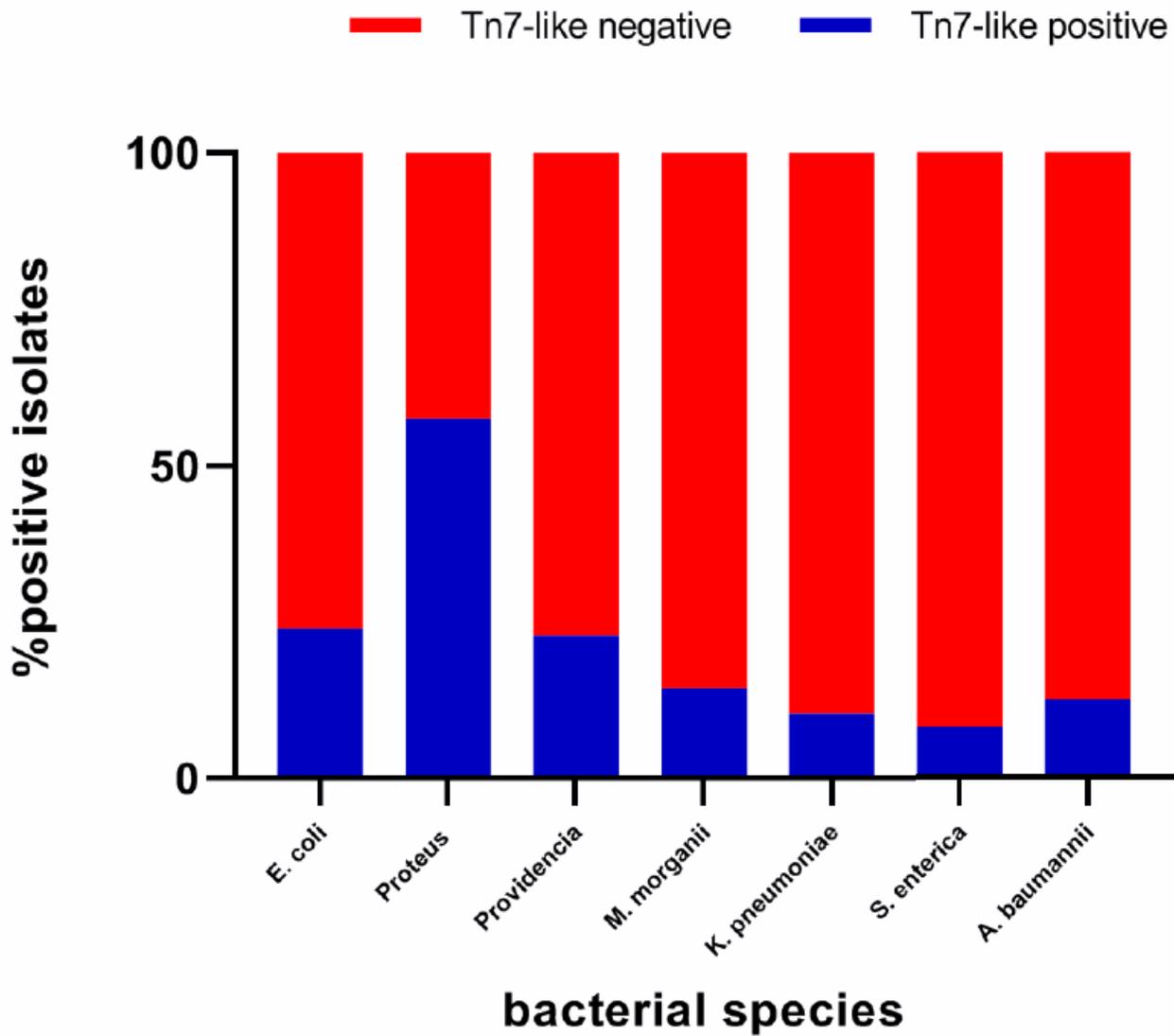


Figure 1

The separation rate of Tn7-like in Enterobacteriaceae of different genera (n= 1482). Tn7-like positive; % of Enterobacteriaceae isolates, which are positive for tnsA gene via PCR, Tn7-like negative; % of isolates negative for tnsA genes via PCR. Comparison of the incidence of Tn7-like transposons in Enterobacteriaceae isolates versus Tn7-like lacking isolates was assessed using the Chi square test. The results are significant. $p < 0.0001 < 0.05$.

