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Genome Note

Draft genome sequence of *Escherichia coli* M51-3: a multidrug-resistant strain assigned as ST131-*H*30 recovered from infant diarrheal infection in Mexico

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ABSTRACT

Objectives: In this study, we report the draft genome sequence of a multidrug-resistant (MDR)*Escherichia coli* strain recovered from stool sample of an outpatient infant girl with acute diarrheal infection in Mexico.
Methods: Antimicrobial susceptibility testing and PCR-based detection of diarrheagenic *E. coli* (DEC) were performed. In addition, genomic DNA from *E. coli* strain M51-3 was sequenced using Ion Torrent PGM platform with 200-bp chemistry and generated reads were de novo assembled using SPAdes v3.11. The draft genome was annotated and analyzed regarding multilocus sequence typing (MLST), serotyping, *fimH* typing, plasmid replicons, acquired antimicrobial resistance and virulence genes using web tools available at the Center for Genomic Epidemiology.
Results: A draft genome comprising 5 088 545 bp in length and 5308 protein-coding sequences was generated. In silico typification revealed that *E. coli* strain M51-3 belongs to ST131-O25:H4-*H*30 pandemic subclone. Several genes associated with resistance to β-lactams [bla_{TEM-1B}], aminoglycosides [*aph(3")-Ib*, *aadA5*, *aph(6)-Id* and *aac(3)-IId*], sulfonamides [*sul1* and *sul2*], trimethoprim [*dfrA17*], and tetracycline [*tet(A)*] were identified. Besides, point mutations in *gyrA*, *parC*, and *parE* genes were detected. Interestingly, the enterotoxin-coding virulence gene *senB* was evidenced.
Conclusions: To our knowledge, this is the first draft genome of an *E. coli* ST131-O25:H4-*H*30 strain recovered from infant diarrheal stool sample in Mexico. The genome sequence of *E. coli* M51-3 presented here will be helpful to understand the genomic diversity of this highly virulent and MDR successfully pandemic bacterial pathogen.

1. Introduction

Escherichia coli is recognized to be one of the major pathogens causing both community and healthcare-associated infections (HAIs). Presently, antibiotic-resistant *E. coli* infections already account for one half of the estimated global burden of antimicrobial resistance [1], and its increase has been established as a consequence of the emergence and dissemination of multidrug-resistant (MDR) single clones associated to sequence types (STs) ST393, ST69, ST73, ST95, and ST131 [2]. In this study, the genome sequence of a MDR *E. coli* recovered from an outpatient infant girl with acute diarrheal infection was analyzed to provide valuable insight into its resistance mechanisms and virulence factors from a genomic panorama.

2. Materials and Methods

From a bacterial collection gathered in 2004, *E. coli* M51-3 was particularly isolated from a stool sample of an 18-month-old outpatient girl with acute diarrheal infection in Culiacan, Sinaloa,

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Mexico. This isolate was the only clinically significant culture result obtained. Species identification was performed using a VYTEK system (bioMérieux Inc, Missouri, USA). Antimicrobial susceptibility to 19 antimicrobial agents (Oxoid, Basingstoke, UK) was evaluated by disk diffusion method and interpreted according to Clinical and Laboratory Standards Institute (CLSI, M100-S25) guidelines. PCR-based typing for diarrheagenic *E. coli* (DEC) was assessed according to López-Saucedo et al [3]. Genomic DNA was extracted using a ZR Fungal/Bacterial DNA Kit (Zymo Research Corp. CA, USA) and was sequenced on an Ion Torrent PGM system (Life Technologies, Carlsbad, CA, USA) with the 200-bp chemistry on a 316 chip yielding a sequence coverage of 20 ×. After adapters removal and read filtering, the generated reads were de novo assembled using SPAdes v3.11 software. Gene prediction and functional annotation were realized by the NCBI Prokaryotic Genome Annotation Pipeline (PGAP, v4.2) and Rapid Annotation using Subsystem Technology (RAST). Multilocus sequence typing (MLST), serotyping, *fimH* allele typing, plasmid replicons, acquired antimicrobial resistance and virulence genes were analyzed using MLST v1.8, SerotypeFinder v1.1, FimTyper v1.0, PlasmidFinder v1.3, ResFinder v3.0, and VirulenceFinder v1.5 web tools, respectively (Center of Genomic Epidemiology; https://cge.cbs.dtu.dk/services/).

