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Emergence and dissemination of epidemic-causing OXA-244 carbapenemase-producing *Escherichia coli* ST38 through hospital sewage in Norway, 2020–2022

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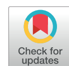

S U M M A R Y

Background: Population-based sewage surveillance has emerged as a promising approach for studying the prevalence of antibiotic resistance in pathogens.

Aim: To determine the temporal prevalence of cefotaxime-resistant *Escherichia coli* in sewage from five sewage treatment plants located in Bergen city, to determine whether ESBL- and carbapenemase-producing *E. coli* are consistently disseminated in the receiving environment through sewage.

Method: A total of 569 cefotaxime-resistant *E. coli* were isolated over a period of 19 months (August 2020 to February 2022) using ECC CHROMagar™ plates from 82 samples, antibiotic sensitivity profiles were determined, using Sensititre™ plates. The draft genome sequences were determined, using Illumina MiSeq-based sequencing. Complete genome sequences were determined, using Oxford Nanopore-based sequencing.

Findings: All 569 strains obtained from influent (*N*=461) and effluent (*N*=108) were multidrug resistant. Most of the sequenced strains (52 of 61) carried $bla_{\text{CTX-M-15}}$ (38.5%) and $bla_{\text{CTX-M-27}}$ (34.6%). The most prevalent sequence types (STs) for ESBL-carrying strains were ST131 (32.8%) and ST38 (21.3%). All CTX-M-27-carrying ST131 strains belonged to clade A or C1, while CTX-M-15-harbouring strains were present in all the clades. Five OXA-244-producing ST38 strains, genetically similar to epidemic-causing strains from Western Norway, France and the Netherlands, were isolated only from raw and treated sewage of the treatment plant receiving hospital sewage.

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Conclusion: This is the first study showing persistent dissemination of OXA-244-producing ST38 clones through sewage in Norway, demonstrating that hospital sewage is the likely source of OXA-244-producing ST38 clones reaching the receiving environment.

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Introduction

Extended spectrum β-lactamase (ESBL)- and carbapenemase-producing Enterobacterales are an emerging public health problem [1]. Although several national surveillance programmes for antibiotic resistance in clinical and veterinary settings exist, surveillance of antibiotic resistance in the community and the environment is largely lacking [2]. Clinically relevant pathogens and antibiotic resistance genes are introduced into the environment via different routes, such as through sewage contamination [3]. Population-based sewage surveillance has emerged as a promising approach for studying the prevalence of ESBL-producing *Escherichia coli* strains by targeting cefotaxime-resistant strains, which are frequently associated with multi-drug resistance [2,4].

Norway represents a low-prevalence country in terms of antibiotic use [5] and occurrence of antibiotic resistance [6]. However, CTX-M-type ESBLs have emerged as a significant clinical problem, also in Norway [7]. Most of the CTX-M-producing *E. coli* isolates obtained from blood samples (N=141) in 2019 belonged to sequence type (ST) 131 (56.7%) [8], suggesting that this high-risk clone is important for the dissemination of CTX-M-type ESBLs in Norway [9]. CTX-M-producing *E. coli* ST131 are also prevalent in clinics in other European countries [10,11]. Moreover, hospital effluent has also been shown to be a source of ST131 strains carrying CTX-M-type ESBLs disseminated into the receiving environment [12,13]. *E. coli* ST131 strains can be subdivided into clades A, B and C, in which clade C is the most dominant globally [14].

Carbapenemases consist of a group of enzymes that hydrolyse almost all β-lactam antibiotics. The clinically relevant enzymes are KPC, NDM, IMP, VIM and OXA-48-like [15]. Community-acquired infections with OXA-244-producing *E. coli* ST38 are increasing in Europe [6]. Recently, outbreaks with OXA-244-producing ST38 clones were reported from Bergen [16]. In a previous study, we detected carbapenemases, such as OXA-244, VIM-1 and NDM-6, in cefotaxime-resistant *E. coli* strains isolated from raw sewage (influent) and treated discharge (effluent) from Bergen city, Norway [4]. However, limited knowledge exists about the persistent dissemination of these strains through sewage in Norway. The aim of the current study was to determine the temporal prevalence of cefotaxime-resistant *E. coli* in influents and effluents from five sewage treatment plants (STPs) located in Bergen city over a period of 19 months, to determine whether there is consistent ongoing dissemination of ESBL- and carbapenemase-producing *E. coli* in the environment through sewage, as well as to identify the potential source of OXA-244-producing *E. coli*.

