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Genome Note

Complete sequence of a new conjugative multidrug-resistance encoding IncFII/IncFIA/IncFIB plasmid carrying NDM-6 metallo-β-lactamase from pathogenic *Escherichia coli* sequence type 167 isolated from sewage in Norway

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ABSTRACT

Objectives: The aim of the current study was to determine the genomic map of resistance genes and to understand the potential for mobility of a new NDM-6-carrying plasmid from a pathogenic *Escherichia coli* strain. A complete and closed genome sequence of the *E. coli* strain was obtained by applying a combination of short-read Illumina and long-read Nanopore-based sequencing.

Methods: Isolation of *E. coli* was performed, using ECC CHROMagar™, and antibiotic sensitivity patterns were determined, using Sensititre™ EUVSEC3 plates. Whole-genome sequencing was performed, using Illumina MiSeq- and Oxford Nanopore MinION-based sequencing. Conjugation experiments were performed, using filter-mating and a green fluorescent protein (GFP)-tagged *E. coli* strain.

Results: Two carbapenem-resistant *E. coli* strains were isolated from sewage. These strains (2–331 and 2–333) belonged to sequence type (ST) 167 and carried an NDM-6 carbapenemase. The complete genome of strain 2–331 (GenBank accession no.: CP110117–22.1) was assembled into six contigs, representing a complete circular chromosome of 4 947 178 bp and five plasmids, ranging from 143 596 bp to 1549 bp. Plasmid p2–331_1 (~144 kb), belonging to the IncFII/IncFIA/IncFIB group, carried multiple antibiotic resistance genes, including *mph(A), mrx(A), bla*$_{TEM-1}$, *rmtB1, bla*$_{NDM-6}$, *ble, sul1, qacE*Δ1, *aadA*Δ, *dfrA12*, and *tet*(A). Plasmid p2–331_1 was transferred from strain 2–331, via conjugation, conferring resistance against eight different classes of antibiotics to a GFP-tagged *E. coli* strain.

Conclusions: Our study showed the emergence of a new conjugative plasmid-carrying NDM-6 carbapenemase from pathogenic *E. coli* ST167 for the first time in Norway. The importance of population-based sewage-surveillance for understanding the antimicrobial resistance situation within the community is highlighted.

Carbapenemase-producing *Enterobacteriaceae* is an emerging global health problem [1]. Carbapenemases comprise a group of enzymes that hydrolyse almost all β-lactam antibiotics. The main clinically important carbapenemases are KPC, NDM, IMP, VIM, and OXA-48-like [2]. During the last decade, variants of the NDM carbapenem resistance gene have rapidly emerged among *Enterobacteriaceae*, usually in coexistence with other antibiotic resistance genes (ARGs) [3]. Although, the clinical prevalence of carbapenem resistance in Norway is low, the incidences of carbapenemases in *Enterobacteriaceae* are increasing [4]. In our previous study, we detected two NDM-6-producing *Escherichia coli* strains belonging to the high-risk clone sequence type (ST) 167, isolated

