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Surface water in Lower Saxony: A reservoir for multidrug-resistant *Enterobacterales*

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ABSTRACT

The emergence of extended-spectrum β-lactamase and carbapenemase-producing Enterobacterales (ESBL-E and CPE, respectively) is a threat to modern medicine, as infections become increasingly difficult to treat. These bacteria have been detected in aquatic environments, which raises concerns about the potential spread of antibiotic resistance through water. Therefore, we investigated the occurrence of ESBL-E and CPE in surface water in Lower Saxony, Germany, using phenotypic and genotypic methods. Water samples were collected from two rivers, five water canals near farms, and 18 swimming lakes. ESBL-E and CPE were isolated from these samples using filters and selective agars. All isolates were analyzed by whole genome sequencing. Multidrug-resistant Enterobacterales were detected in 4/25 (16%) water bodies, including 1/2 rivers, 2/5 water canals and 1/18 lakes. Among all samples, isolates belonging to five different species/species complexes were detected: *Escherichia coli* ($n = 10$), *Enterobacter cloacae* complex ($n = 4$), *Citrobacter freundii* ($n = 3$), *Citrobacter braakii* ($n = 2$), and *Klebsiella pneumoniae* (n = 2). Of the 21 isolates, 13 (62%) were resistant at least to 3rd generation cephalosporins and eight (38%) additionally to carbapenems. CPE isolates harbored $bla_{\text{KPC-2}}$ ($n = 5$), $bla_{\text{KPC-2}}$ and $bla_{\text{VIM-1}}$ ($n = 2$), or $bla_{\text{OXA-181}}$ ($n = 1$); additionally, *mcr-9* was detected in one isolate. Two out of eight CPE isolates were resistant to cefiderocol and two to colistin. Resistance to 3rd generation cephalosporins was mediated by ESBL ($n = 10$) or AmpC ($n = 3$). The presence of AmpC-producing Enterobacterales, ESBL-E and CPE in northern German surface water samples is alarming and highlights the importance of aquatic environments as a potential source of MDR bacteria.

1. Introduction

Multidrug-resistant (MDR) Enterobacterales pose a significant threat to global public health [1]. Among them, extended-spectrum β-lactamase and carbapenemase-producing Enterobacterales (ESBL-E and CPE, respectively) are considered high-priority pathogens by the World Health Organization (WHO) on the global list of antibiotic-resistant bacteria (ARB) [2]. Of concern, these bacteria are not limited to clinical settings, but have also been detected in various environmental sources, including surface waters [3,4]. The interplay between humans, animals, and the environment leads to an accumulation of ARB, highlighting the need for a One Health approach to address this emerging health issue [5].

The diversity of bacteria in aquatic environments is vast, and it has been hypothesized that these environments not only harbor, but also serve as a source for ARB and antibiotic-resistance genes (ARGs) [6,7]. This facilitates the spread of ARGs via horizontal gene transfer, particularly in surface waters, which communicate with water from multiple sources such as healthcare facilities, industry, agriculture, livestock, and urban wastewater, that are rich in antibiotics and ARB [7–9]. Depending on the exposure of surface waters to these sources, a variety of ARB and ARGs can be expected in different water bodies. The flow of surface

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water to croplands and their proximity to recreational water bodies further underscores the role that aquatic environments may play in exposing humans to ARB [6].

In the last decade, the dissemination of ESBL-E and, more rarely, CPE, has been reported in surface waters throughout Europe [8,10–12]. However, there have been few studies that have analyzed surface waters in Germany and data on the epidemiological distribution of ARBs and ARGs in aquatic environments is scarce [13–15]. Therefore, in the present study, we aim to investigate the occurrence of ESBL-E and CPE and the diversity of ARGs in rivers, water canals near farms, and official swimming lakes in Lower Saxony, Germany.

2. Materials & methods

2.1. Sampling procedure

Between September and October 2022, 31 water samples were collected from 25 water bodies, including two rivers, five water canals near farms and 18 official bathing lakes in Lower Saxony, Germany (Fig. 1).

