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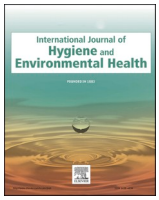

Sewage-based surveillance shows presence of *Klebsiella pneumoniae* resistant against last resort antibiotics in the population in Bergen, Norway

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ABSTRACT

The aim of this study was to understand the prevalence of antibiotic resistance in *Klebsiella pneumoniae* present in the population in Bergen city, Norway using city-scale sewage-based surveillance, as well as the potential spread of *K. pneumoniae* into the marine environment through treated sewage. From a total of 30 sewage samples collected from five different sewage treatment plants (STPs), 563 presumptive *K. pneumoniae* isolates were obtained on Simmons Citrate Agar with myo-Inositol (SCAI) plates, and 44 presumptive *K. pneumoniae* isolates on SCAI plates with cefotaxime. Colistin resistance was observed in 35 isolates, while cefotaxime resistance and tigecycline resistance was observed in only five isolates each, out of 563 presumptive *K. pneumoniae* isolates. All 44 isolates obtained on cefotaxime-containing plates were multidrug-resistant, with 25% (n = 11) showing resistance against tigecycline. Clinically important acquired antibiotic resistance genes (ARGs), like $bla_{\text{CTX-M-14}}$, $bla_{\text{CTX-M-15}}$, *qnrS1*, *aac(3)-IIe*, *tet*(A), and *sul1*, were detected in several sequenced *Klebsiella* spp. isolates (n = 53). All sequenced colistin-resistant isolates (n = 13) had a mutation in the *mgrB* gene with nucleotide substitution at position C88T creating a premature stop codon. All sequenced tigecycline-resistant isolates (n = 4) harbored a Tet(A) variant with 22 amino acid (aa) substitutions compared to the reference protein. The sequenced *K. pneumoniae* isolates (n = 44) belonged to 22 different sequence types (STs) with ST730 (29.5%) as most prevalent, followed by pathogenic ST307 (11.4%). Virulence factors, including aerobactin (*iutA*), enterobactin (*entABCDEFS* and *fepABCDG*), salmochelin (*iro*), and yersiniabactin (*ybt*) were detected in several sequenced *K. pneumoniae* isolates, suggesting pathogenicity potential. Heavy metal resistance genes were common in sequenced *K. pneumoniae* isolates (n = 44) with silver (*silABCEFPRS*) and copper (*pcoABDRS*) resistance genes present in 79.5% of the isolates. Sewage-based surveillance can be a useful tool for understanding antibiotic resistance in pathogens present within a population and to provide up-to date information on the current resistance situation. Our study presents a framework for population-based surveillance of resistance in *K. pneumoniae*.

1. Introduction

Klebsiella pneumoniae is an opportunistic human pathogen known for causing both hospital and community acquired infections (Lepuschitz et al., 2020; Navon-Venezia et al., 2017; Rodrigues et al., 2018; Shon et al., 2013; Siu et al., 2012). Most infections caused by the classical *K. pneumoniae* have historically occurred in hospitals (Shon and Russo, 2012). Recently, hypervirulent *K. pneumoniae* (hvKp) isolates are responsible for causing community acquired infections among healthy people (Russo and Marr, 2019; Shon and Russo, 2012). The *K. pneumoniae* species complex (KpSC) consists of several closely related species including *K. pneumoniae sensu stricto*, *K. quasipneumoniae* subsp. *quasipneumoniae*, *K. quasipneumoniae* subsp. *similipneumoniae*, *K. variicola* subsp. *tropica*, *K. variicola* subsp. *variicola*, *K. quasivariicola*, and *K. africana*, with *K. pneumoniae sensu stricto* as the major species (Wyres et al., 2020). The members of the KpSC are widespread in nature and commonly found in humans, animals, and plants (Lepuschitz et al., 2020; Wyres et al., 2020). Within the KpSC, *K. pneumoniae*, *K. quasipneumoniae*, and *K. variicola* are most frequently reported from clinical samples (Fostervold et al., 2021; Maatallah et al., 2014;

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Rodrigues et al., 2018).

Antibiotic-resistant *K. pneumoniae* caused more than 91,000 infections and 7,400 deaths in Europe in 2015 (Cassini et al., 2019; Wyres et al., 2020). *K. pneumoniae* is known for harboring multiple antibiotic resistance genes (ARGs) thus, making treatment challenging (Rodrigues et al., 2018; Wyres and Holt, 2018). Carbapenem-resistant and extended-spectrum β-lactamase (ESBL)-producing *K. pneumoniae* are an emerging threat to the public health with urgent need for new antibiotics according to the World Health Organization (WHO) (WHO, 2017). Resistance against third generation cephalosporins and carbapenems are increasing worldwide in *K. pneumoniae* (ECDC, 2020). Colistin and tigecycline are last resort antibiotics used for treatment of carbapenem-resistant *K. pneumoniae* infections (Xu et al., 2021). Colistin has long been used in veterinary medicine to prevent and treat infections, as well as in animal feed to promote growth in food producing animals (Poirel et al., 2017). By contrast, tigecycline has not been approved or used in livestock (Martelli et al., 2022). However, the European Union (EU) banned the use of colistin for growth promotion in animal food in 2006 (Maron et al., 2013). In Norway, resistance against colistin in isolates of *K. pneumoniae* collected from different animals between 2017 and 2020 was low, around 1.4%, whereas all isolates were sensitive to tigecycline (NORM/NORM-VET, 2020).

Surveillance of antimicrobial resistance (AMR) is mostly carried out in clinical or veterinary settings, but is largely lacking in community setting or in the environment (Marano et al., 2020). There is limited knowledge on the prevalence of resistant *K. pneumoniae* within the population in Norway (Raffelsberger et al., 2021). Sewage contains collection of stools from the population connected to the sewage system and may therefore reflect what the population could be carrying (Hutinel et al., 2019). Sewage-based surveillance has been successfully demonstrated for understanding resistance in *Escherichia coli* from the population, but little is known about other important pathogens like *K. pneumoniae* (Grevskott et al., 2021; Hutinel et al., 2019; Marano et al., 2020; Puljko et al., 2022).

The aim of our study was to understand the prevalence of antibiotic resistance in *K. pneumoniae* present in the population in Bergen city, Norway using city-scale sewage-based surveillance, as well as understand the prevalence of resistance in *K. pneumoniae* entering the marine environment through treated sewage. Further, we aimed at determining the genetic basis of observed resistance and the pathogenic potential of these isolates using whole genome sequencing (WGS). Here, we show higher prevalence of colistin resistance compared to third generation cephalosporins in *K. pneumoniae* isolates from both raw and treated sewage.

2. Materials and methods

2.1. Collection of influent and effluent samples

Twenty-four-hour composite samples of raw sewage (influent) and treated sewage (effluent) were collected on four different occasions (March 1st, May 3rd, July 5th, and September 6th, 2021) from five different sewage treatment plants (STPs) serving Bergen city, Norway. Flesland is the largest STP serving 152,000 inhabitants, followed by Holen (serving 132,000 inhabitants), Knappen (serving 63,000 inhabitants), Kverneviken Åsane (serving 56,000 inhabitants), and Ytre-Sandviken (serving 44,000 inhabitants) as described in Supplementary Table S1.

