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ORIGINAL RESEARCH

Whole-Genome Sequencing of an *Escherichia coli* ST69 Strain Harboring *bla*$_{CTX-M-27}$ on a Hybrid Plasmid

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Objective: *Escherichia coli* is a common Gram-negative human pathogen. The emergence of *E. coli* with multiple-antibiotic-resistant phenotypes has become a serious health concern. This study reports the whole-genome sequences of third-generation cephalosporin-resistant (3GC-R) and multidrug-resistant (MDR) *E. coli* EC6868 and explores the acquired antibiotic-resistance genes (ARGs) as well as their genetic contexts.

Methods: *E. coli* EC6868 was isolated from a vaginal secretion sample of a pregnant patient in China. The antimicrobial susceptibility was assessed, and whole-genome sequencing was conducted. The acquired ARGs, insertion sequence (IS) elements, and integrons within the genome of *E. coli* EC6868 were identified, and the genetic contexts associated with the ARGs were analyzed systematically.

Results: *E. coli* EC6868 was determined to belong to ST69 and harbored a 144.9-kb IncF plasmid (pEC6868-1) with three replicons (Col156, IncFIB$_{AP001918}$, and IncFII). The ESBL gene *bla*$_{CTX-M-27}$ was located on the structure "ΔIS*Ecp1*-*bla*$_{CTX-M-27}$-IS*903B*", which was widely present in the species of Enterobacteriales. Other ARGs carried by plasmid pEC6868-1 were mainly located on the 18.9-kb IS*26*-composite transposon (five copies of intact IS*26* and one copy of truncated IS*26*) composing of IS*26*-*mphA*-*mrx(A)*-*mphR(A)*-IS*6100*, ΔTn*As3*-*eamA*-*tet(A)*-*tetR(A)*-*aph(6)-Id*-*aph(3")-Ib*-*sul2*-IS*26*, and a class 1 integron, which was widely present on IncF plasmids of *E. coli*, mainly distributed in ST131, ST38, and ST405. Notably, pEC6868 in our study was the first report on a plasmid harboring the 18.9-kb structure in *E. coli* ST69 in China.

Conclusion: The 3GC-R *E. coli* ST69 strain with an MDR IncF plasmid carrying *bla*$_{CTX-M-27}$ and other ARGs, conferring resistance to aminoglycosides, macrolides, sulfonamides, tetracycline, and trimethoprim, was identified in a hospital in China. Mobile genetic elements including IS*Ecp1*, IS*903B*, IS*26*, Tn*3*, IS*6100* and class 1 integron were found within the MDR region, which could play important roles in the global dissemination of these resistance genes.

Keywords: *Escherichia coli*, vaginal secretion, plasmid, *bla*$_{CTX-M-27}$, genetic context

Introduction

Both Gram-negative and Gram-positive bacteria are responsible for urogenital tract infections. However, among Gram-negative bacterial agents, *Escherichia coli* is the most common causative agent.[1,2] Based on data from the China Antimicrobial Surveillance Network (CHINET), in 2022, *E. coli* had the highest detection rate, comprising 18.97% of clinical isolates. Uropathogenic *E. coli* strains were recognized as typical bacterial agents for urinary tract infections,

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these groups of infectious diseases are more common among women rather than men, as well as female reproductive tract.[3,4] In 2021, a meta-analysis of 82 studies, with a sample size of 33,118, indicated that the prevalence of *E. coli* in vaginal samples from pregnant women is 32% and that of extended-spectrum-lactamase (ESBL)-producing *E. coli* in pregnant women is 34%.[5] Perinatal infections caused by *E. coli* can result in premature birth, a low birth weight, and an increased risk of neonatal infection and death.[6,7] Notably, due to the misuse or overuse of antibiotics, antimicrobial resistance (AMR) is considered to feature among the top 10 threats to global health.[8] The emergence and global spread of antibiotic-resistant *E. coli* have caused difficulties in clinical treatment and have become a public health threat.[9] Third-generation cephalosporins are the most commonly used class of antibiotics in gynecology/obstetrics and pediatrics departments to treat infections caused by *E. coli*;[10,11] however, the prevalence of *E. coli* resistant to third-generation cephalosporins is increasing.[12]

Enterobacteriales bear different antibiotic resistance genes encoding Extended-Spectrum-ß-Lactamases (ESBLs) and Metallo-ß-Lactamases (MBLs) on their chromosomes and mobile genetic elements such as plasmids and transposons.[13] In *E. coli* (typical representative of Enterobacteriales), ESBLs mainly confer third-generation cephalosporin resistance.[14] TEM- and SHV-type ESBLs were initially described in the 1980s.[15,16] The first CTX-M-type ESBL (CTX-M-1), with higher hydrolytic activity against cefotaxime than against ceftazidime, emerged in 1995.[17] Since then, several variants of CTX-M-1 have been found,[18] and these are now the most prevalent ESBLs all over the world, with *E. coli* as their major bacterial host.[19]