One of the rivers was sampled at six different locations: two were located upstream of a larger city, two within the city, and two downstream of the city. The other river was sampled at two different locations where swimming was officially allowed. Each location was sampled once. Using a glass bottle attached to a stick, 500 mL of water was sampled 20–30 cm below the water level at a site with a water depth of 1.0–1.5 m (in accordance with the Lower Saxony Bathing Water Ordinance) [18]. All samples were transported on ice and processed within 24 h.

2.2. Sample processing

Water samples were centrifuged (3 min, 500 x *g*) to remove larger compounds when necessary. One hundred mL of water was filtered through mixed cellulose ester membrane filters with a pore size of 0.45 µm (Whatman, Buckinghamshire, UK) using a water suction unit (Sartorius, Goettingen, Germany). For the selection of ESBL-E and CPE, one filter from each sample was transferred to CHROMagar ESBL plate (MAST Diagnostica, Reinfeld, Germany) and one filter to MTC (meropenem-ticarcillin-cloxacillin) agar, which is an in-house CRE agar suitable for analysis of water samples. MTC suppresses non-fermentative gram-negatives, gram-positives, and fungi, which are frequently present in surface waters and could therefore mask CRE. The agar is based on the chromogenic ORI agar (CHROMagar Orientation, CHROMagar, Paris,