Methods

Isolation and identification of cefotaxime-resistant E. coli

Twenty-four-hour, time-proportional, composite sewage samples were collected on nine occasions between August 2020 and February 2022 from five STPs located in Bergen city, in Norway (Supplementary Table S1). For February 2021, sewage samples (influent and effluent) were only collected from two of the five treatment plants (Holen and Flesland). The sewage samples were kept in sterile containers at 4 °C and processed within 6 h after collection. The samples were serially diluted 10-fold with sterile saline (0.85% NaCl) before plating 100-μL aliquots from the original, 10^{-1} and 10^{-2} dilutions on ECC (CHROMagar™, France) chromogenic media containing 2 μg/mL cefotaxime (Sigma-Aldrich, Germany), and incubated at 37 °C, for 20–24 h. From each sample, 10–15 isolated colonies were picked depending on growth, restreaked and incubated at 37 °C overnight. Identification of the strains was performed, using matrix-assisted laser desorption ionization-time of flight mass spectrometry (MALDI-TOF MS) (Bruker Daltonics, Germany), at the Institute of Marine Research (IMR). Subsequently, confirmed *E. coli* strains were stored at -80 °C in Mueller–Hinton (MH) broth (Oxoid, UK) with 2 μg/mL cefotaxime and 20% glycerol until further analysis.

Antibiotic sensitivity testing

The resistance profile of 569 cefotaxime-resistant *E. coli* strains was determined against up to 15 antibiotics (Supplementary Table S2), using a broth microdilution assay with Sensititre® EUVSEC or EUVSEC3 plates (Thermo Scientific, USA), following the manufacturer's protocol. The plates were incubated at 37 °C for 20–22 h. The strains were defined as, 'susceptible' or 'resistant', according to the EUCAST clinical breakpoints tables v.12.0 [17]. The *E. coli* strains were separated into three groups before analysing the resistance rates, based on the type of sewage input the treatment plant received: (1) Municipal-Airport, receiving municipal sewage and sewage from airport and industries (Flesland); (2) Municipal-Hospital, receiving hospital and municipal sewage (Holen); and 3) Municipal, receiving only municipal sewage (Ytre-Sandviken, Kvernevik and Knappen).

Genome sequencing, assembly, and sequencing analysis

We selected 61 cefotaxime-resistant *E. coli* strains, from both influent (N=49) and effluent (N=12), representing

different phenotypic resistance profiles and/or having reduced susceptibility against carbapenems for WGS, as described previously [4]. Briefly, genomes were annotated, using the Prokaryotic Genome Annotation Pipeline (PGAP) v.4.13 at the National Centre for Biotechnology Information (NCBI) (https://www.ncbi.nlm.nih.gov/genome/annotation_prok/). Sequence types were identified, using the PubMLST database (https://pubmlst.org/bigsdb?db=pubmlst_escherichia_seqdef&page=sequenceQuery). *E. coli* strains with novel STs were submitted to Enterobase v.1.1.3 (https://enterobase.warwick.ac.uk/species/index/ecoli), in order to get new ST numbers. The presence of antibiotic resistance genes (ARGs) was analysed, using ResFinder v.4.1 (https://cge.food.dtu.dk/services/ResFinder/), and the CARD v.3.2.2 (https://card.mcmaster.ca/analyze/rgi). Complete overviews of plasmids were obtained, using CGView (https://proksee.ca/). In addition, plasmids with the highest nucleotide similarities from BLAST analysis were aligned to the plasmid sequences, using CGView. Plasmid replicons were typed, using PlasmidFinder 2.0 (https://cge.food.dtu.dk/services/PlasmidFinder/). Only four of five OXA-244-producing ST38 strains, isolated at three different time points, were subjected to Oxford Nanopore long-read sequencing, with subsequent hybrid de-novo assemblies, as previously described [18], because two strains from the same time point were identical clones based on Illumina sequencing.

Core genome analysis of OXA-244-producing E. coli *ST38 and ST131 strains*

Single nucleotide polymorphism (SNP)-based analyses of the OXA-244-producing *E. coli* ST38 strains detected in this study, compared with other ST38 strains from different sources and countries was performed (Supplementary Table S3), as described by Sabat *et al*. [19]. The assembled genome sequences were analysed, using CSI Phylogeny v.1.4 [20]. The parameters minimum depth at SNP positions, minimum relative depth at SNP positions, minimum distance between SNPs and minimum SNP quality, minimum read mapping quality and z-score were used, as previously described [18]. For understanding the distribution of ST131 strains in the respective clades, we used a core-genome-based phylogenetic tree, constructed using the UBCG, i.e., up-to-date bacterial core-gene pipeline v3.0 [21]. We used the WGS of 20 strains belonging to ST131 from our study (Supplementary Table S4) and reference strains for each clade (clade A: strain SE15 (GenBank accession number: AP009378), clade B: Strain S250 (LXQL01000001), clade C1: Strain 81009 (CP021179), and clade C2: Strain EC958 (HG941718)) for UBCG analysis. Finally, a phylogenetic tree was produced with FastTree v.2.1.10.

