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Dissemination of carbapenemase-producing Enterobacterales through wastewater and gulls at a wastewater treatment plant in Sweden

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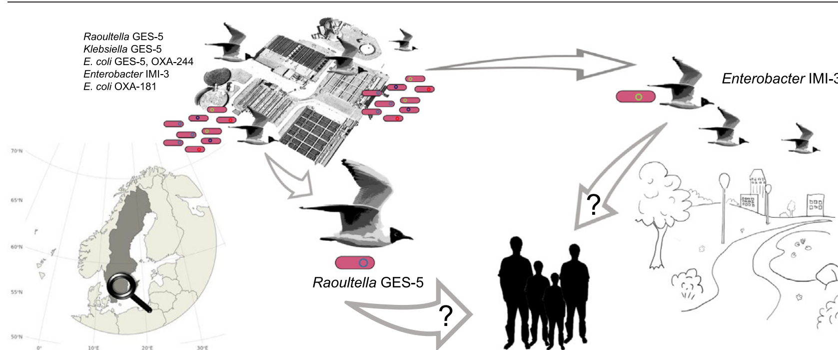
HIGHLIGHTS

- First report of carbapenemase-producing Enterobacterales in animals in Sweden
- Detection of likely pathogens with carbapenemases genes on MGEs in birds and environment
- WWTP sediment basins appear to be a point source for acquiring AMR among gulls.
- WWTPs impact the dissemination of AMR via connected aquatic environments and birds.

GRAPHICAL ABSTRACT



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ABSTRACT

Here we report the detection of carbapenemase-producing Enterobacterales (CPE) isolated from Swedish wastewater and gull faeces. CPE have not been detected in samples from animals in Sweden preceding this report. Sampling of wastewater treatment plant (WWTP) inlet and outlet, sedimentation basins, surface seawater from key aquatic bird habitats and freshly deposited gull faeces was done on six separate occasions during May to September 2021. Following broth enrichment, selective screening of putative CPE was performed on mSuperCarba™ (CHROMagar). Species identification was done with MALDI-TOF. Antimicrobial susceptibility testing was performed according to EUCAST. In total, seventeen CPE were verified by genome sequencing carrying $bla_{\text{GES-5}}$, $bla_{\text{IMI-3}}$, $bla_{\text{OXA-181}}$ or $bla_{\text{OXA-244}}$. The $bla_{\text{GES-5}}$ was carried on IncP plasmids in four different species; *Escherichia coli* ST10 isolated from WWTP outlet, *Raoultella ornithinolytica* isolated from WWTP inlet, outlet and sedimentation basins as well as gull faeces collected at the WWTP and *Klebsiella* spp. isolates from WWTP inlet and outlet. The genetic environment surrounding $bla_{\text{GES-5}}$ was similar in two *Citrobacter freundii* causing human infections. The $bla_{\text{IMI-3}}$ was carried on IncFII(Yp) plasmids in four *Enterobacter ludwigii*, isolated from WWTP outlet and gull faeces collected at a recreational city park 2 km from the WWTP. The $bla_{\text{OXA-181}}$ was located on a COLKP3 plasmid found in an *E. coli*, while $bla_{\text{OXA-244}}$ was chromosomally

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located in an *E. coli* ST10, both isolated from WWTP inlet. Phylogenetic analysis of *R. ornithinolytica* and *E. ludwigii* isolates indicate that the gulls carried strains related to those identified in the WWTP samples. The results thus add to the increasing evidence of WWTPs as anthropogenic reservoirs for mobile genetic elements with antibiotic-resistance functionality. Such environments could profoundly impact the dissemination and spread of such genetic elements via for example aquatic birds, thereby warranting further study and surveillance.

1. Introduction

The emergence of carbapenemase-producing Enterobacterales (CPE) is a threat to both human and animal health (van Duin and Doi, 2017). This since carbapenems are used to treat severe or prolonged infections by extended-spectrum β-lactamase-producing (ESBL) Enterobacterales with few remaining options if also carbapenemases have been acquired (van Duin and Doi, 2017). Carbapenemases are classified into Ambler class A, B or D, depending on their hydrolytic mechanisms (Bonomo et al., 2018). Currently, there are >1000 sequence entries classified as genes encoding carbapenemases in the National Center for Biotechnology Information (NCBI) curated reference gene database (Feldgarden et al., 2019). The five most frequently clinically reported carbapenems are the class A *Klebsiella pneumoniae* carbapenemase (KPC), class B New Delhi metallo-β-lactamase (NDM), Verona integron-encoded metallo-beta-lactamase (VIM), and imipenemase metallo-beta-lactamase (IMP) and class D Oxacillinase (OXA-48) beta-lactamases (Bonomo et al., 2018). Less frequent class A enzymes includes imipenem-hydrolyzing beta-lactamase (IMI) and Guiana extended spectrum (GES), which have been isolated from several water sources (Zhang et al., 2017; Cherak et al., 2021).

CPE infections are mostly associated with health-care settings but community-acquired infections do occur (van Duin and Doi, 2017). Community carriages also occur, with a higher prevalence in for example Asia compared to Europe (Chen et al., 2022; Vendrik et al., 2021; Barbadoro et al., 2021). In Sweden all detected CPE isolates are notifiable both for humans and animals and should be reported to the Public Health Agency of Sweden (PHAS) and the Swedish Board of Agriculture, respectively. For humans, CPE isolates should be sent to PHAS, while for animals they should be sent to the National Veterinary Institute (SVA), for confirmation and typing. Sweden has an overall low incidence of CPE infections in humans, with 137 cases reported in 2021, which corresponds to 1.3 cases/100000 inhabitants (2021 SS, 2021). The most commonly detected carbapenemase was OXA-48 and worth noticing is that 63 % of the CPE cases in 2021 were reported to be acquired abroad (2021 SS, 2021). In Europe, incidences of CPE have been reported in livestock in Germany and Italy, while CPE appear to be more common in animals in reports from non-European countries (Borowiak et al., 2017; Diaconu et al., 2020; Wang et al., 2017; Li et al., 2019; Irrgang et al., 2020). The first report of CPE in animals was however from a black kite (*Milvus migrans*) in Germany (Fischer et al., 2013). As of 2021, there were no reports of CPE in animals in Sweden (2021 SS, 2021). There are however reports of *Klebsiella oxytoca* carrying $bla_{\text{VIM-1}}$ and $bla_{\text{IMP-29}}$ and of Enterobacterales spp. positive for $bla_{\text{OXA-48}}$ and bla_{NDM} isolated from Swedish wastewater and recipient waters (Khan et al., 2018; Flach et al., 2021). In addition, a $bla_{\text{IMI-2}}$ positive *Enterobacter asburiae* isolate has also recently been detected in the environment of a Swedish feed mill (Borjesson et al., 2022).

CPE have been detected in aquatic environments all over the world with water and wastewater treatment plants (WWTPs) being suggested to play important roles in the dissemination of antimicrobial resistant bacteria (ARB) and antibiotic resistance genes (ARG) (Cherak et al., 2021; Kunhikannan et al., 2021; Manaia et al., 2016). In addition, CPE detection has been reported from wild animals, such as white storks and gulls interacting with aquatic environments (Bouaziz et al., 2018; Dolejska et al., 2015; Vittecoq et al., 2017). There are also reports of detection of genetically related antibiotic resistant *E. coli* from wastewater, human clinical samples and aquatic environments, but also a large genetic variation within each sample type (Varela et al., 2015). ARB are thus being detected in several different environments and from diverse hosts with the potential risk of intersectional and interspecies transmission. A joint effort between different sectors including public and animal health together with the environment and including different professions using a One Health approach is therefore needed in order to combat the problem (Organization WH, 2014).

The objective of this study was to investigate presence of CPE at and around a Swedish WWTP and among aquatic birds; Black headed gulls (*Chroicocephalus ridibundus*) interacting with environments impacted by the WWTP and other anthropogenic influence.

2. Materials and methods

2.1. Sampling

Sampling was done on six separate occasions during May to September 2021 (Supplementary Table 1) and included WWTP inlet, outlet, and samples from the sedimentation basins part of the biological treatment phase, seawater from key habitats and freshly deposited gull faeces (Supplementary Table 1). The WWTP is in southern Sweden, and utilizes mechanical, two chemical and biological treatment processes. It has a total load of ~80,000 person equivalents, a mean daily flow of ~18,000 m^3 and a yearly circulation of 6.7×10^6 m^3 of wastewater.

WWTP inlet and outlet and the water samples were filtrated through Sterivex-GP PES 0.22 μM (Merck Millipore) until clogged within 24 h from sampling (see Supplementary Table 1 for volumes). The filters were stored in LB broth (Lennox) with 15 % glycerol at −80 °C. Faecal samples from Black headed gulls (*Chroicocephalus ridibundus*) were taken by swabbing freshly deposited faecal matter from gulls circulating the WWTP sedimentation basins and a key habitat in a recreational city park located 2 km east of the WWTP (Supplementary Table 1). The swabs were put in LB broth (Lennox) with 15 % glycerol and frozen at −80 °C. Storage in −80 °C of both filters and swabs was less than three months prior to culturing.