2.2. Isolation and identification of K. pneumoniae

The samples were collected in sterile containers, stored at 4 °C and transported to the laboratory where they were processed within 6 h of collection. Serial dilutions (ten-fold) of sewage samples were prepared with sterile saline (0.85% NaCl) and plated on Simmons Citrate Agar with myo-Inositol (SCAI) (Sigma-Aldrich, Germany) for March, May, and July samples. Additionally, samples from May, July, and September were plated on SCAI with cefotaxime (2 mg/L) in order to selectively isolate ESBL-producing *K. pneumoniae*. The plates were incubated aerobically at 37 °C for 24–48 h, followed by estimating the number of presumptive *K. pneumoniae* isolates by counting yellow colonies on the SCAI plates. For each sample, up to 30 isolated, yellow colonies were randomly picked from the SCAI plates with and without cefotaxime and transferred to Mueller-Hinton broth (Sigma-Aldrich, Germany) with or without cefotaxime and incubated at 37 °C for 24 h. After incubation, 20% glycerol was added, and the isolates were stored at −80 °C until further analysis.

2.3. Antibiotic susceptibility testing (AST)

The isolates were re-streaked onto SCAI plates, with and without cefotaxime, from the glycerol stocks and incubated at 37 °C for 24–48 h. After incubation, the isolates were identified using matrix-assisted laser desorption ionization-time of flight mass spectrometry (MALDI-TOF MS) at the Institute of Marine Research (IMR, Norway) (Bruker Daltonics, Germany). The resistance profile of 607 presumptive *K. pneumoniae* isolates were determined against 15 antibiotics using the broth microdilution assay with Sensititre™ EUVSEC 3 plates (Thermo Scientific, USA) following manufacturer's protocol. Minimum inhibitory concentrations (MICs) were determined for ampicillin (AMP), azithromycin (AZM), cefotaxime (CTX), ceftazidime (CAZ), meropenem (MEM), nalidixic acid (NAL), ciprofloxacin (CIP), trimethoprim (TMP), sulfamethoxazole (SMX), tetracycline (TET), tigecycline (TGC), gentamicin (GEN), chloramphenicol (CHL), colistin (CST), and amikacin (AMK). The plates were incubated at 37 °C for 22–24 h. Resistance or sensitivity was determined using the EUCAST clinical breakpoint tables v.12.0 (EUCAST, 2022). For some antibiotics not having defined breakpoints for *Enterobacterales* (like NAL, SMX, and TET), the isolates were considered resistant if growth was observed at the highest concentration of the antibiotic. We are aware that this would underestimate the prevalence of resistance against these antibiotics and hence resistance rates for these antibiotics were not included in the statistical analyzes. Standard sensitive *E. coli* strain CCUG 17620 (ATCC 25922) was used as negative control, while *E. coli* CCUG 73937 resistant against ampicillin, cefotaxime, ceftazidime, nalidixic acid, tetracycline, gentamicin, sulfamethoxazole, and trimethoprim was used as a positive control for EUVSEC 3 plates (Grevskott et al., 2020).

2.4. Extraction of genomic DNA and sequencing

Based on phenotypic resistance profiles, 53 presumptive *K. pneumoniae* isolates were selected for WGS. All 53 isolates were regrown on Mueller-Hinton agar (Oxoid, UK), with or without cefotaxime, overnight at 37 °C, followed by genomic DNA extraction using DNeasy Blood and Tissue kit (Qiagen, Germany) according to the manufacturer's instructions. Quantification of extracted genomic DNA was performed using NanoDrop™ 2000 Spectrophotometer (Thermo Scientific, USA) and Qubit™ 2.0 Fluorometer with dsDNA BR Assay Kit (Thermo Scientific, USA). The extracted genomic DNA was sent for sequencing to the Norwegian Sequencing Centre (Oslo University Hospital, Ullevål, Oslo, Norway) at 4 °C. Sequencing libraries were prepared using Nextera DNA Flex Library Prep kit (Illumina, USA) and sequencing was performed using an Illumina MiSeq platform (Illumina, USA), with 2 x 300 bp chemistry.

2.5. Genome assembly and sequencing analysis

Adapters were removed from the obtained raw reads generated by Illumina MiSeq and the reads were quality filtered, assembled using SPAdes (v.3.13.0) (Bankevich et al., 2012), and annotated using National Center for Biotechnology Information (NCBI) Prokaryotic Genomes Automatic Annotation Pipeline (PGAAP) (Tatusova et al., 2013),

as previously described (Radisic et al., 2020). The sequencing coverage was calculated using sequencing depth and assembly size. Kleborate v.2.1.0 (Lam et al., 2021) was used for identification of species, sequence types (STs), ARGs, and important key virulence genes. Further identification of ARGs was performed using ResFinder v.4.1 (Bortolaia et al., 2020) and the comprehensive antibiotic resistance database (CARD) v.3.1.4 (Alcock et al., 2020). Heavy metal resistance genes (HMRGs) were examined using AMRFinderPlus v.3.10.24 (Feldgarden et al., 2019) and the BacMet database v.2.0 (Pal et al., 2014) using default settings. PlasmidFinder v.2.1 was used for identification of plasmids (Carattoli et al., 2014). Virulence genes were also checked using VFanalyzer and the Virulence Factor Database (VFDB) (Liu et al., 2019). We used default settings for all the programs unless otherwise specified.

2.6. Comparative genome analysis of colistin-resistant K. pneumoniae ST730 isolates

A single nucleotide polymorphism (SNP)-based comparison was performed in order to check for clonal relatedness for colistin-resistant isolates belonging to ST730 (n = 12) isolated from the same STP. *K. pneumoniae* ST730 (isolate 2016–567) obtained from blue mussels (*Mytilus edulis*) was used as reference (accession number WPBP00000000) to construct a SNP-based phylogenetic tree (Håkonsholm et al., 2022). The assembled genomes were analyzed with CSI Phylogeny v.1.4 at the web site of the Center for Genomic Epidemiology (https://cge.food.dtu.dk/services/CSIPhylogeny/) (Kaas et al., 2014), using the following parameters: min. depth at SNP positions: disabled; min. relative depth at SNP positions: disabled; min. distance between SNPs: disabled; min. SNP quality: disabled; min. mapping quality: 25; min. z-score: 1.96.

2.7. String test

The string test was performed to determine hypermucoviscosity phenotype of ten sequenced *K. pneumoniae* isolates carrying the virulence gene *rmpA* (Rakotondrasoa et al., 2022). A 1 μl loop was used to stretch a mucoviscous string from each colony grown at 37 °C for 24 h on both SCAI plates and Tryptic Soy Agar (TSA) plates with 5% sheep blood (VWR BDH Chemicals, Belgium). A hypermucoviscous phenotype was defined as positive if a string >5 mm was formed (Rakotondrasoa et al., 2022; Shon et al., 2013).

