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Short Communication

First report of mobile tigecycline resistance gene *tet*(X4)-harbouring multidrug-resistant *Escherichia coli* from wastewater in Norway

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Objectives: The mobile tigecycline resistance gene *tet*(X4), conferring resistance to all tetracyclines, is largely reported from China, however the global spread of such a novel resistance mechanism is a concern for preserving the efficacy of these last-resort antibiotics. The aim of our study was to determine the genetic basis of resistance in a tigecycline-resistant *Escherichia coli* strain (2-326) isolated from sewage in Bergen, Norway, using whole-genome sequencing (WGS).
Methods: WGS was carried out using Illumina MiSeq-based sequencing. *In vitro* conjugation assays were performed to determine the potential of isolate 2-326 to transfer tigecycline resistance to other strains.
Results: Escherichia coli isolate 2-326 belongs to pathogenic sequence type 167 (ST167) and carries several clinically important antibiotic resistance genes including *tet*(X4), $bla_{CTX-M-14}$, *dfrA12, sul2, qnrS1* as well as several aminoglycoside resistance genes. Tigecycline resistance along with resistance to tetracycline, sulfamethoxazole, chloramphenicol and azithromycin was transferred to green fluorescent protein (GFP)-encoding *E. coli* strain CV601-GFP by conjugation.
Conclusion: To the best of our knowledge, this is the first report of *E. coli* carrying mobile *tet*(X4) gene from Norway. Our study demonstrates the ongoing spread of new mechanisms of resistance against last-resort antibiotics and the need for surveillance of such resistance factors in the population in order to mitigate their spread.

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Tigecycline is one of the last-resort antibiotics used to treat infections caused by multidrug-resistant pathogens [1]. Recently, plasmid-mediated mobile tigecycline resistance gene *tet*(X4), conferring resistance to all tetracyclines, was discovered in *Escherichia coli* isolates from China [2]. Although *tet*(X4) is largely reported from China, the global spread of such a novel resistance mechanism is a concern for preserving the efficacy of these last-resort antibiotics [3]. Here we report a multidrug-resistant *E. coli* isolate carrying *tet*(X4) from Norway for the first time.

We obtained 685 *E. coli* isolates from influent and effluent samples collected from wastewater treatment plants in Bergen, Norway, using ECC (CHROMagar, Paris, France) chromogenic media. All of the isolates were checked for antimicrobial susceptibility (data not shown) using EUVSEC Sensititre® plates (Thermo Fisher Scientific, Germany) and were identified by matrix-assisted laser desorption/ionisation time-of-flight mass spectrometry (MALDI-TOF/MS). One of the *E. coli* isolates (isolate 2-326) was resistant to tigecycline and nine other tested antibiotics (Table 1). We applied Illumina MiSeq-based sequencing for whole-genome sequence (WGS) analysis of isolate 2-326, in order to understand the genetic basis of resistance. WGS data was assembled using SPAdes v.3.13.0 [4] with default parameters as previously described [5]. Genome annotation was performed using the National Center for Biotechnology Information (NCBI) Prokaryotic Genome Annotation Pipeline (PGAP) [6]. The draft genome of isolate 2-326 (GenBank accession no. **JADEZU000000000**) has a size of 5 185 374 bp with a total of 135 contigs (>500 bp), an N_{50} value of 131 135, 60X coverage and a G+C content of 50.6%.

Multilocus sequence typing (MLST) analysis (https://pubmlst.org/bigsdb?db=pubmlst_ecoli_achtman_seqdef) showed that isolate 2-326 belongs to sequence type 167 (ST167), which represents an epidemic clone of significant public-health concern that usually encodes β-lactamases [7]. ResFinder 4.1 (https://cge.cbs.dtu.dk/services/ResFinder) showed presence of several acquired antimicrobial resistance genes in the genome of isolate 2-326, including *tet*(X4) and extended-spectrum β-lactamase (ESBL) gene $bla_{CTX-M-14}$ (Table 1) [8]. Isolate 2-326 also carried mutations in