Mobile genetic elements (*eg* plasmid, insertion sequence (IS), transposon, and integron) play a major role in facilitating the spread of resistance genes.[20] ESBL genes are mainly located on plasmids, and among the ESBL-carrying plasmids from *E. coli*, the most common replicon types are IncF, IncI1, IncN, IncHI1, and IncHI2.[21] ESBL genes are always associated with many ISs (such as IS*26*, IS*CR1*, IS*Ecp1*, and IS*10*), transposons (such as Tn*2*), and integrons.[22–24] Here, we report a multidrug resistant (MDR) *E. coli* ST69 strain carrying an IncF plasmid harboring $bla_{\text{CTX-M-27}}$ and other antibiotic resistance genes (ARGs) and present a comparative analysis of the MDR region of the IncF plasmid.

Materials and Methods

For this study, the data collection and analysis are shown in Figure S1.

Bacterial Strains and Identification

The strain EC6868 was isolated from the vaginal secretions of a 39-year-old pregnant woman at the Zhuhai People's Hospital in December 2021. Species identification and antimicrobial susceptibility testing were conducted using a fully automatic VITEK 2 COMPACT system (BioMérieux, France) according to the manufacturer's instructions. The results of antimicrobial sensitivity were interpreted based on the Clinical and Laboratory Standards Institute guidelines (M100-S24). The identity of EC6868 was further confirmed through 16S rRNA gene sequencing by using bacterial universal primers (27F/1492R).

Whole-Genome Sequencing, Assembly, and Annotation

Whole-genome sequencing of strain EC6868 was conducted by Genewiz Biotechnology Co. Ltd. (Suzhou, China) by using the Illumina paired-end sequencing and PacBio long reads sequencing. PacBio reads were assembled using HGAP4.0/Falcon 0.3 of WGS-Assembler 8.2.[25] The genome assembly was then polished with the software Pilon 1.22[26] using Illumina reads. The assembled genome (both chromosome and plasmids) of strain EC6868 was submitted to the GenBank database[27] and annotated using the NCBI Prokaryotic Annotation Pipeline (PGAP).[28]

Bioinformatics Analysis of the Genome of Strain EC6868

Acquired ARGs in the genome of strain EC6868 were identified using the websever ResFinder 4.1,[29] with a minimum coverage of 60% and a minimum identity of 90%. Multilocus sequence typing (MLST) of strain EC6868 was performed using MLST 2.0,[30] selecting the database as "Escherichia coli #1" and using seven housekeeping genes, including *adk, fumC, gyrB, icd, mdh, purA*, and *recA*. Replicon types of the plasmids contained by strain EC6868 were determined using PlasmidFinder 2.1,[31] with the database "Enterobacteriales", minimum coverage of 60%, and minimum identity of 95%.

The ISs adjacent to the ARGs in the EC6868 genome were identified using ISfinder.[32] Sequence similarity search was performed using MegaBLAST[33] scans against the GenBank nr database. BLAST Ring Image Generator (BRIG) 0.95[34] and Easyfig2.2.5[35] were used for plasmid comparisons.

Nucleotide Sequence Accession Numbers

The genome sequence of strain EC6868, which contained a chromosome and two plasmids, was submitted to GenBank under the accession numbers CP095083–CP095085.

Results

Identification and Antimicrobial Susceptibility Testing of Strain EC6868

Strain EC6868 isolated from vaginal secretions of a pregnant woman, was identified as *E. coli* by the automatic VITEK 2 COMPACT system, which was then confirmed via 16SrRNA gene sequencing. *E. coli* strain EC6868 showed resistance to cephalosporins, including cefuroxime, cefuroxime axetil, and ceftriaxone, but conferred intermediate-level ceftazidime resistance (Table S1). In addition, it showed resistance to trimethoprim/sulfamethoxazole and intermediate-level resistance to levofloxacin (Table S1).

Genomic Features of the *E. coli* Strain EC6868

Genomic analysis revealed that the genome of *E. coli* strain EC6868 comprised a 5.25-Mb chromosome and two plasmids with sizes of 144,934 bp (pEC6868-1) and 7919 bp (pEC6868-2). MLST analysis indicated that *E. coli* strain EC6868 belonged to sequence type (ST) 69. PlasmidFinder results indicated that plasmid pEC6868-1 contained three replicons (Col156, $IncFIB_{AP001918}$, and IncFII), whereas plasmid pEC6868-2 was an untypeable plasmid.

