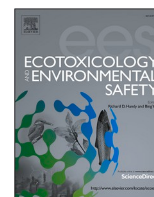
Ecotoxicology and Environmental Safety 226 (2021) 112788

Contents lists available at ScienceDirect

Ecotoxicology and Environmental Safety

journal homepage: www.elsevier.com/locate/ecoenv



Resistance profiles and diversity of β-lactamases in *Escherichia coli* strains isolated from city-scale sewage surveillance in Bergen, Norway mimic clinical prevalence

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ARTICLE INFO

Edited by: Dr Hyo-Bang Moon

Keywords:
Norway
Sewage-based surveillance
Escherichia coli
Antibiotic resistance genes
ESBLs

ABSTRACT

The aim of this study was to examine antibiotic resistance profiles and diversity of β-lactamases in *Escherichia coli* present within the population and the potential spread of resistant *E. coli* into the receiving environment using city-scale sewage surveillance. In *E. coli* isolates from ECC plates without antibiotics from ten influent samples (n = 300), highest resistance was observed against ampicillin (16.6%), sulfamethoxazole (9.7%) and trimethoprim (9.0%), while in effluent samples (n = 262) it was against sulfamethoxazole (11.8%), ampicillin (11.5%) and tetracycline (8.8%). All isolates (n = 123) obtained on cefotaxime-containing plates were multidrug-resistant. Several clinically important antibiotic resistance genes (ARGs) were detected in 46 *E. coli* isolates subjected to whole-genome sequencing, including carbapenemases like NDM-6, VIM-1 and OXA-48-variant, as well as tigecycline resistance gene *tet*(X4). CTX-M-15 was the most prevalent (42.9%) extended-spectrum β-lactamase among cefotaxime-resistant isolates, followed by CTX-M-27 (31.4%) and CTX-M-14 (17.1%), resembling clinical prevalence in Norway. Most of the sequenced isolates carried other clinically relevant ARGs, such as *dfrA17, sul1, sul2, tet*(A)*, aph(6)-Id, aph(3'')-Ib* and *aadA5*. Sixteen different sequence types (STs) were identified, including ST131 (39.1%), ST38 (10.9%) and ST69 (8.7%). One *E. coli* isolate belonging to novel ST (ST11874) carried multiple virulence factors including genotoxin, salmochelin, aerobactin and yersiniabactin, suggesting that this isolate has potential to cause health concerns in future. Our study reveals presence of clinically relevant ARGs like $bla_{\text{NDM-6}}$ and *tet*(X4) in pathogenic strains, which have so far not been reported from the clinics in Norway. Our study may thus, provide a framework for population-based surveillance of antibiotic resistance.

1. Introduction

Clinically relevant β-lactamases carried by the members of family *Enterobacteriaceae* represent an emerging public health threat, for which research and development of new antibiotics is urgently needed (WHO, 2017). Extended-spectrum β-lactamases (ESBLs) and carbapenemases are groups of enzymes that hydrolyze critical important β-lactam antibiotics, such as third-generation cephalosporins (3GCs) and carbapenems (Pfeifer et al., 2010). ESBL-producing *Escherichia coli* are a major cause of community-acquired infections, especially in Europe (Mathers et al., 2015). *E. coli* are mostly commensals and usually part of human gut microbiota, while some opportunistic, pathogenic strains can cause both common and severe infections. Pathogenic strains belonging to sequence type (ST) 131, 38 and 405, represents high-risk clones of significant public health concern that usually encode β-lactamases (Manges et al., 2019; Peirano and Pitout, 2019). Dissemination of specific clones and epidemic plasmids in clinical and community settings is important factor contributing towards the emergence of ESBLs in Europe (Bevan et al., 2017).

In Europe, the prevalence of clinical *E. coli* isolates resistant to 3GCs

Abbreviations: AMP, Ampicillin; ARGs, antibiotic resistance genes; AZI, azithromycin; BMRGs, biocide/metal resistance genes; CFU, colony-forming unit; CHL, chloramphenicol; CIP, ciprofloxacin; COL, colistin; ESBLs, extended-spectrum β-lactamases; Fle, Flesland; FOT, cefotaxime, GEN, gentamicin; Hol, Holen; Kn, Knappen; KvÅ, Kverneviken Åsane; MDR, multidrug resistance; MERO, meropenem; MIC, minimum inhibitory concentration; NAL, nalidixic acid; QRDRs, quinolone resistance-determining regions; SMX, sulfamethoxazole; ST, sequence type; STPs, sewage treatment plants; TAZ, ceftazidime; ET, tetracycline; TGC, tigecycline; MP, trimethoprim; YS, Ytre-Sandviken; 3GCs, third-generation cephalosporins.

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https://doi.org/10.1016/j.ecoenv.2021.112788
Received 2 July 2021; Received in revised form 7 September 2021; Accepted 14 September 2021
Available online 24 September 2021
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is higher in south-eastern countries compared to northern countries (ECDC, 2020). For instance, Italy represents a high-risk country in terms of antibiotic resistance due to, in part, extensive use of antibiotics (EMA, 2020). The prevalence of invasive *E. coli* isolates resistant to 3GCs in Italy was 30.9% in 2019 (ECDC, 2020). In contrast, Norway is a country with a low burden of antibiotic resistance in clinics (ECDC, 2020). In Norway, the clinical prevalence of ESBL-positive *E. coli* isolates in 2019 was 7.1% and 3.0% from blood and urine, respectively (NORM/-NORM-VET, 2020). Accordingly, the occurrence of ESBL-producing *E. coli* in the environment in Norway was also low (Bevan et al., 2017; Grevskott et al., 2017).

Sewage contains a collection of stools from the population connected to the sewage system (Hutinel et al., 2019; Huijbers et al., 2020). Efficient treatment is crucial for limiting the load of untreated sewage released into receiving environments. A major cause for the spread of antibiotic resistance is the partial lack of proper infrastructure for sewage treatment, especially in low- and middle-income countries (Lamba et al., 2018). Sewage serves as an important source for introducing clinically relevant pathogens and antibiotic resistance genes (ARGs) into the environment (Karkman et al., 2019; Marathe et al., 2017, 2013). This is of particular concern, since the efficiency for removing bacteria in sewage treatment systems in Norway is low (VKM, 2020).