2.2. Selective screening for CPE

Samples were enriched in 2 mL brain heart infusion (BHI) broth (Becton Dickinson, USA) with vancomycin (8 mg/L, V2002, Sigma-Aldrich, Germany) for 18–24 h at 36 ± 1 °C. Selective screening of putative CPE was performed by streaking 10 μL enriched BHI broth on mSuperCarba™ (CHROMagar™, EMM Life Science, Sweden) and incubating for 18–24 h at 36 ± 1 °C. *K. pneumoniae* CCUG 64452 was used as positive control and *E. coli* CCUG 17620 was used as negative control. Species identification of putative CPE were done with matrix-assisted laser desorption ionization time-of-flight (MALDI-TOF) (MALDI Biotyper® Bruker Daltonik, Germany) according to manufacturer's instructions (Seng et al., 2009). Antimicrobial susceptibility testing (AST) according to EUCAST (v 11.0) was performed on all verified Enterobacterales (https://eucast.org/clinical_breakpoints/, 2021) using 10 μg ampicillin, 30 μg cefadroxil, 5 μg chloramphenicol, 5 μg ciprofloxacin, 10 μg gentamicin, 10 μg mecillinam, 30 μg nalidixic acid, 100 μg nitrofurantoin, 30/6 μg piperacillin/tazobactam, 30 μg tetracycline, 5 μg trimethoprim, 1,25/23,75 μg trimethoprim-sulfamethoxazole and 10 μg meropenem (all Thermo Fisher Scientific Oxoid Ltd., Hants, UK). Breakpoints for inhibition zone diameters were interpreted according to EUCAST v. 11.0 or the Normalized resistance interpretations method (https://eucast.org/clinical_breakpoints/, 2021; Kronvall and Smith, 2016). Isolates showing reduced susceptibility to meropenem were selected for whole genome sequencing.

2.3. Genotypic characterization of isolated CPE

DNA was extracted from suspected CPE using MagnaPure compact Total NA kit according to manufacturer's instructions (Roche, Sweden). Library preparation was performed with the Illumina Nextera XT kit (Illumina, USA) and libraries verified with the Bioanalyzer High Sensitivity DNA method (Agilent, USA). Sequencing was performed as 2 × 250 bp paired-end using a V3 run kit (Illumina) on a MiSeq instrument (Illumina). Raw reads were trimmed and filtered using fastp 0.23.2 on Galaxy 22.01 with default settings (Chen et al., 2018).

Long-read sequencing was performed following DNA extraction using MagAttract HMW DNA kit according to manufacturer's instructions (Qiagen, Sweden). Library preparation and barcoding was performed using Rapid sequencing gDNA-barcoding chemistry and protocol (Oxford Nanopore Technologies, UK, SQK-RBK110.96, version RPK_9126_v110_revK_24Mar2021). Sequencing was performed using MinION™ MK-1B with FLO-MIN106 R9.4.1 Flow Cell and High-accuracy basecalling with read filtering at Q score < 9 and trimming of barcodes, MinKnow 22.03.6 and Guppy 6.0.7. Hybrid assembly of short- and long-reads was done using Unicycler v0.4.8 through conda (dependencies: SPAdes v 3.13.1, racon v1.4.7, makeblastdb v2.9.0+, bowtie2-build v2.3.5, bowtie2 2.3.5, samtools v1.9, java v11.0.1 and pilon v1.23) with default parameters on Galaxy v 22.01 (Wick et al., 2017; Galaxy, n.d.).

2.4. Comparison to human clinical samples

Phenotypically carbapenem resistant Enterobacterales (CRE) with negative NG-test® CARBA-5 (NG Biotech, France), collected from human clinical samples at the clinical microbiology laboratory in the same county as the WWTP, during 2021 and first six months of 2022 were investigated for the presence of carbapenemases not belonging to the five most prevalent (KPC, NDM, VIM, IMP or OXA).

From the national collection of human clinical CPE at PHAS, three isolates were identified carrying the same carbapenemase genes as the CPE from the gulls. Genome sequencing had already been performed using IonTorrent™ platform. For long-read sequencing, DNA extraction was performed according to the protocol described by Hendrickx et al. (2020). Library construction was performed using the SQK-RBK110-96 kit according to manufacturer instructions. The resulting libraries were sequenced on a FLO-MIN106D (R9.4.1) flowcell and subsequently basecalled using Guppy, V6.3.9 with the dna_r9.4.1_e8.1_hac configuration. Sequencing and basecalling were performed on a GridION running Ubuntu 20.04, and GridION release 22.08.6 (minknow-core-gridion v5.2.2) with an Nvidia Quadro GV100 for GPU acceleration.

Initial read QC was performed using nanoq (limiting reads to those above 1000 bp and removing low-quality reads) (Steinig and Coin, 2022). Primary assembly was performed using Flye, with standard settings, and subsequent polishing of the assembly was performed using Medaka with the medaka_consensus wrapper and the r941_e81_hac_g514 model (Kolmogorov et al., 2019).

The resulting genomes were inspected using Bandage and then processed for annotation using Platon and Bakta (Schwengers et al., 2020, 2021).

2.5. Bioinformatic analysis

Assembled sequences were investigated for resistance genes (ResFinder), mobile genetic elements (MGE) and plasmids (PlasmidFinder) by uploading fasta files to Centers for Genomic Epidemiology (Bortolaia et al., 2020; Zankari et al., 2017; Camacho et al., 2009; Johansson et al., 2021; Carattoli et al., 2014). Sequences were also analyzed using SeroTypeFinder, MLST, in silico 16S (Joensen et al., 2015; Larsen et al., 2012; Wirth et al., 2006; Larsen et al., 2014), Average Nucleotide Identity (ANI) (Richter et al., 2015) and PubMLST (Jolley et al., 2018). Annotation was done with RAST and Prokka 1.14.6 (Overbeek et al., 2005; Aziz et al., 2008; Seemann, 2014). For ANI analysis JSpeciesWS was used with tetra correlation search identifying the best type strain matches in the database, which were then included in a pairwise ANIb analysis with any scores >95 % considered a match (Richter et al., 2016). ANI was used for final species assignment. Comparison, alignment and creation of phylograms of identified plasmids in this study with previously published plasmids (Supplementary Table 2) was done with CLC genomic workbench 22.0.2 using the Neighbor Joining tree construction method with the Jukes-Cantor model and 100 replicates of bootstrap analysis. Using the same strategy, subgrouping the plasmids was done by *trf*A alignment using previously published sequences (Dang et al., 2016a). Analysis of plasmid coverage was done using blast ring image generator (BRIG) (Alikhan et al., 2011). Comparison of the genetic environments surrounding identified carbapenemases was performed using Clinker (Gilchrist and Chooi, 2021).

3. Results

3.1. Detection of CPE in wastewater and gull faeces samples

In total, eight WWTP samples, nine surface seawater samples (grab samples) and two samples of waste activated sludge were collected (Supplementary Table 1). In addition, a total of 49 gull faecal samples were collected around the WWTP sedimentation basins and at a recreational city park 2 km east of the WWTP, which is a key habitat. During the sampling period we frequently observed hundreds of black headed gulls feeding from the WWTP sedimentation basins including all steps of the wastewater treatment.

Eighteen putative CPE were isolated from WWTP inlet ($n = 6$), outlet ($n = 7$), sedimentation basins ($n = 1$) and gull faeces ($n = 4$), while no putative CPE were detected in the seawater or sludge samples. Seventeen of the 18 putative CPE isolates carried genes encoding carbapenemases; three *E. coli*, seven *R. ornithinolytica*, three *Klebsiella* spp. and four *E. ludwigii* which were subjected to long-read sequencing.

Among the 17 isolates four different genes encoding carbapenemases, bla_{GES-5}, bla_{IMI-3}, $bla_{OXA-181}$ and $bla_{OXA-244}$, were identified. Three of the carbapenemases were found on plasmids belonging to different replicon types; bla_{GES-5} on IncP ($n = 12$), bla_{IMI-3} on IncFII(Yp) (n = 1) and $bla_{OXA-181}$ on ColKP3 (n = 1), while the $bla_{OXA-244}$ was chromosomally located (Table 1). IncP/GES-5 plasmids were carried by one *E. coli* ST10 from WWTP outlet, in seven *R. ornithinolytica* from WWTP inlet, outlet and pool and gull faeces collected at the WWTP, and in three *Klebsiella* spp. isolates from WWTP inlet and outlet (Fig. 1). An IncFII(Yp)/IMI-3 plasmid was carried by one isolate and IncFII(Yp)/IMI-3 replicons were identified in an additional three isolates, all belonging to *E. ludwigii* isolated from WWTP outlet and gull faeces collected at a recreational city park with a key habitat located 2 km from the WWTP (Fig. 1). Both bla_{OXA} genes were detected in *E. coli* from WWTP inlet (Table 1).