ResFinder results indicated that *E. coli* EC6868 carried multiple ARGs located on both chromosomes and the plasmid pEC6868-1. The chromosome of *E. coli* EC6868 was found to harbor disinfectant-resistance genes (*sitABCD*). Further, the multidrug-resistance plasmid pEC6868-1 harbored ARGs conferring resistance to extended-spectrum cephalosporins ($bla_{CTX\text{-}M\text{-}27}$), aminoglycosides (*aph(6)-Id, aph(3")-Ib*, and *aadA5*), macrolides (*erm(B)* and *mph(A)-mrx(A)-mphR(A)*), sulphonamides (*sul1* and *sul2*), tetracycline (*tet(A)-tetR(A)*), and trimethoprim (*dfrA17*). Plasmid pEC6868-1 also harbored the antiseptic-resistance gene *ΔqacE*.

Comparative Analysis of the MDR Region of Plasmid pEC6868-1

The ARGs, as well as the adjacent IS elements carried by plasmid pEC6868-1, were located on the ~31.2-kb fragment of the plasmid, forming a large MDR region. The MDR region of pEC6868-1, flanked by ΔIS*1R* and ΔIS*1R*-ΔIS*26*, which was nearly identical to part of the *E. coli* plasmid pB16EC0698-3 (100.00% coverage and 99.78% identity; Figure 1). In the MDR region of pEC6868-1, IS*26* was found to be located upstream of the macrolide-resistance gene *ermB* and its leading peptide gene *ermB(L)* (Figure 1). The ESBL gene $bla_{CTX\text{-}M\text{-}27}$ was located on an IS*Ecp1*-mediated transposition unit, with a truncated IS*Ecp1* (ΔIS*Ecp1*) located upstream of $bla_{CTX\text{-}M\text{-}27}$ and IS*903B* located downstream of $bla_{CTX\text{-}M\text{-}27}$, forming the genetic structure ΔIS*Ecp1*-$bla_{CTX\text{-}M\text{-}27}$-IS*903B* (Figure 1). Based on the region ΔIS*Ecp1*-$bla_{CTX\text{-}M\text{-}27}$-IS*903B*, the BLAST search hit from the database of GenBank (on November 16th, 2023), with the minimum coverage of 100% and minimum identity of 99%, showed that 1109 strains contain the structure (Figure 2). The structure was widely present in the species of Enterobacteriales, including *Klebsiella pneumoniae* (549), *E. coli* (347), *Salmonella enterica* (106), *Proteus mirabilis* (44) and the other 23 species (63) (Figures 2 and S2).

Interestingly, except for *ermB-ermB(L)* and $bla_{CTX\text{-}M\text{-}27}$, other ARGs carried by plasmid pEC6868-1 were located on the 18.9-kb IS*26*-composite transposon. Overall, the 18.9-kb IS*26*-composite transposon was composed of three parts as follows: (1) the *mphA-mrx(A)-mphR(A)* operon conferring macrolide resistance was flanked by IS*26* and IS*6100*, forming the IS*26-mphA-mrx(A)-mphR(A)*-IS*6100* transposable structure (Figure 1); (2) one fragment of pEC6868-1 containing *tet(A)-tetR(A)*, *aph(6)-Id*, *aph(3")-Ib*, and *sul2* located adjacent to IS*26*-ΔTn*As3-eamA* and IS*26-mphA-mrx(A)-mphR(A)*-IS*6100* (Figure 1); (3) notably, a complex class 1 integron, including *intI1, dfrA17, aadA5*, Δ*qacE*, and *sul1*, was also found in the MDR region of plasmid pEC6868-1, located adjacent to IS*26*-ΔIS*Kpn72*-ΔIS*1R*-ΔIS*26* (Figure 1).

