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SHORT REPORT

Genomic Analysis of an *Escherichia coli* Sequence Type 167 Isolate Harboring a Multidrug-Resistant Conjugative Plasmid, Suggesting the Potential Transmission of the Type Strains from Animals to Humans

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Purpose: The *E. coli* ST167 clone is the globally dominant ST among extraintestinal pathogenic *E. coli* (ExPEC) and is frequently associated with carbapenem resistance. This study reports genomic characterization of a pandrug-resistant *E. coli* ST167 isolate (ECO3183) and the possibility of the type strains' transmission.

Materials and Methods: Antibiotic susceptibility testing was performed using disk diffusion and the VITEK 2 automated system. The *E. coli* ECO3183 genome was sequenced. We used the genome to analyze the phylogenetic relationship, phylogenetic group, sequence type (ST), acquired antibiotic resistance genes (ARGs), IS elements, genomics islands, the replicon type and transferability of the plasmids. The conjugative transfer of plasmids was assessed using filter mating experiments.

Results: ECO3183 contained a 4.87-Mb chromosome and two plasmids [pECO3183-1 (167.63 Kb) and pECO3183-2 (46.16 Kb)]. It belonged to phylogenetic group A, clonal complex 10 (CC10), and ST167. ECO3183 is a pandrug-resistant strain nonsusceptible to 24 tested antimicrobials representing 8 different antimicrobial classes. Among 55 *E. coli* isolates phylogenetically related to ECO3183, 47% (26/55) were from humans, while 35% (19/55) were from animals. Further analysis revealed that among 1140 ST167 isolates (in the EnteroBase database), 4% (47/1140) originated from environments, 17% (192/1140) were isolated from humans, and 78% (890/1140) were obtained from animals. The pECO3183-1 contained two identical repeats of a 9633 bp region (IS6100-*sul1*-Δ*aadA16*-*dfrA27*-*arr-3*-*aac(6')-Ib-cr*-IS26) and a 17.88-kb resistance island (*sul2*-*aph(3")-Ib*-*aph(6)-Id*-IS26-Δ*aph(3')-Ia*-IS26-*tet*(A)-Δ*floR*-ΔISVsa3-IS26-Δ*aac(3)-IId*-IS26-*mph*(A)), and these three regions contained most of ECO3183 carrying ARGs. It was identified as a conjugative plasmid, which confers MDR resistance and has the potential to spread.

Conclusion: ECO3183 exhibited pandrug-resistance phenotype that was mediated by pECO3183-1 carrying MDR ARGs and pECO3183-2 carrying bla_{NDM-5}. Source analysis of strains indicated that ST167 *E. coli* might be transmitted between species from animals to humans, which needs continued monitoring.

Keywords: *Escherichia coli*, ST167, bla_{NDM-5}, phylogenetic analysis, MDR conjugative plasmids

Introduction

Infections caused by *Enterobacterales*, including *Escherichia coli, Klebsiella pneumoniae*, and *Proteus mirabilis*, are associated with a variety of clinical illnesses, such as urinary tract infections, septicemia, pneumonia, intra-abdominal infections, and meningitis.[1] Multidrug-resistant (MDR) and carbapenem-resistant *Enterobacterales* are not only resistant to a majority of commonly used antibiotics, but they are also known to rapidly spread from person to person, making them harder

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to control.[1] The emergence of multidrug-resistant (MDR) and carbapenem-resistant *Enterobacterales* poses a serious challenge for clinical treatment.

E. coli, a predominant member of *Enterobacterales*, is frequently encountered and isolated in clinical laboratories. Based on the data obtained from the China Antimicrobial Resistance Surveillance System (http://www.carss.cn/), *E. coli* exhibited an average isolation rate of 20.6% in recent five years (2017–2021), with 1.6% of the strains being carbapenem-resistant. In recent years, the *E. coli* sequence type (ST) 167 clone, as the globally dominant ST among extraintestinal pathogenic *E. coli* (ExPEC), was frequently reported for its association with carbapenem resistance.[2] It has been documented that carbapenem-resistant *E. coli* ST167 strains harboring $bla_{\text{NDM-5}}$ are capable of infecting both humans and animals.[3] However, current knowledge regarding the transmission risk of *E. coli* ST167 strains between animals and humans remains limited. A total of four ST167 isolates exhibited MDR phenotypes among 411 *E. coli* isolates in our previous study.[4] In this study, we sequenced the entire genome of a pandrug-resistant *E. coli* ST167 isolate (ECO3183), analyzed antibiotic resistance genes (ARGs) conferring antibiotic resistance, and explored its potential dissemination risk.

