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ORIGINAL RESEARCH

Genomic Characteristics of a Multidrug-Resistant ST648 *Escherichia coli* Isolate Co-Carrying bla_{KPC-2} and $bla_{CTX-M-15}$ Genes Recovered from a Respiratory Infection in China

Xianhong He, Liwei Xu, Hangdong Dai, Minxia Ge, Jufang Zhu, Hangyu Fu, Shuilong Zhu, Jiayu Shao

Department of Clinical Laboratory, The Third People's Hospital of Xiaoshan, Hangzhou, People's Republic of China

Correspondence: Jiayu Shao; Shuilong Zhu, Department of Clinical Laboratory, The Third People's Hospital of Xiaoshan, 76 Zhishan North Road, Hangzhou, Zhejiang, People's Republic of China, Tel +86 18505812918; +86 18057155360, Email 583744598@qq.com; zslong1976@163.com

Background: The transmission of carbapenem-resistant Enterobacterales pose a significant threat to global public health, which weakens the effectiveness of most antimicrobial agents. The aim of this study is to present the genomic characteristics of a multidrug-resistant *Escherichia coli*, which contains both bla_{KPC-2} and $bla_{CTX-M-15}$ genes, discovered from a respiratory infection in China.

Methods: The antimicrobial susceptibility of *E. coli* isolate 488 was measured by using the broth microdilution method. The Oxford Nanopore MinION and Illumina NovaSeq 6000 platforms were applied to determine the whole-genome sequence of this isolate. De novo assembly of short Illumina reads and long MinION reads were performed by Unicycler. In silico multilocus sequence typing (MLST), antimicrobial resistance genes and plasmid replicon types were determined using the genome sequencing data. Additionally, a pairwise core genome single nucleotide polymorphism (cgSNP) comparison between *E. coli* 488 and all ST648 *E. coli* strains retrieved from NCBI GenBank database were conducted using the BacWGSTdb 2.0 server.

Results: *E. coli* 488 was resistant to aztreonam, levofloxacin, cefepime, fosfomycin, amikacin, imipenem, cefotaxime, and meropenem. The complete genome sequence of *E. coli* 488 (belong to ST648) is made up of eleven contigs totaling 5,573,915 bp, including one chromosome and ten plasmids. Eight antimicrobial resistance genes were identified, including bla_{KPC-2} located in a 46,161 bp IncI1-type plasmid and the $bla_{CTX-M-15}$ gene situated in the chromosome. Other two *E. coli* S617-2 and R616-1 isolates, recovered from China in 2018, are the closest relatives of *E. coli* 488, with only 52 SNPs difference. The genome also contains at least 57 genomic islands and several IS elements.

Conclusion: Our study reveals the first ST648 *E. coli* isolate containing both bla_{KPC-2} and $bla_{CTX-M-15}$ in China. These results could provide valuable insights into the genetic characteristics, antimicrobial resistance mechanisms, and transmission dynamics of carbapenem-resistant Enterobacterales in clinical settings.

Keywords: bla_{KPC-2}, $bla_{CTX-M-15}$, whole genome sequencing, multidrug-resistant, *Escherichia coli*

Introduction

Escherichia coli, a member of the Enterobacterales family, is a common opportunistic pathogen that can cause various infectious illnesses, including urinary tract infections (UTIs) and other infections acquired in the community.[1,2] The presence of carbapenemases in *E. coli* poses a serious threat to public health. These carbapenemase genes may be present in the bacterial chromosome, but are usually located on mobile elements like plasmids or transposons that can spread across bacterial strains, species, and even different types of organisms.[3] Carbapenemases are a type of β-lactamase that can hydrolyze carbapenems. The Ambler classification categorizes β-lactamases into four classes (A, B, C, and D), with class A, B, and D being the most common carbapenemases. Class A carbapenemases include GES, IMI, NMC, KPC, and SME; Class B carbapenemases are metalloenzymes, including GIM, VIM, IMP, NDM, and SIM; and Class D carbapenemases are primarily oxacillinases, such as

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OXA-48. Enterobacterales that synthesize expanded-spectrum β-lactamases may become resistant to other β-lactam drugs, which may also resistant to several other classes of antibiotics, such as quinolones, cephalosporins and macrolides. The most common carbapenemases among nosocomial and community Enterobacterales isolates are NDM, OXA-48, and KPC-type β-lactamases, while CTX-M-type extended-spectrum β-lactamases (ESBLs) is the most predominant ESBLs globally.[4]