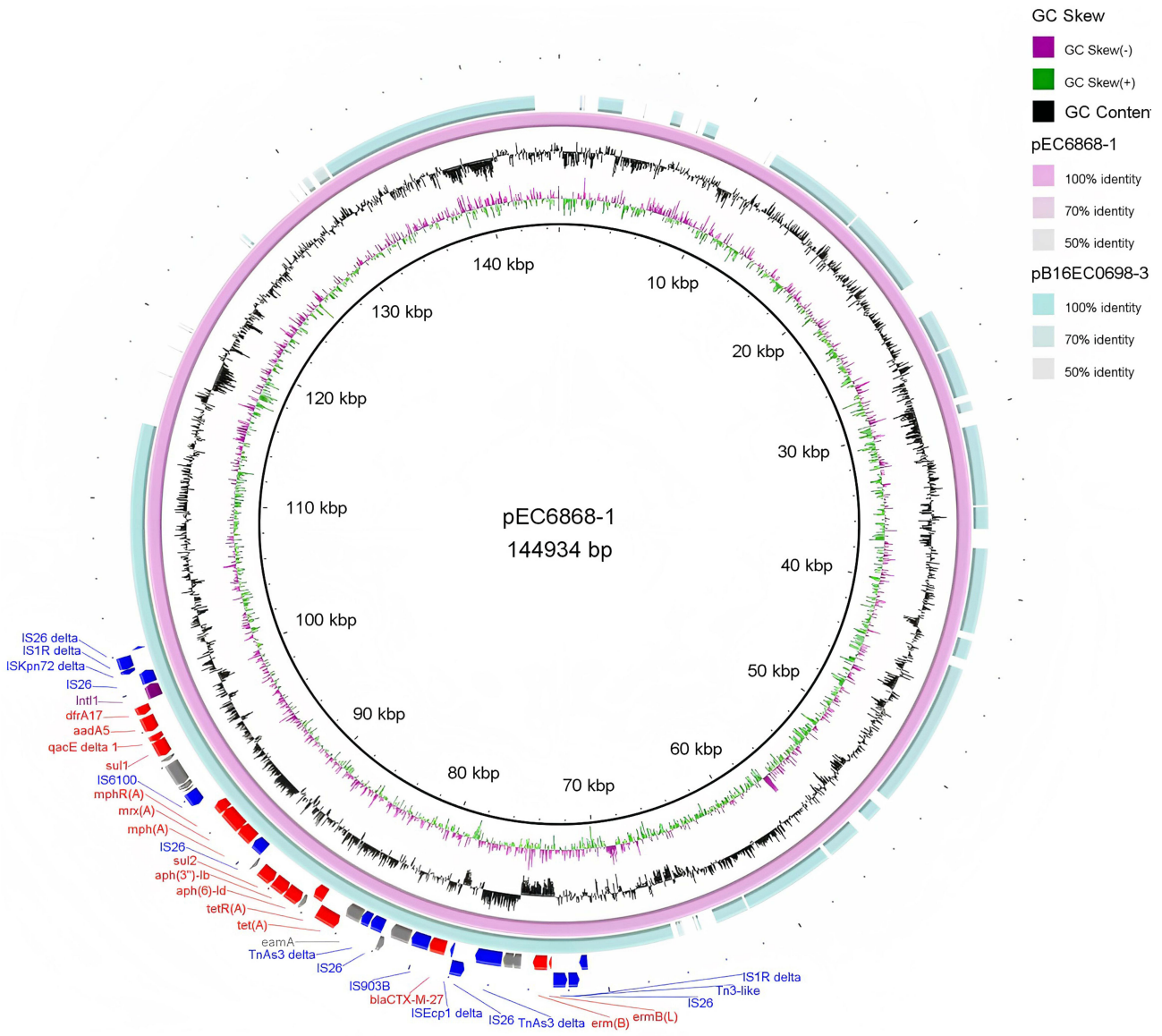

Figure 1 Comparative analysis of multidrug-resistance (MDR) region of plasmid pEC6868-1 and that of *Escherichia coli* plasmid pB16EC0698-3 generated using BRIG. Resistance, transposase, and integrase genes are shown in red, blue, and purple, respectively.

Based on the 18.9-kb IS*26*-composite transposon of plasmid pEC6868-1, the BLAST searches against the nr database of GenBank showed that the 18.9-kb region was widely present on plasmids of *E. coli* (77 plasmids), mainly distributed in ST131, ST38, and ST405 (Figure 3). Notably, pEC6868 in our study was the first report on a plasmid harboring the 18.9-kb IS*26*-composite transposon in *E. coli* ST69 in China. Another ST69 strain of *E. coli* containing the 18.9-kb structure was found in the United States, 2017 (Table S2). Surprisingly, 75 of the 77 plasmids harboring the 18.9-kb IS*26*-composite transposon were found to be the IncF plasmids, including IncFIA, IncFIB and IncFII subtypes, accounting for 97.4% of all the 18.9-kb structure-harboring plasmids of *E. coli*. In addition, four plasmids harboring the 18.9-kb IS*26*-composite transposon were also found in *K. pneumoniae* (Figures 3 and S2).

Moreover, we found that the region within this 18.9 kb IS*26*-composite transposon, containing IS*26-mphA-mrx(A)-mphR(A)*-IS*6100* and the complex class 1 integron, was not only present on the plasmids but also on the chromosomes of some pathogens, such as *E. coli, K. pneumoniae, Klebsiella michiganensis, Proteus mirabilis, Shigella flexneri*, and *Morganella morganii* (Figure 4).