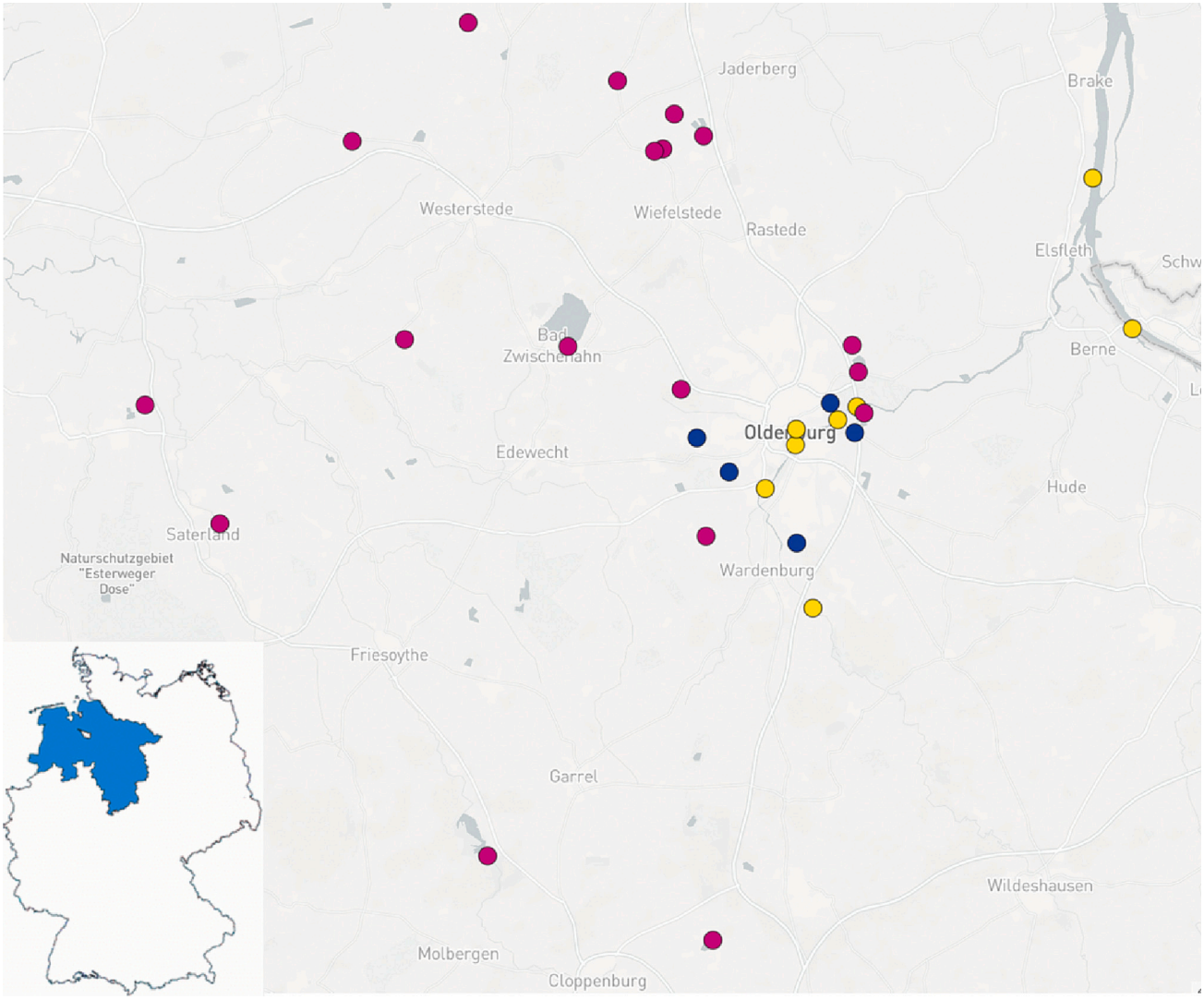

Fig. 1. Sampling sites in Lower Saxony, Germany. Pink dots: lakes, yellow: rivers, blue: water canals near farms (created using Microreact [16]). The map of federal state of Lower Saxony [17]. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

France) which was supplemented with meropenem (0.125 mg/L), ticarcillin (50 mg/L), cloxacillin (400 mg/L), zinc sulfate (50 mg/L), vancomycin (64 mg/L), and amphotericin B (20 mg/L). Extensive testing with quality control and clinical strains was performed to determine the appropriate concentrations of antibiotics in the agar. After inoculation, plates were incubated at 37 °C for 18–24 h.

2.3. Phenotypic characterization

All colonies suspicious of Enterobacterales that grew on the selective plates were identified by MALDI-ToF mass spectrometry (Biotyper, Bruker, Bremen, Germany). The isolates were subjected to antibiotic susceptibility testing (AST) using the Vitek2 system with AST-N428 cards (bioMérieux, Nürtingen, Germany) for the following antibiotics: ampicillin, ampicillin/sulbactam, piperacillin, piperacillin/tazobactam, cefuroxime, cefotaxime, ceftazidime, ertapenem, imipenem, meropenem, gentamicin, ciprofloxacin, tigecycline and trimethoprim/sulfamethoxazole. Results were interpreted according to EUCAST guideline version 12.0 [19]. Additionally, the minimal inhibitory concentrations (MICs) of imipenem, meropenem, aztreonam/avibactam, ceftazidime/avibactam, colistin, ceftolozane/tazobactam, imipenem/relebactam, and temocillin for carbapenem-resistant isolates were determined by broth microdilution assays (MICRONAUT-S, Merlin Diagnostics, Bornheim, Germany). Susceptibility to cefiderocol was assessed by broth microdilution (UMIC cefiderocol, Bruker, Germany) [20], meropenem-vaborbactam by gradient test (bioMérieux). Isolates with an ESBL phenotype were further investigated by the CLSI combination disk test (MAST diagnostics, Reinfeld, Germany) as previously described [21–23]. MDR Enterobacterales were defined as isolates with resistance to 3rd generation cephalosporins and/or carbapenems.

2.4. Multiplex PCR for carbapenemase encoding genes

DNA of all isolates with elevated carbapenem-MICs was extracted from overnight cultures using the DNeasy® Blood & Tissue Kit (QIAGEN, Hilden, Germany) according to the manufacturer's instructions. Identification of carbapenemase encoding genes (bla_{VIM}, bla_{KPC}, bla_{NDM}, bla_{OXA-48}, bla_{OXA-23}, bla_{IMP}, bla_{GIM}, bla_{IMI}, bla_{OXA-58}) in all CPE isolates was conducted by multiplex PCR as described previously [24].