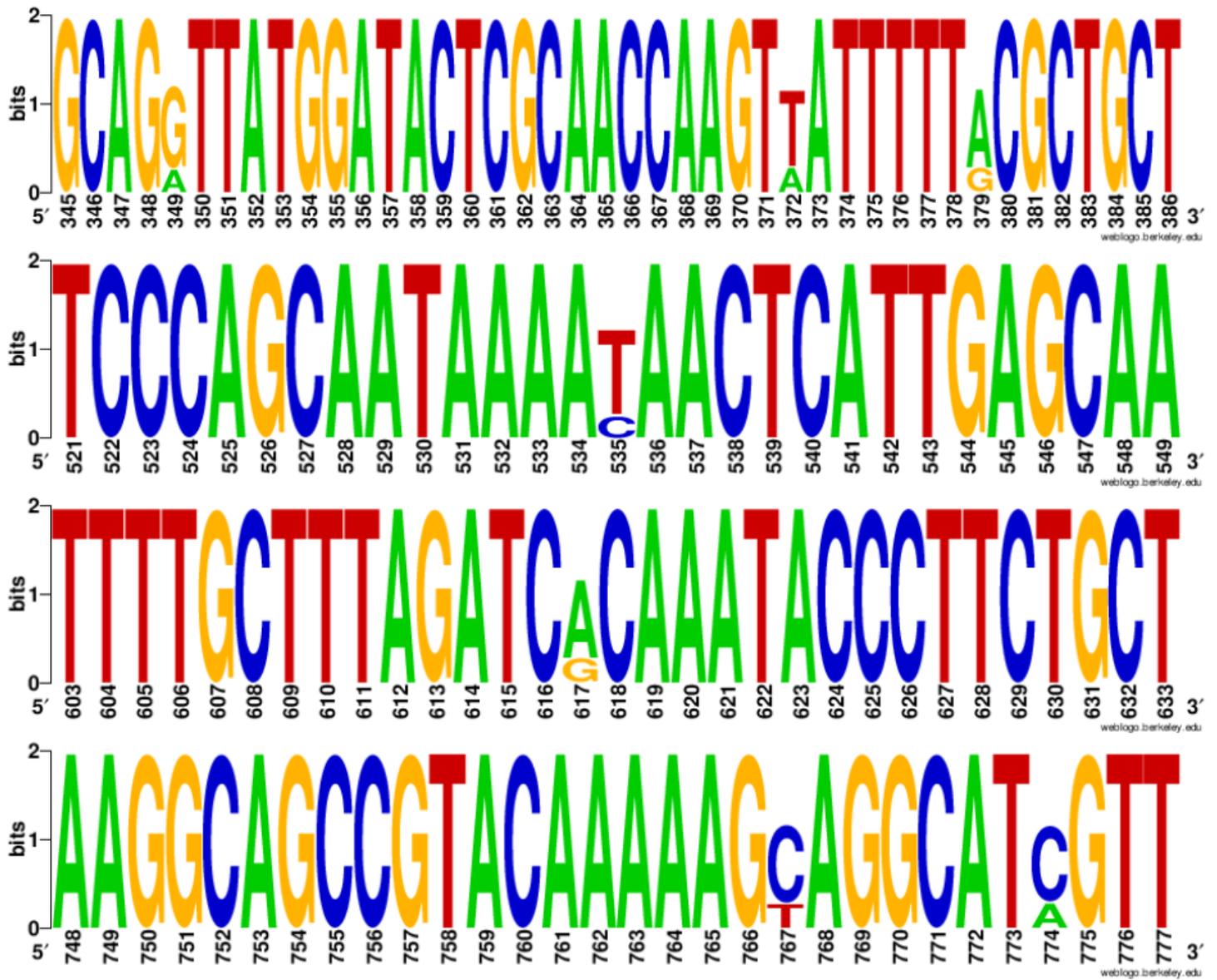


Figure 2

The Weblogo of repeats of int12. The sequence were each mutation site of the integrase gene of class 2 integrons.