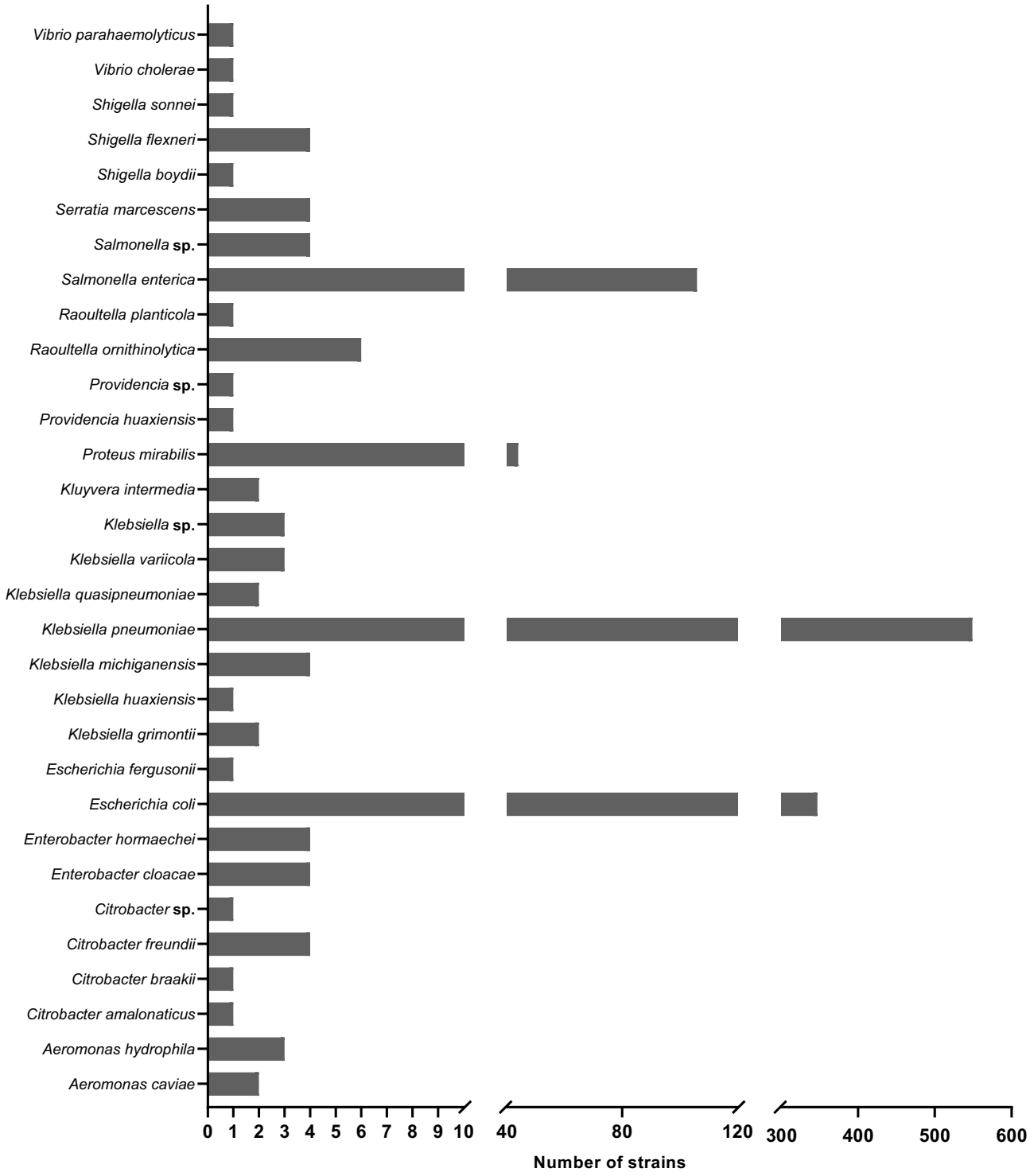

Figure 2 An overview of the species of Enterobacteriales harboring the structure "ΔIS*Ecp1*-bla$_{CTX-M-27}$-IS*903B*.".

Discussion

In this study, we report a third-generation cephalosporin-resistant strain, *E. coli* ST69 (EC6868), isolated from the vaginal secretions of a 39-year-old pregnant woman. *E. coli* ST*69* was determined to be one of the multidrug resistant *E. coli* clones of phylogenetic group D, which are widespread among different hosts, often causing urinary tract infections and exhibiting resistance to antibiotics.[36–38] ST*69 E. coli* strains have been reported to carry the