Klebsiella pneumoniae carbapenemase (KPC) is a type of class A serine β-lactamase that can effectively break down most β-lactam antimicrobial agents. It is one of the most prevalent transmissible carbapenem resistance mechanisms found worldwide. The emergence of KPC-producing Enterobacterales presents a significant challenge to healthcare systems around the globe. Among the various KPC variants, KPC-2 is the most identified type, which is a leading cause of carbapenem resistance in Enterobacterales. The $bla_{\text{KPC-2}}$ gene is typically located on a mobile transposon, often situated on conjugative plasmids, which can spread both vertically within clonal lineages and horizontally across different strains and species. Moreover, the co-occurrence of carbapenem resistance and the production of ESBLs is frequently observed.[5] It is important to note that a single pathogenic bacterial strain that carries a large number of antibiotic resistance genes can pose a significant obstacle for effective treatment.[6] Here, we describe the genomic features of a multi-drug resistant *E. coli* strain that carries both $bla_{\text{KPC-2}}$ and $bla_{\text{CTX-M-15}}$ genes isolated from a respiratory infection in China.

Materials and Methods

The isolate of *E. coli* 488 was recovered from a respiratory infection of a 65-year-old male patient. The purified isolate was initially cultured on Columbia blood agar for 16–18 h at 37°C. Afterward, a single colony of the target strain was cultured in Mueller Hinton broth (Oxoid Ltd., Basingstoke, UK) for 16–18 h at 37°C, then analyzed using a MALDI Biotyper (Bruker Daltonics, Billerica, MA, USA) and 16S rRNA gene sequencing. Antimicrobial susceptibilities were evaluated by using the broth microdilution method to the following antimicrobial agents: amikacin, aztreonam, levofloxacin, fosfomycin, cefoxitin, cefepime, cefotaxime, imipenem, meropenem, colistin, and tigecycline. The breakpoints of colistin and tigecycline for Enterobacterales