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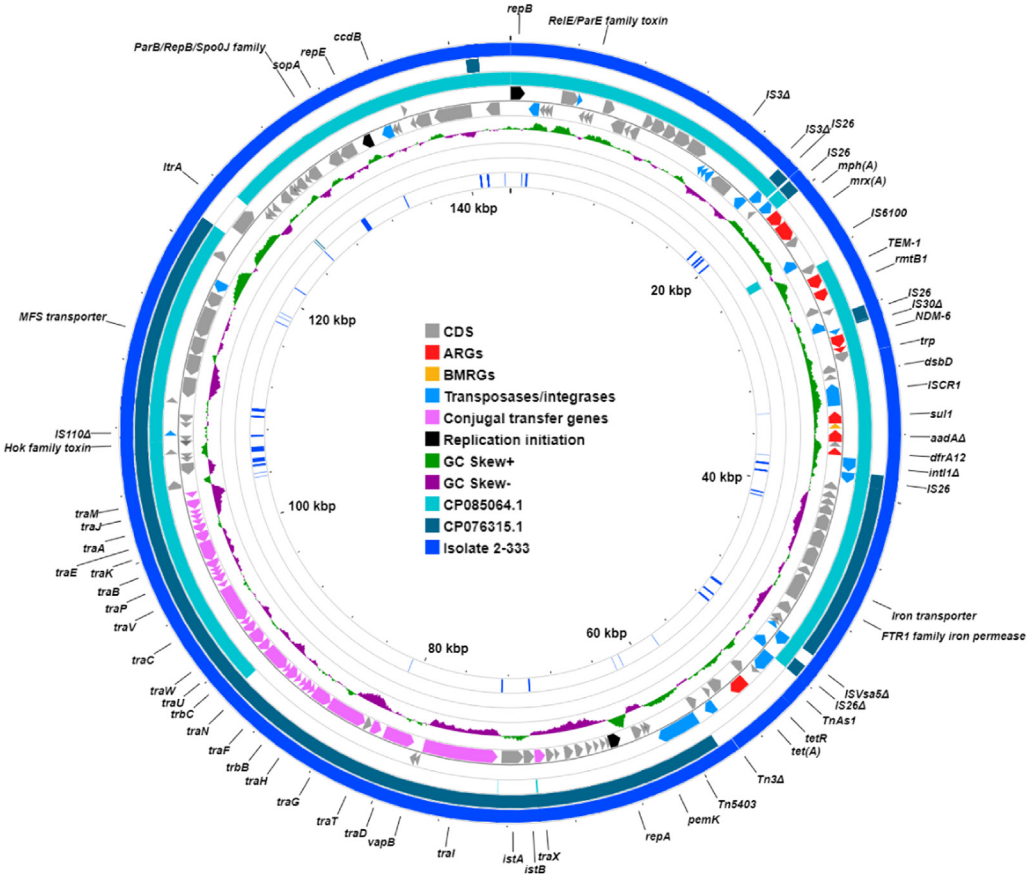

Fig. 1. Genomic map of plasmid p2–331_1 (GenBank accession number: CP110118.1); alignment with *Escherichia coli* plasmid pNDM_P31_YHI_02.21 (CP085064.1), *E. coli* plasmid pS045-CMY-42 (CP076315.1), and contigs from the draft genome sequence of isolate 2–333 (JADZIY000000000.1). Arrows indicate the sizes of the open reading frames and their orientations. Antibiotic resistance genes (ARGs) are highlighted in red; biocide/metal resistance genes (BMRGs), in orange; transposases/integrases, in blue; conjugal transfer genes, in purple; replication initiation, in black; and other genes, in grey. Δ represents truncated genes.

from sewage [5]. The aim of the current study was to determine the genomic map of resistance genes and to understand the potential for mobility of these genes, using a combination of short-read Illumina and long-read Oxford Nanopore sequencing to obtain a complete, closed genome sequences of NDM-6-carrying *E. coli* strain.

The *E. coli* strains 2–331 and 2–333 were isolated from raw sewage collected from a treatment plant in Bergen, Norway, in August 2020 [5]. The genomes of the two strains were sequenced, using short-read Illumina MiSeq, as described previously [5]. Single nucleotide polymorphism (SNP) comparative analysis of the whole-genome sequence (WGS), using CSI phylogeny v.1.4 (https://cge.food.dtu.dk/services/CSIPhylogeny/), indicated that strains 2–331 and 2–333 have a genome sequence difference of only seven SNPs and, thus, belong to the same clone. The strains exhibited resistance to 12 different antibiotics of eight different classes, including meropenem (Supplementary Table S1). For long-read sequencing, only strain 2–331 was selected and grown overnight on Mueller-Hinton agar containing 2 mg/L cefotaxime at 37°C. Whole-genome sequencing was carried out, using an Oxford Nanopore MinION device, and hybrid *de novo* genome assemblies combining the short Illumina and long Oxford Nanopore reads were performed, as previously described [6].

