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Genome Note

Draft genome sequence of a bla_{NDM-1}- and $bla_{OXA-244}$-carrying multidrug-resistant *Escherichia coli* D-ST69 clinical isolate from Egypt

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ABSTRACT

Objectives: This study describes the first draft genome sequence of a multidrug-resistant (MDR) *Escherichia coli* D-ST69 clinical isolate from Egypt carrying bla_{NDM-1} and $bla_{OXA-244}$.
Methods: The strain was isolated in December 2014 from a wound pus swab of a male patient in the city of Kafr El-Sheikh using MacConkey agar containing 2 μg/mL meropenem. The strain was subjected to antimicrobial susceptibility testing, conjugation experiments, and whole-genome sequencing using an Illumina MiSeq platform.
Results: The draft genome of the strain (HR14_AS) was 5.08 Mbp in size containing a total of 90 contigs encoding 4677 predicted genes with an average G+C content of 50.7%. Strain HR14_AS belongs to sequence type 69 (ST69), phylogroup D and exhibits an MDR phenotype, with minimum inhibitory concentrations (MICs) of 64 μg/mL and 32 μg/mL for meropenem and doripenem, respectively. Multiple acquired antimicrobial resistance genes conferring resistance to macrolides [*mdf(A)*], fluoroquinolones [*aac(6')-Ib*-cr], quinolones (*qnrS1*), trimethoprim (*dfrA14*), β-lactams (bla_{NDM-1}, $bla_{OXA-244}$, $bla_{CTX-M-15}$, bla_{OXA-9} and bla_{TEM-1B}) and aminoglycosides [*aac(3)-IId*, *aac(6')-Ib*, *aadA1* and *aph(3')-VI*] were detected. The $bla_{OXA-244}$ and bla_{NDM-1} genes were located on the chromosome (Tn*6237*) and on an IncI1-type self-conjugative plasmid of >93 kb in size, respectively.
Conclusions: Here we report the first draft genome sequence of a MDR *E. coli* D-ST69 isolate carrying bla_{NDM-1} and $bla_{OXA-244}$. Besides clonal expansion of the *E. coli* ST38 pandemic clone, this study further identified that the spread of OXA-244-producing *E. coli* could be related to mobilisation of the IS*1R*-made composite transposon (Tn*6237*) carrying $bla_{OXA-244}$.

The Ambler class D OXA-244 carbapenemase is a variant of OXA-48 with a single (Arg214Gly) substitution that results in decreased carbapenemase activity [1]. OXA-244 was initially reported in a clinical *Klebsiella pneumoniae* isolate recovered in Malaga, Spain, in 2012 [2]. It has since been reported from many other countries, including Germany, Russia, France, Egypt, the UK, the Netherlands, Turkey, Colombia, Algeria and Lebanon [1,3]. The $bla_{OXA-244}$ gene has been detected on a plasmid in *K. pneumoniae* and *Enterobacter aerogenes* as well as integrated into the chromosome of *Escherichia coli* sequence type 38 (ST38) [1,3]. OXA-244-producing *E. coli* do not grow on ChromID® Carba Smart plates because of the reduced carbapenemase activity of OXA-244 [1]. They are therefore difficult to detect, leading to silent dissemination [1]. The aim of this work was to describe the first

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draft genome sequence of a bla_{NDM-1}- and $bla_{OXA-244}$-carrying multidrug-resistant (MDR) *E. coli* D-ST69 clinical isolate from Egypt.

Isolate HR14_AS was recovered from a wound pus swab of a male patient admitted to the orthopaedic department of a hospital in the city of Kafr El-Sheikh, Egypt, on 10 December 2014. The isolate was selected on MacConkey agar supplemented with 2 μg/mL meropenem and was identified using an API 20E system (bioMérieux, Marcy-l'Étoile, France) followed by PCR sequencing of the small subunit ribosomal RNA (16S rRNA) gene as previously described [4]. Antimicrobial susceptibility testing was performed by the disk diffusion assay and broth microdilution method and the results were interpreted in accordance with the 2018 guidelines of the Clinical and Laboratory Standards Institute (CLSI). Isolate HR14_AS showed resistance to meropenem [minimum inhibitory concentration (MIC) = 64 μg/mL], doripenem (MIC = 32 μg/mL), aztreonam, ampicillin, amikacin, amoxicillin/clavulanic acid, cefotaxime, ceftazidime, ceftriaxone, cefoxitin, gentamicin, nalidixic acid, ciprofloxacin, kanamycin and sulfamethoxazole/trimethoprim but remained susceptible to fosfomycin, tigecycline, chloramphenicol and colistin (MIC < 0.5 μg/mL). The isolate was also positive for carbapenemase production by the modified carbapenem inactivation method [4].