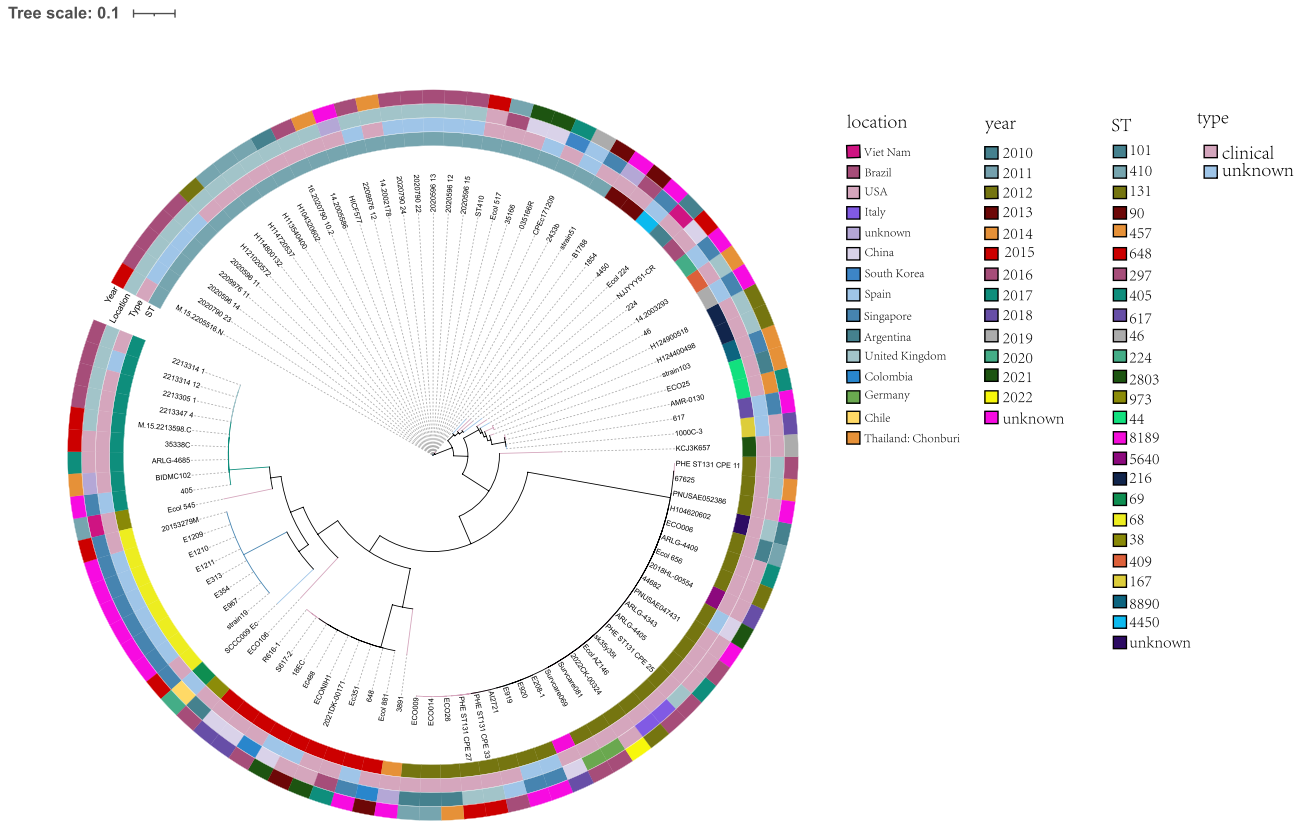

Figure 1 The phylogenetic relationship between EC488 and a total of 99 *E. coli* strains co-carrying $bla_{\text{KPC-2}}$ and $bla_{\text{CTX-M-15}}$ genes currently deposited in the NCBI GenBank database.

were interpreted in accordance with the European Committee on Antimicrobial Susceptibility Testing (EUCAST) criteria (version 9.0), minimum inhibitory concentrations (MICs) of other antimicrobial agents were determined by reference to Clinical and Laboratory Standards Institute (CLSI) 2019 standards. *E. coli* ATCC 25922 was used as a quality control strain.

Genomic DNA was extracted from the isolate using a Genomic DNA Purification Kit (QIAGEN, Valencia, CA, USA). DNA purity and concentration were determined using a NanoDrop spectrophotometer (Thermo Scientific, Waltham, MA, USA). Whole-genome sequencing of *E. coli* 488 was performed both using the Illumina NovaSeq 6000 (Illumina Inc., San Diego, CA, USA) with the 150-bp paired-end protocol and Oxford Nanopore MinION (Oxford Nanopore Technologies, Oxford, UK) platforms. The hybrid assembly of short Illumina and long Nanopore reads was performed using Unicycler v.0.4.8.[7] The genome sequence was automatically annotated by the NCBI Prokaryotic Genomes Annotation Pipeline and then subjected to further analysis to identify the sequence type (ST), capsular type, and antibiotic resistance genes. Antimicrobial resistance genes, plasmid incompatibility (Inc) groups and serotype were determined using ABRicate 1.0.1 in conjunction with ResFinder 4.1, CARD 2020 and PlasmidFinder 2.1 databases.[8,9] BacWGSTdb 2.0 server was used to perform core genome single nucleotide polymorphism (cgSNP) strategy-based bacterial source tracking and in silico multilocus sequence typing (MLST) analysis.[10,11] Using Easyfig 2.2.5, the genetic context of antimicrobial resistance genes and the homologous areas between plasmids were examined.[12] IslandViewer 4, ISfinder 1.0, PHASTER 2016, CRISPRCasFinder 1.0, and antiSMASH 5.2.0 were used to predict genomic islands, insertion sequence (IS) elements, prophage sequences, clustered regularly interspaced short palindromic repeat (CRISPR) sequences, and secondary metabolite gene clusters, respectively.[13–15] OriTfinder was used to identify the presence of conjugative transfer-related modules of the bla_{KPC-2}-carrying plasmid, including oriT region, relaxase gene, the type IV coupling protein (T4CP) gene and the *tra* gene cluster for the type IV secretion system (T4SS).[16] The genome sequences of *E. coli* 488 have been deposited in NCBI GenBank under the accession number CP109875-CP109884.

