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Genome Note

Genomic characterisation of a multidrug-resistant TEM-52b extended-spectrum β-lactamase-positive *Escherichia coli* ST219 isolated from a cat in France

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ABSTRACT

Objectives: TEM-52 extended-spectrum β-lactamases (ESBLs) have been detected in members of the Enterobacteriaceae isolated from human and non-human reservoirs, mainly in European countries. Here we report the first draft genome of a multidrug-resistant TEM-52b-positive *Escherichia coli* isolated from a companion animal in France.
Methods: Whole genomic DNA from *E. coli* 39590 was extracted and was sequenced using an Illumina NextSeq platform. De novo genome assembly was performed using Velvet v.1.2.10 and the draft genome was annotated using the NCBI Prokaryotic Genome Annotation Pipeline v.3.2. Genomic analyses were performed through bioinformatics tools from the Center for Genomic Epidemiology.
Results: The genome size was calculated as 5 362 108 bp, with 5268 protein-coding sequences and a GC content of 50.5%. *E. coli* strain 39590 belonged to ST219, serotype O4:H34 and phylogroup E. The antimicrobial resistome consisted of genes encoding resistance to β-lactams ($bla_{TEM-52b}$), aminoglycosides [*aph(3″)-Ib*, *aph(6)-Id*, *aadA2*, *aadA24*], phenicols (*catA1*), sulfonamides (*sul1*, *sul2*), trimethoprim (*dfrA1*, *dfrA14*), lincosamides (*lnuG*) and tetracycline (*tetA*) as well as mutations in *gyrA* (Ser83Leu, Asp87Asn) and *parC* (Ser80Ile) conferring resistance to quinolones. Virulome analysis revealed *iss*, *astA* and *eilA* genes, and IncQ1, IncX4, IncX1, IncFIB and IncFIC plasmid incompatibility groups were identified.
Conclusion: This draft genome can be used as a reference sequence for comparative studies using human and non-human *E. coli* isolates to identify genetic events that have contributed to pathogenicity and adaptation of TEM-52-producing *E. coli* clones at the human–animal interface as well as to elucidate dynamics of the spread of bla_{TEM-52} ESBL genes.
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Extended-spectrum β-lactamase (ESBL)-producing Enterobacteriaceae have been classified as critical priority pathogens by the World Health Organization (WHO). Another important issue is the transmission of ESBL-producing isolates between humans and animals as well as the growing number of companion animals colonised and/or infected by ESBL-positive pathogens [1]. Currently, TEM-, SHV- and CTX-M-type enzymes are the main ESBLs reported in Enterobacteriaceae. Although TEM and SHV were the first ESBL enzymes, CTX-M enzymes have become widespread, causing a shift in the epidemiology of ESBLs. Nevertheless, TEM-type ESBLs remain clinically relevant, being identified in bacterial isolates from animal and human sources [1–5]. Specifically, the TEM-52 ESBL variant, which differs from the narrow-spectrum β-lactamase TEM-1 by three point mutations (Glu102 → Lys, Met180 → Thr and Gly236 → Ser), was identified for the first time in a *Klebsiella pneumoniae* strain isolated in 1996 from a French

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patient [2]. Since then it has been detected in members of the Enterobacteriaceae family (mostly *Escherichia coli* and *Salmonella* isolates) from humans, pets, wild animals and food-producing animals in European, Asian and North American countries [1–5].

Although many of the reports on TEM-52-producing bacteria come from ESBL prevalence studies, whole-genome sequencing data of bacterial lineages carrying the $bla_{\text{TEM-52}}$ gene remain limited. Here we present the first draft genome sequence of a TEM-52b-producing *E. coli* isolated from a companion animal in France.

E. coli strain 39590 was recovered from a rectal swab culture of a healthy 7-month-old female cat collected during a surveillance study conducted from January–June 2015 at the Veterinary School of Maisons-Alfort (Maisons-Alfort, France). The cat had no previous report of infectious diseases or hospitalisation, whilst the owner of the animal had no history of travel or previous hospitalisation during the past 12 months before collection sample; furthermore, no other pets were kept with the cat.