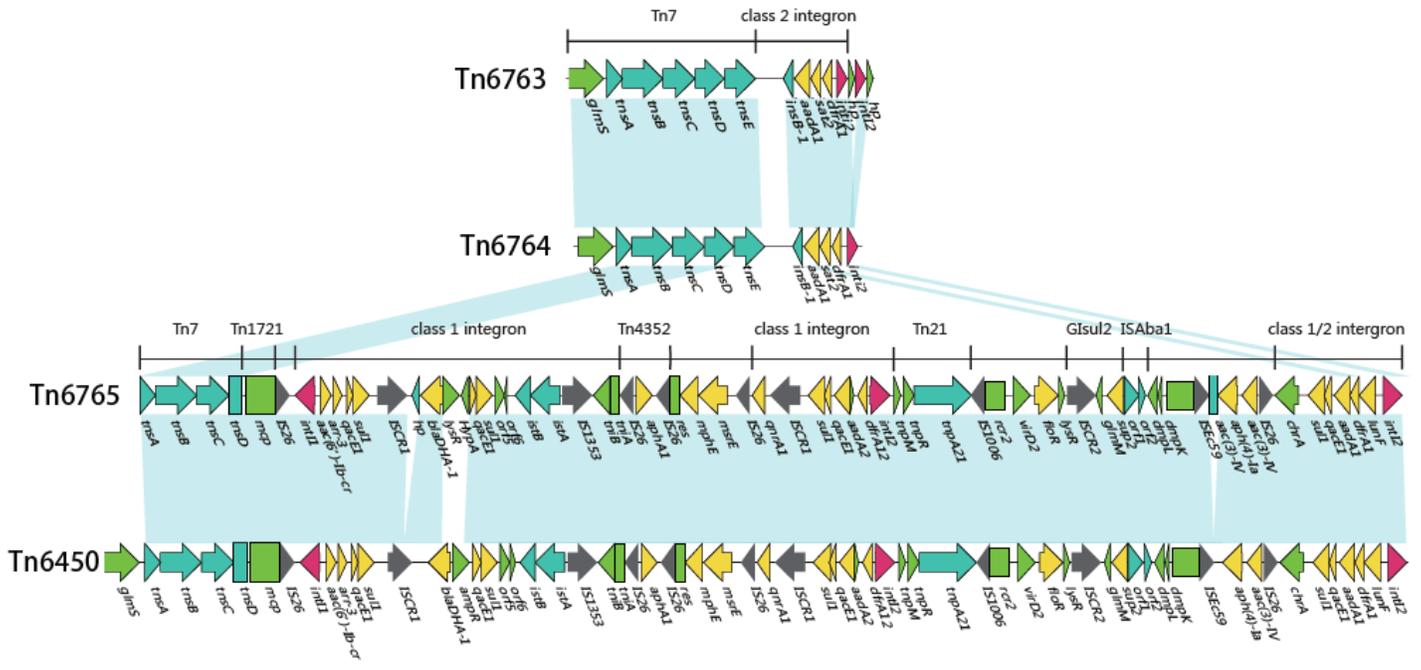


Figure 3

Genetic structure of Tn6763, Tn6764 and Tn6765. The physical maps were generated using Easyfig 2.2.3 and DNAMAN Version 8.0. Linear comparison of Tn6765 region in p1.1.2 with Tn6763 region in *P. mirabilis* strain, Tn6764 region in *E. coli* strain and Tn6450 region in *P. mirabilis* strain SNYG17 (GenBank accession number MF805806). Genes and ORFs are shown as arrows, and their orientations of transcription are indicated by the arrowheads. Horizontal lines, different regions corresponding to Tn7, Tn1721, Tn21, and integrons. Antimicrobial resistance genes are in yellow. transposase are in blue and integrase genes are in red. The IS elements are indicated by gray arrows. Other functions or putative proteins are in green. Shared regions with 99% identity are indicated by shading.