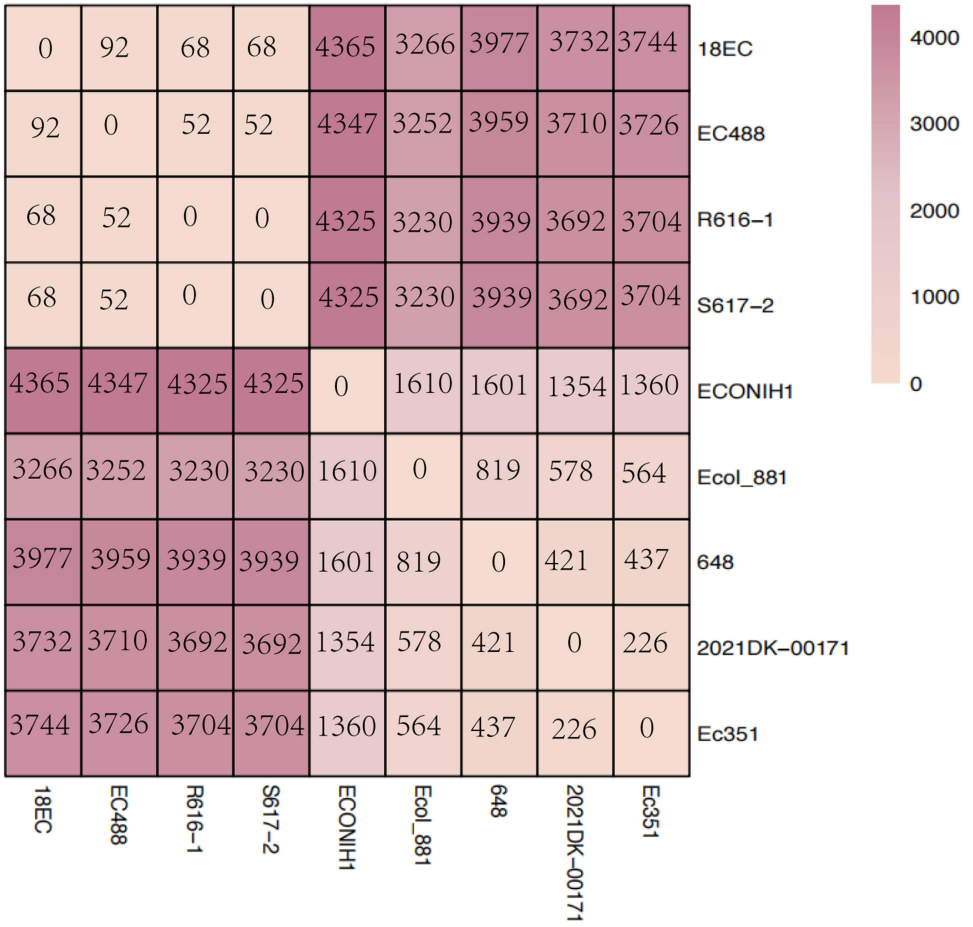

Figure 2 Single nucleotide polymorphisms (SNPs) numbers between *E. coli* 488 and a total of 9 ST648 *E. coli* strains co-carrying bla_{KPC-2} and $bla_{CTX-M-15}$ genes.

Results and Discussion

The complete genome sequence of *E. coli* 488 is made up of eleven contigs that have been closed and circularized, totaling 5,573,915 bp. Contig 1 (5,173,170bp) belonged to the chromosome, whereas the others belonged to different plasmids (contig 2, 133,748 bp; contig 3, 126,200 bp; contig 4, 70,018 bp; contig 5, 49,150 bp; contig 6, 5167 bp; contig 7, 4072 bp; contig 8, 4063 bp; contig 9, 3373 bp; contig 10, 3255 bp; contig 11, 1459 bp). The total GC content of the strain was 50.63%, and 5367 coding sequences (CDSs) and 119 RNAs (92 tRNA, 22 rRNA, and 5 ncRNA) genes were identified. Antimicrobial susceptibility testing showed that the isolate was resistant to aztreonam (MIC = 64 mg/L), cefoxitin (MIC =128 mg/L), levofloxacin (MIC = 8 mg/L), cefepime (MIC = 8 mg/L), fosfomycin (MIC = 64 mg/L), amikacin (MIC = 2 mg/L), imipenem (MIC = 16 mg/L), cefotaxime (MIC =128 mg/L), and meropenem (MIC =64 mg/L). The resistome of *E. coli* 488 is made up of genes that are resistant to tetracyclines (*tet(B)*), macrolides (*mdf(A)* and *mph(A)*), trimethoprim (*dfrA17*), β-lactams ($bla_{\text{CTX-M-15}}$ and $bla_{\text{KPC-2}}$), aminoglycosides (*aadA5*), and sulphonamides (*sul1*).