Currently, several national surveillance programs for antibiotic resistance in clinical and veterinary settings exists. However, surveillance of antibiotic resistance in the community and the environment is lacking (Marano et al., 2020). Population-based sewage surveillance has emerged as a promising approach for studying the prevalence of antibiotic resistance in the community (Hutinel et al., 2019; Huijbers et al., 2020). Sewage makes it possible to analyze *E. coli* and associated antibiotic resistance within the population living in a specific area. The prevalence of ESBL-positive *E. coli* strains can be examined by targeting cefotaxime-resistant strains, which are frequently associated with multidrug-resistance (Marano et al., 2020). Thus, this approach can provide up-to-date surveillance of antibiotic resistance in *E. coli* present in the population, which can be used to alert the local authorities in case of emergence of rare or new resistance threats. In Norway, surveillance of antibiotic resistance in *E. coli* isolates obtained from sewage is lacking (VKM, 2020). The Norwegian surveillance program for antibiotic resistance is largely based on a collection of data from human and animal health sectors (NORM/NORM-VET, 2020), thus, systematic data on the prevalence of resistance in the community and environmental settings is lacking.

The aim of this study was to understand the prevalence of antibiotic resistance and diversity of ESBLs in *E. coli* present in the population in Bergen, Norway using city-scale sewage surveillance, as well as study the potential spread of resistant *E. coli* into receiving aquatic environment through analysis of the treated effluents. We further compared the resistance profiles of *E. coli* isolates from sewage and the clinical *E. coli* isolates from Norway.

2. Materials and methods

2.1. Collection of sewage samples

Composite samples, representing a 24-hour period, from both influent and treated effluent were collected on two occasions (August 5th and October 7th, 2020) from five sewage treatment plants (STPs) located in Bergen city in Norway (Table 1). The largest STP is Flesland (Fle) serving 152,000 inhabitants, which receives sewage from industries as well as the international airport. Holen (Hol) is the second largest STP serving 132,000 inhabitants and receives sewage from a 933-bed hospital. However, the proportion of industry, airport and hospital input is unknown. Knappen (Kn), Kverneviken Åsane (KvÅ) and Ytre-Sandviken (YS) serve 60,000, 56,000 and 44,000 inhabitants, respectively.

2.2. Isolation and identification of Escherichia coli

Sewage samples were transported to the laboratory in sterile containers at 4 °C, and the cultivation-based analyses were initiated within 6 h after collection. The samples were serially diluted ten-fold with sterile saline (0.85% NaCl) before plating on ECC (CHROMagar™, France) chromogenic media, with and without 2 μg/mL cefotaxime (Sigma-Aldrich, Germany), and incubated at 37 °C for 20–22 h. *E. coli* CCUG 17620 and *E. coli* CCUG 73937 (Grevskott et al., 2020) were included as negative and positive controls, respectively, for verifying the ECC plates with cefotaxime. The amount of *E. coli* was estimated by counting the number of blue colonies on the ECC plates. Subsequently, the ratio of cefotaxime-resistant *E. coli* to total *E. coli* was calculated. Isolated, blue colonies were randomly picked from the ECC plates, with and without cefotaxime, and restreaked on Mueller-Hinton (MH) Orientation (CHROMagar™, France) chromogenic media and incubated at 37 °C for 20–22 h. From each sample, ten and 30 isolates were picked from ECC plates with and without cefotaxime, respectively. Identification of isolates was performed using matrix-assisted laser desorption ionization-time of flight mass spectrometry (MALDI-TOF MS) at Institute of Marine Research (IMR) (Bruker Daltonics, Germany). Subsequently, confirmed *E. coli* isolates were stored at −80 °C in MH broth (Oxoid, UK) with 20% glycerol until further use.

2.3. Antibiotic sensitivity testing

The resistance profile of 685 *E. coli* isolates was determined against 14 antibiotics using a broth microdilution assay with Sensititre® EUVSEC plates (Thermo Scientific, USA) following manufacturer's protocol. Each isolate was tested for ampicillin (AMP), cefotaxime (FOT), ceftazidime (TAZ), meropenem (MERO), gentamicin (GEN), nalidixic acid (NAL), ciprofloxacin (CIP), trimethoprim (TMP), sulfamethoxazole (SMX), tetracycline (TET), tigecycline (TGC), azithromycin (AZI), chloramphenicol (CHL), and colistin (COL). The plates were incubated at 37 °C for 20–22 h. The isolates were defined as susceptible or resistant according to the EUCAST clinical breakpoints tables v.10.0 (EUCAST, 2020). For AZI, the minimum inhibitory concentration (MIC) breakpoint for *Salmonella* Typhi ($\geq$16 mg/L for wild-type isolates) was

Table 1
Overview of sewage treatment plants (STPs), capacity and type of treatment.

STP	Area	Capacity (nr. of inhabitants)	Type of treatment		
			Moving bed biofilm reactor (MBBR)	Activated sludge	Chemical removal
Ytre Sandviken	Centrum and Sandviken	44,000	yes	no	no
Flesland	Bergen South[a]	152,000	no	yes	no
Kverneviken Åsane	Åsane	56,000	no	no	yes
Knappen	Minde and Fyllingsdalen	60,000	no	no	yes
Holen	Centrum and Nordnes[b]	132,000	no	yes	no

[a] Receives sewage from industries and international airport
[b] Receives sewage from a 933-bed hospital

used when defining cut-off for resistant. For the remaining antibiotics not included in the EUCAST tables for *E. coli* (*i.e.* NAL, SMX and TET), the isolates were defined as resistant when growing in the highest corresponding antibiotic concentration. *E. coli* CCUG 17620 and *E. coli* CCUG 73937 (Grevskott et al., 2020) were included as negative and positive controls, respectively.

