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Genome Note

Whole genome sequence of an *Escherichia coli* ST410 isolate co-harbouring bla_{NDM-5}, bla_{OXA-1}, $bla_{CTX-M-15}$, bla_{CMY-2}, *aac(3)-IIa* and *aac(6')-Ib*-cr genes isolated from a patient with bloodstream infection in China

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ABSTRACT

Objectives: Mortality associated with carbapenemase-producing Enterobacteriaceae is high as there are few therapeutic options. *Escherichia coli* sequence type 410 (ST410) is currently an international high-risk clone and is responsible for a large number of clinical infections. Here we report the draft genome sequence of a ST410 clinical *E. coli* isolate (ECS9) co-harbouring bla_{NDM-5}, bla_{OXA-1}, $bla_{CTX-M-15}$, bla_{CMY-2}, *aac(3)-IIa* and *aac(6')-Ib*-cr genes, obtained from a patient with bloodstream infection in China.
Methods: The genome of *E. coli* ECS9 was sequenced using an Illumina HiSeq™ 4000 instrument with a 150-bp paired-end approach. Generated sequence reads were assembled using Velvet 1.2.10. Contigs were annotated using Rapid Annotation using Subsystem Technology (RAST), and further whole-genome sequence data analyses were performed.
Results: Escherichia coli ECS9 belongs to multilocus sequence typing (MLST) ST410. The total number of assembled bases was 4 935 145 bp, with 5077 protein-coding sequences. The presence of the bla_{NDM-5}, bla_{OXA-1}, $bla_{CTX-M-15}$ and bla_{CMY-2} genes was detected in addition to other antimicrobial resistance genes conferring resistance to fluoroquinolones, aminoglycosides, trimethoprim, sulfonamides and tetracyclines.
Conclusion: To our knowledge, this is the first report of an*E. coli* ST410 strain co-harbouring bla_{NDM-5}, bla_{OXA-1}, $bla_{CTX-M-15}$, bla_{CMY-2}, *aac(3)-IIa* and *aac(6')-Ib*-cr, obtained from a bloodstream infection in China. The presented genome sequence of carbapenemase-producing *E. coli* strain ST410 could provide further insight into the acquisition of multiple resistance genes by this successful lineage.

1. Introduction

Carbapenemase-producing Enterobacteriaceae (CPE) pose a serious global public-health threat as mortality associated with CPE invasive infections is high [1]. CPE are resistant to almost all β-lactams and most other antibiotics except tigecycline and colistin [2]. *Escherichia coli*, a facultative anaerobic Gram-negative bacterium, is one of the major life-threatening pathogens causing nosocomial infections. Notably, *E. coli* sequence type 410 (ST410) has been identified as an international high-risk clone causing a wide variety of clinical infections [3]. However, clinical isolates co-harbouring the bla_{NDM-5}, bla_{OXA-1}, $bla_{CTX-M-15}$, bla_{CMY-2}, *aac(3)-IIa* and *aac(6')-Ib*-cr genes are rarely described.

Here we describe the draft genome sequence of an *E. coli* ST410 strain (ECS9) isolated from a patient with bloodstream infection in China in order to understand antimicrobial resistance and pathogenic mechanisms in this strain.

2. Materials and methods

Escherichia coli strain ECS9 was obtained from a blood sample of a 70-year-old male with cerebral haemorrhage hospitalised in Jinhua Central Hospital (Jinhua, Zhejiang Province, China) in 2017. The strain was preliminarily identified as *E. coli* by matrix-assisted

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laser desorption/ionisation time-of-flight mass spectrometry (MALDI-TOF/MS) (Bruker Daltonik GmbH, Bremen, Germany) and was further confirmed by 16S rRNA gene sequencing analysis.

Antimicrobial susceptibility testing showed strain ECS9 was resistant to most antimicrobial agents, including ampicillin/sulbactam, aztreonam, ceftazidime, cefepime, ceftriaxone, cefotetan, piperacillin/tazobactam, ciprofloxacin, levofloxacin, trimethoprim/sulfamethoxazole, gentamicin, ertapenem, imipenem, meropenem, nitrofurantoin and tobramycin, but remained susceptible to colistin and tigecycline according to European Committee on Antimicrobial Susceptibility Testing (EUCAST) breakpoints for Enterobacteriaceae (http://www.eucast.org/clinical_breakpoints). Strain ECS9 was classified as multidrug-resistant according to the recently proposed international classification scheme [4].