To investigate the genomic epidemiological characteristics of *E. coli* strains in a worldwide context, the phylogenetic relationships between EC488 and a total of 99 *E. coli* strains co-carrying $bla_{\text{KPC-2}}$ and $bla_{\text{CTX-M-15}}$ genes currently deposited in the NCBI GenBank database were investigated. The isolation dates ranged from 2010 to 2022, and 2016 (24%, n = 24) is the main separation year. The major country of isolation is the United Kingdom (33%, n = 33), followed by Singapore (18%, n = 18), the USA (15%, n = 15), China (7%, n = 7) and Argentina (6%, n = 6). These isolates belong to a variety of sequence types, including ST410 (n = 27, 27%), ST131 (n = 24, 24%), ST405 (n = 9, 9%), ST648 (n = 9, 9%), and ST68 (n = 8, 8%). In addition, the majority of these strains come from clinical samples (n = 68, 68%) (Figure 1). In silico MLST analysis indicated that *E. coli* strain 488 belongs to ST648 *E. coli* 488 and a total of 9 ST648 *E. coli* strains currently deposited in the NCBI GenBank database were compared phylogenetically in order to examine the genomic epidemiological traits of *E. coli* strains in a global context. *E. coli* strain S617-2 and R616-1, which were isolated from a nasopharyngeal swab and anal swab sample in China in 2018, were shown to be the closest relative of *E. coli* 488, which differed by 52 SNPs (Figure 2).

The $bla_{\text{KPC-2}}$ gene was discovered in pEC488, a 46,161 bp IncI1-I-type plasmid. Analysis of the genetic environment revealed that an insertion sequence IS*Kpn6* exists downstream of $bla_{\text{KPC-2}}$ harboring a truncated fragment IS*1182* and IS*15*. The $bla_{\text{KPC-2}}$ gene is downstream of the TraC-RfaH-TraA-IS*5*-TnpR-IS*Kpn27* elements and upstream of the ΔIS*1182*-IS*Kpn6*-IS*15*-Trab-TrbI-VirB8/TrbF, which may mediate the horizontal transfer of $bla_{\text{KPC-2}}$ (Figure 3). A BLAST analysis revealed that the pEC488-KPC plasmid share 63% coverage and 98.60% identity to another plasmid pC271-KPC-2 (Figure 4).

The genome also contains at least 57 genomic islands and several IS elements, the majority belonging to the IS*3* and IS*66* families. Similarly, 10 prophage sequence and 7 CRISPR sequences can be predicted in the genome. The presence of three putative secondary metabolite gene clusters, including the turnerbactin, yersiniabactin and O-antigen biosynthetic gene clusters, can also be predicted. The presence of potential transfer-related modules, including the origin of transfer (oriT) region, the relaxase gene, T4CP genes and the type IV secretion system gene cluster (T4SS) were detected in pEC488-KPC, which was defined as a putative conjugative plasmid.

Conclusion

To summarize, we have presented information on the genomic features of an *E. coli* strain belonging to the ST648 lineage in China, which carries both $bla_{\text{KPC-2}}$ and $bla_{\text{CTX-M-15}}$ genes. These findings will aid in enhancing comprehension

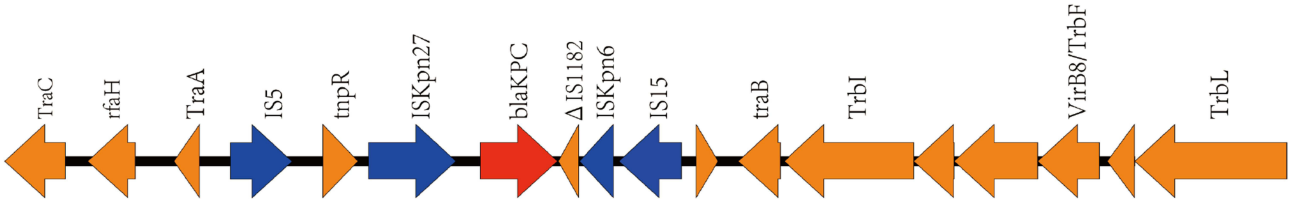

Figure 3 The genetic environment of the $bla_{\text{KPC-2}}$ gene on the plasmid pEC488. The red arrow represents the antimicrobial resistance gene $bla_{\text{KPC-2}}$, the Orange arrows signify other coding sequences (CDSs), and the blue arrows represent insertion sequences.