2.4. DNA extraction and sequencing

Based on phenotypic resistance profiles, 46 *E. coli* isolates were selected for genome sequencing. These isolates were grown overnight on MH agar (Oxoid, UK), either with or without 2 μg/mL cefotaxime (Sigma-Aldrich, Germany) based on the resistance profiles, at 37 °C. Genomic DNA was extracted from the isolates, using the DNeasy Blood and Tissue kit (Qiagen, Germany) following the manufacturer's protocol. The extracted DNA was quantified, using Qubit™ 2.0 Fluorometer with the dsDNA BR (Broad-Range) kit and NanoDrop™ 2000 Spectrophotometer (Thermo Scientific, USA) assay. Sequencing libraries were prepared using Nextera DNA Flex Library Prep kit (Illumina, USA). Sequencing was performed using Illumina MiSeq platform (Illumina, USA), with 2 × 300 bp chemistry, at the Norwegian Sequencing Centre (Ullevål University Hospital, Oslo, Norway).

2.5. Genome assembly and sequencing analysis

The raw reads generated by Illumina MiSeq were quality trimmed and assembled, using methods previously described (Radisic et al., 2020). Draft genomes were annotated using the Prokaryotic Genome Annotation Pipeline (PGAP) v.4.13 at the National Center for Biotechnology Information (NCBI) (Tatusova et al., 2016). Sequence types (STs) were identified, using the PubMLST database (https://pubmlst.org/bigsdb?db=pubmlst_ecoli_achtman_seqdef). *E. coli* isolates with novel STs were submitted to Enterobase v.1.1.2 (Zhou et al., 2020) in order to get new ST number. The presence of ARGs was analyzed, using the ResFinder v.4.1 (Bortolaia et al., 2020) and comprehensive antibiotic resistance database (CARD) v.3.1.0 (Alcock et al., 2020). Virulence genes were examined using the database of "core dataset of protein sequences" at the virulence factors database (VFDB) (Liu et al., 2019), while biocide/metal resistance genes (BMRGs) were examined using the BacMet database v.2.0 (Pal et al., 2014). For the BacMet database, BMRGs were analyzed using the script *BacMet-Scan.pl* against the database of "experimentally confirmed resistance genes".

2.6. Collection of clinical data from the Norwegian surveillance report for year 2019

Resistance data for clinical *E. coli* isolates, obtained from blood and urine, was retrieved from the Norwegian surveillance report (NORM/NORM-VET, 2020), which publishes annual antibiotic resistance results for specific microorganisms from primary diagnostics and national references laboratories.

3. Results

3.1. E. coli counts in influent and effluent samples

Cefotaxime-resistant *E. coli* were detected in all five influent samples (median 200 colony-forming unit (CFU) per mL), and three effluent samples (median 60 CFU/mL) in August 2020 (Supplementary Table S1) and all five influent and two effluent samples in October 2020 (median 420 CFU/mL and 50 CFU/mL, respectively). Total CFU for *E. coli* were higher in influents than in effluents for all five STPs in both August and October sampling. The ratio of cefotaxime-resistant *E. coli* to total *E. coli* in influents varied between 0.3% and 4.4% and for effluents between 0% and 5.0%, respectively, in August samples and between 1.4% and 13.3% and 0–1.1%, respectively, in October samples. During the two sampling occasions, 685 of the 687 isolates (99.7%) were identified as *E. coli*. One isolate was identified as *Citrobacter freundii*, while the other isolate was unidentifiable, and these isolates were removed from subsequent analyses. Out of 685 *E. coli* isolates obtained in this study, 562 were isolated on non-selective plates and 123 were isolated on plates containing cefotaxime.

3.2. Resistance rates in E. coli isolates

A total of 300 and 262 *E. coli* isolates obtained from influent and effluent samples on non-selective plates, respectively, were analyzed. The highest prevalence of resistance among the *E. coli* isolates obtained from influents in August was observed against AMP (12.7%), followed by SMX (9.3%) and TMP (8.0%), while no resistance was observed for MERO, TGC and COL (Fig. 1). For October samples, the highest resistance rate was observed for AMP (20.0%), followed by TET (10.7%), TMP (10.0%) and SMX (10.0%), while no resistance was observed for MERO, TGC and COL. Multidrug resistance (MDR), *i.e.* resistance against three or more antibiotic classes, was observed to be 7.3% for *E. coli* isolates obtained from influents during both sampling occasions.

E. coli isolates obtained from effluents from August showed highest resistance against SMX (13.1%), followed by AMP (9.8%) and AZI (9.0%), while no resistance was observed for MERO, TGC and COL (Fig. 1). Among the *E. coli* isolates obtained from effluents collected in October, the highest resistance rate was observed for AMP (12.9%), followed by SMX (10.7%) and TET (10.0%), while no resistance was observed for FOT, TAZ, MERO, TGC and COL. MDR was observed in 4.1% and 6.4% of *E. coli* isolates obtained from effluent samples from August and October, respectively. The resistance rates of *E. coli* isolates from five STPs did not differ between August and October sampling.

3.3. Comparison of resistance patterns in E. coli isolates from sewage and the clinics

Although lower overall prevalence of resistance was observed in isolates from sewage compared to clinical isolates, the patterns of resistance profiles of *E. coli* isolates from sewage (influents) were similar to those observed in isolates from clinics (blood and urine) (Supplementary Table S2). In all sample types, the highest prevalence of resistance was observed against AMP (43.3% for blood, 34.6% for urine and 16.3% for influents), followed by TMP (not reported for blood, 23.8% for urine and 9.0% for influents) and TMP/SMX (24.6% for blood, 20.5% for urine and 7.0% for influents). The resistance rates against FOT and TAZ were higher in blood (7.2% and 6.0%, respectively) compared to urine (3.2% and 2.3%, respectively) and influents (2.3% and 1.3%, respectively). No resistance was detected against TGC, except for isolates from blood (0.4%). In addition, no resistance was observed against MERO in isolates from all sample types.