Conjugation assay

In-vitro conjugation assays were performed in triplicate, using filter-mating, to investigate the potential of the four OXA-244-producing *E. coli* ST38 strains for transferring ARGs to a green fluorescent protein (GFP)-tagged *E. coli* strain CV601, according to a previously described method [22]. Equal volumes of the prepared donors and recipient were mixed, and the conjugation mixtures were vacuum-filtered on to S-Pak filters (pore size 0.22 μm) (Millipore, USA), before the filters were placed on MH agar plates (Oxoid, UK). The plates were incubated at 30 °C for 3 h. The filters were transferred to tubes containing 10 mL sterile saline (0.85% NaCl) and glass beads and vortexed for 1 min. The samples were serially diluted with sterile saline (0.85% NaCl) before plating 100-μL aliquots on MH Orientation (CHROMagar™, France) chromogenic media with 50 μg/mL kanamycin, 50 μg/mL rifampicin and 2 μg/mL cefotaxime (Sigma-Aldrich, Germany), for selection of transconjugants. In addition, MH plates with 50 μg/mL kanamycin and 50 μg/mL rifampicin were used to estimate the number of recipients. The plates were incubated at 37 °C overnight. Transconjugants were identified based on GFP expression when exposed to UV light.

Results

Resistance rates in cefotaxime-resistant E. coli *isolates*

Cefotaxime-resistant *E. coli* isolates were detected in all five influent samples collected (except for Knappen in March 2021), and in four out of five effluent samples from all treatment plants (Supplementary Table S5). Cefotaxime-resistant *E. coli* isolates were not detected in effluent samples from Knappen at any time points. The concentrations of cefotaxime-resistant *E. coli* in all influent samples ranged from 0 to 2,900 colony-forming units (cfu) per mL, while the effluent samples ranged from 0 to 200 cfu/mL, during all sampling occasions (Supplementary Table S5).

A total of 461 and 108 cefotaxime-resistant *E. coli* strains obtained from influent and effluent samples, respectively, were analysed. The highest resistances observed among the *E. coli* strains (*N*=99) obtained from Municipal-Hospital influent were to ampicillin (100%), sulphamethoxazole (69.7%), tetracycline (58.6%), trimethoprim (56.6%) and ceftazidime (54.5%) (Supplementary Table S2A). For strains (*N*=107) obtained from Municipal-Airport influent, the highest resistances observed were to ampicillin (100%), nalidixic acid (60.7%), trimethoprim (55.1%), sulphamethoxazole (54.2%) and tetracycline (44.9%). The highest resistances observed among the *E. coli* strains (*N*=255) obtained from Municipal influent were against ampicillin (100%), nalidixic acid (68.2%), tetracycline (40.8%), sulphamethoxazole (40.0%) and ciprofloxacin (39.6%). Phenotypic resistance to meropenem (2.0%), tigecycline (1.0%) and colistin (1.0%) was observed only in strains from Municipality-Hospital group.

E. coli strains (*N*=50) obtained from Municipal-Hospital effluent exhibited the highest resistances against ampicillin (100%), trimethoprim (80.0%), sulphamethoxazole (80.0%), tetracycline (72.0%) and ceftazidime (60.0%) (Supplementary Table S2B). Among the *E. coli* strains (*N*=10) obtained from Municipal-Airport effluent, the highest resistances were observed for ampicillin (100%), nalidixic acid (80.0%), tetracycline (60.0%), ceftazidime (60.0%) and azithromycin (60.0%). *E. coli* strains (*N*=48) from Municipal influent showed the highest resistances to ampicillin (100%), nalidixic acid (75.0%), trimethoprim (56.3%), sulphamethoxazole (52.1%) and ceftazidime (52.1%). No resistance was observed against meropenem, tigecycline or colistin in strains obtained from all effluent samples, although, strains displaying reduced susceptibility for meropenem, were detected only from the effluents in the Municipality-Hospital group.