In addition, whole-genome sequencing was performed to understand the human pathogenicity-associated genes in this strain. Genomic DNA (gDNA) was extracted using a DNeasy Blood and Tissue Kit (QIAGEN, Hilden, Germany) according to the manufacturer's instructions. The purity and concentration of gDNA were evaluated by agarose gel electrophoresis and using a Qubit fluorometer (Thermo Fisher Scientific). A DNA library was prepared using a Nextera XT DNA Library Preparation Kit (Illumina Inc., Cambridge, UK), and gDNA was sequenced on an Illumina HiSeq™ 4000 instrument (Illumina Inc.) with a 150-bp paired-end approach at a depth of approximately 200×. Raw reads of *E. coli* ECS9 were assembled into draft genomes using Velvet 1.2.10. Contigs were annotated using Rapid Annotation using Subsystem Technology (RAST), and further whole-genome sequence data analyses were performed using bioinformatics tools available from the Center for Genomic Epidemiology (http://www.genomicepidemiology.org/), i.e. ResFinder v.3.2, MLST v.2.0 (multi-locus sequence typing), ISfinder, VirulenceFinder v.2.0, PlasmidFinder v.2.0, pMLST v.2.0 and FimTyper v.1.0.

3. Results and discussion

The draft genome yielded 353 contigs with an N_{50} value of 72 604 bp and the total number of assembled bases was 4 935 145 bp. The overall GC content of *E. coli* strain ECS9 was 50.50%.

Using RAST analysis, 5077 open-reading frames (ORFs) and 98 RNA genes were predicted, 2211 of which could be functionally annotated. RAST server-based annotation of the whole genome described the distribution of subsystems in this strain. Proteins responsible for carbohydrate utilisation pathways (367 ORFs), amino acids and derivatives (312 ORFs), and cofactors, vitamins, prosthetic groups and pigments (174 ORFs) were abundant among the subsystem categories.

Analysis of the draft genome sequence demonstrated that carbapenem-resistant *E. coli* ECS9 strain belonged to ST410, whereas identification of plasmid replicons revealed that it carried IncQ1 and IncX3 plasmids, with an IncF sequence belonging to the IncFIA/FIB type. The genome also contained several insertion sequence (IS) elements, with the majority belonging to the IS*1*, IS*1380*, IS*200* and IS*5* families.

Using a 90% identity threshold, acquired antimicrobial resistance genes to aminoglycosides [*aph(6)-Id*, *aph(3″)-Ib*, *aadA5 and aac(6′)-Ib*-cr], β-lactams (*bla*$_{CMY-2}$, *bla*$_{CTX-M-15}$, *bla*$_{TEM-1B}$, *bla*$_{NDM-5}$ and *bla*$_{OXA-1}$), phenicols (*catB3*), fluoroquinolones [*aac(6′)-Ib*-cr], sulfonamides (*sul1* and *sul2*), tetracyclines (*tetB*), macrolides [*mdf(A)* and *mph(A)*] and trimethoprim (*dfrA17*) were identified.

In addition, analysis of the quinolone resistance-determining regions of the *gyrA*, *parC* and *parE* genes revealed the presence of multiple and diverse mutations in *gyrA* (S83L and D87N), *parC* (S80I) and *parE* (S458A). Mutations in *gyrA* and *parC* have been reported as major mechanisms of fluoroquinolone/quinolone resistance involving DNA gyrase and topoisomerase IV alterations and often associated with high-level quinolone resistance in Enterobacteriaceae.

In addition, *E. coli* strain ST410 was assigned in silico to serotype 08:H9, suggesting that the isolate belongs to clade C, which is a ST410 clade associated with humans and companion animals [5]. Potential virulence genes, including *lpfA* (long polar fimbriae) and *yidE* (mediator of hyperadherence), were detected. The isolate also harboured the ferrichrome and ferrous iron uptake operons (*fhuABCD* and *feoABCD*), the iron(III) dicitrate uptake operon (*fecRI-ABCDE*) as well as the enterobactin siderophore operon (*entABCDEFH*, *entS*, *fepABCDEG*, *fes* and *ybdZ*), suggesting that ST410 may have significant pathogenic potential.

In general, both the whole genome sequence and the results of bioinformatics analysis of ST410 *E. coli* strain ECS9 could provide further insight into the acquisition of multiple antimicrobial resistance genes by this successful lineage. It is important to monitor *E. coli* ST410 strains to prevent the emergence of pandrug-resistant isolates with epidemic potential.

4. Nucleotide sequence accession no

The complete genome sequence of the *E. coli* ST410 isolate reported here has been deposited at DDBJ/ENA/GenBank under the accession no. VBQE00000000. The version described in this paper is the first version VBQE00000000.

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Competing interests

None declared.

Ethical approval

Not required.

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