3.4. Resistance profiles of cefotaxime-resistant E. coli isolates obtained from sewage

A total of 98 and 25 *E. coli* isolates obtained on cefotaxime-containing plates from influent and effluent samples, respectively, were analyzed. The highest rates of resistance among the *E. coli* isolates (n = 98) obtained from influents in August and October sampling were observed against AMP (100%), followed by FOT (98%) and NAL (64.3%), while no resistance was observed for COL (Supplementary Table S3). For isolates (n = 25) obtained from effluent samples, highest prevalence of resistance was observed towards AMP (100%) and FOT (100%), followed by NAL (64.0%), while no resistance was observed against MERO, TGC and COL (Supplementary Table S3). A higher prevalence of MDR *E. coli* isolates was observed in effluents (100%) compared to influents (86.7%).

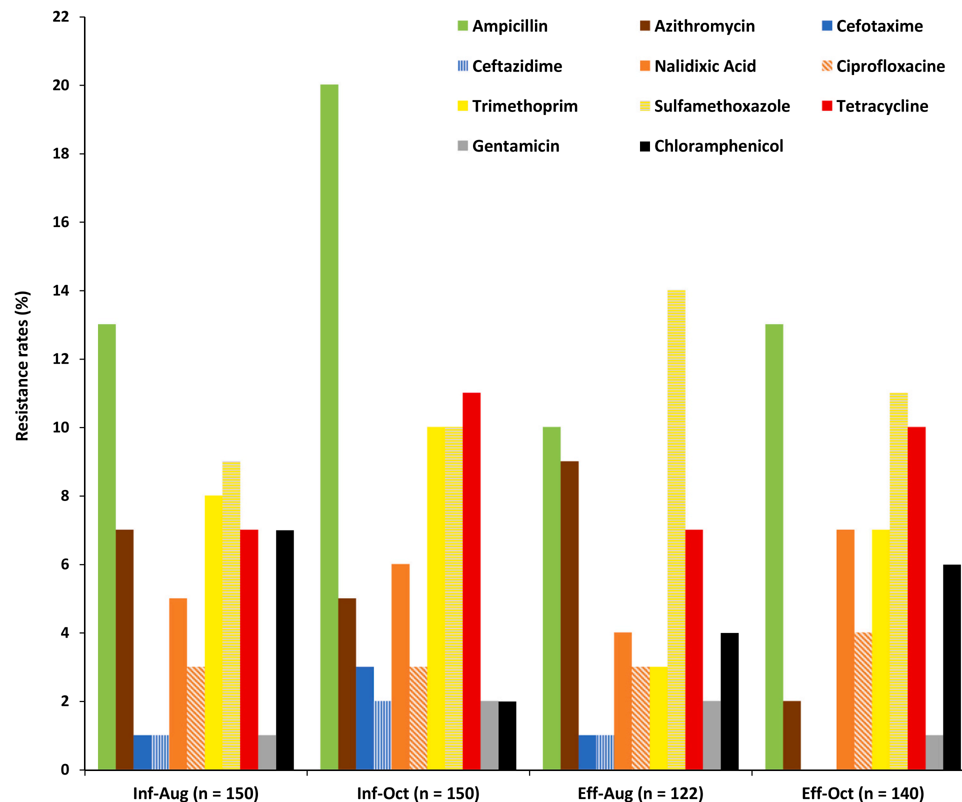

Fig. 1. Resistance rates (%) of *Escherichia coli* isolated from influent (n = 300) and effluent (n = 262) samples collected from five sewage treatment plants in Bergen, Norway in August 2020 and October 2020, respectively. No resistance was observed for meropenem, tigecycline or colistin, hence these antibiotics were not included in the figure.

3.5. Diversity of E. coli STs and clinically relevant β-lactamases

Genome assembly statistics of the draft genome sequences of the sequenced *E. coli* isolates (n = 46) are presented in Supplementary Table S4. Among these, the prevalent sequence type was ST131 (39.1%), followed by ST38 (10.9%) and ST69 (8.7%) (Table 2). Four isolates were assigned to two novel STs, three isolates belonged to ST11873 and one belonged to ST11874. Most isolates carried ESBL and/or carbapenemase genes (Table 2). Isolate 2–331 and 2–333 obtained from influents carried bla_{NDM-6}, while isolate 2–338 and 3–349 obtained from effluents carried $bla_{OXA-244}$ and bla_{VIM-1}, respectively. Among the CTX-M-carrying isolates (n = 35), the majority harbored $bla_{CTX-M-15}$ gene (42.9%), followed by $bla_{CTX-M-27}$ (31.4%) and $bla_{CTX-M-14}$ (17.1%), resembling the clinical prevalence of ESBLs in *E. coli* in Norway (NORM/NORM-VET, 2020). Three isolates carried $bla_{CTX-M-55}$, $bla_{CTX-M-24}$ and $bla_{CTX-M-3}$, respectively. In addition, several other β-lactamase genes were detected among the isolates, such as bla_{TEM-1B}, bla_{TEM-40}, $bla_{TEM-135}$, bla_{SHV-2}, bla_{DHA-1}, bla_{CMY-42} and bla_{OXA-1}. Twelve CTX-M-producing *E. coli* strains also carried TEM-1B. VIM-1-producing isolate 3–349 carried several β-lactamases, such as CTX-M-15, TEM-1B, SHV-2 and OXA-1.

3.6. Other clinically relevant ARGs, virulence genes and BMRGs detected in sequenced strains

Among the sequenced *E. coli* isolates (n = 46), apart from β-lactamases, the majority carried genes conferring resistance to sulfonamides (73.9%), aminoglycosides (71.7%), macrolides (65.2%), tetracyclines (63.0%), trimethoprim (60.9%), fluoroquinolones (32.6%) and phenicols (26.1%) (Table 2). The most prevalent combination of ARGs detected in the sequenced isolates (n = 46) was observed in ten CTX-M-producing isolates, carrying *dfrA17*, *sul1*, *sul2*, *tet*(A), *aph(6)-Id, aph (3'')-Ib, aadA5*, and *mph(A)*. Notably, one CTX-M-14-producing isolate carried *tet*(X4) gene conferring resistance to tigecycline (Marathe et al., 2021). In addition, 70% of the isolates (n = 32) had mutations in quinolone resistance-determining regions (QRDRs) of *gyrA* and *parC* (Table 2), such as *gyrA*(S83L), explaining the high level of resistance observed against NAL and CIP (Hooper and Jacoby, 2015).