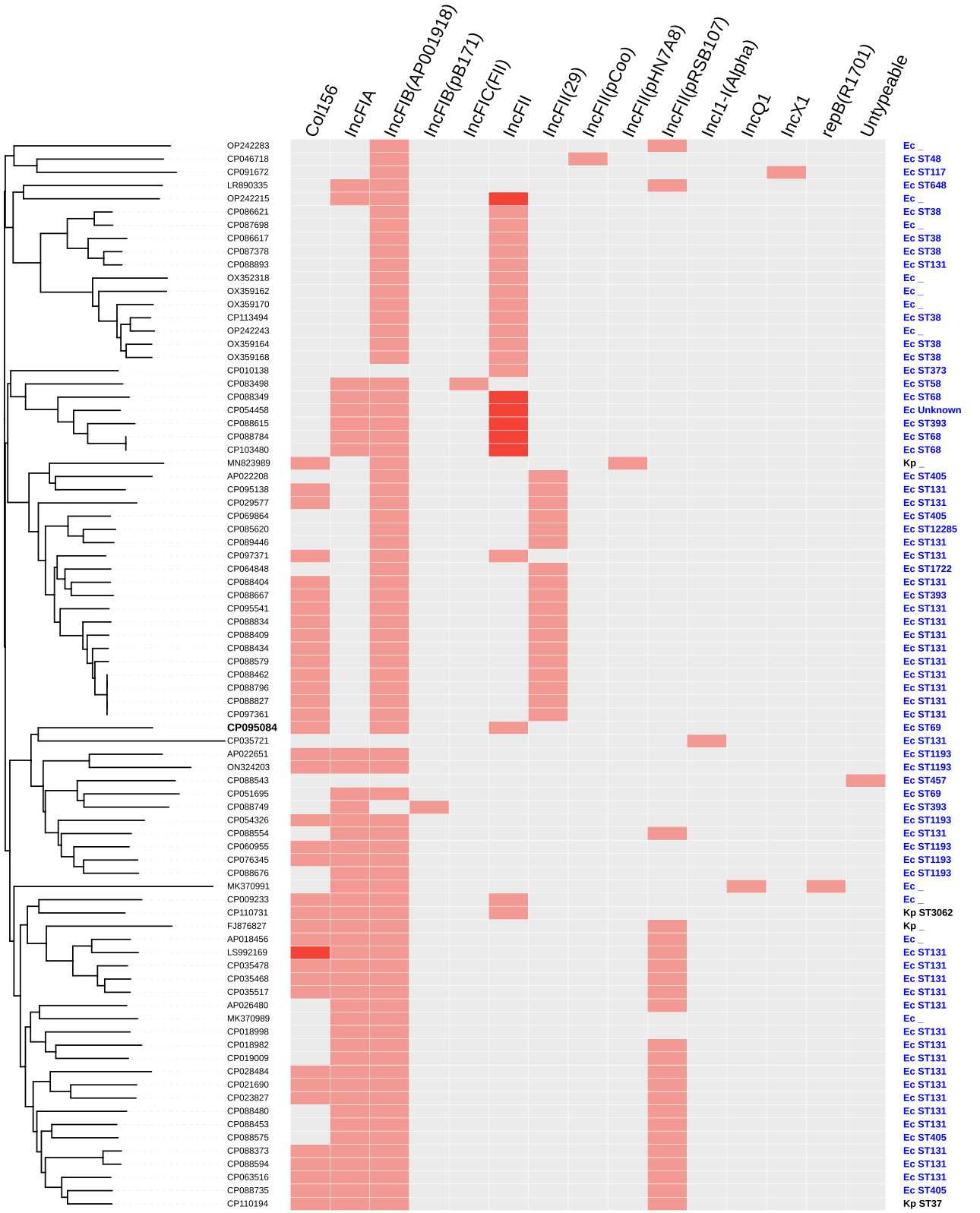

Figure 3 Details of replicon types and STs of host strains of the 81 plasmids harboring 18.9-kb IS*26*-composite transposon. Phylogenetic cladogram based on the presence/absence of orthologous gene families of the 81 plasmids harboring 18.9-kb IS*26*-composite transposon were constructed. Ec and Kp represent *E. coli* and *K. pneumoniae*, respectively.