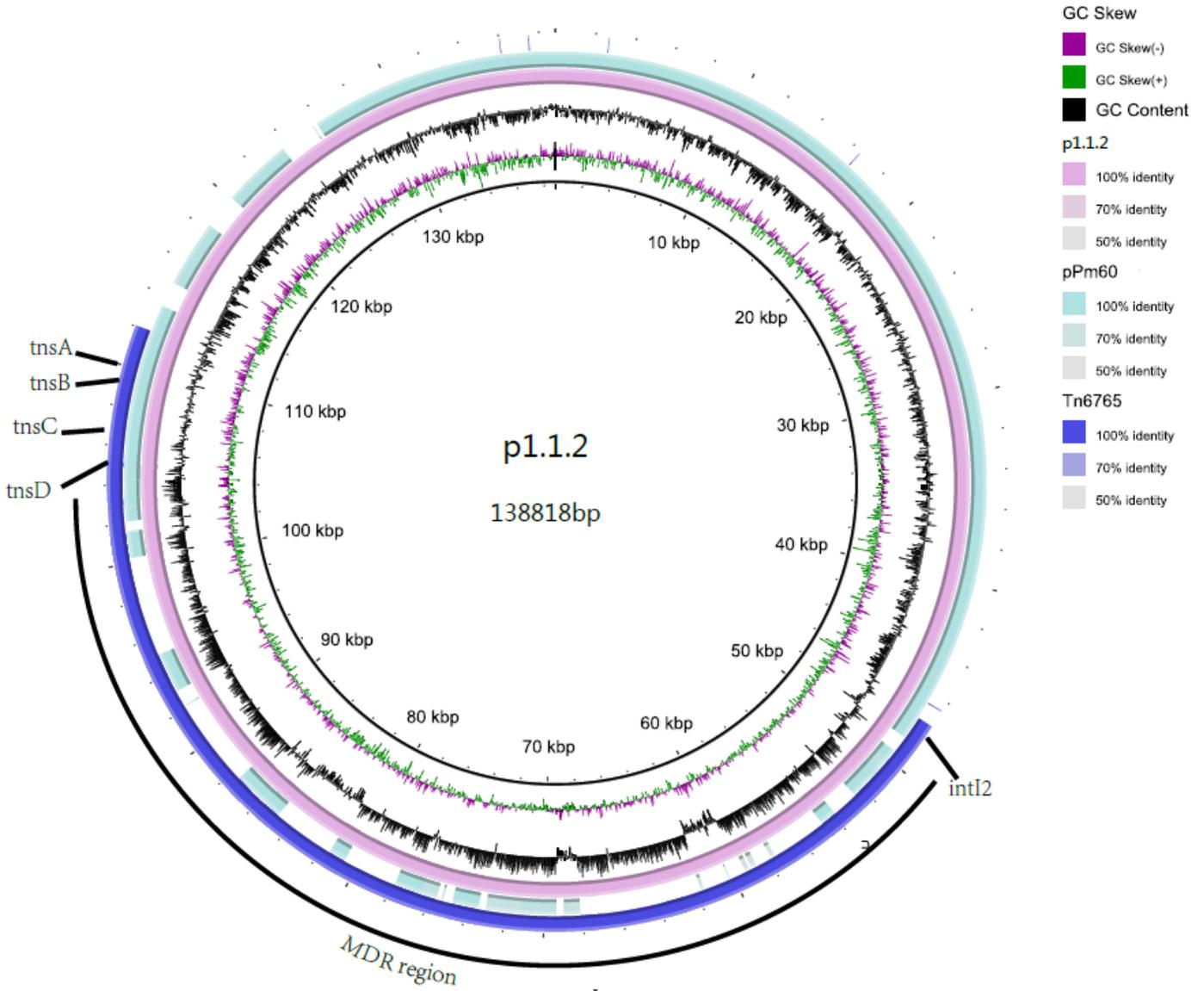


Figure 4

Circular representation of plasmid p1.1.2. The physical map of p1.1.2 was generated using BRIG v0.95.