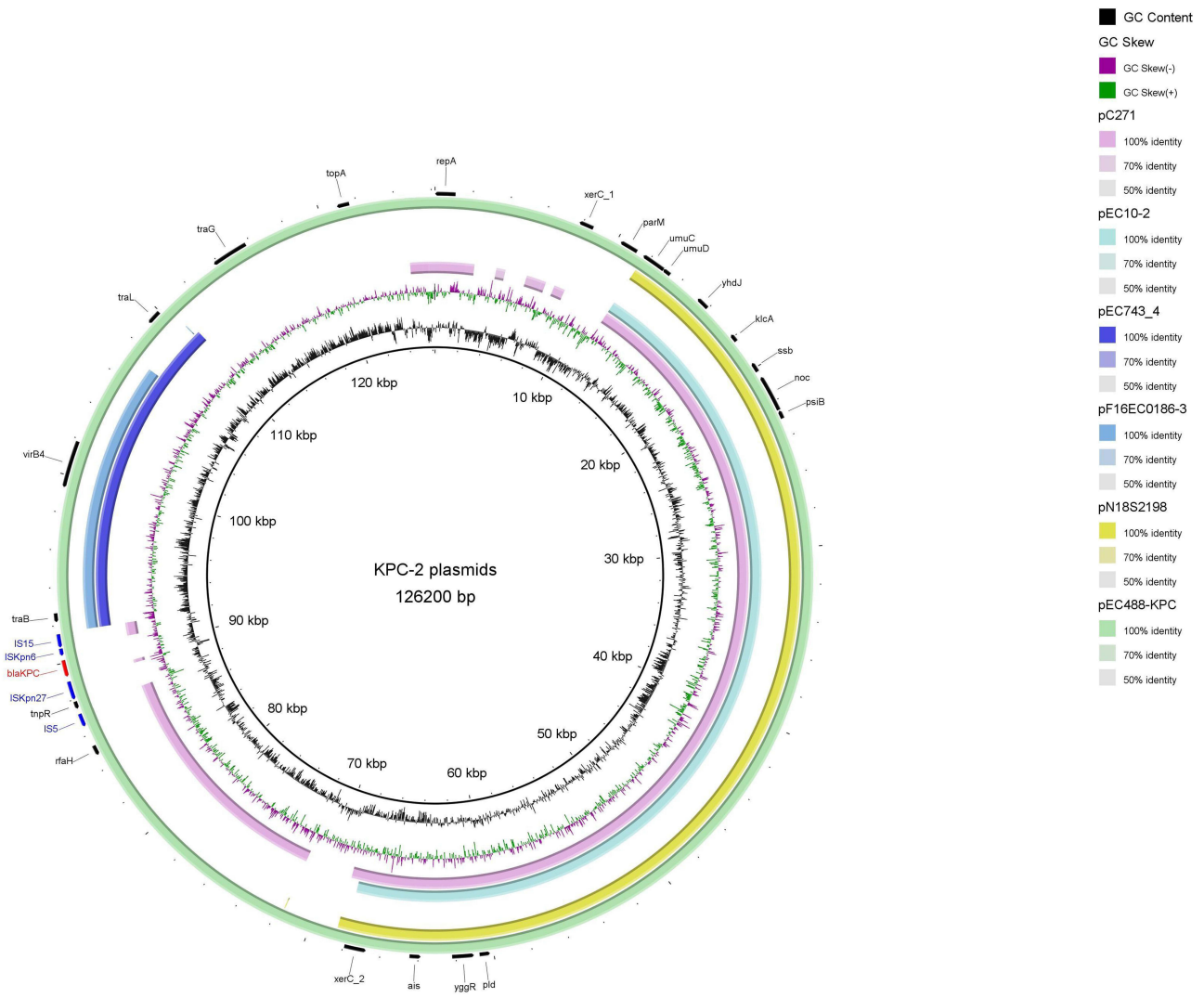

Figure 4 Circular comparative analysis of the *bla*$_{KPC-2}$ bearing plasmids characterized in this study and deposited in GenBank database. Antimicrobial resistance genes and insertion sequence elements were labeled at the outmost ring.

of the mechanisms underlying the multidrug resistant and transmission patterns of carbapenem-resistant *E. coli* in clinical settings. It is imperative to conduct global monitoring and prevention measures to curb its continued spread.

Ethics Approval and Consent to Participate

This study was conducted in accordance with the Declaration of Helsinki and obtained approval from the Medical Ethics Committee at the Third People's Hospital of Xiaoshan, Hangzhou, China (Ethics 2022-2). All isolates were the result of regular laboratory procedures, and no patient-identifiable information was acquired. Therefore, informed consent was not needed.

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Disclosure

The authors declare that they have no competing interests.

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