Draft genome sequence of a multidrug-resistant CTX-M-15 β-lactamase-producing uropathogenic Escherichia coli isolate (ST131-O25b-H30) from Pakistan exhibiting high potential virulence

Draft genome sequence of an MDR, CTX-M-15 *β*-lactamase producing Uropathogenic *Escherichia coli* (UPEC) isolate (ST131-O25b-H30) from Pakistan exhibiting high potential virulence

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ABSTRACT

Objectives: Uropathogenic *Escherichia coli* (UPEC) is the leading cause of urinary tract infections (UTI). The pandemic ST131 clonal type is associated with multidrug resistance and virulence. We report the first draft genome of an ST131-O25b-H30 strain from Pakistan, isolated from a patient with community-acquired UTI.

Methods: Next-generation sequencing was performed using MiSeq and HiSeq 2500 platforms. De novo assembly of the reads was performed using SPAdes (v 3.11.). The genomic features were determined with PATRIC and RAST tool kit.

Results: The 5,327,975 bp draft genome sequence has 5433 CDS and 82 tRNAs, an array of resistance genes (*bla*$_{\text{CTXM-15}}$, *bla*$_{\text{OXA-1}}$, *bla*$_{\text{CMY-2}}$, *sul2, catB, dfrA17, mph*(*A*)), one class 1 integron, 77 IS elements, one transposon (Tn*3*-like), multiple virulence markers and seven intact prophage loci.

Conclusion: In conclusion, the genome sequence of this new UPEC isolate from Pakistan provides novel insight into the genetic attributes of an epidemic clone associated with high level of resistance and virulence.

Keywords: ST131, UPEC, MDR, virulence, Pakistan, whole-genome

Uropathogenic *Escherichia coli* (UPEC) is the major etiological agent of urinary tract infections (UTI) in females and is responsible for 80-90% of community acquired UTI (CA-UTI). UPEC isolates display increasing resistant to all frontline antibiotics deployed to treat UTIs. ST131 is an intriguing sequence type of extra-intestinal pathogenic *E. coli* (EXPEC) that is not only multidrug resistant (MDR) but also possesses an arsenal of virulence factors [1]. This alarming combination imposes a major challenge for treatment of UTI. Only two studies have reported on ST131 prevelence in Pakistan, giving 18 or 46% of *E. coli* isolates from blood and UTIs, or UTIs, respectively, as ST131 strains; in both cases, a strong association with the presence of CTX-M-15 type extended-spectrum-β-lactamases (ESBL) was found [2, 3]. Approximately half of the ST131 isolates were designated as ST131-O25b-H30-R and were fluoroquinolone resistant [3]. The global prevalence of CA-UTI is 0.7% [4], and although UTI frequency is not known for Pakistan it is reported that 65.5 to 85% of UPEC isolates from CA-UTI in Pakistan are multidrug resistant (MDR) [5,6]. Whole-genome sequencing of UPEC strains gives insight on resistance, virulence and plasmid profile, however, as yet, no genome sequence of a UPEC strain from Pakistan is available. This report sheds light on a geographically-distinct UPEC strain from Pakistan enabling a comparative genomic analysis with other strains.

UEC11 is a ST131 UPEC strain collected from a 23 year old female patient (Islamabad, Pakistan) suffering from a CA-UTI. Antimicrobial susceptibility testing performed in accordance with CLSI guidelines, revealed that UEC11 is ESBL-producing and resistant to ampicillin, cephalosporins, gentamicin, fluoroquinolones and trimethoprim. This characterises UEC11 as an MDR strain. Phenotypic assays showed that UEC11 is non-hemolytic, strongly adherent, biofilm producing and serum resistant. Invasion assays in the uroepithelial cell-line ATCC HTB5637 showed that UEC11

is moderately invasive giving similar levels of invasion (data not shown) to those previously reported for other UPEC strains e.g. NA101 (an ST131 strain from India) [7] the ST131 strains UT18 and UT188 (from the UK) [8] and EC958 (from Australia) [9].