2.5. Whole genome sequencing (WGS)

Whole genome sequencing of all 22 isolates was performed as previously described [25]. Briefly, sequencing data was collected for entire genomes, including plasmids. Indexed PCR-free fragment libraries were generated from extracted genomic DNA with an average fragment length of 400 bp. Paired-end sequencing with 2x150bp reads was carried out on the NovaSeq6000 system (Illumina, San Diego) using an S4-flowcell. Demultiplexing was accomplished with BCL-convert (V.4.0.3). All genomes had an average coverage of at least 150×.

Raw reads were quality trimmed with Trimmomatic and assembled using SPAdes [26,27]. Bacterial species were genetically determined via Centrifuge [28]. Assembled genomes were examined for resistance genes with the ResFinder database, using ABRicate [29,30]. The MLST types were determined with the PubMLST database [31] using mlst 2.19.0 [32].

3. Results

In 4/25 (16%) water bodies MDR Enterobacterales were detected, including 1/2 rivers, 2/5 water canals and 1/18 bathing lakes (Table 1).

3.1. Detection of bacteria in water samples

MDR Enterobacterales were detected in 9/31 water samples (29%), yielding a total of 21 different isolates which were further analyzed. Of these, 18 were samples from one river and obtained at six different locations (R-1.1 to R-1.6), two were from two different water canals near farms (WC-1/WC-5), and one was from a swimming lake (L-6), Table 1. Five different Enterobacterales species were identified, including *E. coli* ($n = 10$), *Enterobacter cloacae* complex ($n = 4$), *Citrobacter freundii* ($n = 3$), *Citrobacter braakii* ($n = 2$) and *Klebsiella pneumoniae* (n = 2) (Table 2).

Table 1
Overview of multidrug-resistant (MDR) Enterobacterales detected in different surface water types in the study area.

Surface water type	Water bodies harboring MDR *Enterobacterales* (%)	Total number of water samples	Samples harboring MDR *Enterobacterales* (%)	Total number of MDR isolates detected
Rivers (n = 2)	1/2 (50%)	8	6/8 (75%)	18
Water canals (n = 5)	2/5 (40%)	5	2/5 (40%)	2
Bathing lakes ($n = 18$)	1/18 (5.5%)	18	1/18 (5.5%)	1
	4/25 (16%)	31	9/31 (29%)	21

MDR, multidrug-resistant.

Interestingly, 18/21 MDR Enterobacterales were found in river 1. Remarkably, the presence and diversity of MDR *Enterobacterales* identified in the river sampled at six different locations varied greatly. While only four ESBL-E were detected upstream of the city, one AmpC-producer, two ESBL-E, and three CPEs were detected within the city limits, and three ESBL-E and five CPEs were detected downstream of the city. The two sampling sites located below the city were downstream from the point where water from urban wastewater treatment plants (WWTP) is discharged into the river, which could potentially explain their presence.

3.2. Antibiotic susceptibility profiles and molecular characterization of isolates

All isolates were resistant to at least one of the 3rd generation cephalosporins and 8/21 were resistant to at least one of the carbapenems (Supplementary Table S1).

Of the 13 carbapenem-susceptible isolates, 10 were ESBL producers and three AmpC-producers based on phenotypic tests. All ESBL-E were *E. coli* and harbored at least one ESBL gene. The most commonly detected ESBL gene was $bla_{CTX-M-15}$ ($n = 5$), followed by $bla_{CTX-M-1}$ ($n = 2$), $bla_{CTX-M-14}$, $bla_{CTX-M-27}$, and $bla_{CTX-M-32}$ ($n = 1$ each). In addition to their ESBL genes, four isolates harbored bla_{TEM-1B} and two harbored bla_{OXA-1}, both narrow spectrum β-lactamases. Among the AmpC producers, bla_{CMY-51}, bla_{ACT-9}, $bla_{CMY-101}$ ($n = 1$ each) were detected (Table 1). All ESBL and AmpC-producers were susceptible to carbapenems.