Subsequently, PCR and DNA sequencing were used to screen for carbapenemase-encoding genes, extended-spectrum β-lactamase (ESBL) genes, plasmid-mediated quinolone resistance genes, integrons, mobile colistin resistance genes (*mcr-1* and *mcr-8*) and 16S rRNA methylase genes as well as for *E. coli* phylogrouping as previously reported [4]. The results demonstrated that HR14_AS carried bla_{NDM-1}, $bla_{OXA-244}$, $bla_{CTX-M-15}$, bla_{TEM-1B} and *qnrS* and was assigned to phylogroup D.

Total genomic DNA was extracted from an overnight culture of the isolate using a GenElute™ Bacterial Genomic DNA Kit (Sigma–Aldrich, St Louis, MO, USA). Sequencing libraries were constructed using a Nextera XT Library Preparation Kit, and paired-end sequencing was performed with an Illumina MiSeq system (Illumina Inc.) using a 500-cycle MiSeq Reagent Kit. Genomes were assembled using the A5-miseq pipeline. The draft genome of HR14_AS was 5.08 Mbp in size containing a total of 90 contigs encoding 4677 predicted genes with an average G+C content of 50.7% and a medium genome depth coverage of 70×, with an N_{50} of 250 881 bp. Multilocus sequence typing (MLST) using MLST 2.0 software (https://cge.cbs.dtu.dk/services/MLST/) assigned the isolate to ST69. To our knowledge, this is the first report of a bla_{NDM-1}- and $bla_{OXA-244}$-carrying MDR *E. coli* D-ST69 globally. ST38 was previously identified as the common sequence type associated with clonal expansion of OXA-244-producing *E. coli* in European countries [3,5,6]. However, other sequence types of OXA-244-producing *E. coli* were detected in Egypt and Algeria (ST361 and ST3541), respectively [1,3].

SerotypeFinder 2.0 (https://cge.cbs.dtu.dk/services/SerotypeFinder/) and FimTyper 1.0 (https://cge.cbs.dtu.dk/services/FimTyper/) were used to analyse the serotype and *fimH* subtype, respectively, and identified the O17:H18-*fimH*27 profile. Furthermore, VirulenceFinder 2.0 (https://cge.cbs.dtu.dk/services/VirulenceFinder/) identified virulence genes including *air*