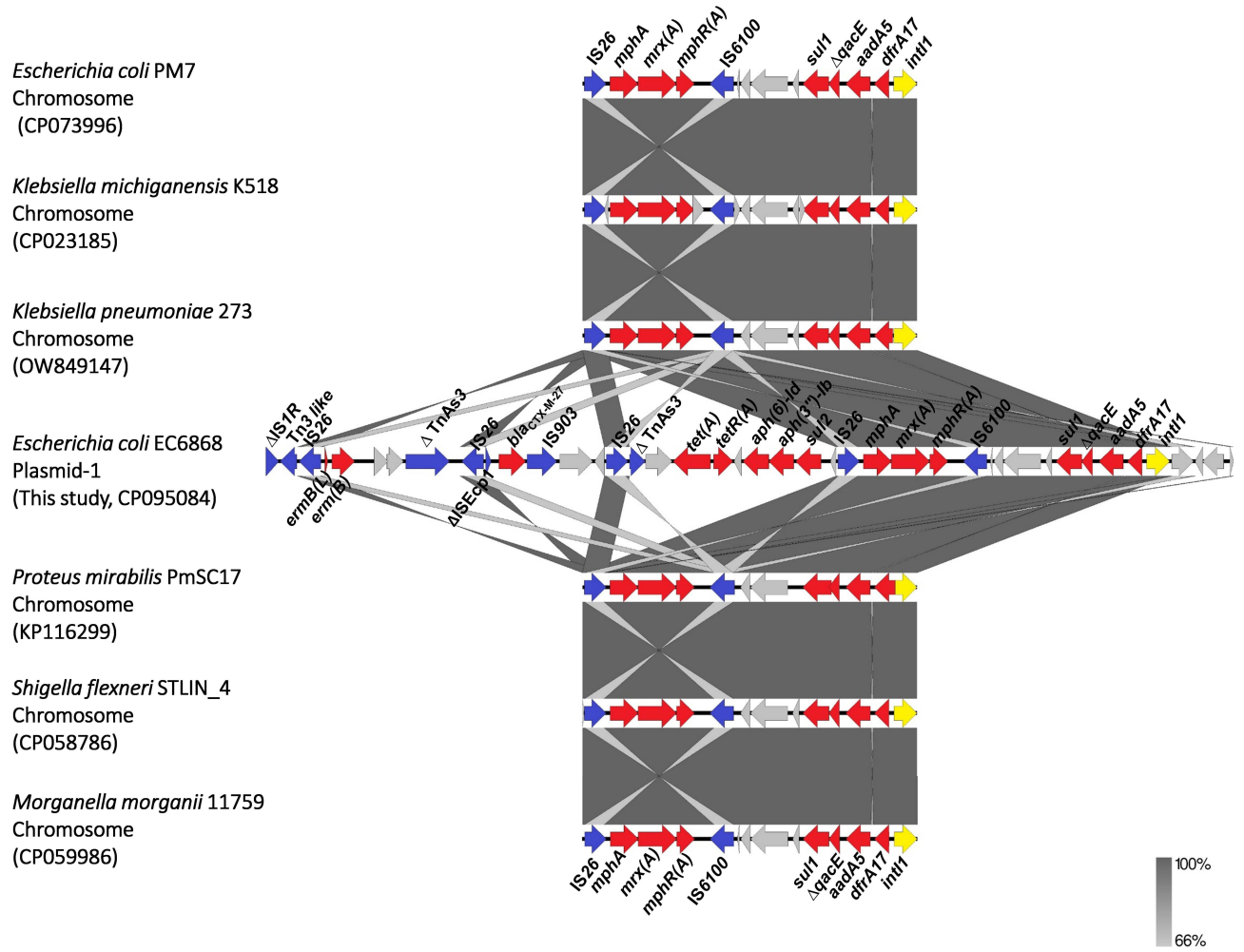

Figure 4 Comparison of the gene cluster containing IS*26*-*mphA*-*mrx(A)*-*mphR(A)*-IS*6100* and the complex class 1 integron carried by plasmid pEC6868-1with chromosomes of other pathogenic bacteria other than *Escherichia coli*. Resistance, transposase, and integrase genes are shown in red, blue, and yellow, respectively.

bla_{VIM}-harboring IncA plasmid,[39] bla_{NDM-1}-harboring IncI1 plasmid,[40] and *mcr-1*-harboring IncHI2 plasmid.[41] In this work, we report that the ST*69 E. coli* strain carries one hybrid plasmid, with three replicons (Col156, $IncFIB_{AP001918}$, and IncFII), harboring $bla_{CTX-M-27}$. IncF plasmids, comprising one of the most common incompatibility types, have been identified globally in Enterobacteriales. Moreover, they vary in size (50–200 kb) and replicon types, which harbor huge amount of ARGs and confer resistance to all major classes of antibiotics, such as beta-lactams, chloramphenicol, aminoglycosides, quinolones, and tetracyclines.[42–45] Notably, the IncF-bla_{CTX-M} association found in *E. coli* EC6868 in our study has been widely observed in *E. coli* isolates of both human and animal origins; for example, the IncF plasmid R100 is responsible for the spread of $bla_{CTX-M-14}$ in the UK and France.[46,47]

Strain EC6868 in our study was found to be a CTX-M-type ESBL-producing *E. coli* isolate. CTX-M-14 and CTX-M-15 are the most common variants of CTX-M, and CTX-M-27 has increased rapidly in terms of its prevalence.[48] Notably, the detection of CTX-M-27 in *E. coli* isolates from patient has been rising and is particularly alarming due to its presence in clonal groups including ST10, ST69, and ST131.[49,50] The ΔIS*Ecp1* marker was located upstream of $bla_{CTX-M-27}$ and carried by pEC6868-1 (ΔIS*Ecp1*-$bla_{CTX-M-27}$-IS*903B*) was found in another study, which points that the conserved region responsible for transmission of $bla_{CTX-M-27}$ was IS*Ecp1*-$bla_{CTX-M-27}$-IS*903B*.[51] IS*Ecp1* belongs to the IS*1380* family,[52] and the IS*Ecp1*-like element is associated with several bla_{CTX-M} gene types in Enterobacteriaceae, including $bla_{CTX-M-14}$, $bla_{CTX-M-24}$, $bla_{CTX-M-22}$, and $bla_{CTX-M-79}$.[53] IS*903B*, located downstream of $bla_{CTX-M-27}$ carried

by pEC6868-1, is a small insertion element of 1057 bp that is transposed predominantly via a conservative "cut-and-paste" mechanism.[54]