3. Results

3.1. K. pneumoniae isolates obtained on non-selective and cefotaxime-containing plates

The total number of presumptive *K. pneumoniae* isolates were higher in influent samples (n = 100, n = 106, and n = 133) compared to effluent samples (n = 65, n = 78, and n = 81) for all five STPs from March, May, and July 2021, respectively. During the three sampling occasions, 563 out of 583 isolates (96.6%) from the non-selective plates were identified as *K. pneumoniae,* 14 out of 583 isolates (2.4%) as *K. variicola,* five out of 583 isolates (0.9%) as *K. oxytoca*, and one out of 583 isolates (0.2%) as *Raoultella ornithinolytica* by MALDI-TOF MS. From the cefotaxime-containing plates obtained from May, July, and September, 44 out of 47 isolates (93.6%) were identified as *K. pneumoniae* and three out of 47 isolates (6.4%) as *K. oxytoca*. Isolates not identified as *K. pneumoniae* by MALDI-TOF MS were excluded from further analysis. Cefotaxime-resistant *K. pneumoniae* were detected on cefotaxime-containing plates in two influent samples from both May (Holen n = 2 and Kverneviken Åsane n = 2) and July (Flesland n = 1 and Holen n = 10), and in three different influent samples from September (Ytre-Sandviken n = 2, Kverneviken Åsane n = 22, and Holen n = 4). Only one cefotaxime-resistant *K. pneumoniae* was isolated from an effluent sample from May 2021 (Holen n = 1).

3.2. Resistance patterns of K. pneumoniae isolates obtained on non-selective plates

A total of 339 and 224 presumptive *K. pneumoniae* isolates were obtained from the influent and effluent samples on non-selective plates, respectively. For isolates from all influent samples (n = 339), highest resistance was observed against ampicillin (82.9%) and azithromycin (63.4%). Colistin and tigecycline resistance was observed in 5.3% (n = 18) and 0.9% (n = 3) of the isolates, respectively, while cefotaxime resistance was observed in 1.5% (n = 5) of the isolates. Similarly, highest resistance was observed against ampicillin (84.8%) and azithromycin (65.6%) in isolates from all effluent samples (n = 224). Colistin and tigecycline resistance was observed in 7.6% (n = 17) and 0.9% (n = 2) of the isolates, while no cefotaxime-resistant isolates were recovered from the effluent samples on non-selective plates. Furthermore, all isolates obtained from the influent and effluent samples were sensitive to meropenem and amikacin. The MICs for individual isolates are presented in Supplementary Table S2.

Apart from resistance against ampicillin and azithromycin, *K. pneumoniae* isolates from March influent also showed resistance against trimethoprim (3%), chloramphenicol (3%), and cefotaxime (1%) (Fig. 1). Similarly, resistance against colistin (15.4%), trimethoprim (1.5%), and chloramphenicol (1.5%) were observed in *K. pneumoniae* isolates from March effluent. Isolates obtained from both influent and effluent samples from May showed highest resistance against ampicillin and azithromycin. The isolates obtained from May influent also showed resistance against colistin (4.7%), tigecycline (2.8%), cefotaxime (1.9%), ceftazidime (1.9%), and trimethoprim (1.9%), while isolates obtained from May effluent showed resistance against colistin (5.1%), tigecycline (2.6%), and trimethoprim (1.3%). Likewise, highest resistance against both ampicillin and azithromycin was also observed in isolates obtained from both influent and effluent from July. We also observed resistance against colistin (9.8%), trimethoprim (8.3%), and cefotaxime (1.5%) in isolates from July influent, and resistance against colistin (3.7%) and trimethoprim (2.5%) in isolates from July effluent.

3.3. Resistance pattern of cefotaxime-resistant K. pneumoniae isolates

A total of 43 and one presumptive *K. pneumoniae* isolates were obtained on cefotaxime-containing plates from influent and effluent samples, respectively, from May, July, and September 2021. All isolates were multidrug-resistant with resistance against at least three different classes of antibiotics (Supplementary Table S3). Resistance against ampicillin (100%), cefotaxime (100%), trimethoprim (86.4%), azithromycin (75%), gentamicin (70.5%), and ceftazidime (70.5%) were observed. Tigecycline resistance was observed in 11 *K. pneumoniae* isolates (25%). No resistance was observed against meropenem, colistin, and amikacin in these isolates.

3.4. Genome sequencing and analysis

Genome sequence assembly statistics of the draft genomes of the sequenced *Klebsiella* spp. isolates (n = 53) are presented in Supplementary Table S4. The total length varied from 5,122,484 bp to 6,017,343 bp and the GC content (%) ranged from 56.4 to 57.7. Nine isolates which were identified as *K. pneumoniae* using MALDI-TOF MS, were later identified as other members of the KpSC such as either *K. quasipneumoniae* subsp. *similipneumoniae* (n = 7) or as *K. quasipneumoniae* subsp. *quasipneumoniae* (n = 2) by Kleborate. The average nucleotide identity based on BLAST (ANIb), calculated using JSpeciesWS (http://jspecies.ribohost.com/jspeciesws/), confirmed that these isolates belonged to either *K. quasipneumoniae* subsp. *similipneumoniae* (ANIb value, ≥ 98.8%) or to *K. quasipneumoniae* subsp. *quasipneumoniae* (ANIb value, 99%), and not to *K. pneumoniae* (ANIb

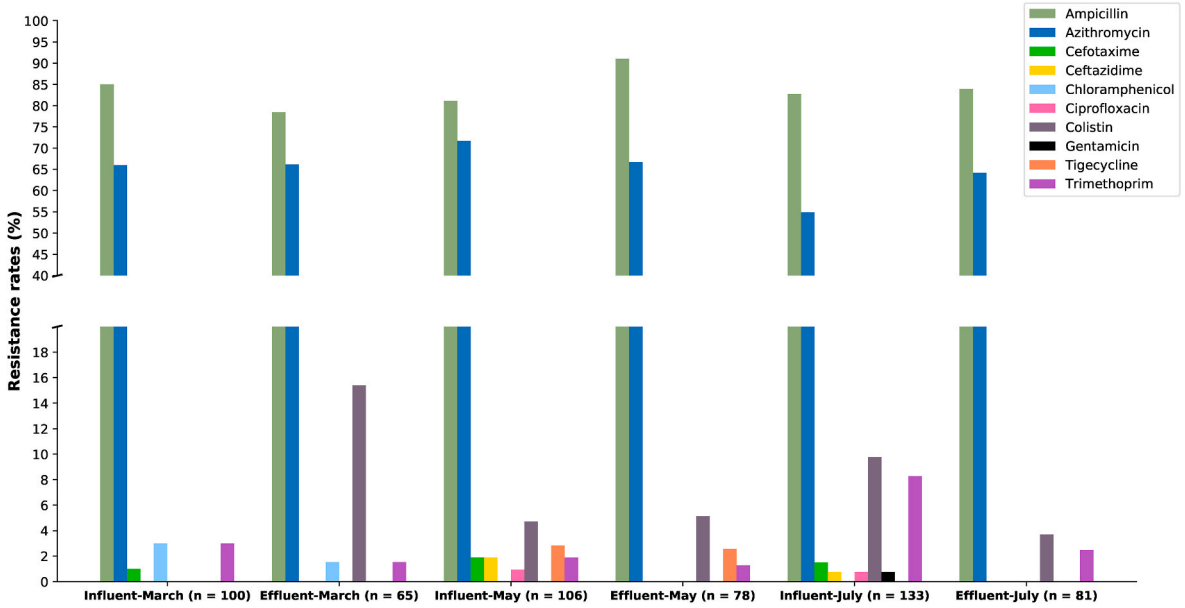

Fig. 1. Resistance rates (%) of *Klebsiella pneumoniae* isolates from influent (n = 339) and effluent (n = 224) samples obtained from five different sewage treatment plants (STPs) in Bergen, Norway, at different time-points (March, May, and July 2021). All isolates were sensitive to meropenem and amikacin, hence these were excluded from the figure. Nalidixic acid, tetracycline, and sulfamethoxazole are also excluded from this figure owing to lack of defined clinical breakpoints.

values ≤ 93.5%) using cut-off values > 95% as recommended in previous studies (Chun and Rainey, 2014; Marathe et al., 2019b).

