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Genomic analysis of two bacterial strains co-isolated from a urinary tract infection: NDM-1-producing *Enterobacter cloacae* accompanied by extended-spectrum β-lactamase-producing *Escherichia coli*

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ABSTRACT

Objectives: *Escherichia coli* and *Enterobacter cloacae* are two major pathogens causing urinary tract infection (UTI). Here we characterised the genome of an NDM-1-producing *E. cloacae* strain and an extended-spectrum β-lactamase (ESBL)-producing *E. coli* strain that were co-isolated from a UTI.
Methods: The genomes of *E. cloacae* strain EC32 and *E. coli* strain EC33 were sequenced using an Illumina HiSeq™ platform. The whole genome sequences were assembled using CLC Genomics Workbench and were annotated by the NCBI Prokaryotic Genome Annotation Pipeline (PGAP) server. Genomic analysis was further performed.
Results: The draft genome sequences of *E. cloacae* EC32 (ST66) and *E. coli* EC33 (ST1139) consisted of 89 contigs comprising 5 178 393 bp and 62 contigs comprising 5 057 666 bp, respectively. Including the bla_{NDM-1} gene, in total 13 resistance genes were identified in *E. cloacae* EC32 conferring resistance to β-lactams, rifampicin, phenicols, fosfomycin, macrolides, quinolones, sulfonamides and tetracycline. Nine resistance genes were identified in *E. coli* EC33 including the ESBL-encoding gene $bla_{CTX-M-27}$.
Conclusion: To our knowledge, this is the first report of genomic characterisation of NDM-1-producing *E. cloacae* and ESBL-producing *E. coli* co-isolated from a UTI in China. The two strains inhabiting in the same environment may allow the possibility of horizontal transfer of bla_{NDM-1} and $bla_{CTX-M-27}$, making clinical treatment even difficult. This report also sheds light on geographically distinct Enterobacteriaceae strains from China enabling a comparative analysis of NDM-1- or ESBL-producing strains.

Escherichia coli and *Enterobacter cloacae* are two major pathogens causing urinary tract infection (UTI). Extended-spectrum β-lactamase (ESBL)-producing *E. coli* have spread both in hospital- and community-acquired UTIs. Carbapenems are very effective antibiotics for treating infections due to ESBL-producing Enterobacteriaceae. However, acquisition of carbapenemases gives rise to resistance to all β-lactams including carbapenems. New Delhi metallo-β-lactamase (NDM) is one of the most important carbapenemases since its first report in 2009 [1]. Co-infection with ESBL-producing *E. coli* and NDM-producing *E. cloacae* causing UTI has been sporadically reported. Here we reported the draft genome sequences of an ESBL-producing *E. coli* and an NDM-producing *E. cloacae* that were co-isolated from a urine sample of a male inpatient in China.

A 50-year-old male patient diagnosed with intracranial haemorrhage and hypertension was hospitalised in a tertiary hospital in Zhejiang Province of China on 21 October 2017. During his hospitalisation, the patient underwent placement of an indwelling urinary catheter. ESBL-producing *E. coli* strain EC33 and NDM-producing *E. cloacae* strain EC32 were co-isolated from a urine sample on 22 November 2017. The strains were preliminarily identified using the VITEK® MS system (bioMérieux, Marcy-l'Étoile, France) and were further confirmed by 16S rRNA gene sequencing.

Antimicrobial susceptibility testing was performed using a VITEK®2 system (bioMérieux) with Gram-negative antimicrobial susceptibility testing card (AST-GN16; bioMérieux) and by Etest following the guidelines of the Clinical and Laboratory Standards Institute (CLSI). Minimum inhibitory concentrations (MICs) of

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Table 1
Antimicrobial resistance genes (ARGs) in isolates *Enterobacter cloacae* EC32 and *Escherichia coli* EC33.

ARG	Contig	Identity (%)	Position	Antimicrobial resistance category
E. cloacae EC32				
bla_{ACT-16}	EC32_contig_2	99.47	19369..20507	β-Lactams
bla_{NDM-1}	EC32_contig_103	100	2962..3774	β-Lactams
bla_{OXA-1}	EC32_contig_70	100	2735..3565	β-Lactams
bla_{TEM-1B}	EC32_contig_125	100	90..950	β-Lactams
arr-3	EC32_contig_70	100	1428..1880	Rifampicin
catB3	EC32_contig_70	99.84	1965..2597	Phenicols
fosA	EC32_contig_2	95.54	182386..182811	Fosfomycin
fosA3	EC32_contig_94	100	2784..3200	Fosfomycin
mph(A)	EC32_contig_83	100	90..995	Macrolides
aac(6')-Ib-cr	EC32_contig_54	100	24753..25337	Quinolones
qnrA1	EC32_contig_100	99.85	1403..2059	Quinolones
sul1	EC32_contig_70	100	25..864	Sulfonamides
tet(B)	EC32_contig_67	100	271..1476	Tetracycline
E. coli EC33				
aadA5	EC33_contig_67	100	1528..2316	Aminoglycosides
aph(3")-Ib	EC33_contig_85	100	3962..4765	Aminoglycosides
aph(6)-Id	EC33_contig_85	100	3126..3956	Aminoglycosides
$bla_{CTX-M-27}$	EC33_contig_88	100	451..1326	β-Lactams
dfrA17	EC33_contig_67	100	924..1397	Trimethoprim
mph(A)	EC33_contig_62	100	2597..3502	Macrolides
sul1	EC33_contig_67	100	2863..3702	Sulfonamides
sul2	EC33_contig_85	100	4826..5641	Sulfonamides
tet(A)	EC33_contig_85	100	873..2072	Tetracycline