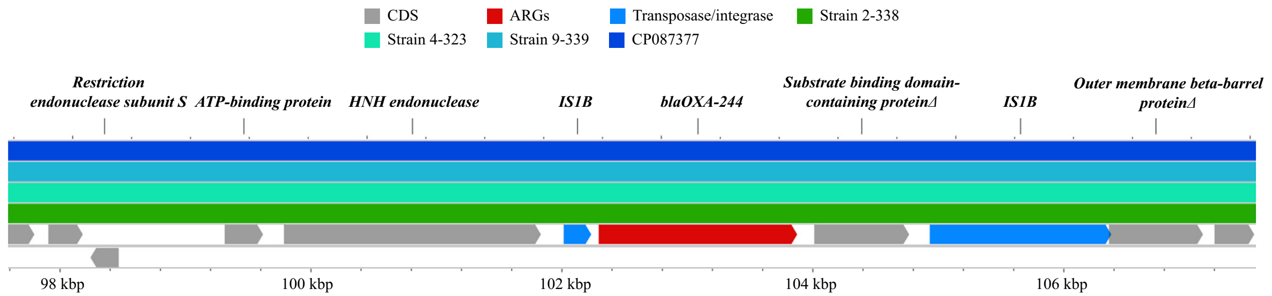

Figure 1. Genomic map of the $bla_{OXA-244}$ gene located between positions 102,056 bp – 105,295 bp, flanked by IS1B transposases on either end, on the chromosome of *Escherichia coli* ST38 strain 4-321 (GenBank accession number: CP101572); alignment with strain 2-338 (CP101326), strain 4-323 (CP101576), strain 9-339 (CP102091), and the clinical *E. coli* ST38 strain RIVM C019217 (CP087377) reported from the Netherlands. Antibiotic resistance genes (ARGs) are highlighted in red, transposases/integrases in blue, and other genes in grey. Δ represents truncated genes. CDS, Coding sequences.

Diversity of cefotaxime-resistant E. coli *and associated ARGs*

Among 61 sequenced strains, the most prevalent STs were ST131 (32.8%) and ST38 (21.3%) (Supplementary Table S4). Five strains were assigned to two novel STs, four strains belonged to ST11873, and one strain to ST12850. Twelve cefotaxime-resistant *E. coli* strains carried carbapenemase genes, of which five strains carried $bla_{OXA-244}$, five carried bla_{VIM-1} [23], and two carried bla_{NDM-6} [24]. Most of the *E. coli*

Figure 2. (a) Genomic map of plasmid p2-338_2 (GenBank accession number: CP101328); alignment with plasmid p4-321_1 (CP101573), p4-323_2 (CP101578), p9-339_1 (CP102092), pKresCPE0314 (OX359164.1), pKresCPE0296 (OX352318.1), pKresCPE0307 (OX359169.1), pRIVM_C019217_2 (CP087378), and *E. coli* plasmid pRIVM_C039205_2 (CP086621). (b) Map of the multi-drug resistance region of the plasmid p2-338_2, located between positions 52,023 bp and 73,819 bp and flanked by IS*26* transposases on either end. Arrows indicate the sizes of the ORFs and their orientations. Antibiotic resistance genes (ARGs) are highlighted in red, biocide/metal resistance genes (BMRGs) in orange, transposases/integrases in blue, conjugal transfer genes in purple, replication initiation in black, and other genes in grey. Δ represents truncated genes. CDS, Coding sequences.

Table I

Minimum inhibitory concentrations (MICs) of different antibiotics for *Escherichia coli* strains 2-338, 4-321, 4-323, 9-339 and transconjugants of green fluorescent protein (GFP)-tagged *E. coli* carrying plasmid p2-338_2, p4-321_1, p4_323_2, and p9-339_1

Strain	Month, year	STP	Type	MALDI-TOF MS	AMP	AZM	CTX	CAZ	MEM	NAL	CIP	TMP	SUL	TET	TGC	GEN	CHL	CST
2–338	Aug, 2020	Holen	Effluent	*E. coli*	**>64**	**32**	**>4**	4	0.12	**128**	0.25	**>32**	**>1024**	**>64**	<0.25	<0.5	<8	<1
4–321	Feb, 2021	Holen	Influent	*E. coli*	**>64**	**>64**	**>4**	4	0.25	<4	0.03	**>32**	**>1024**	**>64**	<0.25	<0.5	<8	<1
4–323	Feb, 2021	Holen	Effluent	*E. coli*	**>64**	**64**	**>4**	4	0.5	<4	0.03	**>32**	**>1024**	**>64**	<0.25	<0.5	<8	<1
9–339	Nov, 2021	Holen	Influent	*E. coli*	**>64**	**64**	**>4**	**8**	0.5	<4	<0.015	**>32**	**>1024**	**>64**	<0.25	<0.5	<8	<1
TC_p2-338_2	NA	NA	NA	*E. coli*	**>64**	**>64**	**>4**	**>8**	<0.03	8	0.03	**>32**	**>1024**	**>64**	<0.25	<0.5	<8	<1
TC_p4-321_1	NA	NA	NA	*E. coli*	**>64**	**>64**	**>4**	4	<0.03	8	0.03	**>32**	**>1024**	**>64**	<0.25	<0.5	<8	<1
TC_p4-323_2	NA	NA	NA	*E. coli*	**>64**	**>64**	**>4**	4	<0.03	8	0.03	**>32**	**>1024**	**>64**	<0.25	<0.5	<8	<1
TC_p9-339_1	NA	NA	NA	*E. coli*	**>64**	**64**	**>4**	**8**	<0.03	8	0.03	**>32**	**>1024**	**>64**	<0.25	<0.5	<8	<1
GFP *E. coli*	NA	NA	NA	*E. coli*	4	4	<0.25	<0.5	<0.03	8	0.03	<0.25	<8	<2	<0.25	<0.5	<8	<1