3.2. Comparisons of IncP/GES-5 plasmids

Four IncP plasmids, not previously described in literature were identified in *E. coli* ($n = 1$, p3274c7a/GES-5), *R. ornithinolytica* (n = 1, p3275c3/GES-5) and *Klebsiella* spp. ($n = 2$, p3336c2/GES-5 and p3336c3/GES-5), all with an *intI1* integrase cassette containing bla_{GES-5} (Fig. 2). The IncP plasmids also shared a common backbone with *parA*, *parB*, *tra* and *trb* genes (Fig. 2). The p3274c7a/GES-5 was the largest plasmid and contained mercury resistance genes and bla_{SHV-1} (Fig. 2A). IncP/GES-5 from *E. coli* and *R. ornithinolytica* also carried sulfonamide resistance gene *sul1* and small multidrug resistance gene *qacE delta 1* (Fig. 2A, B) while IncP/GES-5 from *Klebsiella* spp. carried *tniABQ/R* but no mercury resistance genes (Fig. 2C, D). All four incP plasmids were classified as subgroup beta through phylogenetic analysis of the IncP *trfA* gene (Supplementary Fig. 1). When compared to previously published bla_{GES-5} plasmids the IncP/GES-5 plasmid from *R. ornithinolytica* from this study showed closest relatedness to pDTC28 detected in river sediment in China (Dang et al., 2016b) and p2020-O-9 carried by *Serratia marcescens* from an nosocomial outbreak in the ICU in Japan 2020 (Fig. 3A) (Nakanishi et al., 2022). The

Table 1
Replicons with antimicrobial-resistance genes and virulence genes detected in CPE isolated in this study.

Sample ID / Isolate ID	Isolate number	Sample type	Date	Species[b]	MLST	Carbapenemase			Other resistance/virulence genes			Accession number
						Gene	Gene location	Sequence size bp & name	Gene	Gene location	Sequence size bp & name	
3303	c2	Gull faeces	June 16	*E. ludwigii*	ST641	*bla*$_{IMI-3}$	n/a	59,363	ACT family β-lactamase, *fosA2*	n/a	3,983,048	JARZAZ000000000
3306	c7	WWTP outlet	June 28	*E. ludwigii*	ST641	*bla*$_{IMI-3}$	n/a	32,207	ACT family β-lactamase, *fosA2*	n/a	2,052,217	JARZBA000000000
3319	c2	WWTP outlet	July 07	*E. ludwigii*	ST641	*bla*$_{IMI-3}$	IncFII(Yp) plasmid[e]	172,488 (p3319c2/IMI-3)	ACT family β-lactamase, *fosA2*	Chromosome[e]	4,857,156	JARZAY000000000[h]
3333	c3	WWTP outlet	July 16	*E. ludwigii*	ST641	*bla*$_{IMI-3}$	IncFII(Yp) replicon[f]	153,197	ACT family β-lactamase, *fosA2*	n/a	4,821,229	JARZAU000000000
3274	c7a	WWTP outlet	May 23	*E. coli*	ST10	*bla*$_{GES-5}$	IncP plasmid[e]	55,428 (p3274c7a/GES-5)	*qacE*, *aac(6')-Ib-cr*, *sul1*, *aac(6')-Ib3*	IncP plasmid[e]	55,428 (p3274c7a/GES-5)	JARZAT000000000[h]
									bla$_{TEM-1B}$, *qacE*, *sul1*, *dfrA12*, *aadA2*	IncFII(Yp) plasmid[e]	107,491 (p3274c7a/TEM-1)	
									sitABCD, *tet(B)*, *catA1*, *mpg(A)*, *drfA14* *sitA*, *iutA*, *traT*, *iucC*	IncFII plasmid[e]	161,186 (p3274c7a-IncFII)	
									fimH, *terC*, *gad*, *hlyE*	Chromosome[e]	4,652,483 (c3274c7a)	
3336	c2	WWTP inlet	Sep 07	*K. pneumoniae*	ST359	*bla*$_{GES-5}$	IncP plasmid[e]	46,226 (p3336c2/GES-5)	*aac(6')-Ib-cr/Ib3*	IncP plasmid[e]	46,226 (p3336c2/GES-5)	JARZAX000000000
									bla$_{SHV-1}$, *fosA*, *OqxB*, *OqxA* *iutA*	Chromosome[e]	5,189,090	
3337	c2	WWTP outlet	Sep 07	*K. pneumoniae*	ST359	*bla*$_{GES-5}$	IncP plasmid[e]	46,226 (p3337c2/GES-5)	*aac(6')-Ib-cr/Ib3*	IncP plasmid[e]	46,226 (p3337c2/GES-5)	JARZAS000000000
									bla$_{SHV-33}$, *fosA*, *OqxB*, *OqxA* *iutA*	n/a	5,106,042	
3336	c3	WWTP inlet	Sep 07	*K. variicola*	ST919[d]	*bla*$_{GES-5}$	IncP plasmid[e]	49,822 (p3336c3/GES-5)	*aac(6')-Ib-cr/Ib3*	IncP plasmid[e]	49,822 (p3336c3/GES-5)	JARZAW000000000[h]
									bla$_{LEN-32}$, *fosA*, *OqxB*, *OqxA* *iutA*	Chromosome[e]	5,593,260	
3275	c3	WWTP inlet	May 23	*R. ornithinolytica*	n/a	*bla*$_{GES-5}$	IncP plasmid[e]	23,145 (p3275c3/GES-5)	*qacE*, *sul1*, *aadA2b*	IncP plasmid[e]	23,145 (p3275c3/GES-5)	JARZAV000000000
									mcr-9[g]	n/a	9815	
									terC	IncHI1A/B (CIT) replicon[f]	43,019	
									tet(A)	IncN replicon[f]	143,019	
									ORN family β-lactamase	n/a	479,703	
									fyuA, *irp2*	n/a	989,709	
3277	c1	Gull faeces	May 24	*R. ornithinolytica*	n/a	*bla*$_{GES-5}$	IncP plasmid[e]	23,145 (p3277c1/GES-5)	*qacE*, *sul1*, *aadA2b*	IncP plasmid[e]	23,145 (p3277c1/GES-5)	JARZBB000000000
									mcr-9[g]	n/a	2174 bp	
									terC	IncHI1A/B (CIT) replicon[f]	213,674	
									ARR-3, *tet(A)*, *qacE*, *dfrA27*, *sul1*, *aadA16*, *aac(6')-Ib-cr*	IncN plasmid[e]	125,861 (p3277c1-IncN)	
									ORN family β-lactamase	n/a	206,609	
									fyuA, *irp2*	n/a	894,407	
3284	c3	Gull faeces	May 24	*R. ornithinolytica*	n/a	*bla*$_{GES-5}$	IncP plasmid[e]	23,145 (p3284c3/GES-5)	*qacE*, *sul1*, *aadA2b*	IncP plasmid[e]	23,145 (p3284c3/GES-5)	JARZAR000000000
									mcr-9[g]	n/a	2174	
									terC	IncHI1A/B (CIT) replicon[f]	143,019	
									ARR-3, *tet(A)*, *qacE*, *dfrA27*, *sul1*, *aac(6')-Ib-cr*	IncN replicon[f]	30,055	
									ORN family β-lactamase	n/a	477,42	
									fyuA, *irp2*	n/a	622,399	

3293	c1	Gull faeces	May 24	*R. ornithinolytica*	n/a	bla_{GES-5}	IncP plasmid[e]	24,472 (p3293c1/GES-5)	*qacE*, *sul1*, *aadA2b*	IncP plasmid[e]	24,472 (p3293c1/GES-5)	JARZBC000000000
									mcr-9[g] *terC*	IncHI1A/B (CIT) plasmid[e]	237,916	
									ARR-3, *tet(A)*, *qacE*, *dfrA27*, *sul1*, *aac(6')-Ib-cr*	IncN plasmid[e]	125,861 (p3293c1-IncN)	
									ORN family β-lactamase, *fosA* *fyuA*, *irp2*	n/a	1,837,937	
3336	c1	WWTP inlet	Sep 07	*R. ornithinolytica*	n/a	bla_{GES-5}	IncP plasmid[e]	23,145 (p3336c1/GES-5)	*qacE*, *sul1*, *aadA2b*	IncP plasmid[e]	23,145 (p3336c1/GES-5)	JARZAQ000000000
									mcr-9[g]	n/a	8974	
									terC	IncHI1A/B (CIT) replicon[f]	225,439	
									ARR-3, *tet(A)*, *qacE*, *dfrA27*, *sul1*, *aac(6')-Ib-cr*	IncN replicon[f]	1,420,848	
									ORN family β-lactamase	n/a	301,638	
									fyuA, *irp2*	n/a	1,254,778	
3341	c1	WWTP sedimentation basins[a]	May 24	*R. ornithinolytica*	n/a	bla_{GES-5}	IncP plasmid[e]	23,145 (p3341c1/GES-5)	*qacE*, *sul1*, *aadA2b*	IncP plasmid[e]	23,145 (p3341c1/GES-5)	JARZAP000000000
									mcr-9[g]	n/a	14,489	
									terC	IncHI1A/B (CIT) replicon[f]	142,311	
									ARR-3, *tet(A)*, *dfrA27*, *aadA16*, *aac(6')-Ib-cr*	IncN replicon[f]	44,794	
									ORN family β-lactamase	n/a	394,692	
									fyuA, *irp2*	n/a	893,564	
3337	c3	WWTP outlet	Sep 07	*R. ornithinolytica*[c]	n/a	bla_{GES-5}	IncP plasmid[e]	23,145 (p3337c3/GES-5)	*qacE*, *sul1*, *aadA2b*	IncP plasmid[e]	23,145 (p3337c3/GES-5)	JARZAO000000000
									mcr-9[g], *terC*	IncHI1A/B (CIT) replicon[f]	115,582	
									ARR-3, *tet(A)*, *qacE*, *dfrA27*, *sul1*, *aac(6')-Ib-cr*	IncN plasmid[e]	49,937 (p3337c3-IncN)	
									ORN family β-lactamase	n/a	530,392	
									fyuA, *irp2*	n/a	990,033	
3275	c5	WWTP inlet	May 23	*E. coli*	ST372	$bla_{OXA-181}$	ColKP3 plasmid[e]	51,479 (p3275c5/OXA-181)	*qnrS1*	ColKP3 plasmid[e]	51,479 (p3275c5/OXA-181)	JARZAM000000000
									sitABCD *iucC*, *iutA*, *mchF*, *ompT*, *traT*, *iroN*, *cia*, *sitA*, *cvaC*, *iss*, *hlyF*	IncFII plasmid[e]	127,746 (p3275c5-IncFII/FIB)	
									sitABCD *terC*, *kpsE*, *gad*, *iss*, *vat*, *vfcV*, *sitA*, *chuA*, *irp2*, *ompT*, *usp*, *fyuA*, *kpsMII*, *ibeA*, *terC*, *hra*	n/a	4,530,924	
3336	c7	WWTP inlet	Sep 07	*E. coli*	ST10	$bla_{OXA-244}$	Chromosome[e]	4,756,317	$bla_{CTX-M-15}$, *qnrS1* *cib*	IncB/O/K/Z plasmid[e]	98,660 (p3336c7/CTX-M-15)	JARZAN000000000[h]
									terC, *gad*, *fyuA*, *irp2*	Chromosome[e]	4,756,317	