3.5. STs of sequenced Klebsiella spp. isolates

The sequenced *K. pneumoniae* isolates (n = 44) belonged to 22 different STs with ST730 (n = 13, 29.5%) as most prevalent, followed by ST307 (n = 5, 11.4%), ST872 (n = 4, 9.1%), ST405 (n = 3, 6.8%), and ST869 (n = 2, 4.5%) (Table 1). The remaining *K. pneumoniae* isolates (n = 17) belonged to one ST each. The sequenced *K. quasipneumoniae* subsp. *similipneumoniae* isolates (n = 7) belonged to three different STs with ST1584 (n = 4, 57.1%) as most prevalent, followed by ST414 (n = 2, 28.6%), and ST1676 (n = 1, 14.3%), whereas the two sequenced *K. quasipneumoniae* subsp. *quasipneumoniae* isolates belonged to ST2010.

3.6. Clinically important ARGs detected in sequenced Klebsiella spp.

All except one isolate (K6-110) of the sequenced *Klebsiella* spp. carried variants of known intrinsic ARGs like *fosA, oqxA*, *oqxB,* and either bla_{SHV} or bla_{OKP-B}. Multiple acquired ARGs were detected in the sequenced *Klebsiella* spp. isolates (n = 23, 43.4%) including different β-lactamase genes, belonging to class A (bla_{TEM-1}, $bla_{CTX-M-14}$, and $bla_{CTX-M-15}$) and class D (bla_{OXA-1}). CTX-M-15 was most prevalent (34%) among the acquired β-lactamases. We identified other clinically acquired ARGs encoding resistance against quinolones (*qnrS1* and *qnrB1*), aminoglycosides (*aac(3)-IIe, aac(6′)-Ib-cr5, aph(3′)-Ia, aph(3″)-Ib, aph(6)-Id, aadA, aadA1,* and *aadA2*) tetracyclines (*tet*(A) and *tet*(C)), sulfonamides (*sul1* and *sul2*), and chloramphenicol (*catII*, *catB3*, and *catB4*) in the sequenced isolates (Table 1). Most of the genotypic data matched with the resistant phenotypes (Supplementary Table S2 and Supplementary Table S3). Some isolates carried $bla_{SHV-187}$ or $bla_{OKP-B-34}$, but were sensitive to ampicillin (K4-32, K4-38, and K4-44). Several isolates (K5-309, K5-314, K6-183, K6-328, K7-301, K7-322, and K7-325) carrying the *catB3* gene were sensitive to chloramphenicol.

Notably, all sequenced colistin-resistant isolates (n = 13) had mutation in the *mgrB* gene with nucleotide substitution at position C88T leading to premature termination of protein. Although they had the same mutation and belonged to the same ST, theses isolates formed different distinct clusters in the SNP-based phylogeny. Thus, suggesting that these may represent different clones (Supplementary Fig. S1). Sequenced tigecycline-resistant isolates carried a Tet(A) variant with 22 amino acid (aa) substitutions (with 94% identity) at positions C12S, S81A, I85V, Y132H, S155G, S156G, M176T, I178C, V206A, I212V, A228V, T231A, T245A, S246T, L247T, T271A, T272A, A289T, L296F, V323M, F389L, and V393A, compared to the reference sequence of Tet (A) for *Enterobacterales* (accession number WP_031942321.1).

3.7. Detected HMRGs in sequenced Klebsiella spp. isolates

HMRGs were widely distributed in all sequenced *Klebsiella* spp. (Supplementary Table S5), with the *corA* gene (100%) as the most prevalent conferring resistance to magnesium and cobalt. Apart from carrying *corA*, most sequenced isolates carried the *acrD* gene (n = 51). Copper resistance genes (*pcoABDRS*) and silver resistance genes (*silABCEFPRS*) were the most common combination (n = 43) in the sequenced *Klebsiella* spp. HMRGs were not detected in seven sequenced *K. pneumoniae* isolates using AMRFinderPlus, but were detected using the BacMet database. Among the sequenced *K. pneumoniae* isolates (n = 44), 79.5% carried HMRGs encoding resistance against silver (*silABCEFPRS*) and copper (*pcoABDRS*). We further detected HMRGs encoding resistance against nickel (*ncrABCY*) (43.2%), arsenic (*arsABDR*) (31.8%), zinc (*znuB/yebI*) (31.8%), and mercury (*merDRT*) (4.5%) in the sequenced *K. pneumoniae* isolates (n = 44). The seven sequenced *K. quasipneumoniae* subsp. *similipneumoniae,* carried multiple HMRGs conferring resistance to silver (*silABCEFPRS*) (85.7%), copper (*pcoABCDRS*) (85.7%), zinc (*znuB/yebI*) (71.4%), and mercury (*merPRT*) (28.6%), while both sequenced *K. quasipneumoniae* subsp. *quasipneumoniae* isolates carried various HMRGs conferring resistance to silver (*silABCEFPRS*), copper (*pcoABCDRS*), and arsenic (*arsABDR*). All sequenced *Klebsiella* spp. isolates carrying acquired ARGs also carried HMRGs, thus signifying the potential for co-selection (Seiler and Berendonk, 2012).

3.8. Plasmid replicons

Plasmid replicons were detected in 90.9% of the sequenced *K. pneumoniae* isolates with 14 different replicon types (Table 1). The most common replicon type was IncFIB(K) (n = 38), followed by IncFII

Table 1
Sequence types (STs), antibiotic resistance genes (ARGs), and plasmid replicons detected in the sequenced *Klebsiella* spp. isolates (n = 53) obtained from influents (Inf.) and treated effluents (Eff.).