Conjugal transfer frequency ranged between 5 and 7×10^{-7} per recipient cells for all four strains. GFP *E. coli*, green fluorescent protein expressing *E. coli* strain CV601-GFP; MALDI-TOF MS, matrix-assisted laser desorption ionization-time of flight mass spectrometry; NA, not applicable; STP, sewage treatment plant; TC_p2-338_2, transconjugant (*E. coli* strain CV601-GFP carrying plasmid p2-338_2) selected on plates containing tetracycline (10 μg/mL); TC_p4-321_1, transconjugant (*E. coli* strain CV601-GFP carrying plasmid p4-321_1) selected on plates containing tetracycline (10 μg/mL); TC_p4-323_2, transconjugant (*E. coli* strain CV601-GFP carrying plasmid p4-323_2) selected on plates containing tetracycline (10 μg/mL); TC_p9-339_1, transconjugant (*E. coli* strain CV601-GFP carrying plasmid p9-339_1) selected on plates containing tetracycline (10 μg/mL). MICs above clinical breakpoints for Enterobacterales have been marked in bold. Antibiotics: ampicillin (AMP); azithromycin (AZM); cefotaxime (CTX); ceftazidime (CAZ); chloramphenicol (CHL); ciprofloxacin (CIP); colistin (CST); gentamicin (GEN); meropenem (MEM); nalidixic acid (NAL); sulphamethoxazole (SMX); tetracycline (TET); tigecycline (TGC); and trimethoprim (TMP).

strains (N=52) carried CTX-M-type ESBLs, of which the majority carried $bla_{\text{CTX-M-15}}$ (38.5%), followed by $bla_{\text{CTX-M-27}}$ (34.6%), while the remaining carried either a carbapenemase ($bla_{\text{NDM-6}}$ or $bla_{\text{VIM-1}}$) or $bla_{\text{CMY-42}}$. Apart from β-lactamases, the majority of the sequenced *E. coli* strains carried genes conferring resistance to sulphonamides (*sul1, sul2*), tetracyclines (*tet*(A)), trimethoprim (*dfrA17, dfrA14*), aminoglycosides (*aph(3'')-Ib, aph(6)-Id*) and macrolides (*mph(A)*). One isolate carried a *tet*(X4) gene conferring resistance to tigecycline [25].

Complete genome sequences of OXA-244-producing E. coli strains

The complete genome of the four isolates were assembled into one contig representing a complete circular chromosome (~5.2 Mb) and several plasmids, ranging from 211,283 bp to 58,274 bp (Supplementary Table S7). All four strains carried the $bla_{\text{OXA-244}}$ gene on the chromosome at the same position (102,056 bp – 105,295 bp), flanked by IS1B transposases on either end (Figure 1). The DNA fragment (3,239 bp) carrying $bla_{\text{OXA-244}}$ and the flanking transposases, detected on the chromosome in the four strains, is identical (>99.9% nucleotide identity) to a segment located on the chromosome of OXA-244-producing *E. coli* strain RIVM C019217 (CP087377) reported from the Netherlands (Figure 1).

The sequenced strains carried all ARGs, except $bla_{\text{OXA-244}}$, on an IncFIB plasmid (~148 kb) (Figure 2a). This plasmid has a multi-drug-resistance region that harbours *dfrA17, sul1, sul2, mph(A), aadA5, aph(3″)-Ib, aph(6)-Id, tet*(A) and $bla_{\text{CTX-M-27}}$, conferring resistance to trimethoprim, sulphonamides, macrolides, aminoglycosides, tetracyclines and third-generation cephalosporins (Figure 2b). In addition, this region also carries genes conferring resistance to quaternary ammonium compounds (*qacE*Δ1) and chromate (*chrA*). The IncFIB plasmid is conjugative and was transferred from the four strains to a GFP-tagged *E. coli* strain, at a transfer frequency of 7×10^{-7} per recipient cell, via conjugation, with transfer of resistance against nine antibiotics (Table I). The IncFIB plasmid (~148 kb) detected in our strains is identical (>99.9% nucleotide identity) to plasmid pKresCPE0314 (OX359164.1), pKresCPE0296 (OX352318.1) and pKresCPE0307 (OX359169.1), reported from three OXA-244-producing ST38 clinical strains from Norway, respectively (Figure 2a). In addition, the IncFIB plasmid detected in our strains is also identical (>99.9%) to plasmid pRIVM_C019217_2 (CP087378) and pRIVM_C039205_2 (CP086621) from two OXA-244-producing ST38 clinical strains, reported from the Netherlands, respectively (Figure 2a).