Materials and Methods

Bacterial Isolate

Similar to our previous study,[4] the *E. coli* strain ECO3183 was isolated from a urine sample of a urinary tract-infected patient in the Second Hospital of Tianjin Medical University (Tianjin, China). The strain was identified using the VITEK MS system (bioMérieux, Marcy l'Etoile, France).

Antimicrobial Susceptibility Testing (AST)

We used a Gram-negative antimicrobial susceptibility testing card (AST-GN13 and AST-GN334) on the VITEK 2 system to perform the susceptibility tests. The Kirby-Bauer disc diffusion assay (K-B) was additionally employed to detect kanamycin, chloramphenicol, polymyxin B, and tetracycline. *E. coli* ATCC 25922 was used as a control strain. Interpretation of the results was performed using the guidelines from the Clinical & Laboratory Standards Institute (CLSI, 2022)[5] or the European Committee on Antimicrobial Susceptibility Testing (EUCAST) breakpoints (2022, https://www.eucast.org/).

Whole Genome Sequencing and Analysis

The whole-genome sequence of ECO3183 was sequenced using Pacific Biosciences (PacBio) Sequel sequencing platform and high-throughput Illumina sequencing platform at the Beijing Novogene Bioinformatics Technology Co., Ltd. Sequence reads were generated from 10 kb SMRT Bell library and 350 bp library. The SMRT Link v.5.0.1 program (Pacific Biosciences of California, Inc., Menlo Park, CA, USA) was used for preliminary genome assembly, Arrow in the SMRT analysis software suite (https://www.pacb.com/support/software-downloads/) was used to align the initial assembly results, and complete circular contigs without any gaps were generated. The assembled genome was submitted to ClermonTyping[6] (http://clermontyping.iame-research.center/), the *Escherichia* typing database (https://pubmlst.org/bigsdb?db=pubmlst_escherichia_seqdef), ResFinder 4.1[7–9] (https://cge.food.dtu.dk/services/ResFinder/), ISfinder[10] (https://isfinder.biotoul.fr/) to identify phylogenetic group, sequence type (ST), acquired antibiotic resistance genes (ARGs), and IS elements, respectively. Core genome multilocus sequence typing (cgMLST) approaches with a 100 threshold for phylogenetic analysis were used to investigate the phylogenetic relationship between ECO3183 and other *E. coli* isolates currently available on the BacWGSTdb server[11] (http://bacdb.cn/BacWGSTdb/). The replicon type and transferability of the plasmids were predicted using PlasmidFinder 2.1[9,12] (https://cge.food.dtu.dk/services/PlasmidFinder/) and OriTfinder[13] (https://tool-mml.sjtu.edu.cn/oriTfinder/oriTfinder.html), respectively. Genomics islands were predicted by the IslandPath-DIOMB method implemented in IslandViewer 4[14] (https://www.pathogenomics.sfu.ca/islandviewer/).

Conjugation

ECO3183 was subjected to conjugation via filter mating with the sodium azide-resistant *E. coli* J53 as the recipient, following the procedure described previously.[15] Transconjugants were selected on LB agar supplemented with 100 mg/L

sodium azide and 50 mg/L tetracycline. Plasmid DNA was purified from the *E. coli* J53 transconjugant using the EZNA® BAC/PAC DNA kit (Omega Bio-Tek Inc., Norcross, GA, USA). PCR was performed to detect the presence of pECO3183-1 among these transconjugants using two sets of primers specific to pECO3183-1 (Table S1). For all reactions, the reaction mixture (25 μL) contained 1 μL of plasmid DNA, 1 μL each of primer, 9.5 μL of ddH_2O and 12.5 μL of 2 × Taq PCR Master Mix (Sangon Biotech Co., Ltd., Shanghai, China) under the following conditions: initial denaturation at 94°C for 4 min, 35 cycles of incubation at 94°C for 30s, 51°C for 30s and 72°C for 18s, and a final extension at 72°C for 5 min. The following primer pairs were used to generate PCR products of different length: aph(6)-Id_F/aph(6)-Id_R (238 bp) and aph(3″)-Ib_F/aph(3″)-Ib_R (207 bp).