Isolate	Species	Sample type	ST	ARGs	Plasmid replicons
K4-32	*K. quasipneumoniae* subsp. *similipneumoniae*	Eff.	ST1584	$bla_{OKP\text{-}B\text{-}34}$, *fosA5*, *oqxA*, *oqxB*	Col440I, Col440II, IncFIB(K), IncFII(K)
K4-38	*K. pneumoniae*	Eff.	ST20	$bla_{SHV\text{-}187}$, *fosA10*, *oqxA*, *oqxB32*	–
K4-41	*K. pneumoniae*	Eff.	ST846	$bla_{SHV\text{-}1}$, *fosA5*, *oqxA*, *oqxB*	Col440I, IncFIA(HI1), IncFIB(K), IncFII(K)
K4-44	*K. quasipneumoniae* subsp. *similipneumoniae*	Eff.	ST1584	$bla_{OKP\text{-}B\text{-}34}$, *fosA5*, *oqxA*, *oqxB*	Col440I, Col440II, IncFIB(K), IncFII(K)
K4-50	*K. pneumoniae*	Eff.	ST872	$bla_{SHV\text{-}11}$, *fosA5*, *oqxA*, *oqxB*	Col440I, IncFIB(K), IncFII(K), IncR
K4-74	*K. pneumoniae*	Inf.	ST730	$bla_{SHV\text{-}1}$, *fosA5*, *oqxA*, *oqxB19*	IncFIB(K), IncFIB, IncFII(K), IncR
K4-130	*K. pneumoniae*	Eff.	ST872	$bla_{SHV\text{-}11}$, *fosA5*, *oqxA*, *oqxB19*	Col440I, IncFIB(K), IncFIB, IncFII(K), IncR
K4-136	*K. pneumoniae*	Eff.	ST872	$bla_{SHV\text{-}11}$, *fosA5*, *oqxA*, *oqxB19*	Col440I, IncFIB(K), IncFIB, IncFII(K)
K4-154	*K. pneumoniae*	Inf.	ST222	$bla_{SHV\text{-}40}$, *fosA5*, *oqxA*, *oqxB19*	IncFIA(HI1), IncFIB(K), IncFIB, IncHI1B, IncR
K4-170	*K. pneumoniae*	Inf.	ST1517-1LV	$bla_{SHV\text{-}11}$, *fosA5*, *oqxA*, *oqxB19*	–
K4-172	*K. pneumoniae*	Inf.	ST721	$bla_{SHV\text{-}1}$, *fosA5*, *oqxA*, *oqxB*	–
K4-180	*K. pneumoniae*	Eff.	ST730	$bla_{SHV\text{-}1}$, *fosA5*, *oqxA*, *oqxB19*	Col, Col440I, IncFIA(HI1), IncFIB(K), IncFIB, IncFII(K)
K4-188	*K. pneumoniae*	Eff.	ST730	$bla_{SHV\text{-}1}$, *fosA5*, *oqxA*, *oqxB19*	Col440I, IncFIA(HI1), IncFIB(K), IncFIB, IncFII(K)
K5-1	*K. quasipneumoniae* subsp. *similipneumoniae*	Inf.	ST1584	$bla_{OKP\text{-}B\text{-}34}$, *fosA5*, *oqxA*, *oqxB*	Col440I, Col440II, IncFIB(K), IncFII(K)
K5-45	*K. quasipneumoniae* subsp. *similipneumoniae*	Eff.	ST1584	$bla_{OKP\text{-}B\text{-}34}$, *fosA5*, *oqxA*, *oqxB*	Col440I, Col440II, IncFIB(K), IncFII(K)
K5-76	*K. pneumoniae*	Inf.	ST200	$bla_{SHV\text{-}1}$, *fosA10*, *oqxA*, *oqxB*	–
K5-122	*K. pneumoniae*	Inf.	ST252	$bla_{SHV\text{-}1}$, *fosA5*, *oqxA*, *oqxB19*	Col440I, IncFIA(HI1), IncFIB(K), IncFIB, IncFII(K)
K5-175	*K. pneumoniae*	Eff.	ST872	$bla_{SHV\text{-}11}$, *fosA5*, *oqxA*, *oqxB19*	Col440I, IncFIB(K), IncFIB, IncFII(K), IncR
K5-204	*K. quasipneumoniae* subsp. *quasipneumoniae*	Inf.	ST2010	$bla_{OKP\text{-}A\text{-}9}$, $bla_{CTX\text{-}M\text{-}15}$, *fosA5, oqxA*, *oqxB*, *aph(3″)-Ib*, *aadA2, qnrS1, sul1, sul2, dfrA12, mphA*	IncFIB(K)
K5-210	*K. pneumoniae*	Inf.	ST104-1LV	$bla_{SHV\text{-}71}$, $bla_{LAP\text{-}2}$, *fosA, oqxA*, *oqxB*, *qnrS1, tet*(A)	ColRNAI, IncFIB(K), IncFII
K5-212	*K. pneumoniae*	Inf.	ST730	$bla_{SHV\text{-}1}$, *fosA5*, *oqxA*, *oqxB19*	Col, Col440I, IncFIA(HI1), IncFIB(K), IncFIB, IncFII(K)
K5-222	*K. pneumoniae*	Inf.	ST966	$bla_{SHV\text{-}1}$, *fosA10*, *oqxA*, *oqxB*	IncHI1B
K5-229	*K. pneumoniae*	Inf.	ST730	$bla_{SHV\text{-}1}$, *fosA5*, *oqxA*, *oqxB19*	Col440I, IncFIA(HI1), IncFIB(K), IncFIB, IncFII(K)
K5-233	*K. pneumoniae*	Eff.	ST730	$bla_{SHV\text{-}1}$, *fosA5*, *oqxA*, *oqxB19*	Col440I, IncFIA(HI1), IncFIB(K), IncFIB, IncFII(K)
K5-307	*K. quasipneumoniae* subsp. *similipneumoniae*	Inf.	ST414	$bla_{OKP\text{-}B\text{-}8}$, $bla_{CTX\text{-}M\text{-}15}$, *fosA5, oqxA*, *oqxB*, *aph(6)-Id*, *aph(3″)-Ib*, *sul2*	IncFIB(K)
K5-309	*K. pneumoniae*	Inf.	ST307	$bla_{SHV\text{-}28}$, $bla_{CTX\text{-}M\text{-}15}$, $bla_{OXA\text{-}1}$, $bla_{TEM\text{-}1}$, *fosA, oqxA*, *oqxB19*, *aac(3)-IIe, aac(6′)-Ib-cr, aph(3″)-Ib, aph(6)-Id, qnrB1, sul2, tet*(A), *catB3, dfrA14*	IncFIB(K), IncFII(K)
K5-310	*K. pneumoniae*	Eff.	ST3691	$bla_{CTX\text{-}M\text{-}15}$, $bla_{OXA\text{-}1}$, $bla_{TEM\text{-}1}$, *fosA, oqxA*, *oqxB*, *aac(3)-IIe, aac(6′)-Ib-cr, aph(3″)-Ib, aph(6)-Id, qnrB1, sul2, tet*(A), *catB3, dfrA14*	Col(MG828), IncFIB(K)
K5-314	*K. pneumoniae*	Inf.	ST405	$bla_{SHV\text{-}76}$, $bla_{CTX\text{-}M\text{-}15}$, $bla_{OXA\text{-}1}$, $bla_{TEM\text{-}1}$, *fosA, oqxA*, *oqxB14*, *aac(3)-IIe, aac(6′)-Ib-cr, aph(3″)-Ib, aph(6)-Id, sul2, catB3, dfrA14*	IncFIB(K), IncFII(K)
K5-322	*K. quasipneumoniae* subsp. *quasipneumoniae*	Inf.	ST2010	$bla_{OKP\text{-}A\text{-}9}$, $bla_{CTX\text{-}M\text{-}15}$, *fosA, oqxA*, *oqxB*, *aph(3″)-Ib*, *aph(6)-Id*, *aadA2, qnrS1, sul1, sul2, dfrA12, mphA*	IncFIB(K)
K6-63	*K. pneumoniae*	Inf.	ST869	$bla_{SHV\text{-}27}$, $bla_{CTX\text{-}M\text{-}14}$, $bla_{LAP\text{-}2}$, *fosA5, oqxA*, *oqxB19*, *qnrS1, sul1, tet*(A), *dfrA1*	IncFIB(K), IncFII
K6-110	*K. pneumoniae*	Eff.	ST25-1LV	*fosA, oqxA*, *oqxB*, *aph(3″)-Ib*, *aph(6)-Id*, *tet*(C)	Col(IMGS31), Col440I, IncFIA(HI1), IncFIB(K)
K6-123	*K. pneumoniae*	Inf.	ST730	$bla_{SHV\text{-}1}$, *fosA5*, *oqxA*, *oqxB19*	Col440I, IncFIA(HI1), IncFIB(K), IncFIB, IncFII(K)
K6-125	*K. pneumoniae*	Inf.	ST730	$bla_{SHV\text{-}1}$, *fosA5*, *oqxA*, *oqxB19*	IncFIA(HI1), IncFIB(K), IncFIB, IncFII(K)
K6-126	*K. pneumoniae*	Inf.	ST730	$bla_{SHV\text{-}1}$, *fosA5*, *oqxA*, *oqxB19*	Col440I, IncFIA(HI1), IncFIB(K), IncFIB, IncFII(K)
K6-129	*K. pneumoniae*	Inf.	ST730	$bla_{SHV\text{-}1}$, *fosA5*, *oqxA*, *oqxB19*	Col440I, IncFIA(HI1), IncFIB(K), IncFIB, IncFII(K)
K6-130	*K. pneumoniae*	Inf.	ST730	$bla_{SHV\text{-}1}$, *fosA5*, *oqxA*, *oqxB19*	Col440I, IncFIA(HI1), IncFIB(K), IncFII(K)
K6-135	*K. pneumoniae*	Inf.	ST730-1LV	$bla_{SHV\text{-}1}$, *fosA5*, *oqxA*, *oqxB19*	Col440I, IncFIA(HI1), IncFIB(K), IncFIB, IncFII(K)