Five copies of intact IS*26* and one ΔIS*26* were found within the MDR region of plasmid pEC6868-1. The 820-bp IS*26* was first described in 1983[55] and was determined to be present in numerous early antibiotic-resistant bacteria, playing a critical role in disseminating ARGs among Gram-negative bacteria.[56] IS*26* is frequently associated with genes encoding antibiotic resistance factors,[57] and it can contribute to the expression of the ARGs by supplying a promoter −35 region which can be coupled with a −10 region in the adjacent ARG.[58]

In this study, two translocatable units conferring macrolide resistance, IS*26-ermB(L)-erm(B)* and IS*26-mphA-mrx(A)-mphR(A)*-IS*6100*, were detected in plasmid pEC6868-1. In IS*26-ermB(L)-erm(B)*, the *ermB* encodes a ribosomal methylase which can reduce the affinity of macrolides for the ribosome, resulting in high level of macrolide resistance.[59] Macrolide antibiotics promote ribosome stalling on the ErmB(L) (regulatory leader peptide), ultimately inducing the expression of *ermB*.[60] In the gene cluster *mphA-mrx(A)-mphR(A)* for high-level macrolide inactivation, *mphA* encodes a phosphotransferase that inactivates erythromycin and *mrx(A)* encodes a protein required for expression of MphA, whereas MphR(A) encoded by *mphR(A)* negatively regulates the expression of *mphA* at the transcriptional level.[61] The IS*26-mph(A)-mrx(A)-mph(R)(A)*-IS*6100* unit, which is linked to the global dissemination of macrolide-resistance genes,[62] is the most common form in plasmids carrying *mphA*.[63]

Class 1 integrons are prevalent genetic elements that play a key role in the spread of antibiotic resistance,[64] allowing bacteria to capture and exchange ARGs embedded in the gene cassettes. Moreover, acquisition of gene cassettes is catalyzed by integrase encoded by *int1*, which is a site-specific recombinase.[65] Class 1 integrons are structurally composed of three core elements, specifically an integrase gene (*intI1*), a primary recombination site (*attI1*), and a common promoter enabling the transcription of gene cassettes.[66] In all integrons, detection rate of class 1 integrons in clinical isolates is highest at 50–70%, which is linked to the production and spread of antibiotic-resistant bacteria.[67,68]

Conclusion

Here, we describe the genomic characteristics of a 3GC-R and ESBL-producing *E. coli* strain EC6868 belonging to ST69 harboring $bla_{\text{CTX-M-27}}$ isolated from a pregnant patient in China. The strain carried multiple-resistance genes conferring resistance to aminoglycosides, macrolides, sulfonamides, tetracycline, and trimethoprim. The $bla_{\text{CTX-M-27}}$ gene located on a hybrid plasmid pEC6868-1 (IncFII/IncFIB/Col156). The ESBL gene $bla_{\text{CTX-M-27}}$ was flanked by the truncated IS*Ecp1* (ΔIS*Ecp1*) and the IS*903B* (ΔIS*Ecp1-*$bla_{\text{CTX-M-27}}$-IS*903B*), which was widely present in the species of Enterobacteriales, especially in *K. pneumoniae, E. coli*, and *Salmonella enterica*. Except for $bla_{\text{CTX-M-27}}$, other ARGs carried by plasmid pEC6868-1 were mainly located on the 18.9-kb IS*26*-composite transposon (five copies of intact IS*26* and one copy of truncated IS*26*). The 18.9-kb structure was widely present on IncF plasmids of *E. coli*, mainly distributed in ST131, ST38, and ST405. Notably, pEC6868 in our study was the first report on a plasmid harboring the 18.9-kb structure in *E. coli* ST69 in China. In addition, Tn*3*, IS*6100* and class 1 integron also play important role in the dissemination of acquired ARGs carried by the plasmid pEC6868-1 of *E. coli* strain EC6868.

Ethical Approval Statement

This study has been approved by the Ethics Committee of Zhuhai People's Hospital. The present study was a study focusing on bacteria and did not contain any sensitive personal information. Therefore, informed consent was not required according to "Measures for the Ethical Review of Biomedical Research Involving Humans" (https://www.gov.cn/gongbao/content/2017/content_5227817.htm).

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Disclosure

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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