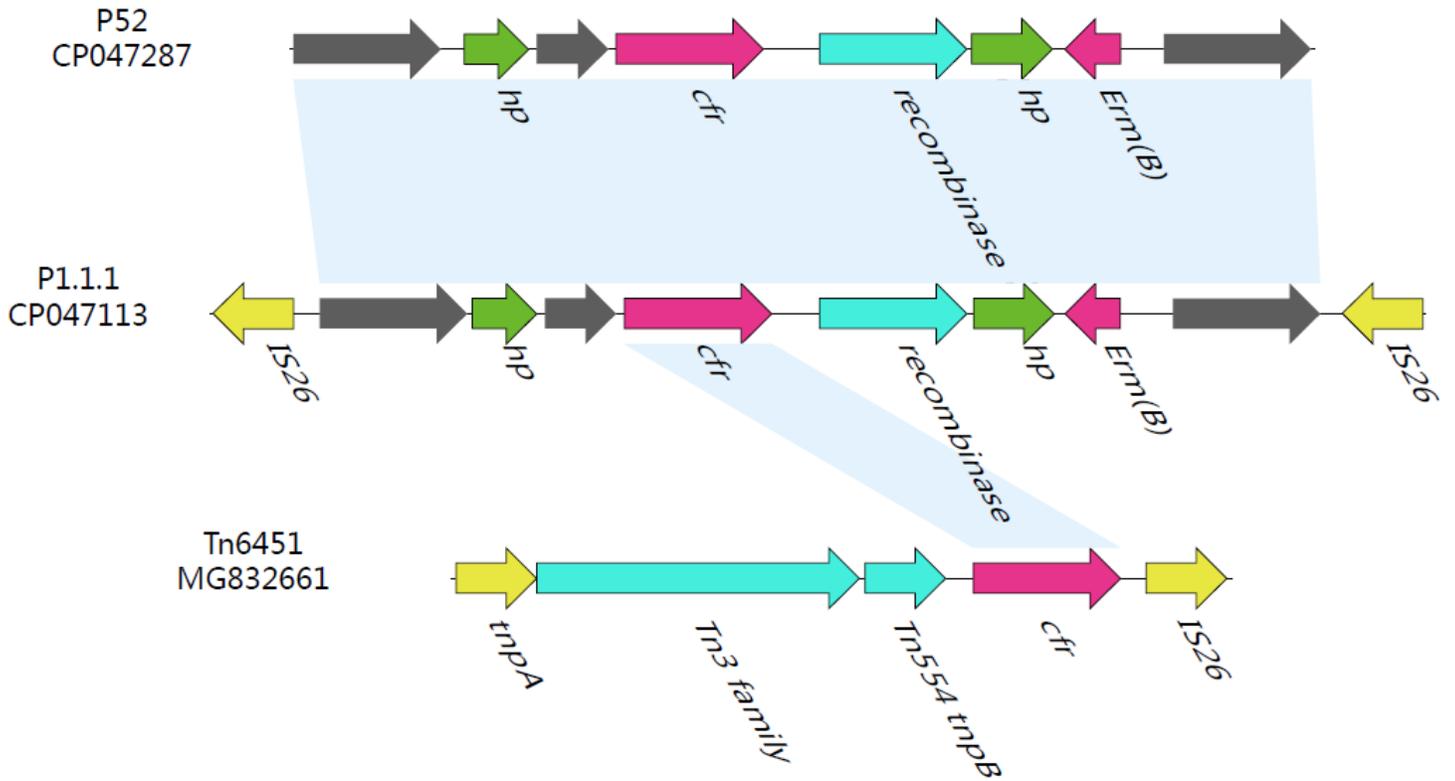


Figure 5

Genetic structure of *cfr*-containing region and in p1.1.1. The physical maps were generated using Easyfig 2.2.3 and DNAMAN Version 8.0. Linear comparison of the *cfr*-containing region in p1.1.1 with the *cfr*-containing region in *P. cibarius* strain G11 (Genebank number CP047287) and in *M. morganii* strain BCMM24 (Genebank number MG832661). Genes and ORFs are shown as arrows, and their orientations of transcription are indicated by the arrowheads. Antimicrobial resistance genes are in red. The IS elements are indicated by yellow arrows. Putative proteins are in green. Shared regions with 99% identity are indicated by shading.