The isolates carried several virulence factors, including aerobactin (*iucA-D, iutA*), ferrienterobactin (*fepA-D, fepG, fes*), salmochelin (*iroB-E, iroN*) and yersiniabactin siderophores (*fyuA*, *irp1–2, ybtA/E/P/Q/S/U/X*), and type III (*espL1/4, espR1/3, espX1–2, espX4–5, espY3–4*) and type VI (*tssA-C, tssF-G, tssJ/L/M*) secretion systems (Supplementary Table S5). In addition, a few isolates harbored important toxins like cytotoxic necrotizing factor (*cnf1*), hemolysin (*hlyA-D)*, genotoxin colibactin (*clbA, clbP*) and *Shigella* enterotoxin (*senB, set1A-1B*), suggesting the pathogenic potential of these isolates (Garcia et al., 2013; Gu et al., 2019; McCarthy et al., 2015). Along with virulence, many isolates harbored multiple BMRGs encoding resistance to acriflavine (*acrA-B, acrE/envC, acrF/envD, acrR/ybaH*), quaternary ammonium compounds *(qacE/F, qacEΔ1, sugE)*, copper (*pcoA, copA, cusA)* and zinc (*zntA, znuA, zraR*) (Supplementary Table S6). A few isolates carried genes conferring resistance to silver (*silA-C, silE-F, silP, silR-S*) and mercury (*merA-B, merD/P/T, merR1-R2*).

4. Discussion

To the best of our knowledge, this is the first study performing a city-scale sewage surveillance of antibiotic resistance in *E. coli* strains, especially from Norway. Our study reveals the presence of clinically relevant carbapenemases and ESBLs like NDM-6, VIM-1, OXA-48-variant, CTX-M-15 and CTX-M-14, as well as tigecycline resistance gene *tet*(X4) in *E. coli* present in the local population in Bergen city, Norway, as well as in treated effluents from STPs. We also show that the resistance profiles and diversity of ESBLs detected in *E. coli* isolates

Table 2
Sequence type (ST), antibiotic resistance genes (ARGs), and mutations in quinolone resistance-determining regions (QRDRs) of *gyrA* and *parC* detected in *Escherichia coli* isolates (n = 46) obtained from influent (Inf.) and effluent (Eff.) samples in this study.