Core genome phylogeny of ST131 strains

Based on the maximum likelihood phylogenetic tree, most of the ST131 strains in our study belonged to clade A, followed by C1 and C2, while we did not detect any strains belonging to clade B (Figure 3). All of strains carrying CTX-M-27 belonged to either clade A or C1, while strains harbouring CTX-M-15 were present in all the clades. An SNP-based core genome phylogenetic tree shows that the OXA-244-producing *E. coli* ST38 strains (N=5) and the clinical OXA-244-producing ST38 strains from Western Norway exhibit between one and 18 SNPs (Figure 4, Supplementary Table S8). Moreover, the five strains

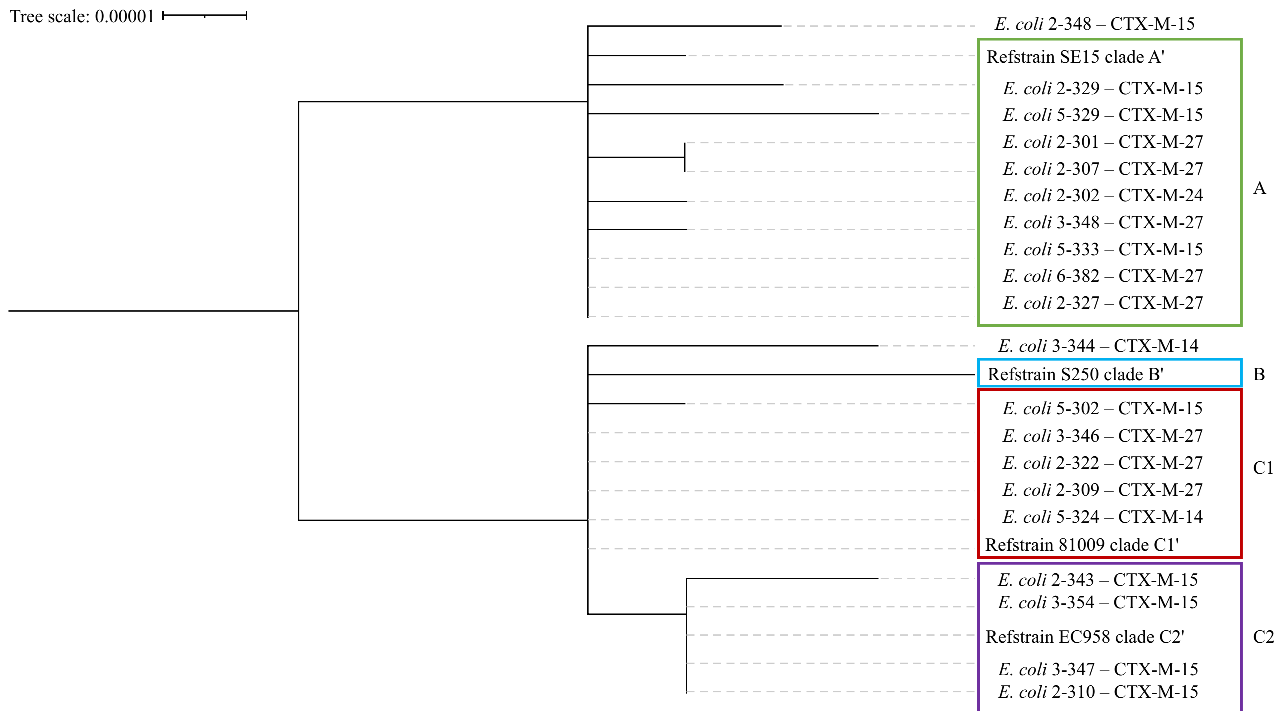

Figure 3. Maximum-likelihood phylogenetic tree of 20 CTX-M-producing *Escherichia coli* sequence type (ST) 131 strains and four reference strains for each clade; clade A: strain SE15 (GenBank accession no.: AP009378), clade B: Strain S250 (LXQL01000001), clade C1: Strain 81009 (CP021179), and clade C2: Strain EC958 (HG941718). Bar, 0.00001 substitution per position.

are clustering closer to clinical OXA-244-producing ST38 strains from France and the Netherlands, compared with other ST38 strains. The number of SNPs matrix is presented in Supplementary Table S8.

Data availability

The genome sequences of the OXA-244-producing strains are deposited and publicly available in GenBank/DDBJ/ENA under the following accession numbers: CP101326-30 (strain 2-338), CP101572-75 (strain 4-321), CP101576-80 (strain 4-323), CP102091-93 (strain 9-339) and JALDRW000000000 (strain 4-328). Accession numbers for other sequenced cefotaxime-resistant *E. coli* strains are presented in Supplementary Table S6. The raw sequencing data have been deposited in the Sequence Read Archive (SRA) under the BioProject accession no. PRJNA681451.

Discussion

This is the first study showing persistent dissemination of cefotaxime-resistant *E. coli* and OXA-244-producing *E. coli* ST38 clones through sewage in Norway. Our study demonstrates that hospital sewage is the likely source of OXA-244-producing *E. coli* ST38 clones reaching the receiving environment through treated sewage.