(*continued on next page*)

Table 1 (*continued*)

Isolate	Species	Sample type	ST	ARGs	Plasmid replicons
K6-138	*K. pneumoniae*	Inf.	ST730	*bla*$_{SHV-1}$, *fosA5*, *oqxA*, *oqxB19*	Col440I, IncFIB(K), IncFIB, IncFII(K)
K6-148	*K. pneumoniae*	Inf.	ST730	*bla*$_{SHV-1}$, *fosA5*, *oqxA*, *oqxB19*	Col440I, IncFIB(K), IncFIB, IncFII(K)
K6-183	*K. pneumoniae*	Inf.	ST405	*bla*$_{SHV-76}$, *bla*$_{CTX-M-15}$, *bla*$_{OXA-1}$, *bla*$_{TEM-1}$, *fosA5, oqxA*, *oqxB14*, *aac(3)-IIe, aac(6′)-Ib-cr, aph(3″)-Ib, aph(6)-Id, sul2, catB4, dfrA14*	IncFIB(K), IncFII(K)
K6-243	*K. quasipneumoniae* subsp. *similipneumoniae*	Eff.	ST1676	*bla*$_{OKP-B-5}$, *fosA5, oqxA*, *oqxB*, *aadA1, sul1, dfrA1*	Col440I, IncFIB(K), IncFIB, IncFII, IncR
K6-308	*K. pneumoniae*	Inf.	ST187	*bla*$_{SHV-11}$, *bla*$_{CTX-M-15}$, *fosA5, oqxA*, *oqxB19*, *aph(3′)-Ia, aph(3″)-Ib, aph(6)-Id, aadA, qnrS1, sul1, sul2, tet*(A), *dfrA12, mphA*	IncFIB(K), IncFIB
K6-320.1	*K. pneumoniae*	Inf.	ST869	*bla*$_{SHV-27}$, *bla*$_{CTX-M-14}$, *bla*$_{LAP-2}$, *fosA5, oqxA*, *oqxB19*, *qnrS1, sul1, tet*(A), *dfrA1*	IncFIB(K), IncFII
K6-320.2	*K. pneumoniae*	Inf.	ST607	*bla*$_{SHV-1}$, *bla*$_{CTX-M-15}$, *bla*$_{OXA-1}$, *fosA5, oqxA*, *oqxB19*, *aac(6′)-Ib-cr5*	ColpVC
K6-322	*K. quasipneumoniae* subsp. *similipneumoniae*	Inf.	ST414	*bla*$_{OKP-B-8}$, *bla*$_{CTX-M-15}$, *fosA5, oqxA*, *oqxB*, *aph(3″)-Ib, aph(6)-Id, sul2*	IncFIB(K)
K6-325	*K. pneumoniae*	Inf.	ST268	*bla*$_{SHV-11}$, *bla*$_{CTX-M-15}$, *bla*$_{TEM-1}$, *fosA5, oqxA*, *oqxB5*, *aac(3)-IId, aph(3″)-Ib, aph(6)-Id, qnrB1, sul2, dfrA14*	IncFIB(K), IncFIB, IncHI1B, IncR
K6-328	*K. pneumoniae*	Inf.	ST405	*bla*$_{SHV-76}$, *bla*$_{CTX-M-15}$, *bla*$_{OXA-1}$, *bla*$_{TEM-1}$, *fosA5*, *oqxA*, *oqxB14*, *aac(3)-IIe, aac(6′)-Ib-cr5*, *aph(3″)-Ib*, *aph(6)-Id*, *sul2*, *catB3*, *dfrA14*	Col440I, IncFIB(K), IncFII(K)
K7-301	*K. pneumoniae*	Inf.	ST753	*bla*$_{SHV-27}$, *bla*$_{CTX-M-15}$, *bla*$_{OXA-1}$, *fosA5, oqxA11*, *oqxB19*, *aac(6′)-Ib-cr5, aph(3″)-Ib, aph(6)-Id, aadA, qnrS1, sul1, sul2, catB3, dfrA12, mphA*	Col, Col440I, IncFIB(K), IncFIB, IncHI1B
K7-302	*K. pneumoniae*	Inf.	ST2167	*bla*$_{SHV-11}$, *bla*$_{CTX-M-15}$, *bla*$_{TEM-1}$, *fosA5, oqxA*, *oqxB*, *aadA, qnrS1, sul1, sul2, tet*(A), *catII, dfrA16*	FIA, IncFIB(K), IncHI1B
K7-318	*K. pneumoniae*	Inf.	ST307	*bla*$_{SHV-28}$, *bla*$_{CTX-M-15}$, *bla*$_{TEM-1}$, *fosA5, oqxA*, *oqxB19*, *aac(3)-IIe, aph(3″)-Ib, aph(6)-Id, sul2, dfrA14*	IncFIB(K), IncFII(K)
K7-322	*K. pneumoniae*	Inf.	ST307	*bla*$_{SHV-28}$, *bla*$_{CTX-M-15}$, *bla*$_{TEM-1}$, *bla*$_{OXA-1}$, *fosA5, oqxA*, *oqxB19*, *aac(3)-IIe, aac(6′)-Ib-cr5, aph(3″)-Ib, aph(6)-Id, qnrB1, sul2, tet*(A), *catB3, dfrA14*	IncFIB(K), IncFII(K)
K7-323	*K. pneumoniae*	Inf.	ST307	*bla*$_{SHV-28}$, *bla*$_{CTX-M-15}$, *bla*$_{TEM-1}$, *fosA5, oqxA*, *oqxB19*, *aph(3″)-Ib, aph(6)-Id, sul2, dfrA14*	IncFIB(K), IncFII(K)
K7-325	*K. pneumoniae*	Inf.	ST307	*bla*$_{SHV-28}$, *bla*$_{CTX-M-15}$, *bla*$_{OXA-1}$, *bla*$_{TEM-1}$, *fosA5, oqxA*, *oqxB19*, *aac(3)-IIe, aac(6′)-Ib-cr5, aph(3″)-Ib, aph(6)-Id, qnrB1, sul2, tet*(A), *catB3, dfrA14*	IncFIB(K), IncFII(K)

Plasmid replicons not detected are marked with '-'.