The *E. coli* isolate was initially identified using an API 20E identification system (bioMérieux SA), and antimicrobial susceptibility testing was performed by the disk diffusion method according to the French Society of Microbiology (https://www.sfm-microbiologie.org/). *E. coli* strain 39590 exhibited a multidrug-resistant profile to amoxicillin, piperacillin, ticarcillin, cefalotin, cefuroxime, streptomycin, chloramphenicol, nalidixic acid, ofloxacin, enrofloxacin, tetracycline, sulfonamides and trimethoprim and was positive by the double-disk synergy test for ESBL production.

Whole genomic DNA was extracted using a PureLink™ Quick Gel Extraction and PCR Purification Combo Kit (Life Technologies, Carlsbad, CA). A 150-bp library for Illumina paired-end sequencing was constructed using a Nextera XT DNA Library Preparation Kit (Illumina Inc.), being further sequenced using an Illumina NextSeq platform (Illumina Inc.). De novo genome assembly was performed using Velvet v.1.2.10, and contig annotation was carried out using the NCBI Prokaryotic Genome Annotation Pipeline (PGAP) v.3.2 (http://www.ncbi.nlm.nih.gov/genome/annotation_prok/).

A total of 16 433 644 paired-end reads were assembled de novo into 366 contigs (500× coverage) using SPAdes 3.13.0 (https://www.ebi.ac.uk/~zerbino/velvet/). The genome size was calculated at 5 362 108 bp, with 5268 protein-coding sequences and a GC content of 50.5%. In total, 70 tRNAs, 9 rRNAs, 7 ncRNAs and 272 pseudogenes were identified.

Analysis of the antimicrobial resistome of *E. coli* 39590 confirmed the presence of the $bla_{\text{TEM-52b}}$ gene, which encodes an ESBL variant that differs from TEM-52 by Ala222→Val mutation (GenBank accession no. **AF126444**). In addition, the presence of genes encoding resistance to aminoglycosides [*aph(3″)-Ib, aph(6)-Id*, *aadA2* and *aadA24*], phenicols (*catA1*), sulfonamides (*sul1* and *sul2*), trimethoprim (*dfrA1* and *dfrA14*), lincosamides (*lnuG*) and tetracycline (*tetA*) was confirmed as well as mutations in the *gyrA* (Ser83Leu, Asp87Asn) and *parC* (Ser80Ile) genes conferring resistance to quinolones (https://cge.cbs.dtu.dk//services/ResFinder/). In this regard, correlation between the genotype and resistance phenotype was confirmed. Furthermore, the presence of the *mdfA* gene was identified, which encodes a multidrug efflux pump (MdfA) conferring resistance to cationic and zwitterionic lipophilic compounds, including disinfectants and clinically important antibiotics (https://www.uniprot.org/uniprot/P0AEY8).

PlasmidFinder 2.0 analyses resulted in the identification of IncQ1, IncX4, IncX1, IncFIB and IncFIC plasmid incompatibility groups (https://cge.cbs.dtu.dk//services/PlasmidFinder/). However, a limitation of this study is that it was not possible to confirm the exact location of the $bla_{\text{TEM-52b}}$ gene since short sequencing read technology was used and, consequently, we could not assemble plasmid sequences.

Moreover, the virulence genes *iss* (increased serum survival), *astA* (EAST1 toxin) and *eilA* (HilA-like regulator, homologous to the *Salmonella* regulator *hilA*) were detected using VirulenceFinder 2.0 (https://cge.cbs.dtu.dk//services/VirulenceFinder/). Molecular epidemiology and phylogenetic analysis using MLST 2.0 (multilocus sequence typing) (https://cge.cbs.dtu.dk/services/MLST/), SerotypeFinder 2.0 (https://cge.cbs.dtu.dk/services/SerotypeFinder/) and Clermont typing (http://clermontyping.iame-research.center/) tools revealed that *E. coli* 39590 belonged to sequence type 219 (ST219), serotype O4:H34 and phylogroup E. TEM-52-producing *E. coli* ST219 have also been identified in humans in Tunisia [5].

In summary, data from this draft genome sequence can provide significant information to be used in comparative studies to better understand the transmission and spread of multidrug-resistant ESBL-producing *E. coli* at the human–animal interface.

This Whole Genome Shotgun project has been deposited at DDBJ/ENA/GenBank under the accession no. **NBSF00000000**. The version described in this paper is version **NBSF00000000.1**.

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Competing interests

None declared.

Ethical approval

Not required.

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