[a] WWTP sediment basins part of the biological treatment phase.
[b] Species determined by MALDI-TOF, in silico 16S, PubMLST and Average Nucleotide Identity (ANI). ANI was used for final species assignment.
[c] *R. planticola* by MALDI-TOF.
[d] MLST for *K. pneumoniae*.
[e] Completed, circular chromosome or plasmid.
[f] Replicons denote identified plasmid replicons in the same sequence as the gene of interest.
[g] Truncated.
[h] Genomes with all replicons circularized.

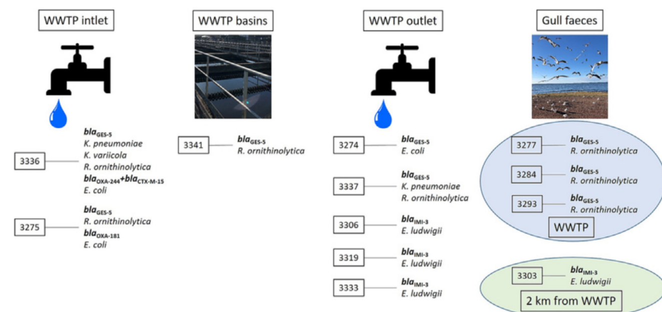


Fig. 1. The CPE collected in the study.
bla$_{GES-5}$ was carried on an IncP plasmid in *E. coli* ST10 from WWTP outlet, in seven *R. ornithinolytica* isolated from WWTP inlet, outlet and sedimentation basins and gull faeces collected at the WWTP and in three *Klebsiella* spp. isolates from WWTP inlet and outlet. *bla*$_{IMI-3}$ was carried on an IncFII(Yp) plasmid in four isolates of *E. ludwigii* isolated from WWTP outlet and gull faeces collected at a recreational city park located 2 km from the WWTP. In addition, *bla*$_{OXA-181}$ located on a COLKP3 plasmid was found in an *E. coli* and *bla*$_{OXA-244}$ was chromosomally located in an *E. coli* also harboring an IncB/O/K/Z plasmid with *bla*$_{CTX-M-15}$.

IncP/GES-5 from *Klebsiella* spp. and *E. coli* from this study showed closest relatedness to pICP-4GES detected in *Pseudomonas aeruginosa* causing upper respiratory infection of a patient in China (Fig. 3A) (Xu et al., 2018). Synteny analysis of the genetic environment surrounding *bla*$_{GES}$/*intI1* show a variable structure with different genes encoding aminoglycoside resistance, variable transposases, presence/absence of *tniQ/B* genes, *sul1* and *qacE delta* (Fig. 3B).

3.3. Comparison of IncFII(Yp)/IMI-3 plasmids

The IncFII(Yp)/IMI-3 was found in four separate isolates of *E. ludwigii* sampled from gull faeces ($n = 1$) and WWTP outlet ($n = 3$), all on different sampling occasions. In one sample (3319c2) an IncFII(Yp) plasmid, not previously described was identified, generating a 172,488 bp IncFII(Yp) plasmid p3319c2/IMI-3, with a GC content of 52.9 % (Fig. 4). In the three other isolates different parts of the pIncFII(Yp)/IMI-3 were located on different contigs, as indicated by BRIG analysis (Supplementary Fig. 2). The overall sequence structure of p3319c2/IMI-3 can be divided into one region constituting conjugational transfer with 21 *tra*- and 3 *trb*-genes, one region with 17 T6SS genes, DNA replication genes (*umuC*, *repA*, *repA2*) and segregation and stability genes (*parA*, *parB*, *klcA*, *RelB/StbD*, *RelE*, *xerD*) (Fig. 4). The plasmid also harbored SOS-response responsible genes (*PsiA*, *PsiB* and *YedK*), type IV toxin-antitoxin genes (*YafZ* and *YkfF*), genes involved in fimbrin biogenesis (*SfmH*, *FimD*, *FimE*, *fimC*) and several mobile genetic elements (Fig. 4). When compared to previously published *bla*$_{IMI}$ plasmids the IncFII(Yp)/IMI-3 plasmid from this study showed closest relatedness to pRJ46C, pGA45 with *bla*$_{IMI-3}$ and N14-0444 plasmid pIMI-6 with *bla*$_{IMI-6}$ (Fig. 5A). Synteny analysis of *bla*$_{IMI-3}$ and surrounding genes from all four *E. ludwigii* isolates as well as the three closely related plasmids pRJ46C, pGA45 and N14-0444 show identical sequences for *bla*$_{IMI-3}$ and *imiR* (with an exception for pGA45 where a deletion of the 36 first bases has occurred) (Fig. 5B). The surrounding IS elements are highly similar with downstream ISEc36 and upstream ISEcl1-like (Fig. 5B). p3319c2/IMI-3 carries the type VI secretion system responsible for facilitating bacterial survival and propagation (Bonnin et al., 2021; Cascales, 2008).

3.4. The COLKP3/OXA-181 plasmid

A 51479 bp COLKP3/OXA-181 plasmid (p3275c5/OXA-181) was identified in an *E. coli* ST372 collected from WWTP inlet (Fig. 6). The plasmid also carried *qnrB* and several *virB/D* genes belonging to T4SS which facilitates horizontal gene transfer and bacterial host survival (Table 1).

3.5. Other resistance genes

The isolate carrying *bla*$_{OXA-244}$ was an ST10 *E. coli* with an IncB/O/K/Z plasmid harboring *bla*$_{CTX-M-15}$ and *qnrS1* (Table 1). Additional detected resistance genes include intrinsic ACT family cephalosporin-hydrolyzing class C beta-lactamase and ORN family beta-lactamase in all four *E. ludwigii* and all seven *R. ornithinolytica*, respectively (Table 1). Furthermore the *R. ornithinolytica* isolates also harbored an IncN replicon with several resistance genes and an IncHI1A/B replicon with a truncated *mcr-9* gene (Table 1).

3.6. Virulence genes

Several putative virulence genes were identified in two of the *E. coli* isolates (Table 1). The ST10 isolate with *bla*$_{GES-5}$ carried genes including *fimH*, *hlyE*, *sitABCD*, *iutA*, *traT*, *iucC*, and *terC*. The ST372 isolate with *bla*$_{OXA-181}$ carried genes including *sitABCD*, *iucC*, *iutA*, *ompT*, *traT*, *iroN*, *sitA*, *hlyF*, *kpsE*, *kpsMII*, *vat* and *ibeA*.

3.7. Phylogenetic relatedness of R. ornithinolytica with IncP/GES-5 and E. ludwigii with IncFII(Yp)/IMI-3

Phylogenetic relatedness for both *R. ornithinolytica* with 10–41 allelic distances (AD) between isolates and the *E. ludwigii* isolates with 2–7 AD, indicate a relatedness between isolates collected from different samples, i.e., gull faeces, WWTP inlet, outlet and sediment basins. There was one exception; 3337c3 where up to 191 AD were detected to the other *R. ornithinolytica*. This sample 3337c3 was typed as *R. planticola* with MALDI-TOF, indicating a difference to the other *R. ornithinolytica*.

3.8. Comparison between gulls/WWTP findings and human clinical isolates

Investigation of phenotypically CRE collected from human clinical isolates at the clinical microbiology laboratory at the local hospital connected to the WWTP identified five isolates for which the NG-test CARBA-5 was negative, four were typed as *E. cloacae* and one as *K. pneumoniae* by MALDI-TOF MS. However, no genes encoding known carbapenemases were detected in these isolates.