Five OXA-244-producing strains (2-338, 4-321, 4-323, 4-328 and 9-339) were isolated only from Municipal-Hospital influent and effluents from three different sampling occasions (August 2020, and February and November 2021). These strains belonged to the high-risk clone, ST38, known to cause extra-intestinal infections [26]. SNP-based comparative analysis of the WGS showed that the five strains and the clinical OXA-244-producing ST38 strains from Western Norway [27] belonged to the same clone (Figure 4, Supplementary Table S8) [28], demonstrating that epidemic-causing OXA-244-producing *E. coli* ST38 strains are disseminated through hospital sewage. Moreover, the OXA-244-producing ST38 strains (N=5) in our study are clustering closer to clinical OXA-244-producing clinical ST38 strains from France and the Netherlands [29,30], compared with other ST38 strains in an SNP-based core genome phylogenetic tree, suggesting that our strains are similar to epidemic-causing strains from Western Europe (Figure 4, Supplementary Table S8) [6]. Outbreaks with OXA-244-producing ST38 clones recently have been reported from Haukeland University Hospital, in Bergen [16]. The repeated detection of OXA-244-producing *E. coli* ST38 isolates in raw and treated sewage from the treatment plant receiving hospital input, shows that this high-risk clone is disseminated into the receiving marine environment through hospital sewage [31—34].

Besides detecting OXA-244-producing *E. coli* strains, several ESBL-producing *E. coli* strains were detected in our study. ESBL-producing *E. coli* is a growing problem in clinics in Norway, with CTX-M-type being the most prevalent ESBL. Among CTX-M-producing clinical strains, ST131 is the most prevalent in Norwegian clinics. Consistent with the clinical data, we detected continuous dissemination of CTX-M-type ESBLs through sewage, with CTX-M-15 and CTX-M-27 being the predominant variants. Among CTX-M-carrying *E. coli*, ST131 was the most prevalent sequence type, which is a worldwide pandemic pathogen associated with ESBL production [14,26]. The majority of the ST131 strains in our study carried $bla_{CTX\text{-}M\text{-}15}$ or $bla_{CTX\text{-}M\text{-}27}$, which is similar to the clinical observations,