Table 2
Sequenced *Klebsiella pneumoniae* (n = 9) isolates carrying yersiniabactin (*ybt*) isolated from sewage in Bergen, Norway.

Isolate	ST	*ybt*	ICE*Kp*/Plasmid
K4-170	ST1517-1LV	*ybt 4*	Plasmid
K5-210	ST104-1LV	*ybt 20*	ICE*Kp10*
K5-222	ST966	*ybt 4*	Plasmid
K5-314	ST405	*ybt 27*	ICE*Kp22*
K6-183	ST405	*ybt 27*	ICE*Kp22*
K6-320.2	ST607	*ybt 15*	ICE*Kp11*
K6-325	ST268	*ybt 10*	ICE*Kp4*
K6-328	ST405	*ybt 27*	ICE*Kp22*
K7-302	ST2167	*ybt 9*	ICE*Kp3*

ST: sequence type, ICE*Kp*: integrative conjugative element of *Klebsiella pneumoniae*.

(K) (n = 28), and IncFIB (n = 22). Among other members of KpSC, only seven different plasmid replicons were identified with IncFIB(K) (n = 9) as most prevalent. Most sequenced *Klebsiella* spp. (81.1%) carried more than one plasmid replicon. All sequenced *Klebsiella* spp. isolates carrying acquired ARGs also carried plasmid replicons.

3.9. Virulence genes

All sequenced *K. pneumoniae* carried virulence factors involved in adherence (type I fimbriae and type 3 fimbriae), regulation (RcsAB), as well as various secretion systems like T6SS-I and T6SS-III (Supplementary Table S6). Salmochelin (*iroE*) and enterobactin (*entABCDEFS* and *fepABCDG*) were present in all sequenced *K. pneumoniae* isolates (n = 44), while aerobactin (*iutA*) was present in 97.7%, and yersiniabactin (*ybtAEPQSTUX*) in 20.5%. Furthermore, five different integrative conjugative elements (ICE*Kp*) and six different *ybt* lineages were detected in nine *ybt* positive *K. pneumoniae* isolates, with ICE*Kp22* (n = 3) and *ybt 27* (n = 3) as the most common combination (Table 2). Two *ybt 4* were plasmid borne. Ten sequenced isolates carried the *rmpA* gene involved in hypermucoid phenotype (Supplementary Table S6). Five out of ten *K. pneumoniae* isolates (K6-125, K6-126, K6-129, K6-130, and K6-135) showed a positive hypermucoviscous phenotype in the string test on both SCAI plates and TSA plates with 5% sheep blood. Nine of the sequenced isolates belonging to the KpSC carried several important virulence factors, including aerobactin (*iutA*), enterobactin (*entABCEFS*, *fepABCDG*, and *fes*), and salmochelin (*iroE*), thus suggesting pathogenicity potential.

4. Discussion

To the best of our knowledge, this is the first study performing city-scale sewage surveillance of *K. pneumoniae,* especially in Norway. We show higher prevalence of resistance against colistin compared to resistance against third generation cephalosporins in *K. pneumoniae* isolated from sewage in Bergen, Norway. Colistin resistance has increased the last years due to excessive use and limited treatment options of carbapenem-resistant *K. pneumoniae* infections (Petrosillo et al., 2019). In this study, 35 out of 563 (6.2%) *K. pneumoniae* isolates from the same STP receiving municipality sewage were resistant against colistin. We sequenced 13 of the 35 colistin-resistant isolates in order to understand the mechanisms of colistin resistance. All sequenced colistin-resistant *K. pneumoniae* isolates (n = 13) belonged to ST730 (n = 12) or ST730-1LV (n = 1) and had a MIC of ≥4 μg/ml for colistin. We further show that the sequenced colistin-resistant *K. pneumoniae* ST730 isolates (n = 12) formed different clusters in the SNP-based phylogenetic tree, suggesting polyclonal dissemination of colistin resistance.

Colistin resistance can be achieved either through genetic modifications (such as insertions, deletions or substitutions) in chromosomal genes (like *mgrB*, *pmrAB*, and *phoPQ*) or through overexpression of efflux pumps, capsule polysaccharides, or acquisition of mobile colistin-resistant genes (*mcr*) (Elias et al., 2021). We detected mutation in the *mgrB* gene with nucleotide substitution at position C88T, leading to a premature stop codon at position 88 (C88T substitution), contributing to a truncated protein of only 29 aa instead of 47 aa long protein. Poirel et al. (2015) showed that truncation of the *mgrB* gene is a key mechanism for development of colistin resistance in *K. pneumoniae*, that is prevalent in *K. pneumoniae* clinical isolates (da Silva et al., 2021). This explains the observed colistin resistance in our study.

Like colistin, tigecycline is a last resort antibiotic used for treatment of carbapenem-resistant *K. pneumoniae* infections (Du et al., 2018; Xu et al., 2021). Phenotypic resistance against tigecycline was detected in five out of 563 (0.9%) *K. pneumoniae* isolates. While 11 out of 44 isolates obtained on cefotaxime-containing plates were tigecycline-resistant. To understand the mechanism of tigecycline resistance four tigecycline-resistant isolates were subjected to WGS (K5-210, K5-309, K6-320.1, and K7-325). Tigecycline resistance in *K. pneumoniae* can be achieved by mechanisms like mutations in the *tet*(A) gene or overexpression of RND efflux pumps like OqxAB and AcrAB (Chiu et al., 2017; Du et al., 2018; Park et al., 2020). Studies have previously suggested that mutations or aa substitutions in the Tet(A) could decrease tigecycline sensitivity in *K. pneumoniae* and other pathogens (Akiyama et al., 2013; Linkevicius et al., 2015; Xu et al., 2021). We detected Tet(A) with 22 amino substitutions in our isolates. The number of substitutions observed in Tet(A) in our study could thus, explain the observed tigecycline resistance in the sequenced *K. pneumoniae* isolates. Although more experiments are needed to confirm this observation.

All sequenced colistin- and tigecycline-resistant isolates carried important virulence factors such as aerobactin (*iutA*), enterobactin (*entABCDEFS* and *fepABCDG*), and salmochelin (*iroE*). Aerobactin has shown to increase the virulence of *K. pneumoniae* (Lawlor et al., 2007), as well as being the most dominant siderophore produced by hvKp (Russo et al., 2015). In addition to aerobactin, enterobactin, and salmochelin are known to be produced by hvKp (Russo et al., 2015). We further detected the virulence gene, *rmpA,* in five sequenced colistin-resistant *K. pneumoniae* isolates (K6-125, K6-126, K6-129, K6-130, and K6-135), and one sequenced tigecycline-resistant *K. pneumoniae* isolate (K7-325). The virulence gene, *rmpA*, is a regulator of mucoid phenotype A and is highly associated with hvKp (Hsu et al., 2011). The five colistin-resistant *K. pneumoniae* isolates carrying *rmpA*, all showed hypermucoviscous phenotype, while the one tigecycline-resistant *K. pneumoniae* isolate carrying *rmpA* did not. The presence of such virulence factors in isolates resistant against last resort antibiotics indicates potential for pathogenicity of these resistant isolates.