Since CPE with *bla*$_{IMI-3}$ and *bla*$_{GES-5}$ were detected in both gulls and WWTP samples incidence of these genes was investigated by PHAS among the isolates collected in the national microbial surveillance program for CPE. From 2015 to 2021, 967 CPE isolates were submitted to and verified at PHAS, among which three were identified to carry *bla*$_{GES-5}$, but no isolates carrying *bla*$_{IMI-3}$ were identified. The PHAS isolates carrying *bla*$_{GES-5}$ were two *Citrobacter freundii*, isolated in 2017 and 2020, and one *Klebsiella oxytoca* isolated in 2018. Genomic comparison of these isolates with the *bla*$_{GES-5}$ carrying isolates identified in this study revealed different genetic environments. The human isolates did not carry any IncP plasmids instead the *bla*$_{GES-5}$ was associated with an IncFII replicon (C080) and an IncW replicon (C122). However, the genetic environment around *bla*$_{GES-5}$ for the two *C. freundii* isolates (C080 and C122) was similar to that described for the IncP plasmids with *intI1* and presence of *sul1* and *qacE delta 1* (Fig. 7). Worth noticing is that C080 and C122 also harbored *bla*$_{IMP-14}$ downstream of *intI1*.

4. Discussion

In this study four different carbapenemases, *bla*$_{GES-5}$, *bla*$_{IMI-3}$, *bla*$_{OXA-181}$ and *bla*$_{OXA-244}$, were identified among 17 isolates of Enterobacterales sampled from WWTP inlet, outlet, sediment basins and gull faeces collected at the WWTP and at a recreational city park 2 km from the WWTP. According to published data, both *bla*$_{GES}$ and *bla*$_{OXA}$ appear to be frequently detected in different aquatic samples including wastewater and are isolated from several different Enterobacterales whereas *bla*$_{IMI}$ genes are mostly detected in Enterobacter spp. isolated from river and lake samples (Cherak et al., 2021; Teixeira et al., 2022). While CPE have been reported from

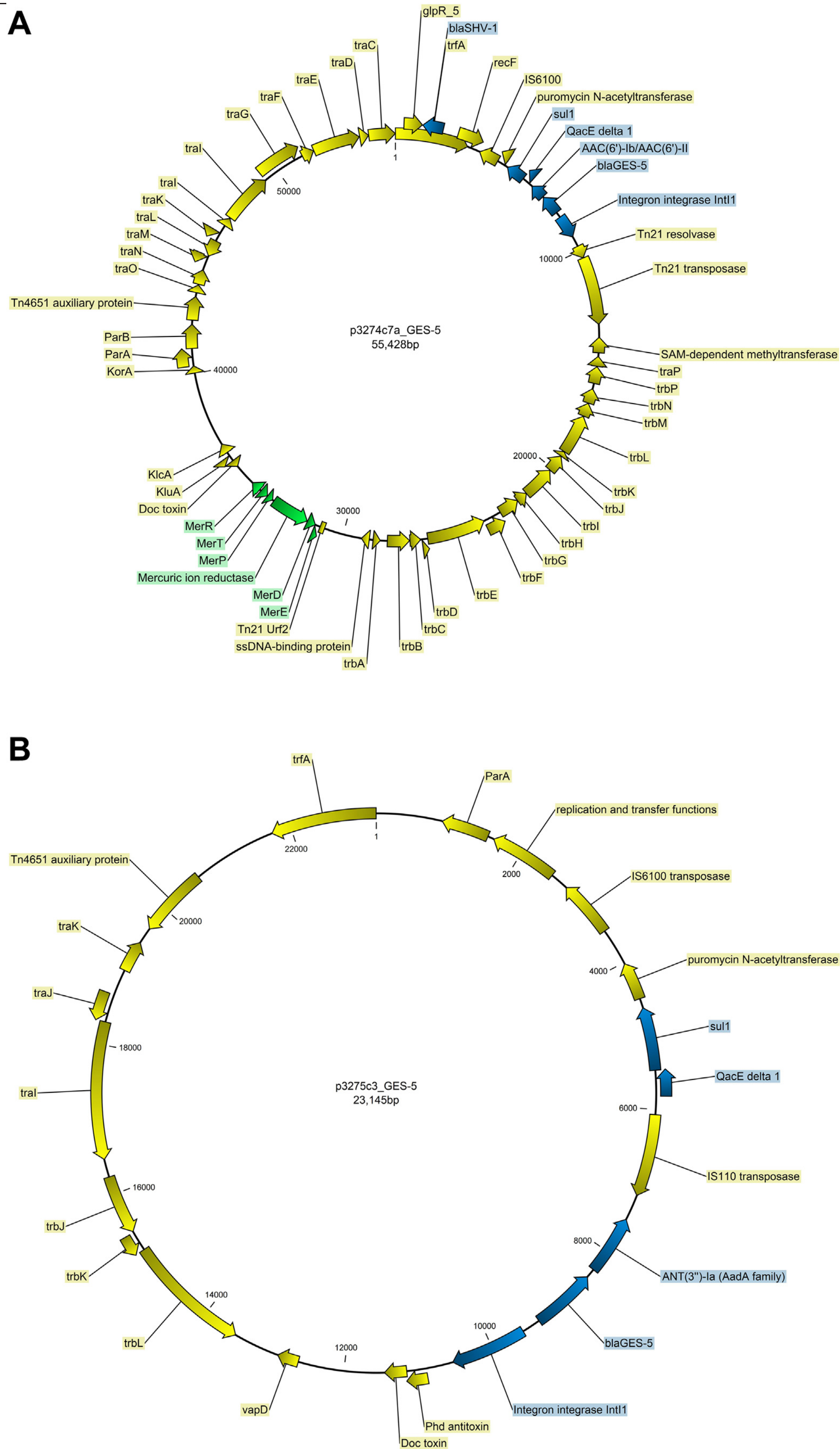
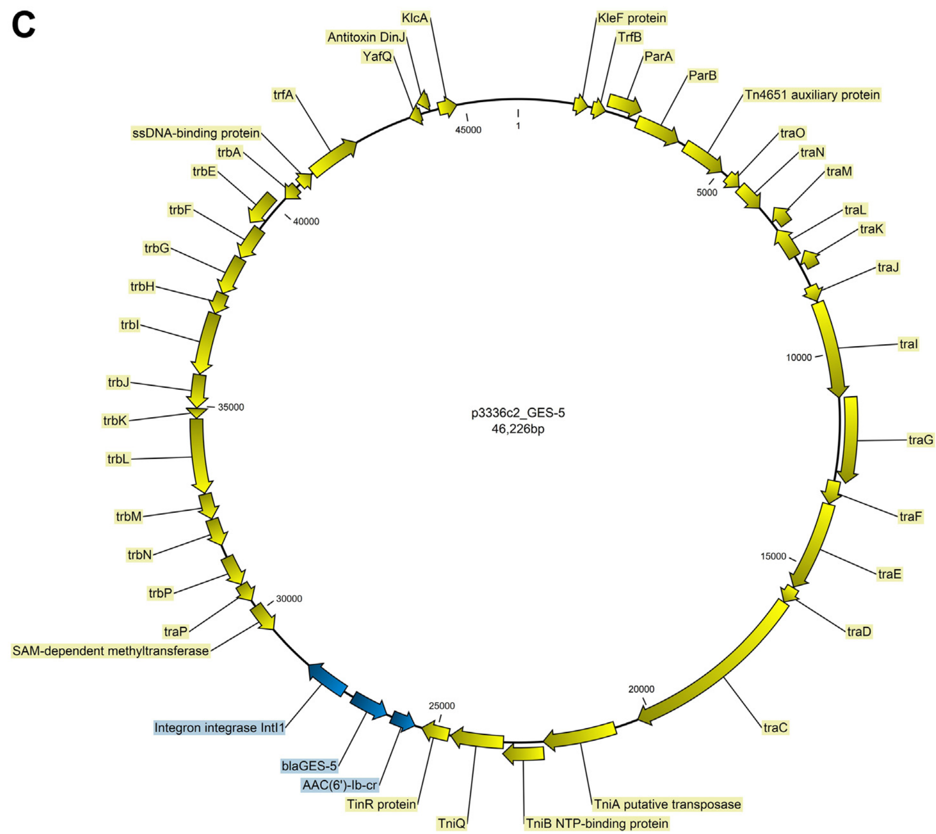


Fig. 2. The overall sequence structure of the novel IncP/GES5 plasmids.
Four IncP plasmids were identified *E. coli* with a GC content of 52.9 % (A), in *R. ornithinolytica* with a GC content of 65.4 % (B) and *Klebsiella* spp. with a GC content of 65.2 and 62.6 % (C, D). All IncP plasmids carried *intI1* integrase cassette containing $bla_{\text{GES-5}}$.