The genome was annotated, using Prokaryotic Genome Annotation Pipeline v.4.13 (https://www.ncbi.nlm.nih.gov/genome/annotation_prok/). The complete genome of *E. coli* strain 2–331 was assembled into six contigs (GenBank accession no.: CP110117–22.1), one representing a complete circular chromosome of 4 947 178 bp and five plasmids, ranging from 143 596 bp to 1549 bp (Supplementary Table S2).

Escherichia coli strain 2–331 belongs to ST167 (https://pubmlst.org/bigsdb?db=pubmlst_escherichia_seqdef&page=sequenceQuery), which represents a globally disseminated high-risk clone of significant public health interest [7].

Plasmid replicons were typed, using PlasmidFinder 2.0 (https://cge.food.dtu.dk/services/PlasmidFinder/), and a complete overview of the plasmid was obtained, using CGView (https://proksee.ca/). Strain 2–331 carried all ARGs, except for bla_{CMY-42}, on a mosaic plasmid p2–331_1 (143 596 bp, CP110118.1) carrying three different replicons, IncFII, IncFIA, and IncFIB (Fig. 1). Most of the ARGs were located between positions 19 149 bp–39 284 bp, flanked by complete IS26 transposases on either end. This region carried *mph(A)*, *mrx(A)*, bla_{TEM-1}, *rmtB1*, bla_{NDM-6}, *ble*, *sul1*, *qacE*Δ1, *aadA*Δ, and *dfrA12*, while *tet*(A) was located outside this multidrug-resistance (MDR) region. The bla_{NDM-6} gene was located in a highly conserved region (IS30Δ-bla_{NDM}-*ble-trpT*-IS26), which has been observed previously in NDM-carrying *E. coli* strains in Europe [8–9]. Moreover, the MDR region containing NDM-6 (positions 24 487 bp–52 043 bp; ~27 kb) was identical to the NDM-5-carrying plasmid pNDM_P31_YHI_02.21 (~134 kb) in *E. coli* (CP085064.1) reported from the UK (Fig. 1). The plasmid p2–331_1 carried three type II toxin-antitoxin systems, RelE/ParE, PemK/I, and CcdA/B, indicating potential for persistence.

Identical plasmids were not detected in the Nucleotide collection (nt) database. Nonetheless, the plasmid backbone (~64 kb) was similar to that of plasmid pS045-CMY-42 (~167 kb) from

E. coli (CP076315.1) reported from Germany. Alignment of contigs from the draft genome sequence of strain 2–333 showed the presence of plasmid p2–331_1, with 100% coverage and identity. *In vitro* conjugation experiments were performed in triplicate, using filter-mating and a green fluorescent protein (GFP)-tagged *E. coli* strain as recipient [10]. The plasmid p2–331_1 was transferred from strain 2–331 to a GFP-tagged *E. coli* strain at a conjugation frequency of 3×10^{-7} per recipient cells, with transfer of resistance against 12 antibiotics belonging to eight different classes (Supplementary Table S1), thus indicating the potential for spread of this NDM-6-carrying plasmid.

The $bla_{\text{CMY-42}}$ gene was located on an IncI plasmid p2–331_2 (72 901 bp), between positions 41 457 bp–43 752 bp, flanked by a complete IS1B transposase on one side. Plasmid p2–331_2 had three fragments (31 819 bp, 16 199 bp, and 14 020 bp) that show high sequence similarities (≥99% nucleotide identity) to segments of plasmid pS045-CMY-42 (∼167 bp) in *E. coli* (CP076315.1), reported from Germany.