3. Results

E. coli M51-3 displayed a MDR phenotype since was resistant to ampicillin, carbenicillin, piperacillin, cefazolin, gentamicin, tobramycin, ciprofloxacin, nalidixic acid, norfloxacin, ofloxacin, sulfamethoxazole/trimethoprim, and ticarcillin/clavulanic acid. In addition, it was non-susceptible to amikacin, cefepime, ceftazidime, cefuroxime, and meropenem; remaining only susceptible to ceftriaxone and nitrofurantoin. On the other hand, PCR amplification of diarrhea-associated genes (*eaeA*, *bfpA*, *stx1*, *stx2*, *st*, *lt*, *ial*) for DEC identification resulted negative.

The draft genome sequence of *E. coli* M51-3 consisted of 133 contigs with a genome size of 5 088 545 bp and a GC content of 50.78%. A total of 5308 protein-coding sequences and 92 RNA genes (17 rRNAs, 70 tRNAs, and 5 ncRNAs) were predicted. According to the Achtman MLST scheme (*adk*-53, *fumC*-40, *gyrB*-47, *icd*-13, *mdh*-36, *purA*-28, *recA*-29 alleles), in silico serotyping, and *fimH* typing, *E. coli* M51-3 belongs to the ST131-O25:H4-*H*30 subclone; the most extensively resistant and epidemiologically successful clone responsible for a large proportion of drug-resistant urinary tract and bloodstream infections worldwide [4]. PlasmidFinder identified five plasmid replicons corresponding to incompatibility (Inc) group F [IncFII, IncFIA, and IncFIB], Col(BS512), and Col156.

Regarding antimicrobial resistance, *E. coli* M51-3 harbored diverse genes conferring resistance to a variety of antimicrobial classes including to aminoglycosides [*aph(3'')-Ib*, *aadA5*, *aph(6)-Id* and *aac(3)-IId*], sulfonamides [*sul1* and *sul2*], and trimethoprim [*dfrA17*]; whereof the *dfrA17-aadA5* gene cassette array were housed in a class 1 integron. The tetracycline resistance gene [*tet (A)*] was also found but was not phenotypically tested. Analysis of quinolone resistance-determining region (QRDR) identified point mutations leading to double mutations in GyrA (S83 L and D87 N), ParC (S80I and E84 V), and a single mutation in ParE (I529 L) suggesting a high-level fluoroquinolone resistance by *E. coli* M51-3. Although ST131 clones are mainly associated with CTX-M type extended-spectrum β-lactamase (ESBL) production, ESBL-negative ST131 isolates have also been reported worldwide [5]. Here none ESBL-encoding gene was detected; instead, the $bla_{\text{TEM-1B}}$ gene which encodes a TEM-1 β-lactamase was identified. Thereby, the in silico determined resistance profile is in line with the antimicrobial susceptibility testing results. VirulenceFinder analysis showed the possession of four potential virulence factors: *sat* (secreted autotransporter toxin), *iha* (adherence protein), *gad* (glutamate decarboxylase), and *senB* (plasmid-encoded enterotoxin). It is noteworthy that *senB* gene product (TieB protein) is implicated in the enterotoxic activity of enteroinvasive *E. coli* (EIEC) and *Shigella* strains, and the loss of *senB* results in a significantly reduction of their enterotoxicity [6]. This suggests that *E. coli* M51-3 might be able to elicit secretory diarrhea, however this feature needs to be addressed.

In summary, we report the first draft genome sequence of a MDR *E. coli* ST131-O25-*H30* subclone isolated from an infant girl with acute diarrheal infection in Mexico. The coexistence of the aforementioned antimicrobial-resistance profile and pathogenic potential in a single *E. coli* strain represents a great health concern, particularly in hospitals given the impact of HAIs on the public health. Surveillance, reinforcement of infection prevention strategies and antimicrobial stewardship should be implemented to control the ongoing dissemination of this highly adapted pathogen worldwide. The *E. coli* M51-3 genome sequence will be helpful to understand the genomic structure, its diversity, and distribution of this successfully pandemic clone.

4. Nucleotide sequence accession no

The Whole Genome Shotgun project has been deposited at DDBJ/ENA/GenBank databases under the accession number PDUF00000000. The version described in this paper is the first version, PDUF01000000.

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Competing Interests

None declared.

Ethical Approval

Not required.

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