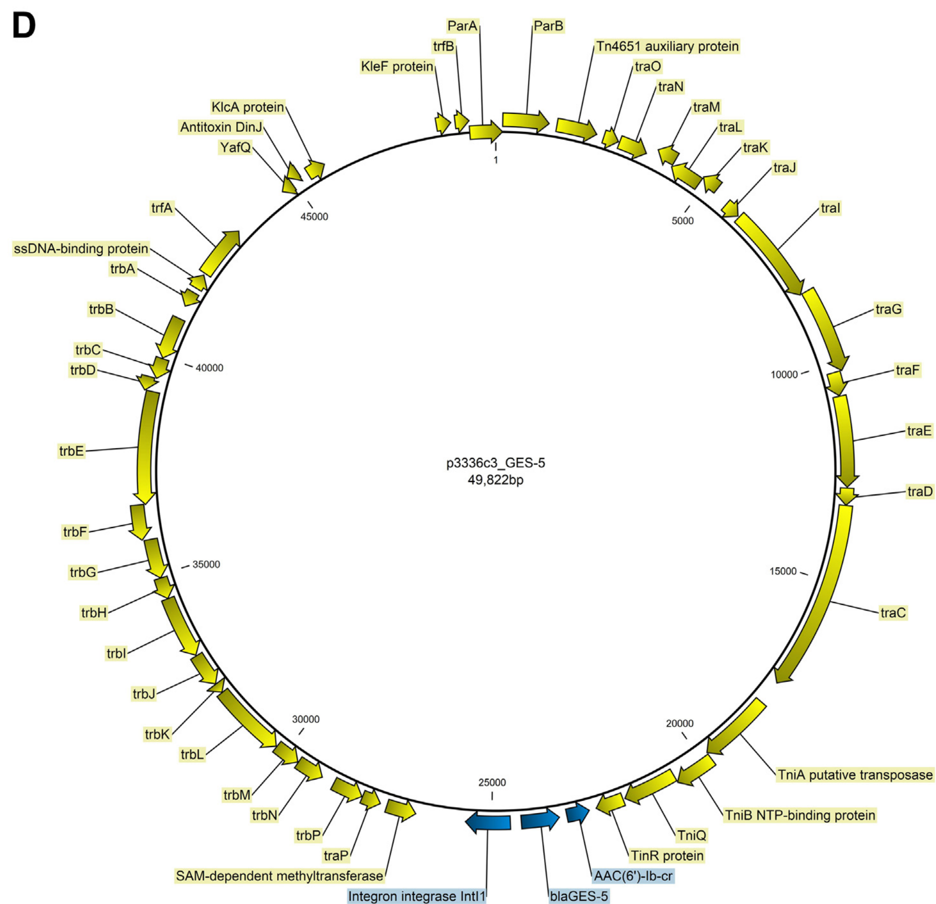


Fig. 2 (*continued*).

environmental samples before in Sweden, although with other genes – bla_{VIM-1}, bla_{IMP-29} and bla_{IMI-2} (Khan et al., 2018; Flach et al., 2021; Borjesson et al., 2022), this is the first report of detection of CPE from animals in Sweden. However, there are previous reports of gulls and other wild birds carrying CPE with bla_{OXA-48}, bla_{VIM-1}, bla_{NDM-1} and bla_{KPC-2} in Europe (Kock et al., 2018; Abbassi et al., 2022; Ahlstrom et al., 2022).

The bla_{GES-5} was located on IncP-plasmids of different lengths (Table 1) in 11 isolates belonging to *E. coli*, *Klebsiella* spp. and *R. ornithinolytica*. GES-type enzymes are being increasingly detected and are associated with several different Inc. replicons from a variety of different Enterobacterales from both clinical and environmental samples (Bonnin et al., 2017, 2021; Moura et al., 2018; Girlich et al., 2012; Cuzon et al., 2016). In a recent report, a 12 kb plasmid harboring bla_{GES-5} was identified in *E. coli* isolated from pigs in Germany 2020 (Irrgang et al., 2020). The IncP/GES-5 plasmids in this study were similar to previously described plasmids derived from river sediments in China, a nosocomial outbreak in Japan of *S. marcescens* and *P. aeruginosa* causing upper respiratory infection in China (Dang et al., 2016b; Xu et al., 2018). However, the genetic environment surrounding the *intI1* integrase cassette containing bla_{GES-5} show a variable structure with different genes encoding additional AMR genes and variable transposases (Moura et al., 2018; Girlich et al., 2012; Cuzon et al., 2016). This data together with previously published data indicate that the bla_{GES-5}/*intI1* cassette can be found in various species and genetic environments (Moura et al., 2018; Girlich et al., 2012; Cuzon et al., 2016). IncP plasmids appears to have a wide host range where data suggests a crucial role in bacterial adaptation (Popowska and Krawczyk-Balska, 2013), and was in this study detected in three different species. These plasmids have frequently been described in bacteria isolated from wastewater but have also been

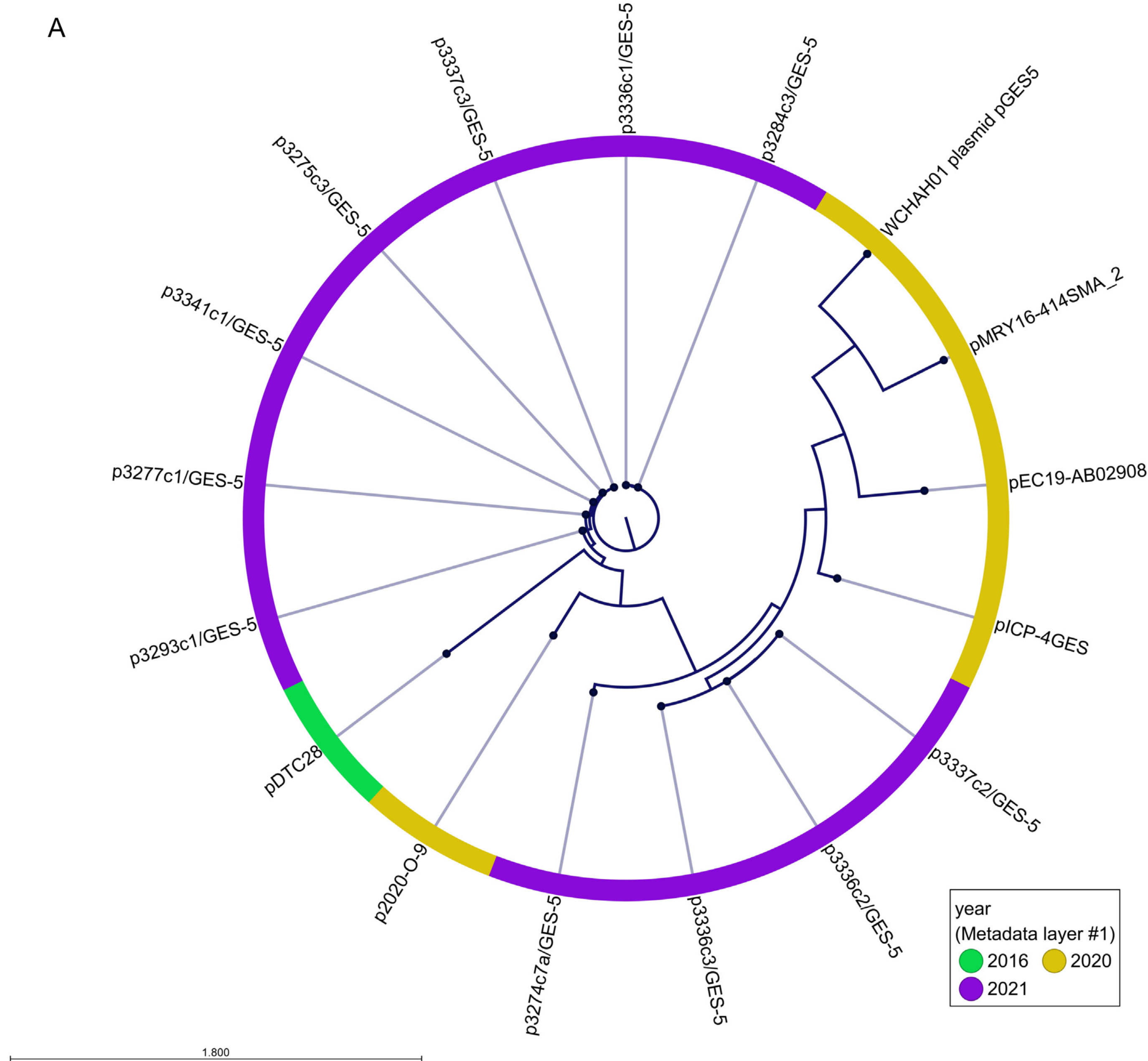


Fig. 3. Sequence comparison of IncP/GES plasmids.
Phylogenetic whole sequence analysis of six previously published IncP/GES plasmids compared to the eleven IncP/GES-5-plasmids isolated in this study (A). Synteny analysis of bla_{GES-5} and surrounding genes from 3275c3, 3274c7a, 3336c3 and 3336c2 from this study as well as the six closely related plasmids; pICP-4GES from *Pseudomonas aeruginosa*, pEC19-AB02908 from *E. coli*, pDTC28, p2020-O-9 and pMRY16-414SMA from *Serratia marcescens* and WCHAH01 plasmid pGES5 from *Aeromonas hydrophila* (B). * & **100 % Identity.

