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Genome Note

Draft genome sequence of an *Escherichia coli* ST410 isolate co-harbouring *bla*$_{\text{CTX-M-15}}$,*bla*$_{\text{CMY-42}}$, *bla*$_{\text{OXA-1}}$, *aac(3)-IIa* and *aac(6′)-Ib*-cr genes with *gyrA* and *parC* mutations isolated from a paediatric patient in Poland

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ABSTRACT

Objectives: *Escherichia coli* is one of the major causative agents of nosocomial infections. Here we report the first draft genome sequence of an *E. coli* strain (no. 158) isolated in Poland carrying *bla*$_{\text{CTX-M-15}}$, *bla*$_{\text{CMY-42}}$, *bla*$_{\text{OXA-1}}$, *aac(3)-IIa* and *aac(6′)-Ib*-cr genes together with mutations in the *gyrA* and *parC* genes.
Methods: Total DNA was sequenced using an Illumina NextSeq 500 platform. The draft genome of *E. coli* strain 158 was assembled using SPAdes 3.9 assembler. Contigs were annotated using the Prokka v.1.12 algorithm. Species confirmation, multilocus sequence typing (MLST), serotyping, molecular virulence and resistance traits, and plasmid replicons were analysed using appropriate bioinformatics tools available at the Centre for Genomic Epidemiology website. Additional *in silico* analyses were also conducted.
Result: The genome size was estimated at 4 883 487 bp, with 4601 predicted coding sequences. The presence of *bla*$_{\text{CTX-M-15}}$, *bla*$_{\text{CMY-42}}$, *bla*$_{\text{OXA-1}}$, *aac(3)-IIa* and *aac(6′)*-Ib-cr genes was detected in addition to other antimicrobial resistance genes as well as mutations in the *gyrA* (Ser83Leu and Asp87Asn) and *parC* (Ser80Ile) genes. The investigated strain *E. coli* 158 belongs to ST410.
Conclusion: To our knowledge, this is the first draft genome of an *E. coli* strain co-harbouring *bla*$_{\text{CTX-M-15}}$, *bla*$_{\text{CMY-42}}$, *bla*$_{\text{OXA-1}}$, *aac(3)-IIa* and *aac(6′)*-Ib-cr genes with mutations in *gyrA* and *parC* reported in Poland. The reported genome sequence contains valuable information on genetic features of antimicrobial resistance mechanisms of *E. coli* in Poland.

Escherichia coli is one of the major pathogens causing nosocomial infections. Increasing antimicrobial resistance, especially to third- and fourth-generation cephalosporins, aminoglycosides and fluoroquinolones, has been reported over the years. Resistance to β-lactams is mainly due to the production of extended-spectrum β-lactamases (ESBLs). CTX-M-15, encoded by the *bla*$_{\text{CTX-M-15}}$ gene, has been recognised as one of the most important ESBLs widely disseminated among *E. coli*. In recent years, the occurrence of plasmid-mediated AmpC β-lactamases (pAmpCs) has been increasingly reported [1]. Plasmid-mediated quinolone resistance has been reported to co-exist with ESBLs and pAmpCs [2,3]. Moreover, high-level resistance to fluoroquinolones is considered a consequence of mutations in the subunits of DNA gyrase (GyrA and GyrB) and DNA topoisomerase IV (ParC and ParE) [3]. Co-existence of the aforementioned resistance mechanisms may pose serious challenges to the treatment of *E. coli* infections.

Escherichia coli strain 158 was recovered during routine diagnostics from a bronchoalveolar lavage sample of a paediatric patient hospitalised in Warsaw, Poland. Species identification and antimicrobial susceptibility testing were performed using a

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Table 1
Characteristics of *Escherichia coli* strain 158.

Serotype	MLST	Antimicrobial agent	MIC (mg/L)	MIC breakpoints (mg/L)[a]	Resistance genotype by WGS
08:H21	ST410	Cefoxitin	32	8 (ECOFF)[b]	$bla_{\text{CTX-M-15}}$
		Cefotaxime	128	S ≤1; R >1	$bla_{\text{CMY-42}}$
		Ceftazidime	512	S ≤1; R >4	$bla_{\text{OXA-1}}$
		Ceftriaxone	256	S ≤1; R >2	
		Cefepime	32	S ≤1; R >4	
		Gentamicin	48	S ≤1; R >4	*aac(3)-IIa*
		Ciprofloxacin	>32	S ≤0.5; R >1	*aac(6′)-Ib*-cr
					GyrA (Ser83Leu, Asp87Asn)
					ParC (Ser80Ile, Glu62Lys)
					ParE (Ser458Ala)
		Other resistance mechanisms detected by WGS analysis			
		Macrolides	Not tested	–	*mphA*
		Tetracyclines	Not tested	–	*tetA*
		Chloramphenicol	Not tested	–	*catB4*

MLST, multilocus sequence typing; MIC, minimum inhibitory concentration; WGS, whole-genome sequencing; ECOFF, epidemiological cut-off value; S, susceptible; R, resistant.

[a] MIC breakpoints according to European Committee on Antimicrobial Susceptibility Testing (EUCAST) clinical breakpoint table 2015.