Isolate	Sample type	ST	ARGs	QRDRs
2–10	Inf.	ST744	$bla_{\text{TEM-1B}}$, *dfrA17, sul1, sul2, tet*(B), *aadA5, aph(3'')-Ib, aph(6)-Id, catA1, mph(A)*	*gyrA*(S83L, D87N), *parC* (S80I)
2–13	Inf.	ST10	$bla_{\text{TEM-1B}}$, *dfrA5, sul2*	–
2–28	Inf.	ST349	$bla_{\text{DHA-1}}$, *dfrA7, dfrA17, sul1, sul2, qnrB4, tet(A), aph(3'')-Ib, aph(6)-Id, mph(A)*	–
2–53	Eff.	ST1380	$bla_{\text{TEM-1B}}$, $bla_{\text{DHA-1}}$, *sul1, qnrB4, mph(A)*	–
2–75	Inf.	ST69	$bla_{\text{CTX-M-14}}$, $bla_{\text{TEM-1B}}$, *dfrA14, mph(A)*	*gyrA*(S83L, D87N), *parC* (S80I)
2–87	Inf.	ST131	$bla_{\text{TEM-1B}}$, *dfrA17, sul1, sul2, tet*(A), *aph(6)-Id, aph(3'')-Ib, aac(3)-Iid, mph(A)*	*gyrA*(S83L)
2–106	Inf.	ST46	$bla_{\text{OXA-1}}$, *tet*(B), *aadA1, catA1*	–
2–116	Inf.	ST58	$bla_{\text{TEM-1B}}$, *dfrA5, sul2, tet*(B), *aph(6)-Id, aph(3'')-Ib*	*gyrA*(S83L)
2–165	Inf.	ST131	$bla_{\text{CTX-M-27}}$, $bla_{\text{DHA-1}}$, *dfrA17, sul1, sul2, qnrB4, tet*(A), *aph(3'')-Ib, aph(6)-Id, mph(A)*	*gyrA*(S83L, D87N), *parC* (S80I)
2–233	Inf.	ST131	$bla_{\text{CTX-M-3}}$, $bla_{\text{TEM-1B}}$	*gyrA*(S83L)
2–301	Inf.	ST131	$bla_{\text{CTX-M-27}}$, *dfrA17, sul1, sul2, tet*(A), *aph(3'')-Ib, aph(6)-Id, aadA5, mph(A)*	*gyrA*(S83L)
2–302	Inf.	ST131	$bla_{\text{CTX-M-24}}$, $bla_{\text{TEM-1B}}$	*gyrA*(S83L)
2–307	Eff.	ST131	$bla_{\text{CTX-M-27}}$, *dfrA17, sul1, sul2, tet*(A), *aph(6)-Id, aph(3'')-Ib, aadA5, mph(A)*	*gyrA*(S83L)
2–309	Inf.	ST131	$bla_{\text{CTX-M-27}}$, *dfrA17, sul1, sul2, tet*(A), *aph(6)-Id, aph(3'')-Ib, aadA5, mph(A)*	*gyrA*(S83L, D87N), *parC* (S80I)
2–310	Inf.	ST131	$bla_{\text{CTX-M-15}}$, $bla_{\text{TEM-1B}}$, $bla_{\text{OXA-1}}$, *aac(6')-Ib-cr, aac(3)-IIe*	*gyrA*(S83L, D87N), *parC* (S80I)
2–318	Inf.	ST295	$bla_{\text{CTX-M-15}}$, *dfrA1, sul2, qnrS1, tet*(A), *aadA1, aph(3'')-Ib, aph(6)-Id*	–
2–320	Inf.	ST69	$bla_{\text{CTX-M-15}}$, *qnrS1*	–
2–322	Inf.	ST131	$bla_{\text{CTX-M-27}}$, *dfrA17, sul1, sul2, tet*(A), *aph(6)-Id, aph(3'')-Ib, aadA5, mph(A)*	*gyrA*(S83L, D87N), *parC* (S80I)
2–324	Inf.	ST38	$bla_{\text{CTX-M-15}}$, $bla_{\text{TEM-1B}}$, *dfrA7, dfrA12, sul1, tet*(D), *aadA2, aac(3)-IId, catA1, mph(A)*	*gyrA*(S83L, D87N), *parC* (S80I)
2–326	Inf.	ST167	$bla_{\text{CTX-M-14}}$, *dfrA12, sul2, qnrS1, tet*(X4), *tet*(M), *aph(6)-Id, aadA1, aadA2, ant(2'')-Ia, aph(3'')-Ib, cml, erm(B), erm (42)*	*gyrA*(S83L, D87N), *parC* (S80I)
2–327	Inf.	ST131	$bla_{\text{CTX-M-27}}$, $bla_{\text{TEM-1B}}$, *dfrA17, sul1, sul2, tet*(A), *aadA5, aph(3'')-Ib, aph(6)-Id, aac(3)-Iid, mph(A)*	*gyrA*(S83L)
2–329	Inf.	ST131	$bla_{\text{CTX-M-15}}$, *dfrA17, sul1, sul2, tet*(A), *aph(3'')-Ib, aadA5, aph(6)-Id, mph(A)*	*gyrA*(S83L)
2–331	Inf.	ST167	$bla_{\text{NDM-6}}$, $bla_{\text{TEM-1B}}$, $bla_{\text{CMY-42}}$, *dfrA12, sul1, tet*(A), *aadA2, rmtB, mph(A)*	*gyrA*(S83L, D87N), *parC* (S80I)
2–333	Inf.	ST167	$bla_{\text{NDM-6}}$, $bla_{\text{TEM-1B}}$, $bla_{\text{CMY-42}}$, *dfrA12, sul1, tet*(A), *aadA2, rmtB, mph(A)*	*gyrA*(S83L, D87N), *parC* (S80I)
2–335	Inf.	ST10	$bla_{\text{CTX-M-15}}$, *qnrS1*	*gyrA*(S83L)
2–338	Eff.	ST38	$bla_{\text{OXA-244}}$, $bla_{\text{CTX-M-27}}$, *dfrA17, sul1, sul2, tet*(A), *aph(6)-Id, aph(3'')-Ib, aadA5, mph(A)*	*gyrA*(S83L)
2–343	Inf.	ST131	$bla_{\text{CTX-M-15}}$, $bla_{\text{OXA-1}}$, *tet*(A), *aac(6')-Ib-cr, aac(3)-IIe*	*gyrA*(S83L, D87N), *parC* (S80I)
2–345	Inf.	ST1193	$bla_{\text{CTX-M-15}}$, $bla_{\text{TEM-1B}}$	*gyrA*(S83L, D87N), *parC* (S80I)
2–346	Inf.	ST5044	$bla_{\text{CTX-M-55}}$, *sul2, qnrS4, tet*(A), *aph(6)-Id, aph(3'')-Ib, floR*	–
2–348	Inf.	ST131	$bla_{\text{CTX-M-15}}$, $bla_{\text{TEM-1B}}$	*gyrA*(S83L)
3–178	Inf.	ST11874[a]	$bla_{\text{TEM-40}}$	–
3–304	Inf.	ST69	$bla_{\text{CTX-M-27}}$, $bla_{\text{TEM-1B}}$, *dfrA17, sul1, tet*(A), *aac(3)-IIe, aadA5, erm(B), mph(A)*	*gyrA*(S83L)
3–315	Inf.	ST38	$bla_{\text{CTX-M-14}}$, $bla_{\text{TEM-1B}}$, *dfrA12, sul1, sul2, tet*(D), *aadA2, aph(3'')-Ib, aph(6)-Id, mph(A)*	*gyrA*(S83L, D87N), *parC* (S80I)
3–316	Inf.	ST38	$bla_{\text{CTX-M-14}}$, $bla_{\text{TEM-135}}$, *tet*(A)	–
3–325	Inf.	ST69	$bla_{\text{CTX-M-27}}$, $bla_{\text{DHA-1}}$, *dfrA17, sul1, qnrB4, aadA5, mph(A)*	*gyrA*(S83L)
3–338	Inf.	ST11873[a]	$bla_{\text{CTX-M-15}}$, $bla_{\text{TEM-1B}}$, $bla_{\text{OXA-1}}$, *sul2, qnrS1, aac(3)-IIe, aac(6')-Ib-cr, aph(3')-Ia, catB3, floR, mph(A)*	–
3–341	Inf.	ST11873[a]	$bla_{\text{CTX-M-15}}$, $bla_{\text{TEM-1B}}$, $bla_{\text{OXA-1}}$, *sul2, qnrS1, aac(3)-IIe, aac(6')-Ib-cr, aph(3')-Ia, catB3, floR, mph(A)*	–
3–343	Inf.	ST11873[a]	$bla_{\text{CTX-M-15}}$, $bla_{\text{TEM-1B}}$, $bla_{\text{OXA-1}}$, *sul2, qnrS1, aac(3)-IIe, aac(6')-Ib-cr, aph(3')-Ia, catB3, floR, mph(A)*	–
3–344	Inf.	ST131	$bla_{\text{CTX-M-14}}$, $bla_{\text{TEM-1B}}$, *dfrA17, sul1, sul2, tet*(A), *aac(3)-IId, aadA5, aph(6)-Id, aph(3'')-Ib, mph(A)*	*gyrA*(S83L, D87N), *parC* (S80I)
3–346	Inf.	ST131	$bla_{\text{CTX-M-27}}$, *dfrA17, sul1, sul2, tet*(A), *aph(6)-Id, aadA5, aph(3'')-Ib, mph(A)*	*gyrA*(S83L, D87N), *parC* (S80I)
3–347	Inf.	ST131	$bla_{\text{CTX-M-15}}$, $bla_{\text{TEM-1B}}$, $bla_{\text{OXA-1}}$, *dfrA17, sul1, tet*(A), *aadA5, aac(3)-IIe, aac(6')-Ib-cr, mph(A)*	*gyrA*(S83L, D87N), *parC* (S80I)
3–348	Inf.	ST131	$bla_{\text{CTX-M-27}}$, $bla_{\text{TEM-1B}}$, *dfrA17, sul1, sul2, tet*(A), *aph(6)-Id, aph(3'')-Ib, aadA5, aac(3)-Iid, mph(A)*	*gyrA*(S83L)
3–349	Eff.	ST635	$bla_{\text{VIM-1}}$, $bla_{\text{CTX-M-15}}$, $bla_{\text{TEM-1B}}$, $bla_{\text{SHV-2}}$, $bla_{\text{OXA-1}}$, *dfrA14, sul1, sul2, qnrB1, tet*(A), *aph(3'')-Ib, aph(6)-Id, aac(6')-Ib4, aph(3')-XV, aac(3)-IIe, catA1*	–
3–354	Eff.	ST131	$bla_{\text{CTX-M-15}}$, $bla_{\text{TEM-1B}}$, $bla_{\text{OXA-1}}$, *dfrA17, sul1, tet*(A), *aac(6')-Ib-cr, aac(3)-IIe, aadA5, mph(A)*	*gyrA*(S83L, D87N), *parC* (S80I)
3–355	Inf.	ST1380	$bla_{\text{TEM-1B}}$, $bla_{\text{DHA-1}}$, *sul1, qnrB4, mph(A)*	–
3–360	Inf.	ST38	$bla_{\text{CTX-M-14}}$, *sul2, aph(3'')-Ib, aph(6)-Id*	*gyrA*(S83L), *parC*(S80I)