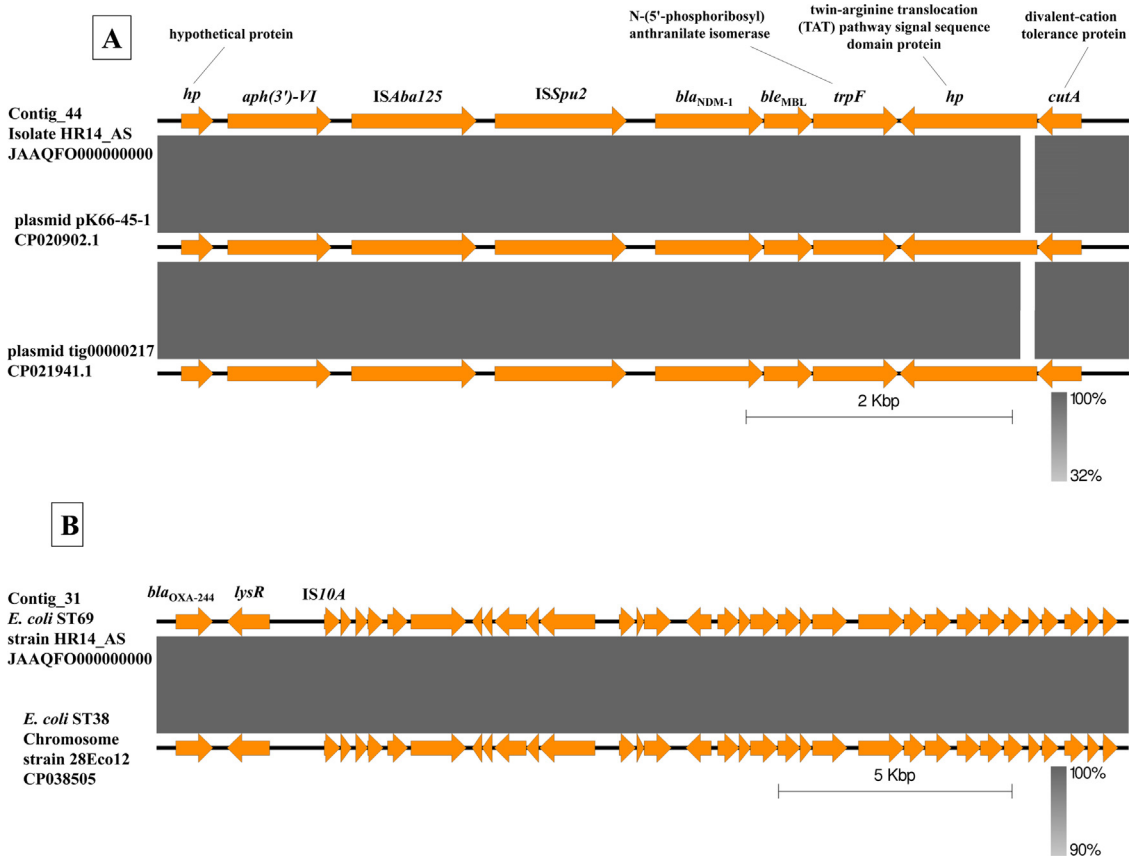

Fig. 1. (A) Schematic representation of the genetic environment of bla_{NDM-1} identified from the draft genome sequence of *Escherichia coli* strain HR14_AS analysed in this study, including linear comparison with plasmid tig00000217 (accession no. CP021941) in *Klebsiella pneumoniae* strain AR_0145 from the USA and plasmid pK66-45-1 (accession no. CP020902) in clinical *K. pneumoniae* strain K66-45 from Norway. (B) Linear comparison of the genetic environment of $bla_{OXA-244}$ identified from *E. coli* strain HR14_AS analysed in this study and the chromosome of a clinical *E. coli* ST38 strain 28Eco12 (accession no. CP038505) isolated in Colombia. The genetic environment of bla_{NDM-1} and $bla_{OXA-244}$ was *aph*(3')-*VI*–IS*Aba125* (IS*30* family)–IS*Spu2* (IS*630* family)–bla_{NDM-1}–ble_{MBL} (A) and IS*1R* (IS*1* family)–$bla_{OXA-244}$–*lysR*–IS*10A* (IS*4* family) (Tn*6237*) (B), respectively. The figure was drawn using Easyfig (http://mjsull.github.io/Easyfig/).

(enteroaggregative immunoglobulin repeat protein), *eilA* (*Salmonella* HilA homologue), *gad* (glutamate decarboxylase) and *lpfA* (long polar fimbriae). ResFinder 3.2 (https://cge.cbs.dtu.dk/services/ResFinder/) identified a diversity of acquired antimicrobial resistance genes conferring resistance to macrolides [*mdf(A)*], fluoroquinolones [*aac(6')-Ib*-cr], quinolones (*qnrS1*), trimethoprim (*dfrA14*), β-lactams (bla_{NDM-1}, $bla_{OXA-244}$, $bla_{CTX-M-15}$, bla_{OXA-9} and bla_{TEM-1B}) and aminoglycosides [*aac(3)-IId*, *aac(6')-Ib*, *aadA1* and *aph(3')-VI*].

PlasmidFinder 2.0 (https://cge.cbs.dtu.dk/services/PlasmidFinder/) identified four different incompatibility groups, including IncI1-Iγ, IncFII(K), IncR and IncY. Conjugation experiments were performed using azide-resistant *E. coli* J53 as recipient as described previously [4]. Transconjugants were selected on Luria–Bertani agar supplemented with sodium azide (100 μg/mL) and ampicillin (100 μg/mL). Several transconjugants were selected for colony PCR and plasmid analysis as described previously [4]. The NDM-positive *E. coli* transconjugant with only one plasmid was selected. It was resistant to meropenem (MIC = 32 μg/mL), doripenem (MIC = 8 μg/mL), amoxicillin/clavulanic acid, aztreonam, ceftazidime, ceftriaxone, cefotaxime, cefoxitin and gentamicin. The bla_{NDM-1} gene was located on an IncI1-Iγ-type self-conjugative plasmid of >93 kb in size as determined by PCR-based replicon typing [4]. Furthermore, bla_{NDM-1}, $bla_{CTX-M-15}$, bla_{TEM-1B} and *qnrS1* genes were co-transferred, indicating that they were located on the same conjugative IncI1 plasmid, which belonged to ST37-CC3 as detected by pMLST (https://cge.cbs.dtu.dk/services/pMLST/). However, the $bla_{OXA-244}$ gene was not successfully transferred by conjugation, transformation or electroporation, suggesting its chromosomal location.