We report here the complete sequence of a new conjugative plasmid-carrying $bla_{\text{NDM-6}}$ from pathogenic *E. coli* ST167 for the first time in Norway. Sewage-based surveillance showed the presence of NDM-6-carrying ST167 already in 2020 [5], this strain was reported a year later in the clinics in Norway in 2021 [4]. Thus, these findings emphasise the importance of population-based sewage surveillance for understanding the antibiotic resistance situation within the community before it becomes a problem in the clinics, especially in low-prevalence settings, such as Norway.

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Data availability: The assembled, complete genome sequence of *E. coli* 2–331 has been submitted to DDBJ/ENA/GenBank under the following genome accession numbers: CP110117–22.1. The assembled, draft genome sequence of *E. coli* 2–333 has been submitted to DDBJ/ENA/GenBank under following genome accession number: JADZIY000000000.1. The raw sequencing data have been deposited in the Sequence Read Archive under the BioProject accession no. PRJNA681451.

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.jgar.2023.04.005.

References

[1] WHO. World Health Organization (WHO) publishes list of bacteria for which new antibiotics are urgently needed 2017, https://www.who.int/news/item/27-02-2017-who-publishes-list-of-bacteria-for-which-new-antibiotics-are-urgently-needed; 2022 [accessed 18.11.22].
[2] Nordmann P, Dortet L, Poirel L. Carbapenem resistance in *Enterobacteriaceae:* here is the storm!. Trends Microbiol 2012;18(5):263–71. doi:10.1016/j.molmed.2012.03.003.
[3] Wu W, Feng Y, Tang G, Qiao F, McNally A, Zong Z. NDM metallo-β-lactamases and their bacterial producers in health care settings. Clin Microbiol Rev 2019;32(2):e00115–18. doi:10.1128/CMR.00115-18.
[4] NORM/NORM-VET Usage of antimicrobial agents and occurrence of antimicrobial resistance in Norway in 2021, Tromsø/Oslo: NIPH; 2022. 2022.
[5] Grevskott DH, Ghavidel FZ, Svanevik CS, Marathe NP. Resistance profiles and diversity of β-lactamases in *Escherichia coli* strains isolated from city-scale sewage surveillance in Bergen, Norway mimic clinical prevalence. Ecotoxicol Environ Saf 2021;226:112788. doi:10.1016/j.ecoenv.2021.112788.
[6] Grevskott DH, Serra-Salvà F, Moore ERB, Marathe NP. Nanopore sequencing reveals genomic map of CTX-M-type extended-spectrum β-lactamases carried by *Escherichia coli* strains isolated from blue mussels (*Mytilus edulis*) in Norway. BMC Microbiol 2020;20(1):1–10. doi:10.1186/s12866-020-01821-8.
[7] Manges AR, Geum HM, Guo A, Edens TJ, Fibke CD, Pitout JDD. Global extraintestinal pathogenic *Escherichia coli* (ExPEC) lineages. Clin Microbiol Rev 2019;32(3) e00135–18. doi:10.1128/CMR.00135-18.
[8] Chakraborty T, Sadek M, Yao Y, Imirzalioglu C, Stephan R, Poirel L, et al. Cross-border emergence of *Escherichia coli* producing the carbapenemase NDM-5 in Switzerland and Germany. J Clin Microbiol 2021;59(3):e02238–20. doi:10.1128/JCM.02238-20.
[9] Turton JF, Pike R, Perry C, Jenkins C, Turton JA, Meunier D, et al. Wide distribution of *Escherichia coli* carrying IncF plasmids containing $bla_{\text{NDM-5}}$ and *rmtB* resistance genes from hospitalized patients in England. J Med Microbiol 2022;71:001569. doi:10.1099/jmm.0.001569.
[10] Jutkina J, Marathe NP, Flach C-F, Larsson DGJ. Antibiotics and common antibacterial biocides stimulate horizontal transfer of resistance at low concentrations. Sci Total Environ 2018;616–7:172–8. doi:10.1016/j.scitotenv.2017.10.312.