Next-generation sequencing was performed (MicrobesNG, University of Birmingham) on MiSeq and HiSeq 2500 platforms and a sequence coverage of 30X was obtained. De novo assembly of the reads was performed using SPAdes (v 3.11.) genome assembler based on de Bruijn graph assembler. The genome assemblies were evaluated, and the metrics were calculated using Quality Assessment Tool for Genome Assemblies (QUAST). The genomic features were determined with Pathosystems Resources Integration Center (PATRIC; https://www.patricbrc.org/), and insertion elements (IS) and integrated and conjugative elements (ICE) were determined with IS finder (https://isfinder.biotoul.fr/) and ICEberg (http://db-mml.sjtu.edu.cn/ICEberg/) . Altogether 144 contigs were obtained after assembly providing a total genome size of 5,327,975 bp and a GC content of 50.72%. The genomic annotation indicated 5433 CDS and 82 tRNA genes. A yersiniabactin synthesis-associated ICE (highly similar to ICEEcoUMN026-1 of UPEC UMN026) of 65,732 bp was also identified. A total of 77 IS elements (belonging to 18 families) were identified. IS3, ISL3, IS200 and IS66 families were those predominantly present.

Analysis of the *adk*, *fumC*, *gyrB*, *icd*, *mdh*, *purA* and *recA* alleles using the Atchman database (https://pubmlst.org/mlst/), confirmed that UEC11 is an ST131 strain. Serotyping was performed by SerotypeFinder (https://cge.cbs.dtu.dk/services/SerotypeFinder/) and FimTyper webtool (https://cge.cbs.dtu.dk/services/FimTyper/) which identified UEC11 as H4-O25b sub-clone H-30, indicating that it is an epidemiologically virulent and MDR strain. Four plasmids were identified

using PLASMIDFinder (https://cge.cbs.dtu.dk//services/PlasmidFinder/) corresponding to incompatibility groups IncFII, IncFIA, IncFIB and Col156. Genome annotation performed using PATRIC and CARD (https://card.mcmaster.ca/analyze/rgi), indicated three β-lactamases: CTXM-15 (class A); OXA-1 (class D); and AmpC (class C). Additionally, genes conferring resistance to tetracycline (*tetA*), chloramphenicol (*catB*), fluoroquinolones (*gyrA* mutations; S83L, D87N), aminoglycosides (*aac(3')-IIc, aac(6')-Ib/aac(6')-II, aph(3'')-Ia*), sulfonamide (*sul2*), trimethoprim (*dfrA17*) and macrolides (*mph(A)*) were identified, explaining the observed ESBL and MDR status. A Tn*3* transposon (carrying a tetracycline resistance gene) and a class 1 integron (carrying a trimethoprim resistance gene), were also identified. UEC11 also encodes a wide array of virulence genes that explains its virulent and invasive nature. Such virulence genes include those encoding adhesins and invasins (*fimH, papGI, papGII, papGIII, papC, papH*), toxins (*cnf1*, RTX, *espC*) and a hypothetical enterotoxin similar to *senB* of *Shigella flexneri* 2a str. 30. UEC11 also encodes various iron-acquisition systems: uptake and synthesis of enterobactin, aerobactin and yersinibactin; uptake of heme; and uptake of ferrous iron (Feo and Efe). The serum-resistance gene (*traT*) and *csgB* (encoding curlin) required for biofilm formation were also identified. Phenotypic detection of virulence factors correlated well with whole genome results. The UEC11 genome sequence most closely matched that of the UPEC urosepsis strain JJ1886 (isolated in the USA in 2007; [10]) and this strain also had highly similar MDR- and virulence-gene profiles. Seven intact (PHAGE_Gordon_Kita, 27.1Kb; PHAGE_Entero_BP, 29.4Kb; PHAGE_Burkho_phiE255, 36.8Kb; PHAGE_Entero_P88, 41.2Kb; PHAGE_Entero_lambda, 28.4Kb; PHAGE_Yersin_L_413C, 33.9Kb; PHAGE_Entero_BP, 25.7kb) and one incomplete (PHAGE_Entero_BP_4795, 26.5kb) prophage regions were detected using PHASTER

(http://phaster.ca/). CRISPRcasfinder showed no strong evidence for a Cas-CRISPR locus (http://crispr.i2bc.paris-saclay.fr/).

In conclusion, the genome sequence of this new UPEC isolate enables novel insight into the genetic characteristics of a virulent CTXM-15 producing ST131-H4:O25b-H30 strain isolated from a patient in Pakistan.

This Whole Genome Shotgun project has been deposited at GenBank under the accession QICE00000000. The version described in this paper is version QICE01000000.

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Declarations

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Competing Interests: We declare that there is no conflict of interest.

Ethical Approval: Not required

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