tigecycline and colistin were determined by standard broth microdilution assay with fresh cation-adjusted Mueller–Hinton broth (Oxoid Ltd., Basingstoke, UK). The MICs of the two isolates are presented in Supplementary Table S1. Strain EC33 is a typical ESBL-producing *E. coli* that was resistant to third-generation cephalosporins but was susceptible to cefoxitin, β-lactam/β-lactamase inhibitor combinations and carbapenems. Strain EC32 is a carbapenem-resistant Enterobacteriaceae that was resistant to all β-lactams tested.

The genomes of strains EC32 and EC33 were sequenced using an Illumina HiSeq™ platform (Illumina Inc., San Diego, CA) following a 2 × 150-bp paired-end protocol. The whole genome sequences were assembled using CLC Genomics Workbench 10.0 software (QIAGEN, Valencia, CA) and were automatically annotated by the NCBI Prokaryotic Genome Annotation Pipeline (PGAP) server. Resistance genes of the isolate was analysed using the BacWGSTdb server [2]. Multilocus sequence typing (MLST), plasmids and virulence genes were analysed using MLST 2.0, PlasmidFinder 1.3 and VirulenceFinder 1.5, respectively. Further bioinformatic analysis, such as identification of insertion sequence (IS) elements and clustered regularly interspaced short palindromic repeat (CRISPR) sequences, were predicted using ISfinder and CRISPR-Finder, respectively [3,4].

The draft genome sequences of *E. cloacae* EC32 and *E. coli* EC33 consisted of 89 contigs comprising 5 178 393 bp and 62 contigs comprising 5 057 666 bp, respectively. In total, 4925 (strain EC32) and 4813 (strain EC33) protein-coding sequences were identified by the PGAP server. According to the MLST scheme, *E. cloacae* EC32 belongs to ST66 and *E. coli* EC33 belongs to ST1139. The genome of *E. coli* EC33 contains five questionable CRISPRs and several IS elements, with the majority belonging to the IS*1*, IS*3*, IS*4* and IS*5* families. The major IS elements in *E. cloacae* EC32 belong to the IS*3*, IS*5* and IS*66* families. Three plasmid replicons were identified in *E. cloacae* EC32 (IncFII, IncHI2 and IncHI2A) and another three in *E. coli* EC33 (ColBS512, IncFIA and IncFIB).

The antimicrobial resistance genes identified in the genome of the isolates are presented in Table 1. Thirteen resistance genes were identified in *E. cloacae* EC32, including genes encoding resistance to β-lactams (bla_{ACT-16}, bla_{NDM-1}, bla_{OXA-1} and bla_{TEM-1B}), rifampicin (*arr-3*), phenicols (*catB3*), fosfomycin (*fosA* and *fosA3*), macrolides [*mph(A)*], fluoroquinolones [*aac(6′)-Ib*-cr and *qnrA1*], sulfonamides (*sul1*) and tetracycline [*tet(B)*]. Nine resistance genes were identified in *E. coli* EC33 including the ESBL-encoding gene $bla_{CTX-M-27}$ (Table 1). Four virulence factors including *gad* (glutamate decarboxylase), *iha* (adherence protein), *sat* (secreted autotransporter toxin) and *vat* (vacuolating autotransporter toxin) were also identified in *E. coli* EC33.

Strains harbouring both carbapenemases and ESBLs have been reported previously [5]. In the current study, strain *E. cloacae* EC32 harboured bla_{NDM-1} but no ESBL gene, whereas strain *E. coli* EC33 harboured $bla_{CTX-M-27}$ ESBL gene but no carbapenemase gene. The two strains inhabiting in the same environment may allow the possibility for horizontal transfer of bla_{NDM-1} and $bla_{CTX-M-27}$. This may be one of the reasons for the emergence of Enterobacteriaceae co-producing carbapenemases and ESBLs, which makes clinical treatment very difficult. Furthermore, this report also sheds light on geographically distinct *E. cloacae* and *E. coli* strains from China enabling a comparative analysis of NDM-1- or ESBL-producing strains.

This Whole Genome Shotgun project has been deposited at DDBJ/EMBL/GenBank under the accession no. SORQ00000000 and SORR00000000. The version described in this paper is the first version.

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Competing interests

None declared.

Ethical approval

Not required.

Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.jgar.2019.04.007.

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