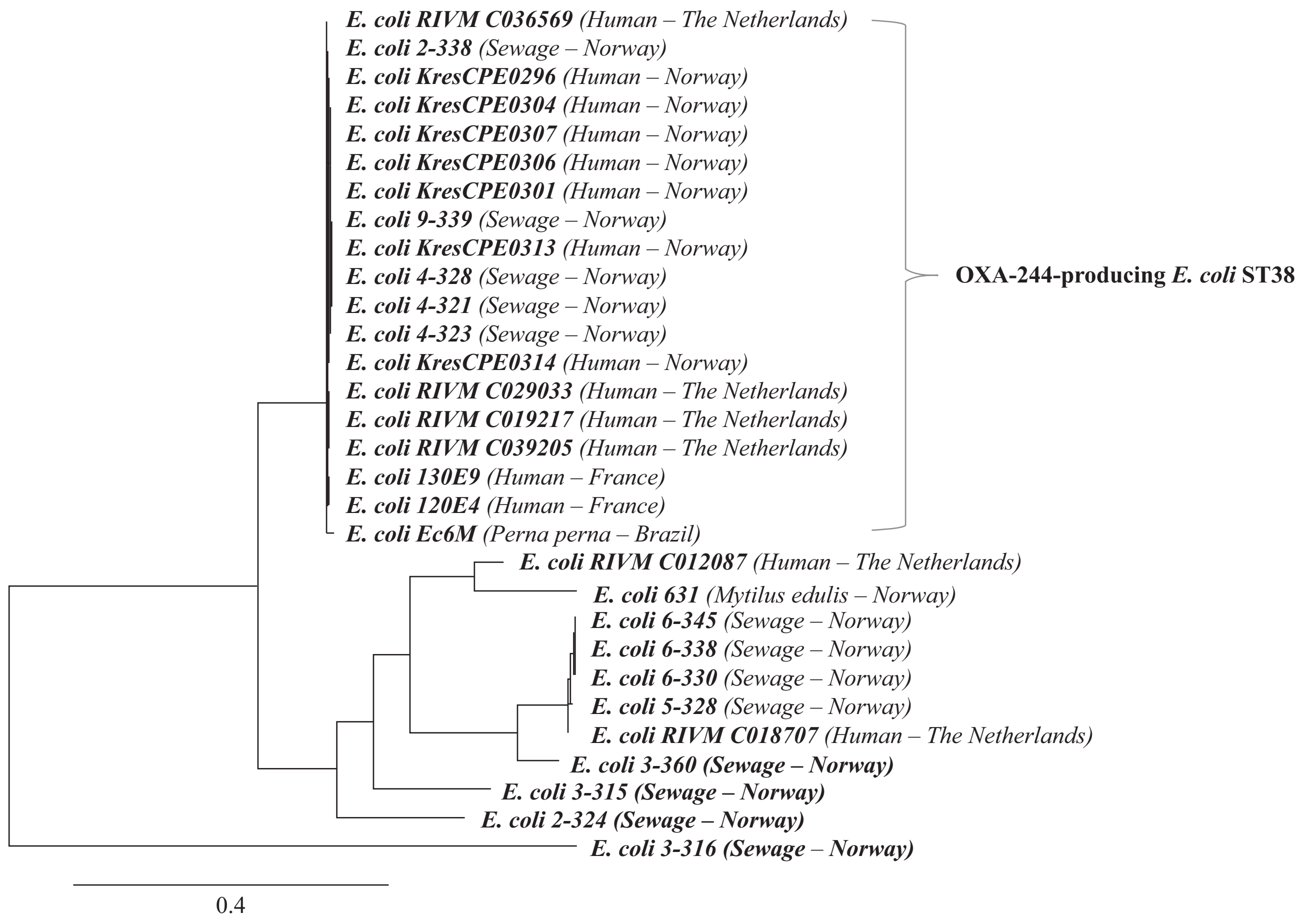

Figure 4. Single nucleotide polymorphism (SNP)-based core genome phylogenetic tree of five OXA-244-producing *Escherichia coli* strains (2-338, 4-321, 4-323, 4-328 and 9-339) and genome sequences of other ST38 strains retrieved from GenBank. This includes clinical OXA-244-producing *E. coli* ST38 strains (120E4, 130E9, Ec6M, KresCPE0296, KresCPE0304, KresCPE0307, KresCPE0306, KresCPE0301, KresCPE0313, KresCPE0314, RIVM C019217, RIVM C029033, RIVM C039205 and RIVM C036569), OXA-48-producing ST38 strains (RIVM C012087 and RIVM C018707), and other ST38 strains from Norway (2-324, 3-315, 3-316, 5-328, 6-330, 6-345 and 631).

suggesting that this clone is important for dissemination of CTX-M-type ESBLs in Norway [35].

Whole-genome phylogeny has allowed the delineation of ST131 into phylogenetic subgroups (clades), with each clade demonstrating differences in antimicrobial resistance profiles [36]. A study from the UK determined that CTX-M-15 was detected only in clades C1 and C2 but was not detected in clades A or B [37]. A longitudinal study (2002–2017) in Norway showed that ST131 clades A, C1 and C2 and were responsible for all the CTX-M positive isolates, while clade B did not carry any ESBLs [35]. Consistent with these results, we detected no clade B ST131 isolate in our study. The study showed that CTX-M-15 was the dominating ESBL in clades A and C2, while CTX-M-27 dominated in clade C1. In contrast, our study shows that CTX-M-27 is the predominant ESBL in clades A and C1, while clade C2 isolates exclusively carry CTX-M-15. Our study thus, suggests that the distribution of CTX-M in ST131 clades might have changed over a time of five years, although extensive clinical data is needed to ascertain the fact.

In conclusion, the prevalence of ESBLs detected in the cefotaxime-resistant *E. coli* strains obtained from sewage resembled the clinical prevalence of ESBL-producing *E. coli* strains in Norway [8], suggesting that the occurrence of CTX-M-type ESBLs observed in our strains potentially reflects the current local resistance situation [38]. The prevalence of carbapenem resistance, as well as the usage of carbapenems in Norway is low [39]. The repeated detection of epidemic-causing OXA-244-producing *E. coli* ST38 strains in the treatment plant receiving hospital sewage is, thus, of great concern. Our study demonstrates the ongoing dissemination of epidemic-causing CTX-M-producing *E. coli* ST131 strains, as well as OXA-244-producing *E. coli* ST38 high-risk clones, through hospital sewage in Norway, thus emphasizing the importance of sewage-based surveillance of resistance in pathogens for understanding the local resistance situation. Our study further highlights the need for mitigation strategies, such as onsite treatment of hospital sewage before it is released to municipal treatment plants, in order to counter environmental dissemination of clinically important resistant pathogens.

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Author contributions

D.H.G.: methodology, investigation, formal analysis, visualization, writing – original draft. V.R.: methodology, investigation, writing – review and editing. F.S.S: methodology, investigation, formal analysis, data curation, writing – review and editing. E.R.B.M.: resources, writing – review and editing. K.S.A: resources, writing – review and editing. M.P.V.: formal analysis, data curation, writing – review and editing. N.P.M.: conceptualization, methodology, investigation, validation, writing – review and editing, supervision, project administration, funding acquisition.

Conflict of interest statement

The authors declare no conflicts of interest.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jhin.2023.12.020.

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