Results and Discussion

E. coli strain ECO3183 is a pandrug-resistant strain nonsusceptible to 24 tested antimicrobials. The 24 antimicrobials tested represent a total of 8 different antimicrobial classes, including gentamicin, kanamycin, tobramycin, amoxicillin/clavulanate, ampicillin, ampicillin/sulbactam, ceftriaxone, cefazolin, cefepime, cefoperazone/sulbactam, cefotetan, cefoxitin, ceftazidime, cefuroxime, ertapenem, imipenem, piperacillin/tazobactam, chloramphenicol, ciprofloxacin, levofloxacin, polymyxin B, nitrofurantoin, trimethoprim/sulfamethoxazole, and tetracycline. The minimum inhibitory concentration (MIC) of the antibiotics tested and associated ARGs are presented in Table 1. The phylogenetic group was determined by querying ClermonTyping with the genome sequence, while the clonal complex and ST were concurrently determined by querying the *Escherichia* typing database with the same genome sequence. ECO3183 belonged to phylogenetic group A, clonal complex 10 (CC10) and ST167, which has been reported to be the 14th of top 20 ExPEC STs.[16] ECO3183 contained a 5.08-Mb genome, including a 4.87-Mb chromosome and two plasmids (pECO3183-1 and pECO3183-2). We analyzed the phylogenetic relationship between ECO3183 and other *E. coli* isolates currently deposited in the BacWGSTdb server (24 April 2023; Figure 1). A total of 55 phylogenetically related strains (with 24–100 different cgMLST alleles) were identified in the database (Table S2), all belonging to ST167, with 69% (38/55) from China, 47% (26/55) from humans, and 35% (19/55) from animals. This data indicates the potential transmission of these 55 strains between animals and humans. More specifically, the closest relatives of ECO3183 (only 24 different cgMLST alleles) were another three ST167 *E. coli* strains carrying $bla_{\text{NDM-9}}$ (490, 494, 707; Accession No. SAMN05928947, SAMN05928958, SAMN05928933), which were isolated from flies in a chicken farm in Shandong, China.[17] The published study linked flies to the widespread dissemination of bla_{NDM}.[17] Similarly, a recent study also reported the clustering of six $bla_{\text{NDM-5}}$-positive ST167 *E. coli* isolates (ECO167624, LR880734.1, 562.30390, 562.50775, 562.50948, and 562.50949) from both human and animal sources within a clade on the phylogenetic tree.[18] The upper and lower right corners of the phylogenetic tree (Figure 1) showed that animal-origin ST167 *E. coli* isolates (upper cluster: 490, 494, 707, 598, 601; lower cluster: ECOL_18, LJ037ch) cluster closely with human-origin isolates (upper cluster: E4903, ECO3183, ECM_49; lower cluster: L665, L610, L612, FDAARGOS_434), suggesting a potential transmission between animals and humans. To further explore the possibility of the type strains' transmission between animals and humans within a larger cohort of ST167 isolates, we conducted an analysis of the isolation sources for all 1480 ST167 *E. coli* isolates found in the EnteroBase database (https://enterobase.warwick.ac.uk/; 7 May 2023; Table S3). Out of the total of 1480 isolates, 1140 strains had well-documented isolation sources. Among these 1140 ST167 isolates, 4% (47/1140) were from environments, 17% (192/1140) from humans, and 78% (890/1140) from animals. The data also suggested that ST167 *E. coli* strains might be transmitted between species from animals to humans.