[a] Novel sequence type.

obtained from sewage resembled the clinical prevalence in Norway.

NDM-6 was detected in two isolates (2–331 and 2–333) from influent (Table 2). They belonged to ST167, which is a known pathogenic sequence type of *E. coli*, usually associated with extra-intestinal infections (Manges et al., 2019). In accordance, these isolates harbor several virulence factors, including ferrienterobactin and yersiniabactin siderophores, type III and type VI secretion systems, suggesting potential for virulence (Johnson et al., 2018; Navarro-Garcia et al., 2019). Although, NDM-5-producing *E. coli* belonging to ST167 has previously been detected, NDM-6 has so far not been reported from the clinics in Norway (NORM/NORM-VET, 2020). Our findings thus, provide important insights into the current resistance situation within the local population, revealing the presence of clinically relevant carbapenemases in pathogenic *E. coli* strains, that are prevalent in the clinics in other countries (Dadashi et al., 2019).

We also detected carbapenemases OXA-244 and VIM-1 in two isolates (2–338 and 3–349, respectively) from effluent (Table 2). The OXA-244-producing isolate belonged to the high-risk clone ST38, which is widespread globally and responsible for causing urinary tract infections (Manges et al., 2019). This isolate also carried multiple virulence factors, such as ferrienterobactin and yersiniabactin siderophores, type III and type VI secretion systems (Supplementary Table S5), indicating the potential for virulence. Outbreaks with OXA-244-producing *E. coli* ST38 has recently been reported from Bergen, Norway (UNN, 2020). This highlights the importance of city-scale sewage surveillance of antibiotic resistance for providing up-to-date knowledge on the local resistance situation. Although the VIM-1-producing isolate belonged to the commensal ST635, $bla_{\text{VIM-1}}$ gene is mobile and can be transferred between different pathogens (Arcari et al., 2020). The clinical prevalence of VIM-variants in Norway have remained largely stable, while NDM-variants and OXA-48-variants have had a gradual increase during the last ten years (NORM/NORM-VET, 2020). Two carbapenemase-producing isolates obtained from effluent suggests that clinically relevant carbapenemases are introduced into the receiving environment through sewage pollution (Lamba et al., 2018; Karkman et al., 2019; Marathe et al., 2019). This may contribute to environmental transmission of clinically important ARGs conferring resistance to last-resort antibiotics (Marathe et al., 2017). Along with large amounts of fecal material, sewage also contains considerable amounts of other antimicrobial agents, such as biocides and heavy metals (Östman et al., 2017). These agents are widely used in healthcare and agriculture, and would inevitably end up in sewage treatment systems as well as the environment (Wang and Liang, 2021; Rehman et al., 2019). Heavy metal pollution has been observed in the marine environment in Norway, especially in areas close to aquaculture and mining activities, thereby contributing to possible selection pressure (IMR, 2021). The VIM-1-producing isolate carried several BMRGs, including genes conferring resistance silver, copper and mercury (Supplementary Table S6), suggesting the potential for persistence and spread of this isolate in the marine environment (Yuan et al., 2019).