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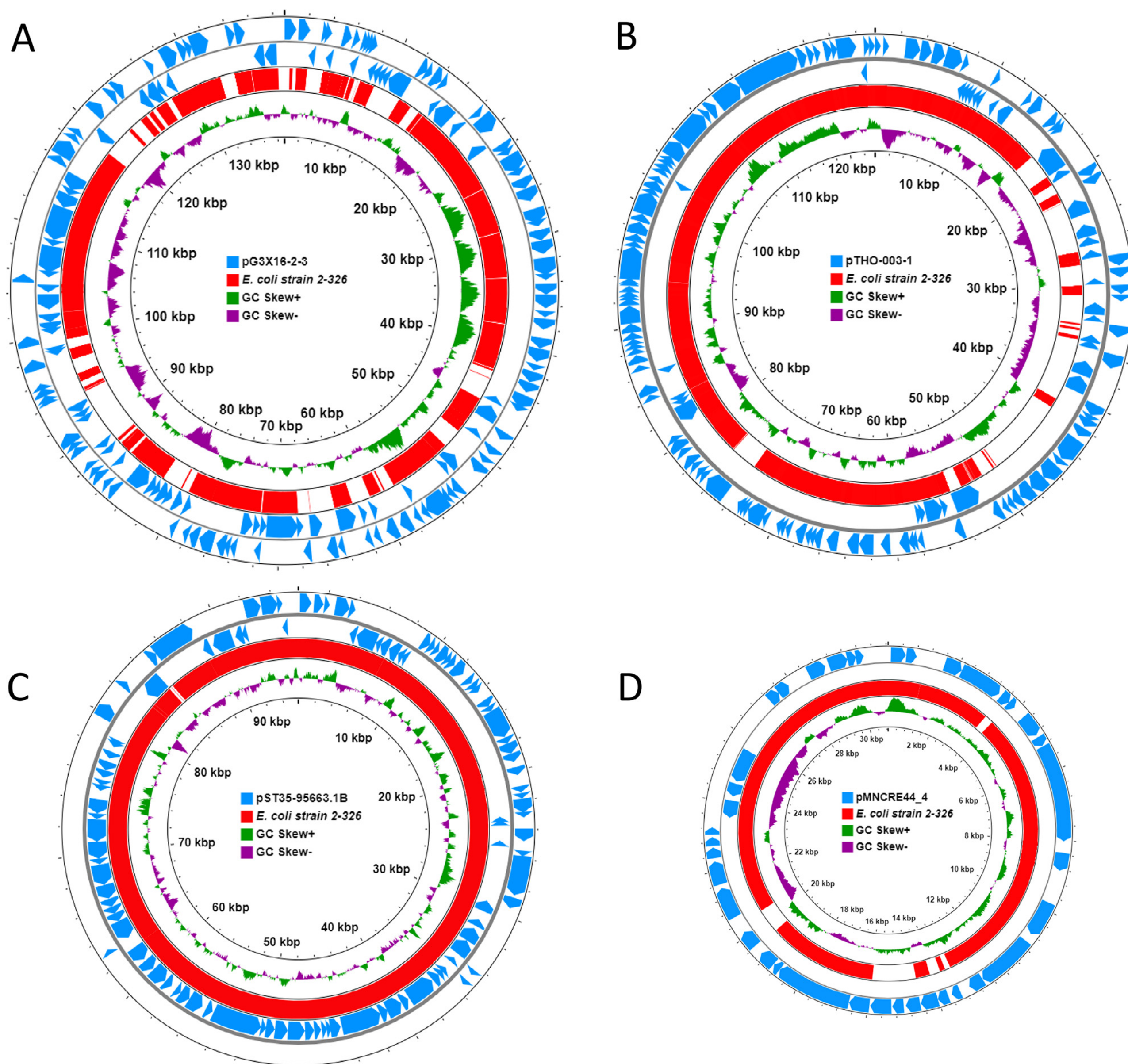

Fig. 1. Alignment of contigs from the draft genome sequence of isolate 2-326 with the reference plasmids: (A) IncFIA/FIB plasmid pG3X16-2-3 (accession no. **NZ_CP038140.1**) of 138.9 kb described in *E. coli* isolated from swine faeces in China in 2017; (B) IncFII plasmid pTHO-003-1 (accession no. **AP022526.1**) of 123.4 kb described in *E. coli* isolated from human sputum in Japan in 2018; (C) IncI1-I(Gamma) plasmid pST35-95663.1B (accession no. **NZ_CP051282**) of 95.8 kb described in *Salmonella enterica* serovar Typhimurium isolated from a bird in Canada in 2020; and (D) IncX4 plasmid pMNCRE44_4 (accession no. **CP010880.1**) of 30.8 kb described in *E. coli* isolated from human sepsis in the USA in 2012. Blue rings in each alignment represent the reference plasmid and the red ring represents contigs from the draft genome sequence of isolate 2-326 that aligned with the reference plasmid with >98% nucleotide identity.

gyrA (D87N and S83L) and *parC* (S80I), thus explaining the high level of ciprofloxacin resistance. Although several aminoglycoside resistance genes were detected in isolate 2-326 (Table 1), no phenotypic resistance to gentamicin was observed. This might be attributed to the fact that most of the aminoglycoside-modifying enzymes detected in isolate 2-326 efficiently modify other aminoglycosides but not gentamicin [9].

VFanalyzer at the Virulence Factor Database [10] (http://www.mgc.ac.cn/cgi-bin/VFs/v5/main.cgi?func=VFanalyzer) showed that isolate 2-326 carried several virulence factors (Supplementary Table S1), including yersiniabactin (*ybt*), *sitABC*, cytolysin A and type VI secretion system, suggesting the potential for pathogenicity of this isolate.