ESBL-producing *K. pneumoniae* is a big clinical problem, also in Norway (Fostervold et al., 2021; NORM/NORM-VET, 2021). All *K. pneumoniae* isolates (n = 44) obtained on cefotaxime-containing plates were multidrug-resistant (Supplementary Table S3). The sequenced ESBL-producing *K. pneumoniae* isolates belonged to nine different ST, with ST307 as most dominant. Acquired ARGs, including clinically important ESBLs like CTX-M-14 and CTX-M-15, were widely distributed among the sequenced cefotaxime-resistant *Klebsiella* spp. isolates (n = 17), with CTX-M-15 as the most prevalent ESBL. CTX-M-type ESBLs is considered as an important emerging problem in the clinics in Norway due to the rapid spread among *Enterobacteriaceae* (NORM/NORM-VET, 2020; Rossolini et al., 2008).

ST307 represents an emerging multidrug-resistant high-risk clone in the clinics worldwide, including Norway, due to its capability for causing serious infections (Fostervold et al., 2021; NORM/NORM-VET, 2019; Peirano et al., 2020; Runcharoen et al., 2017). ST307 is often associated with ESBLs and carbapenemases, hence treatment is challenging (Peirano et al., 2020). Recent studies have suggested increasing prevalence of ST307, particularly carrying $bla_{\text{CTX-M-15}}$, causing ESBL-positive *K. pneumoniae* infections in Norway (Fostervold et al., 2021; Wyres et al., 2019). All detected ST307 isolates in our study harbored several clinically acquired ARGs encoding resistance against

aminoglycosides, sulfonamides, quinolones, and tetracyclines along with $bla_{CTX\text{-}M\text{-}15}$. Important virulence factors, such as aerobactin, enterobactin, and salmochelin siderophores, as well as type I fimbriae, type 3 fimbriae, and type 6 secretion system (T6SS-III) were detected in all sequenced multidrug-resistant isolates (Zhu et al., 2021).

A high diversity of STs of *K. pneumoniae* was observed from sewage in Norway, including the globally distributed ST20 (Wyres et al., 2020). Recently, Raffelsberger et al. (2021) found ST20 as the most common *K. pneumoniae* ST present in humans in Norway. Our study is not comparable with the previous study for *K. pneumoniae* ST diversity in human carriers in Norway, as we did not sequence all isolates, but selected a few isolates based on differences in resistance patterns. Thus, we cannot comment on the true ST diversity of *K. pneumoniae* found in sewage and this can be regarded as a limitation of our study. However, we still detected ST307 to be a dominant ST among the ESBL-producing *K. pneumoniae*, which is consistent with the clinical data (NORM/-NORM-VET, 2019).

Recently, Puljko et al. (2022) showed that *K. pneumoniae* was mostly removed from STPs, due to no or rare detection from the treated effluents. In contrast, we show presence of *K. pneumoniae* isolates (n = 224) in effluent samples from various STPs in Bergen municipality collected on several occasions, indicating their potential to spread into the receiving environment through treated sewage discharge. Colistin- and tigecycline-resistant isolates, as well as one isolate carrying $bla_{CTX\text{-}M\text{-}15}$, were isolated from effluent. These isolates also carried multiple HMRGs along with acquired ARGs. Sewage contains significant amounts of antimicrobial agents, including heavy metals along with fecal matter (Östman et al., 2017). Heavy metals are widely used as antimicrobial agents in hospitals (Östman et al., 2017), and agriculture (Rehman et al., 2019), thus may eventually end up in the STPs. Heavy metals are also present in antifouling paints used in aquaculture (Emenike et al., 2021) and on ships (Wang et al., 2008), in turn contributing to selection pressure in the marine environment. The presence of such heavy metals in the environment may contribute to persistence and co-selection (Seiler and Berendonk, 2012) of resistant *K. pneumoniae* in the marine environment (Yuan et al., 2019).

Although, sensitive to ampicillin, $bla_{SHV\text{-}187}$ or $bla_{OKP\text{-}B\text{-}34}$ were detected in two isolates (K4-38 and K4-44). Previous studies have shown SHV-carrying *K. pneumoniae* to be sensitive to ampicillin, owing to differential expression of the bla_{SHV} gene (Fu et al., 2007; Lu et al., 2017). Additionally, one of the sequenced ampicillin-sensitive *K. pneumoniae* isolate (K6-110) showed absence of bla_{SHV}. This is in accordance with a previous study showing *K. pneumoniae* lacking the bla_{SHV} gene (Håkonsholm et al., 2020). This may explain the observed sensitivity against ampicillin in the isolates in our study. Similarly, sensitivity to chloramphenicol was observed in several isolates even though the *catB3* gene was detected. Our results are in accordance with previous studies showing chloramphenicol sensitivity in isolates carrying the *catB3* gene (Altayb et al., 2022; Zankari et al., 2012).

5. Conclusion

Our study shows a higher prevalence of resistance against colistin compared to resistance against third generation cephalosporins present in *K. pneumoniae* isolated during a city-scale sewage surveillance in Bergen, Norway. Sewage effluent from hospitals is shown to be a source for releasing pathogens into the receiving environment (Lamba et al., 2017; Marathe et al., 2019a). In contrast, we found colistin- and tigecycline-resistant isolates, as well as pathogenic ST307 carrying ESBLs in influents from a treatment plant that receives only municipal sewage, indicating that these clones are prevalent within the population. Our study thus, underlines the importance of using sewage surveillance as a tool to obtain up-to-date information on the current resistance situation within the population connected to STPs. Our study provides a framework for sewage-based surveillance of antibiotic resistance in *K. pneumoniae* species complex present in the population.

Author contributions

Vera Radisic: Methodology, Investigation, Bioinformatic analysis, Analysis of data, Visualization, Writing – draft, Review, and Editing. **Didrik H. Grevskott:** Methodology, Investigation, Writing – Review, and Editing. **Bjørn Tore Lunestad:** Critical inputs, Writing – Review, and Editing. **Lise Øvreås:** Critical inputs, Writing – Review, and Editing. **Nachiket P. Marathe:** Conceptualization, Methodology, Investigation, Validation, Writing – Review, Editing, Funding acquisition, and Responsible for the overall direction of the project.

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Data availability

The assembled genome sequences have been submitted to GenBank under following genome accession numbers: JAKVAK000000000, JAKVAJ000000000, JAKVAI000000000, JAKVAH000000000, JAKVAG000000000, JAKVAF000000000, JAKVAE000000000, JAKVAD000000000, JAKVAC000000000, JAKVAB000000000, JAKVAA000000000, JAKUZZ000000000, JAKUZY000000000, JAKUZX000000000, JAKUZW000000000, JAKUZV000000000, JAKUZU000000000, JAKUZT000000000, JAKUZS000000000, JAKUZR000000000, JAKUZQ000000000, JAKUZP000000000, JAKUZO000000000, JAKUZN000000000, JAIFJX000000000, JAIFJV000000000, JAIFJU000000000, JAIFJS000000000, JAIFJR000000000, JAJOHF000000000, JAJOHE000000000, JAKUZM000000000, JAJOHD000000000, JAJOHC000000000, JAJOHB000000000, JAJOHA000000000, JAJOGZ000000000, JALDPU000000000, JALDPV000000000, JALDPW000000000, JALDPX000000000, JAJOGY000000000, JAJOGX000000000, JAJOGW000000000, JALDPY000000000, JAJOGV000000000, JAJOGU000000000, JAJOGT000000000, JAJOGS000000000, JAJOGR000000000, JAJOGQ000000000, JAJOGP000000000, and JAJOGO000000000.

Declaration of competing interest

The authors have no conflict of interest.

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Appendix A. Supplementary data

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