Two plasmids pECO3183-1 (167,630 bp) and pECO3183-2 (46,155 bp) belonged to different incompatibility groups IncC and IncX3, respectively. The pECO3183-1 was most similar to another four multidrug resistance plasmids (≥ 99.9% identity and ≥ 84% coverage) available on the NCBI nucleotide collection (nr/nt) database (24 April 2023) (Figure 2). Two plasmids pECY53 (GenBank accession no. KT997783.1) and pUnnamed3 (CP083494.1) from *E. coli* clinical isolates contained the same ARGs (*aph(6)-Id, aph(3")-Ib, aac(3)-IId, aph(3')-Ia, floR, sul2*, and *tet*(A)), while the other two plasmids pJNE2-NDM (CP096170.1) and pJNE10-2-NDM (CP096164.1) from environmental isolates of *S. putrefaciens* also contained the same ARGs (*aph(6)-Id, aph(3")-Ib, aac(3)-IId, aph(3')-Ia,*$bla_{\text{NDM-1}}$, *mph(A), sul1, sul2, arr-3, aadA5*, and *dfrA17*).[22] Interestingly, the plasmid pECO3183-1 contained two identical repeats of a 9633 bp region (IS6100-*sul1-ΔaadA16-dfrA27-arr-3-aac(6')-Ib-cr*-IS26) and a 17.88-kb resistance island (*sul2-aph(3")-Ib-aph(6)-Id*-IS26-*Δaph(3')-Ia*-IS26-*tet*(A)-*ΔfloR*-ΔISVsa3-IS26-*Δaac(3)-IId*-IS26-*mph*(A)), and the three regions contributed

Table 1 Antimicrobial Susceptibility Patterns and Resistance Genes of ECO3183

Class and Antimicrobial(s)[a]	MIC(s) (μg/mL) or Inhibition Zone Diameter (mm)	Interpretation[b]	Associated Resistance Gene(s)[c]
Aminoglycoside			
Amikacin	≤ 2	S	*aac(6')-Ib-cr*
Gentamicin	≥ 16	R	*aac(3)-IId* mutation
Kanamycin	6	R	*aph(3')-Ia* mutation
Tobramycin	8	I	*aac(6')-Ib-cr, aac(3)-IId* mutation
Beta-lactam			
Amoxicillin/clavulanate	≥ 32/16	R	bla_{NDM-5}
Ampicillin	≥ 32	R	bla_{NDM-5}
Ampicillin/sulbactam	≥ 32/16	R	bla_{NDM-5}
Aztreonam	2	S	None
Ceftriaxone	≥ 64	R	bla_{NDM-5}
Cefazolin	≥ 32	R	bla_{NDM-5}
Cefepime	16	R	bla_{NDM-5}
Cefoperazone/sulbactam	≥ 64/32	R	bla_{NDM-5}
Cefotetan	≥ 64	R	bla_{NDM-5}
Cefoxitin	≥ 64	R	bla_{NDM-5}
Ceftazidime	≥ 64	R	bla_{NDM-5}
Cefuroxime	≥ 64	R	bla_{NDM-5}
Ertapenem	≥ 8	R	bla_{NDM-5}
Imipenem	8	R	bla_{NDM-5}
Piperacillin/tazobactam	≥ 128/4	R	bla_{NDM-5}
Chloramphenicol			
Chloramphenicol	6	R	*floR* mutation
Fluoroquinolone			
Ciprofloxacin	≥ 4	R	*aac(6')-Ib-cr*
Levofloxacin	≥ 8	R	Unknown
Lipopeptides			
Polymyxin B	4	R	Unknown
Nitrofuran			
Nitrofurantoin	64	I	Unknown
Sulfonamide			
Trimethoprim /sulfamethoxazole	≥ 16/304	R	*sul1, sul2, dfrA27*
Tetracycline			
Tetracycline	7	R	*tet*(A)
Tigecycline	≤ 0.5	S	None

Notes: [a]Kanamycin, chloramphenicol, polymyxin B and tetracycline (underlined) were tested by the K-B method; Other antimicrobials were tested using AST-GN13 and AST-GN334 cards on the VITEK 2 system. [b]Interpretations were based on CLSI guidelines, except for cefoperazone/sulbactam interpreted using cefoperazone CLSI breakpoints[19,20] and tigecycline were interpreted by EUCAST breakpoints. [c]Identification of the acquired ARGs and predictions of phenotypes were performed by ResFinder 4.1 (https://cge.food.dtu.dk/services/ResFinder/). The cellular location for bla_{NDM-5} was plasmid pECO3183-2; the cellular location for another 13 genes was plasmid pECO3183-2; among these 13 genes, *aph(6)-Id, aph(3'')-Ib, aadA16* mutation (*ΔaadA16), mph(A)*, and *arr-3* were not shown in the table. Mutations (Δ) reached the following conditions: > 98% sequence identity and > 99.5% alignment coverage. Although *aac(6')-Ib-cr* could be responsible for the amikacin resistance,[21] four experiments showed that *E. coli* strain ECO3183 carrying *aac(6')-Ib-cr* was susceptible to amikacin. This is possibly related to the expression level of the *aac(6')-Ib-cr* gene in ECO3183. In addition, *gyrA* (p.S83L) mutation located on the chromosome was predicted to be responsible for the ciprofloxacin resistance (data not shown).