A BLASTn (https://blast.ncbi.nlm.nih.gov/Blast.cgi) search in combination with identification of insertion sequence (IS) elements using ISFinder (https://www-is.biotoul.fr/blast.php) illustrated the genetic environment of bla_{NDM-1} and $bla_{OXA-244}$, which was *aph(3')-VI*–IS*Aba125* (IS*30* family)–IS*Spu2* (IS*630* family)–bla_{NDM-1}–ble_{MBL} (Fig. 1A) and IS*1R* (IS*1* family)–$bla_{OXA-244}$–*lysR*–IS*10A* (IS*4* family) (Tn*6237*) (Fig. 1B) for bla_{NDM-1} and $bla_{OXA-244}$, respectively.

A BLASTn search using the whole bla_{NDM-1} contig identified similar sequences from plasmid tig00000217 (GenBank accession no. CP021941) in *K. pneumoniae* strain AR_0145 from the USA and plasmid pK66-45-1 (GenBank accession no. CP020902) in clinical *K. pneumoniae* strain K66-45 from Norway (100% query coverage and 99.99% sequence identity) (Fig. 1A). Moreover, a BLASTn search using the whole $bla_{OXA-244}$ contig identified an identical sequence from the chromosome of clinical *E. coli* ST38 strain 28Eco12 (GenBank accession no. CP038505) isolated in Colombia (100% query coverage and 100% sequence identity) (Fig. 1B) [7]. These results suggest the probable activity of Tn*6237* transposon by its movement to different *E. coli* clones or different sites in the chromosome of *E. coli*.

In conclusion, here we report the first draft genome sequence of a bla_{NDM-1}- and $bla_{OXA-244}$-carrying MDR *E. coli* D-ST69 globally. Besides clonal expansion of the *E. coli* ST38 pandemic clone, this study further identified that the spread of OXA-244-producing *E. coli* could be related to mobilisation of the IS*1R*-made composite transposon (Tn*6237*) carrying $bla_{OXA-244}$.

The draft genome sequence of *E. coli* strain HR14_AS has been deposited at DDBJ/ENA/GenBank under BioProject no. PRJNA612707 and the accession no. is JAAQFO000000000.

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Conflict of interest

None declared.

Ethical approval

Not required.

References

[1] Hoyos-Mallecot Y, Naas T, Bonnin RA, Patino R, Glaser P, Fortineau N, et al. OXA-244-producing *Escherichia coli* isolates, a challenge for clinical microbiology laboratories. Antimicrob Agents Chemother 2017;61: e00818-17.
[2] Oteo J, Hernández JM, Espasa M, Fleites A, Sáez D, Bautista V, et al. Emergence of OXA-48-producing *Klebsiella pneumoniae* and the novel carbapenemases OXA-244 and OXA-245 in Spain. J Antimicrob Chemother 2013;68:317–21.
[3] European Centre for Disease Prevention and Control (ECDC). OXA-244-producing *Escherichia coli* in the european Union/European economic area and the UK since 2013—18 February 2020. Stockholm, Sweden: ECDC; 2020.
[4] Soliman AM, Zarad HO, Nariya H, Shimamoto T, Shimamoto T. Genetic analysis of carbapenemase-producing Gram-negative bacteria isolated from a university teaching hospital in Egypt. Infect Genet Evol 2020;77:104065.
[5] Potron A, Poirel L, Dortet L, Nordmann P. Characterisation of OXA-244, a chromosomally-encoded OXA-48-like β-lactamase from *Escherichia coli*. Int J Antimicrob Agents 2016;47:102–3.
[6] Findlay J, Hopkins KL, Loy R, Doumith M, Meunier D, Hill R, et al. OXA-48-like carbapenemases in the UK: an analysis of isolates and cases from 2007 to 2014. J Antimicrob Chemother 2017;72:1340–9.
[7] Abril D, Bustos Moya IG, Marquez-Ortiz RA, Josa Montero DF, Corredor Rozo ZL, Torres Molina I, et al. First report and comparative genomics analysis of a $bla_{OXA-244}$-harboring *Escherichia coli* isolate recovered in the American continent. Antibiotics (Basel) 2019;8:222.