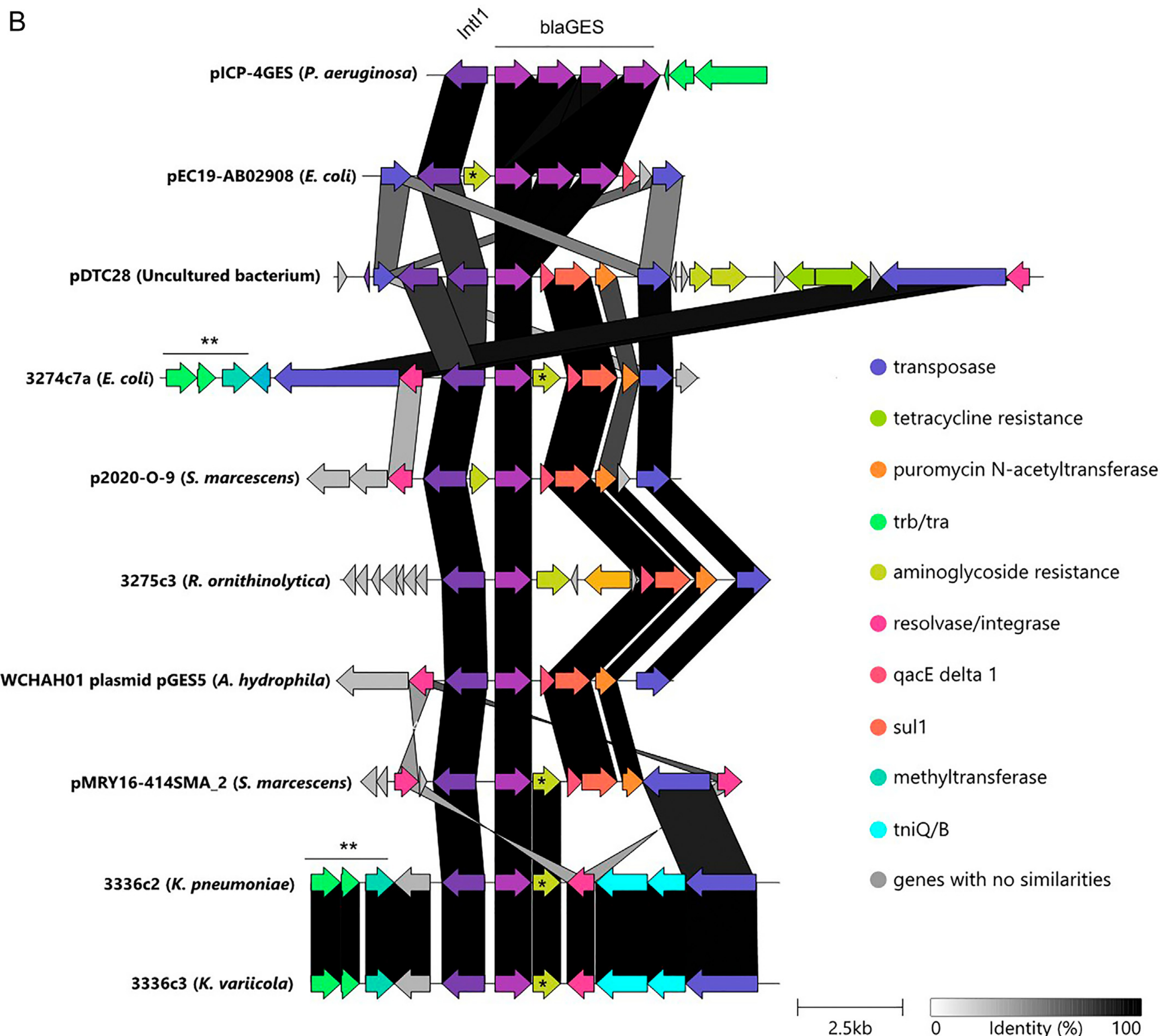


Fig. 3 (*continued*).

found in human clinical CPE isolates (Zhao et al., 2017; Lalaoui et al., 2019; Yong et al., 2022). The IncP plasmids appear to have great diversity with several different MGEs with resistance and virulence genes, but they share a common backbone consisting of genes encoding efficient replication, conjugational transfer and maintenance (Popowska and Krawczyk-Balska, 2013). Thus, a larger additional sampling campaign from the same locations could probably result in identification of additional IncP plasmids.

The IncFII(Yp)/IMI-3 was found in *E. ludwigii* isolated from gull faeces and WWTP outlet. The first reports of $bla_{\text{IMI-3}}$ are from China in 2017 where *R. ornithinolytica* harbored an IncFII(Yp)/IMI-3 plasmid and France in 2015 where *E. cloacae* harbored the gene (Zhang et al., 2017; Dupont et al., 2016). The IncFII(Yp)/IMI-3 plasmid described in this study was similar to previously described plasmids derived from river sediments in China (pGA45) and human clinical samples in China (pRJ46C) and Canada (N14-0444) (Zhang et al., 2017; Dang et al., 2016a; Boyd et al., 2017). In addition, the genetic environment surrounding $bla_{\text{IMI-3}}$ and *imiR* was highly similar with the same IS elements and the type VI secretion system responsible for facilitating bacterial survival and propagation (Bonnin et al., 2021; Cascales, 2008). Early reports mention $bla_{\text{IMI-1}}$ as being chromosomally located in Enterobacter spp. and rarely associated with plasmids (Diene and Rolain, 2014; Bush and Bradford, 2020). However, in more recent reports other bla_{IMI} genes have been described to be associated to plasmids with IncFII replicons (Boyd et al., 2017; Bush and Bradford, 2020). IncFII plasmids have a complex structure with great diversity and highly variable resistance and virulence genes and are frequently detected in different Enterobacterales isolated from human, animal and environmental samples (Rozwandowicz et al., 2018). Thus, further highlighting the potential risk of environmental and interspecies transmission (Juhas et al., 2008).

The COLKP3/OXA-181 plasmid was identical to pEc-042-T5-MAC_1 (CP086539), pEc-042-T20-MAC_1 (CP086527) and pEC-042-T20-ESBL_1 (CP086521) all derived from the same clinically isolated *E. coli* Ec-042 collected from an employee at a Swiss veterinary clinic in 2018 (ST410, OXA-181 producer) (Bioproject PRJNA769291) (Moser et al., 2022).

The data in this study supports that ARG dissemination in the environment is spread by both possible horizontal gene transfer and by clonal propagation. This because the $bla_{\text{GES-5}}$/*IntI1* cassette could be found in different species as well as in slightly different genetic environments and in related

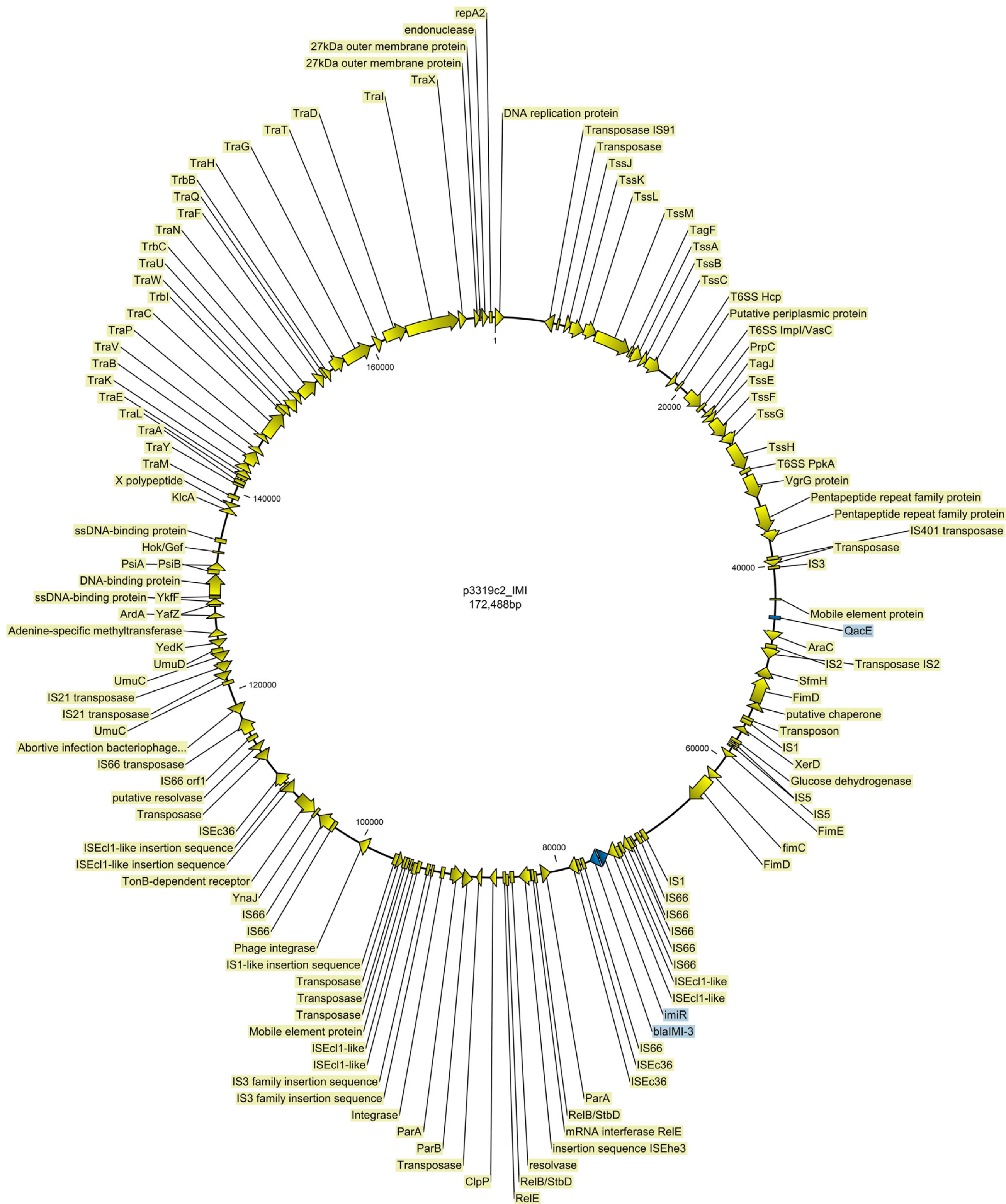


Fig. 4. The overall sequence structure of p3319c2/IMI-3.