Among carbapenemase-producing isolates five isolates harbored bla_{KPC-2}, two isolates both bla_{KPC-2} and bla_{VIM-1}, and one isolate $bla_{OXA-181}$. All CPEs except one isolate additionally carried either an ESBL gene ($bla_{CTX-M-15}$) or an AmpC gene (bla_{ACC-1}, bla_{ACT-10}, bla_{CMY-66}, bla_{CMY-70}), Table 2. Interestingly, one of the *C. freundii* isolates that carried bla_{KPC-2} and bla_{VIM-1} (R-1.5.2) was found to also harbor the colistin resistance gene *mcr-9*. All carbapenemase-producing isolates were susceptible to the new β-lactam/β-lactamase inhibitor combinations aztreonam/avibactam and imipenem/relebactam, and resistant to ceftolozane/tazobactam. Resistance to other antibiotics was observed for temocillin (6/8, 75%), ceftazidime/avibactam (2/8, 25%), meropenem-vaborbactam (2/8, 25%), cefiderocol (2/8, 25%), and colistin (2/8, 25%), Table 3.

The two colistin-resistant *Enterobacter cloacae* complex isolates belong to the *Enterobacter* cluster I, based on molecular comparison of the partial *hsp60* sequence as described by Hoffmann and Roggenkamp [33]. Members of this cluster have been associated with heteroresistance towards colistin [34]. We furthermore detected the presence of the *erc* gene in both isolates. The presence of this gene in *Enterobacter* spp. has

Table 2
Genetic characterization of ESBL-E, CPE and AmpC-producing Enterobacterales detected in surface water in Lower Saxony.

Surface water	Sampling site	Isolate	Species	β-lactamase type by phenotypic tests	Sequence type (MLST)	β-lactamase according to WGS
Rivers						
River 1	1	R-1.1.1	*Escherichia coli*	ESBL	ST-44	CTX-M-1, TEM-210, OXA-1
		R-1.1.2	*Escherichia coli*	ESBL	ST-1266	CTX-M-32, TEM-1B
	2	R-1.2.1	*Escherichia coli*	ESBL	ST-12779	CTX-M-14, TEM-1B
		R-1.2.2	*Escherichia coli*	ESBL	ST-10	CTX-M-15
	3	R-1.3.1	*Citrobacter braakii*	carbapenemase	ST-568-like	**KPC-2, VIM-1,** CMY-70, TEM-1B, OXA-1
		R-1.3.2	*Enterobacter cloacae* complex	carbapenemase	ST-23	**OXA-181**, CTX-M-15, ACT-2, OXA-1
		R-1.3.3	*Escherichia coli*	ESBL	ST-167	CTX-M-15, TEM-1B
	4	R-1.4.1	*Escherichia coli*	ESBL	ST-131	CTX-M-27
		R-1.4.2	*Citrobacter freundii*	AmpC	ST-307	CMY-51
	5	R-1.5.1	*Citrobacter freundii*	carbapenemase	ST-11	**KPC-2**, CMY-66, TEM-1B, OXA-1
		R-1.5.2	*Citrobacter freundii* complex	carbapenemase	ST-955-like	**KPC-2, VIM-1,** ACC-1, TEM-1B, OXA-1
		R-1.5.3	*Enterobacter cloacae* complex	carbapenemase	ST-29	**KPC-2**, TEM-1B, OXA-1
		R-1.5.4	*Klebsiella pneumoniae*	carbapenemase	ST-782	**KPC-2,** CTX-M-15, SHV-28
		R-1.5.5	*Escherichia coli*	ESBL	ST-131	CTX-M-15, OXA-1
		R-1.5.6	*Escherichia coli*	ESBL	ST-93	CTX-M-1, TEM-1B
		R-1.5.7	*Escherichia coli*	ESBL	ST-2741	CTX-M-15
	6	R-1.6.1	*Klebsiella pneumoniae*	carbapenemase	ST-1401	**KPC-2,** SHV-26, TEM-1B, OXA-1
		R-1.6.2	*Enterobacter cloacae* complex	carbapenemase	ST-29	**KPC-2,** TEM-1B, OXA-1
Water canals						
Water canal 1	1	WC-1	*Escherichia coli*	ESBL	ST-517	CTX-M-15
Water canal 5	1	WC-5	*Enterobacter cloacae* complex	AmpC	ST-1001	ACT-9
Swimming lakes						
Lake 6	1	L-6	*Citrobacter braakii*	AmpC	ST-386-like	CMY-101