[b] The cefoxitin ECOFF (8 mg/L) has a high sensitivity but poor specificity for identification of AmpC-producing *Enterobacteriaceae* as this agent is also affected by permeability alterations and some carbapenemases. Classical non-AmpC-producers are wild-type, whereas plasmid AmpC-producers or chromosomal AmpC-hyperproducers are non-wild-type (EUCAST clinical breakpoint table 2015).

VITEK®2 system (bioMérieux, Marcy-l'Étoile, France). In addition, antimicrobial susceptibility was evaluated by the agar dilution method (cefoxitin, ceftazidime, cefotaxime and ceftriaxone) and Etest (cefepime, gentamicin and ciprofloxacin). The results were interpreted according to European Committee on Antimicrobial Susceptibility Testing (EUCAST) guidelines (http://www.eucast.org/). The isolate was resistant to all antimicrobials tested. ESBL production was confirmed by the double-disk synergy test (DDST) test. AmpC β-lactamases production was confirmed by the DDST and disk potentiation test (DPT) [4].

All whole-genome sequencing (WGS) steps (DNA isolation, DNA purification, library preparation and sequencing) were performed at BioBank Lab. Co. (Łódź, Poland). Libraries was prepared using a Nextera XT DNA Library Preparation Kit (Illumina Inc., San Diego, CA). Total DNA was sequenced on an Illumina NextSeq 500 platform (Illumina Inc.) using 140-bp paired-end reads. The genome of the investigated strain was assembled using SPAdes 3.9 assembler (https://cge.cbs.dtu.dk/services/SPAdes/). Contigs were annotated using the Prokka v.1.12 algorithm (http://www.vicbioinformatics.com/software.prokka.shtml). Species confirmation, multilocus sequence typing (MLST), serotyping, virulence and resistance genes, and plasmid replicons were analysed using SpeciesFinder 1.2, MLST 1.8, SerotypeFinder 1.1, VirulenceFinder 1.5, ResFinder 3.0 (database version 30 November 2017) and PlasmidFinder 1.3, respectively, available from the Centre for Genomic Epidemiology (https://cge.cbs.dtu.dk/services/). All analysis were performed around December 2017 using actual at that time databases. In addition, the ISfinder database (https://isfinder.biotoul.fr/) was used to search and identify insertion sequences (IS) elements.

The draft genome sequence of the investigated *E. coli* strain 158 consisted of 150 contigs comprising 4 883 487 bp, with 4601 predicted coding sequences. The average coverage was estimated at 50×. The overall G + C content of this strain was 50.57%. The main results from WGS and the minimum inhibitory concentrations (MICs) of the tested antimicrobial agents are summarised in Table 1. *In silico*, MLST revealed that *E. coli* strain 158 belongs to ST410 (*adk–fumC–gyrB–icd–mdh–purA–recA*, allele number 6-4-12-1-20-18-7, respectively). Noteworthy, *E. coli* ST410 has been identified as a potential pandemic clone found in humans, animals and the environment [5]. Furthermore, *E. coli* ST410 clinical isolates have been found to harbour resistance genes such as bla_{NDM}, bla_{KPC}, $bla_{\text{CTX-M}}$ and bla_{OXA} as well as their combinations with other resistance determinants [5]. Resistance genes to a variety of antimicrobial agents were identified in the genome sequence of *E. coli* strain 158, including to β-lactams ($bla_{\text{CTX-M-15}}$, $bla_{\text{CMY-42}}$ and $bla_{\text{OXA-1}}$), aminoglycosides [*aac(3)-IIa*], aminoglycosides and fluoroquinolones [*aac(6′)-Ib*-cr], macrolides (*mphA*), tetracyclines (*tetA*) and chloramphenicol (*catB4*). In addition, a double mutation in GyrA (Ser83Leu and Asp87Asn) as well as a single mutation in ParC (Ser80Ile) were detected. The number and location of these mutations may suggest high resistance to fluoroquinolones in the investigated *E. coli* strain [3]. Additional mutations in ParE (Ser458Ala) and ParC (Glu62Lys) were also identified. Five plasmid replicons [Col(BS512), IncI1, IncFII, FIA and FIB] were detected, in line with the aforementioned plasmid-borne resistance genes. Moreover, the investigated genome sequence revealed several IS elements. Among others, there were IS*Ecp1* and IS*1* considered to be involved in the mobilisation of $bla_{\text{CTX-M-15}}$ and $bla_{\text{CMY-42}}$ and of $bla_{\text{CMY-42}}$, respectively [1,2]. *Escherichia coli* strain 158 was assigned *in silico* to serotype 08:H21, and two potential virulence genes, namely *gad* (glutamate decarboxylase) and *lpfA* (long polar fimbriae), were found.

Here we report the first draft genome sequence of an *E. coli* ST410 strain co-harbouring $bla_{\text{CTX-M-15}}$, $bla_{\text{CMY-42}}$, $bla_{\text{OXA-1}}$, *aac(3)-IIa* and *aac(6′)-Ib*-cr genes as well as mutations in *gyrA* and *parC* isolated in Poland. The resistance profile determined *in silico* is in line with *in vitro* antimicrobial susceptibility testing results. The reported co-existence of the aforementioned resistance mechanisms in an *E. coli* strain in Poland is alarming.

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Competing interests

None declared.

Ethical approval

Not required.

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