R. ornithinolytica isolates and that the $bla_{\text{IMI-3}}$ could be found in similar *E. ludwigii* isolates. Previous limited data also suggests direct anthropozoonotic and zooanthroponotic transmission of CPE, and members of our group have shown that gulls can acquire ARB from anthropogenic sources and disperse across large areas (Kock et al., 2018; Ramsamy et al., 2022; Ahlstrom et al., 2021). That data together with our current detection in gulls and wastewater, indicate a great host variability with a potential risk of environmental, anthropozoonotic and zooanthroponotic transmission. The detection of different CPE in gulls in Sweden calls for further studies including telemetry collection of the birds' movement patterns to both identify key habitats and possible mitigation of ARG and ARB dissemination. Thus, further studies investigating the gulls' movement patterns could play an important role on providing data to perform risk assessment of environmental and interspecies transmission of CPE.

Fig. 5. Sequence comparison of IncFII(Yp)/IMI plasmids.
Phylogenetic whole sequence analysis of previously published IncFII(Yp) plasmids compared to p3319c2/IMI-3 (A). Synteny analysis of bla_{IMI-3} and surrounding genes from all four *E. ludwigii* as well as the three closely related plasmids; pRJ46C from *R. ornithinolytica*, N14-0444-plasmid IMI-6 from *E. cloacae* and pGA45 (B). N14-0444, 3333c3, pGA45 reversed for illustrative purposes.

R. ornithinolytica was earlier thought of as an environmental, relatively harmless bacteria but there are increasing numbers of reports of resistance (Zou et al., 2022; Hajjar et al., 2020). Aside from being a possible vector for resistance *R. ornithinolytica* is an emerging human pathogen, and the history with low incidence of reported human infections might depend on difficulties in correctly identify the species with previously phenotypic-based laboratory tests (Hajjar et al., 2020). Bacteria belonging to the *Enterobacter* genus can be found in diverse environmental habitats and are also implicated in hospital-acquired infections and outbreaks (Davin-Regli et al., 2019). *Enterobacter* spp. have intrinsic resistance towards several antibiotics and can easily acquire additional resistance genes (Davin-Regli et al., 2019). A recent study reports of the detection of bla_{IMI-2} *E. asburiae* collected from Swedish feed mill and several studies have described *E. cloacae complex* isolates with bla_{IMI} in environmental samples, primarily aquatic-related ones (Borjesson et al., 2022).

The *E. coli* ST10 isolate with bla_{GES-5} carried several genes which are common in both avian pathogenic *E. coli* (APEC) and extraintestinal pathogenic *E. coli* (ExPEC) (Sarowska et al., 2019). The ST10 complex is known for its broad host range and in some cases extraintestinal pathogenic potential in both animals and humans (Kocsis et al., 2022). The *E. coli* ST372 isolate with $bla_{OXA-181}$ also had genes indicative of APEC/ExPEC. ExPEC and particularly strains of uropathogenic *E. coli* (UPEC) including CPE have been shown to occur in wastewater (Calhau et al., 2015; Zhi et al., 2020; Adefisoye and Okoh, 2016; Hoelle et al., 2019). As both virulence determinants and sequence types overlap between human ExPEC and APEC (Mehat et al., 2021), wild birds could potentially be particularly susceptible to

Fig. 6. The overall sequence structure of p3275c5/OXA-181.

being infected by such strains and likely to disseminate them. The isolation of a *bla*$_{GES-5}$-carrying ST10 *E. coli* with traits consistent with the ExPEC pathotype from a WWTP outlet is particularly worrisome. This since this finding shows that *bla*$_{GES-5}$ is not restricted to environmental bacteria and

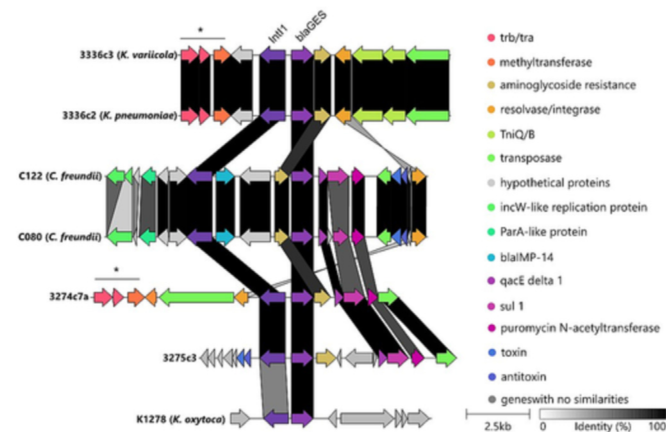


Fig. 7. Sequence comparison of genetic environment surrounding *bla*$_{GES-5}$. Synteny analysis of *bla*$_{GES-5}$ and surrounding genes from 3275c3, 3274c7a, 3336c3 and 3336c2 from this study as well as the three identified human clinical isolates C080, C122 and K1287. There is 100 % sequence identity for *IntI1* for all isolates but K1287 where *IntIPac* is present instead. C080 and C122 also carries *qacE delta 1*, *sul1*, and the gene for puromycin *N*-acetyltransferase as both 3274c7a and 3275c3. *100 % Identity. K1278 was reversed for illustrative purposes.

indicates that there might be a potential that plasmids carrying these more environmental related genes could be horizontally spread to likely pathogens in WWTPs.

In clinical settings the occurrence of Enterobacterales with the *bla*$_{GES}$ and *bla*$_{IMI}$ genes has remained infrequent compared to other gene groups encoding carbapenemases and the reason for this remains ambiguous, but this and other recent studies show that they are readily identified in the environment and WWTPs. However, the IMI and GES might also be underdiagnosed at clinical laboratories in Sweden, so the true prevalence might have been underestimated in human and animal settings, because some laboratories do not include them in their confirmation of CPE and thus, they are not submitted to the PHAS CPE surveillance program. The findings in this study where *bla*$_{GES-5}$ was not associated with IncP in the two human *C. freundii* isolates but that the genetic environment was similar to that described for the IncP plasmids with *intI1* and presence of *sul1* and *qacE delta 1* indicate a common ancestor for the clinical, the environmental and the gull isolates. Thus, this study further supports to the hypothesis that the environment is a potential reservoir for carbapenem-resistant Enterobacterales and these genes further highlighting the importance of MDR monitoring within human and animal health care as well as in the environment using a One Health approach.

5. Conclusion

This is the first report of CPE in animals in Sweden. The incidence of clinical CPE is still low in Sweden, but the repeated detection in anthropogenic affected environments is concerning. We often observed hundreds of black headed gulls feeding from the WWTP sedimentation basins and we

have previously showed that gulls can acquire ARB from anthropogenic sources and disperse across large areas. This, together with the fact that we found the same CPE in both birds and the wastewater sedimentation basin points towards the actual WWTP sedimentation basins as a point source for acquiring AMR among feeding gulls and, in this particular case, a risk of further dissemination to a popular city park.

The results add to the increasing evidence of WWTPs as likely anthropogenic reservoirs for mobile genetic elements with antibiotic-resistance functionality. Such environments could profoundly impact the dissemination and spread of such genetic elements via for example connected aquatic environments and aquatic birds, thereby warranting further study and surveillance.

Abbreviations

AD	allelic distances
ANI	Average Nucleotide Identity
AMR	antimicrobial resistance
ARB	antibiotic resistant bacteria
CPE	carbapenemase-producing Enterobacterales
CRE	carbapenem-resistant Enterobacterales
ESBL	extended-spectrum beta-lactamase-producing
EUCAST	The European Committee on Antimicrobial Susceptibility Testing
MALDI-TOF	Matrix-assisted Laser Desorption/Ionization Time of Flight
MGE	mobile genetic elements
MLST	multilocus sequence typing
SVA	National Veterinary Institute
PHAS	Public Health Agency of Sweden
WWTP	wastewater treatment plant

CRediT authorship contribution statement

Hanna Woksepp: Conceptualization, Methodology, Software, Formal analysis, Investigation, Resources, Data curation, Writing – original draft, Visualization, Supervision, Project administration. **Klara Karlsson:** Investigation, Resources, Data curation, Writing – review & editing. **Stefan Börjesson:** Investigation, Data curation, Writing – review & editing, Funding acquisition. **Oskar Karlsson Lindsjö:** Software, Investigation, Data curation, Writing – review & editing. **Robert Söderlund:** Software, Investigation, Data curation, Writing – review & editing. **Jonas Bonnedahl:** Conceptualization, Methodology, Resources, Writing – original draft, Supervision, Funding acquisition.

Data availability

All sequences are publicly accessible under accession number PRJNA961034 at National Center for Biotechnology Information (https://www.ncbi.nlm.nih.gov/bioproject/PRJNA961034).

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.scitotenv.2023.163997.

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