most of the ARGs (Figure 2). IS26, IS6100, and ΔISVsa3 were interspersed between these ARGs, highlighting their possible role in the dissemination of these MDR ARGs. The plasmid pECO3183-1 was defined as a putative conjugative plasmid because it carried the oriTfinder-predicted oriT region and genes encoding relaxase, T4CP, and T4SS. Filter mating experiments further showed that *E. coli* J53 transconjugant exhibited a multidrug resistance profile (Table S4). PCR amplification of fragments from the *aph(3'')-Ib* and *aph(6)-Id* genes of pECO3183-1 confirmed that the antibiotic

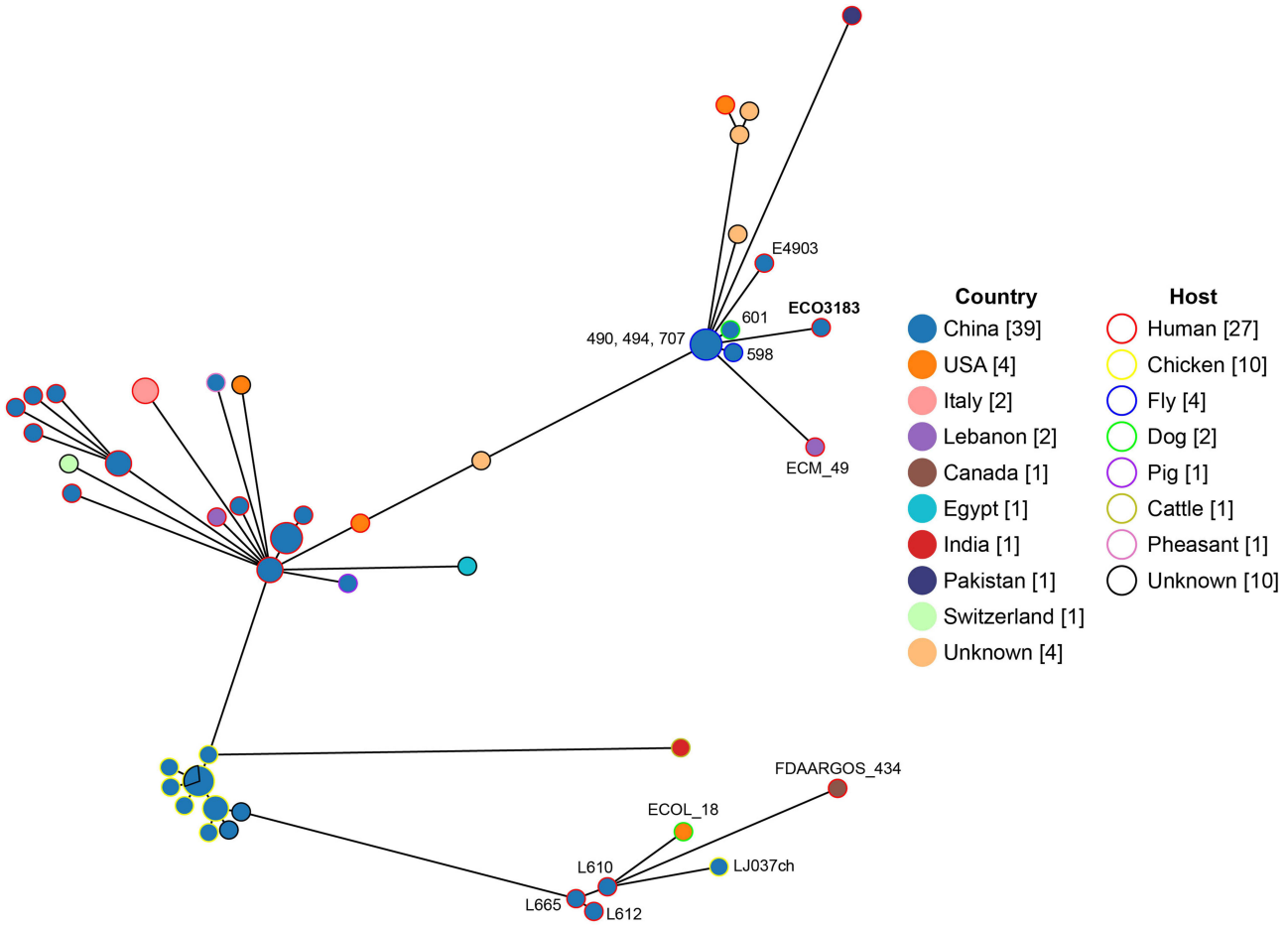

Figure 1 Phylogenetic tree of ECO3183 and other *E. coli* isolates retrieved from the BacWGSTdb server (http://bacdb.cn/BacWGSTdb/). The line length connecting each circle depicts clonal relationships between different isolates. The fill and stroke color of the circle denotes a specific country and host, respectively. The numbers in square brackets are the number of corresponding isolates.

resistance dissemination transfer was mediated by pECO3183-1 (Figure S1). The literature also suggests that the large and broad host range of IncC plasmids is a significant contributor to the spread of ARGs.