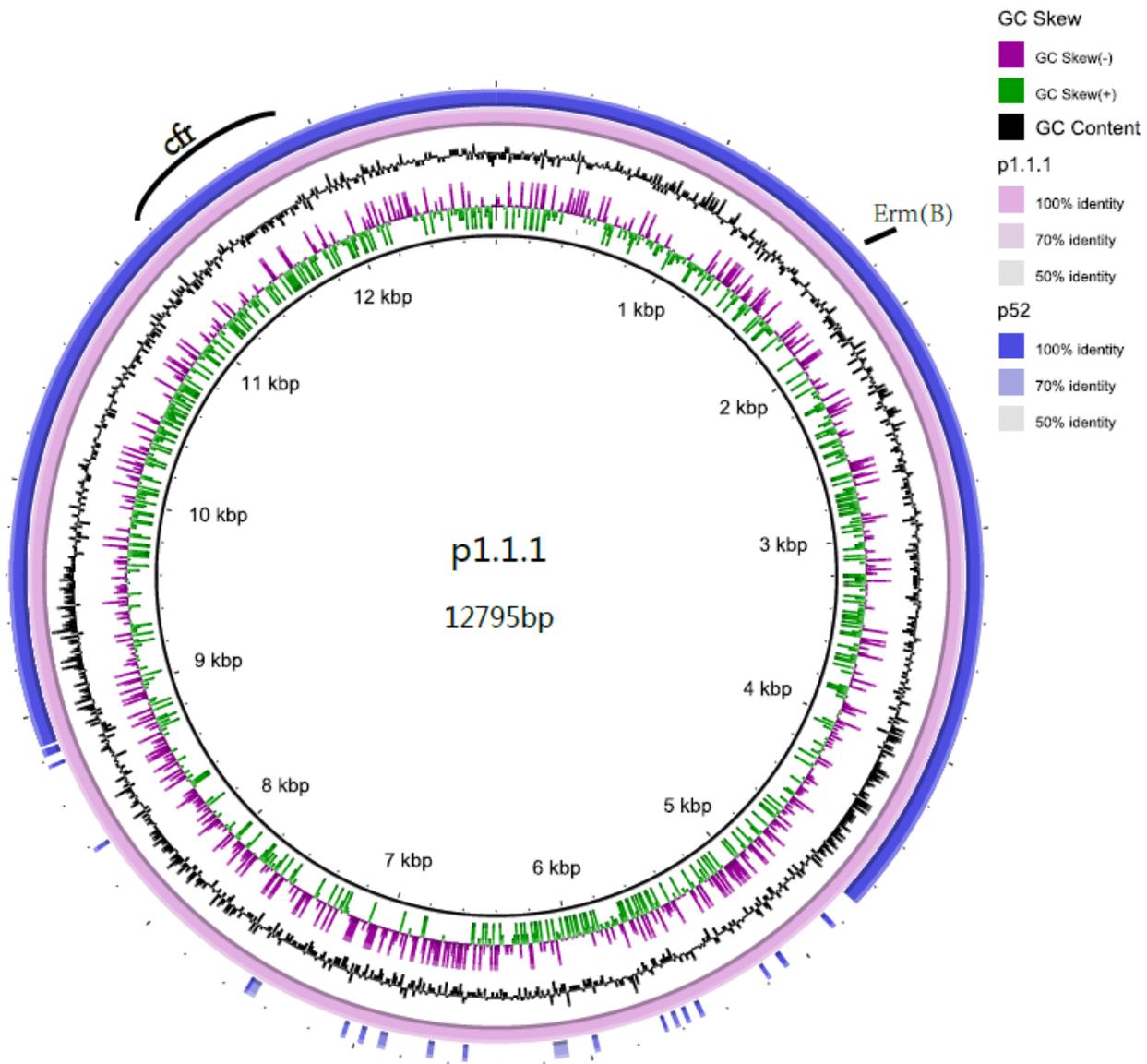


Figure 6

Circular representation of plasmid p1.1.1. The physical map of p1.1.1 was generated using BRIG v0.95.

Supplementary Files

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