Besides detecting carbapenemase-producing *E. coli*, pathogenic strains carrying different CTX-M-type ESBLs were also detected in influents and treated effluents that are entering the receiving environment. In Norway, CTX-M-type ESBLs have emerged as a significant clinical problem (NORM/NORM-VET, 2020). Among CTX-M-producing isolates in our study, $bla_{\text{CTX-M-15}}$ was most frequently detected, followed by $bla_{\text{CTX-M-27}}$ and $bla_{\text{CTX-M-14}}$. The prevalence of CTX-M-type ESBLs detected in our isolates was similar to the pattern reported from the clinical *E. coli* isolates from 2019 in Norway (NORM/NORM-VET, 2020), suggesting that the diversity of ESBLs observed among the *E. coli* isolates obtained from influent, thus, potentially reflects the current local resistance situation (Hutinel et al., 2019; Huijbers et al., 2020). Most of the CTX-M-producing isolates in our study belonged to pathogenic STs, such as 131, 38 and 69 that are known to cause extra-intestinal infections (Manges et al., 2019). In Norway, most of the clinical isolates of ESBL-producing *E. coli* obtained from blood samples (n = 141) in 2019 belonged to ST131 (56.7%), followed by ST38 (5.0%) (NORM/NORM-VET, 2020), suggesting that these high-risk clones are important for the dissemination of CTX-M-type ESBLs in Norway (Grevskott et al., 2020; Paulshus et al., 2019). In addition, four isolates belonging to two novel STs of *E. coli* were detected in our study (Table 2). Among these, one isolate (3−178) belonging to novel ST11874 carried multiple virulence factors, including cytotoxic necrotizing factor 1, genotoxin colibactin ClbA-Q, hemolysin A-D, as well as ferrienterobactin, salmochelin, aerobactin and yersiniabactin siderophores, indicating that this isolate belonging to novel ST may represent a potential pathogen that may cause significant public health concern in future (Gu et al., 2019; McCarthy et al., 2015; Johnson et al., 2018).

Reduction in the number of cefotaxime-resistant *E. coli* and total *E. coli* in the effluents was observed for all five STPs (Supplementary Table S1). For Fle and Kn, there was complete reduction in the number of cefotaxime-resistant *E. coli* isolates during both occasions, while for KvÅ there was complete reduction in the number of cefotaxime-resistant *E. coli* isolates in effluent from October sampling. Although Hol is the only site that receives hospital sewage (Table 1), the number of cefotaxime-resistant *E. coli* isolates in influent and effluent of this STP did not differ compared to the other STPs. However, clinically important metallo-β-lactamases like NDM-6 and VIM-1, as well as tigecycline resistance gene *tet*(X4), were detected only in this treatment plant. The resistance profiles of *E. coli* isolates obtained from influent samples were similar, but a fold lower than, the clinical prevalence in Norway (Supplementary Table S2). A possible explanation for the observed differences in resistance rates is that the clinical prevalence of resistance is based on *E. coli* strains causing infections, while isolates obtained from influents predominantly originate from the local population connected to the sewage system and may represent both pathogenic and commensal *E. coli* strains (Clermont et al., 2017). Nevertheless, the resistance pattern observed in the *E. coli* isolates obtained from influents in August and October sampling mimicked the clinical prevalence in Norway (NORM/NORM-VET, 2020). Our study thus, provides important insights into the resistance situation in the local population.

5. Conclusion

Our study highlights the importance of sewage-based surveillance of antibiotic resistance, in order to understand the current resistance situation in the population. It demonstrates the presence of clinically relevant carbapenemases, such as NDM-6, VIM-1 and OXA-244, in *E. coli* isolates obtained from influents and effluents collected from Bergen city in Norway. Norway has a very low prevalence of carbapenem-resistance, as well as a low usage of last-resort antibiotics (NORM/NORM-VET, 2020). Detection of clinically important carbapenemases in sewage emphasizes the need for population-based sewage surveillance of resistance in order to understand the local resistance situation and to predict future local outbreaks. Our study may thus, provide a framework for population-based surveillance of antibiotic resistance, especially in Norway.

CRediT author contribution statement

Didrik H. Grevskott: Methodology, Investigation, Formal analysis, Visualization, Writing – original draft. Fatemeh **Z. Ghavidel:** Formal analysis, Data curation, Writing – review & editing. **Cecilie S. Svanevik:** Resources, Writing – review & editing. **Nachiket P. Marathe:** Conceptualization, Methodology, Investigation, Validation, Writing – review & editing, Supervision, Project administration, Funding acquisition.

Declaration of Competing Interest

The authors declare that no conflict of interest exists.

Data availability

The assembled genome sequences have been submitted to GenBank under the following accession numbers: JADZIC000000000, JADZID 000000000, JADZIE000000000, JADZIF000000000, JADZIG000000000, JADZIH000000000, JADZII000000000, JADZIJ000000000, JADZIK 000000000, JADZIL000000000, JADZIM000000000, JADZIN000000000, JADZIO000000000, JADZIP000000000, JADZIQ000000000, JADZIR 000000000, JADZIS000000000, JADZIT000000000, JADZIU000000000, JADEZU000000000, JADZIV000000000, JADZIW000000000, JADZIX 000000000, JADZIY000000000, JADZIZ000000000, JADZJA000000000, JADZJB000000000, JADZJC000000000, JADZJD000000000, JADZJE00 0000000, JAFKEB000000000, JADZJF000000000, JADZJG000000000, JADZJH000000000, JADZJI000000000, JADZJJ000000000, JADZJK 000000000, JADZJL000000000, JADZJM000000000, JADZJN00000 0000, JADZJO000000000, JADZJP000000000, JADZJQ000000000, JADZJR000000000, JADZJS000000000, JADZJT000000000.

Acknowledgements

The study was financially supported by the Institute of Marine Research under the Ocean Health initiative (project number: 15495) and the Research Council of Norway (grant number 315266) to Nachiket P. Marathe. We are grateful to Sandra McCarley from Bergen Vann, Kristine S. Akervold from Bergen municipality for their support and permission for sample collection. We also thank Fereidun Akhoundzadeh from Bergen municipality for his help with arranging sampling, and the people working at wastewater treatment plants for help with collecting samples. The sequencing service was provided by the Norwegian Sequencing Centre (www.sequencing.uio.no), a national technology platform hosted by the University of Oslo and supported by the "Functional Genomics" and "Infrastructure" programs of the Research Council of Norway and the South eastern Regional Health Authorities. We thank Kateryna S. Natvik and Vera Radisic for providing technical assistance.

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.ecoenv.2021.112788.

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