[23] In addition, the plasmid pECO3183-2 sequence was BLASTed against the NCBI nucleotide collection (nr/nt) database using blastn via the NCBI BLAST web interface (https://blast.ncbi.nlm.nih.gov/Blast.cgi) with default parameter values (12 July 2023). It displayed a high similarity (≥ 99% identity and coverage) to 200 plasmids (containing pECO3183-2). Out of these 200 plasmids, 122 are labeled with the abbreviation "NDM" in their names, indicating their possession of the bla_{NDM} gene (data not shown). Due to pECO3183-2 belonging to the IncX3 incompatibility group, most of the 122 highly homologous plasmids to pECO3183-2 also belong to IncX3. Consistent with our analysis findings, multiple reports in the literature have documented the dissemination of bla_{NDM} via the IncX3 plasmids.[24–26]

In conclusion, *E. coli* strain ECO3183 is a pandrug-resistant strain nonsusceptible to 24 tested antimicrobials representing 8 different antimicrobial classes. Its pandrug-resistance phenotype was mediated by pECO3183-1 carrying MDR ARGs and pECO3183-2 carrying bla_{NDM-5} (Table 1). The plasmid pECO3183-1 contained two identical repeats of a 9633 bp region and a 17.88-kb resistance island, and the three regions contributed most of the ARGs. Source analysis of strains indicated that ST167 *E. coli* might be transmitted between species from animals to humans, which needs continued monitoring.