PlasmidFinder 2.1 (https://cge.cbs.dtu.dk/services/PlasmidFinder/) showed the presence of several plasmid replicons such as IncFIA, IncFIB, IncFII, IncI1-I(Gamma) and IncX4 in isolate 2-326 [11]. We performed nucleotide BLAST (https://blast.ncbi.nlm.nih.gov/Blast.cgi) using the contigs containing antimicrobial resistance genes and plasmid replicons from the genome of isolate 2-326 against NCBI nucleotide collection database (nr/nt), in order to identify putative plasmids present in our isolate. We further aligned plasmids with the highest nucleotide identity from BLAST analysis to the draft genome sequence of isolate 2-326 using CGView [12]. We identified putative presence of four different previously reported plasmids in isolate 2-326 (Fig. 1). The genome sequence of isolate 2-326 indicated putative presence of IncF1A/F1B plasmid pG3X16-2-3 (accession no. **NZ_CP038140.1**) with >70% sequence coverage and nucleotide identity of >99%. This plasmid carrying *tet*(X4) was first described in an *E. coli* strain isolated from swine faeces in China.

Table 1
Minimum inhibitory concentrations (MICs) of different antimicrobial agents tested against *Escherichia coli* isolate 2-326, the transconjugant (TC) of *gfp E. coli* and the recipient strain *gfp E. coli*, as well as antimicrobial resistance genes (ARGs) detected in the draft genome sequence of *E. coli* isolate 2-326

Antimicrobial agent	MIC of isolate 2-326 (μg/mL)	ARGs detected in isolate 2-326	MIC of TC-*gfp E. coli* [a] (μg/mL)	MIC of *gfp E. coli* [a] (μg/mL)
Ampicillin	**>64**	$bla_{CTX-M-14}$	4	4
Azithromycin	**>64**	*erm(B), erm(42)*	**>64**	8
Cefotaxime	**>4**	$bla_{CTX-M-14}$	<0.25	<0.25
Ceftazidime	1	$bla_{CTX-M-14}$	<0.5	<0.5
Meropenem	<0.03	–	<0.03	<0.03
Nalidixic acid	**>128**	*qnrS1*	8	8
Ciprofloxacin	**>8**	*qnrS1*	0.03	0.03
Trimethoprim	**>32**	*dfrA12*	<0.25	<0.25
Sulfamethoxazole	**>1024**	*sul2*	**>1024**	<8
Tetracycline	**64**	*tet*(X4), *tet*(M)	**64**	<2
Tigecycline	**8**	*tet*(X4)	**8**	<0.25
Gentamicin	2	*aph(6)-Id, aadA1, aadA2, ant(2'')-Ia, aph(3'')-Ib*	<0.5	<0.5
Chloramphenicol	**>128**	*cml*	**16**	<8
Colistin	<1	–	<1	<1

NOTE: MICs above clinical breakpoints for *Enterobacterales* have been marked in bold (https://www.eucast.org/clinical_breakpoints/).
[a] Green fluorescent protein-expressing *E. coli* strain CV601-GFP.

Tigecycline resistance along with tetracycline, sulfamethoxazole, chloramphenicol and azithromycin resistance was transferred to green fluorescent protein (GFP)-encoding *E. coli* strain CV601-GFP via conjugation carried out according to the previously described method [13]. Transconjugants were obtained with a conjugation frequency of 2×10^{-5} transconjugants per recipient cell using tigecycline (2 μg/mL), kanamycin (50 μg/mL) and rifampicin (50 μg/mL) for selection. Two transconjugants from the selective plates were picked and antimicrobial susceptibility using EUVSEC Sensititre® plates was performed. Both of the transconjugants showed an identical resistance profile, being resistant to tigecycline, tetracycline, sulfamethoxazole, chloramphenicol and azithromycin (Table 1), further suggesting the possible presence of *tet*(X4)-carrying plasmid pG3X16-2-3 (Fig. 1).

Moreover, isolate 2-326 carried three other plasmids similar to: (i) IncFII plasmid pTHO-003-1 from *E. coli* isolated from human sputum in Japan (accession no. **AP022526.1**) with >86% sequence coverage and 98% nucleotide identity; (ii) IncI1-I(Gamma) plasmid pST35-95663.1B from *Salmonella enterica* serovar Typhimurium isolated from a bird in Canada (accession no. **NZ_CP051282**) with >96% sequence coverage and >99.6% sequence identity; and (iii) IncX4 plasmid pMNCRE44_4 from *E. coli* causing human sepsis in the USA (accession no. **CP010880.1**) with >86% sequence coverage and 99.4% identity.

Norway has very low prevalence of tigecycline resistance in clinical *E. coli* isolates [14]. Recently, Pan et al. described that Tet(X) enzymes have been detected in 23 countries across five continents [3]. Detection of the plasmid-mediated *tet*(X4) gene in Norway suggests the ongoing global spread of this tigecycline resistance mechanism. Our study thus highlights the need for surveillance of resistance against last-resort antibiotics, especially in a low-prevalence setting such as Norway, in order to mitigate further spread of such resistance factors. It also highlights the importance of sewage-based surveillance for understanding the prevalence of antibiotic resistance in the population.

Data availability

This Whole Genome Sequence for *E. coli* isolate 2-326 has been deposited in DDBJ/ENA/GenBank under the accession no. **JADEZU000000000**. The raw sequencing data have been deposited in the Sequence Read Archive (SRA) under the accession no. **SRR14611341**.

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