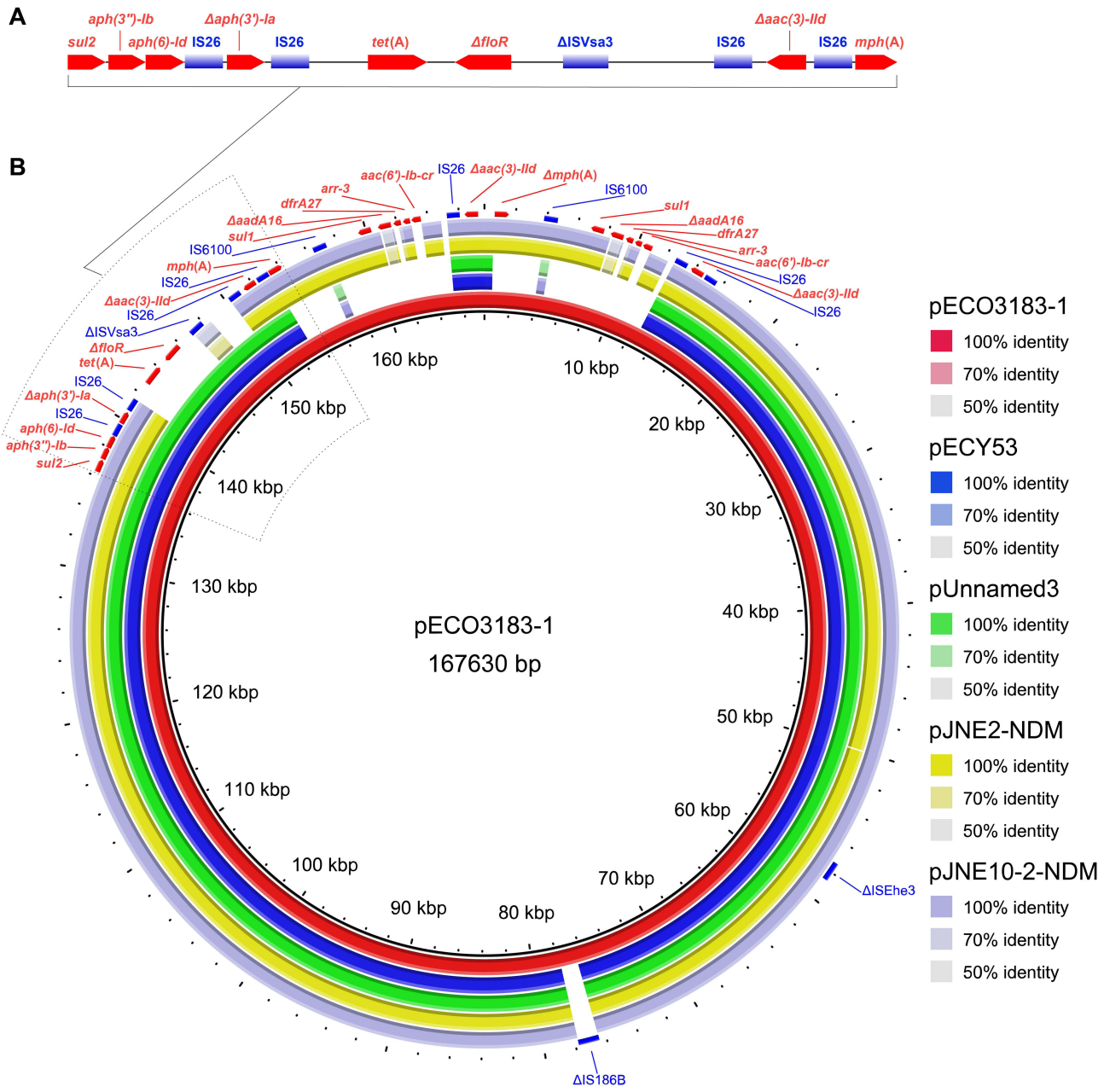

Figure 2 (**A**) A 17.88-kb resistance island carrying 8 ARGs and 5 IS elements was predicted using the IslandPath-DIOMB method. (**B**) BRIG analysis of the conjugative multidrug resistance plasmid pECO3183-1. Comparative analysis of pECO3183-1 with four closely related plasmids was performed using the BLAST Ring Image Generator (BRIG).[27] The concentric rings display similarity between the pECO3183-1 sequence in the inner ring and the other sequences in the outer rings. The various color levels indicate a BLAST result with a matched degree of shared regions, as shown to the right of the ring. The predicted ARGs are shown in red, and the IS elements are shown in blue.

Abbreviation: IS, insertion sequence.

Ethics Statement

The ECO3183 strain was generated as part of routine clinical laboratory procedures. This study complied with the Declaration of Helsinki and the Ethics Committee of the Second Hospital of Tianjin Medical University exempted this study from review because it focused only on bacteria.

Nucleotide Sequence Accession Numbers

The ECO3183 chromosome, pECO3183-1, and pECO3183-2 plasmid sequences were deposited under GenBank accession numbers CP104721, CP104722, and CP104723, respectively.

Author Contributions

All authors made a significant contribution to the work reported, whether that is in the conception, study design, execution, acquisition of data, analysis and interpretation, or in all these areas; took part in drafting, revising or critically reviewing the article; gave final approval of the version to be published; have agreed on the journal to which the article has been submitted; and agree to be accountable for all aspects of the work.

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Disclosure

The authors report no conflicts of interest in this work.

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