ESBL, extended-spectrum β-lactamase; MLST, multilocus sequence typing; ST, sequence type; WGS, whole-genome sequencing. Carbapenemase genes are marked in bold.

Table 3
Minimal inhibitory concentrations (MIC) of carbapenemase-producing isolates to carbapenems, new β-lactam/β-lactamase inhibitor combinations, temocillin, colistin and cefiderocol.

		MIC (mg/L)									
Isolate	Species	MEM	IMP	AZA	CZA	CZT	IMR	MEV	TEM	COL	FDC
R-1.3.1	*Citrobacter braakii* (KPC-2, VIM-2)	≥ 16	≥ 16	≤ 0.5	≥ 32	≥ 16	2	8	32	≤ 0.5	0.25
R-1.3.2	*Enterobacter cloacae* complex (OXA-181)	1	2	≤ 0.5	≤ 2	≥ 16	1	1	≥ 64	≥ 8	4
R-1.5.1	*Citrobacter freundii* (KPC-2)	8	2	≤ 0.5	≤ 2	≥ 16	≤ 0.5	0.06	32	1	2
R-1.5.2	*Citrobacter freundii* complex (KPC-2 VIM-1)	≥ 32	16	≤ 0.5	≥ 32	≥ 16	2	>64	≥ 64	≤ 0.5	0.25
R-1.5.3	*Enterobacter cloacae* complex (KPC-2)	≥ 32	16	≤ 0.5	≤ 2	≥ 16	≤ 0.5	0.06	32	≥ 8	1
R-1.5.4	*Klebsiella pneumoniae* (KPC-2)	8	8	≤ 0.5	≤ 2	16	≤ 0.5	0.03	16	1	2
R-1.6.1	*Klebsiella pneumoniae* (KPC-2)	≥ 32	≥ 16	≤ 0.5	≤ 2	8	1	0.5	16	1	0.5
R-1.6.2	*Enterobacter cloacae* complex (KPC-2)	≥ 32	≥ 16	≤ 0.5	≤ 2	≥ 16	≤ 0.5	0.06	32	2	8

AZA: aztreonam/avibactam, CZA: ceftazidime/avibactam, COL: colistin, CZT: ceftolozane/tazobactam, FDC: cefiderocol, IPM: imipenem, IMR: imipenem/relebactam, MEM: meropenem, MEV: meropenem/vaborbactam TEM: temocillin.

been shown to promote heteroresistance towards colistin via increased expression of the PhoP-PhoQ two-component system, which in turn upregulates the *arnBCADTEF* operon [35].

The resistance genes other than β-lactamases carried by the isolates are summarized in Supplementary Table S2.

4. Discussion

Our study revealed that 3rd generation cephalosporin resistant Enterobacterales and CPE were present in 4 out of 25 (16%) sites and in 9 out of 31 (29%) water samples in Lower Saxony, Germany, a state known for its intensive agriculture and livestock production. This indicates a high prevalence of these isolates in surface waters in this region, particularly in the studied river. Furthermore, the genome-based analyses identified a broad spectrum of β-lactamase genes in these strains.

Our results regarding ESBL-E species and ESBL genes were consistent with those of previous studies on surface water throughout Europe [12,15]. All ESBL-E were *E. coli* and $bla_{\text{CTX-M-15}}$ was the most frequently detected ESBL gene. Interestingly, $bla_{\text{CTX-M-32}}$ was detected in a river upstream of the city, a gene that had not previously been reported from surface waters. It has been identified in wild mallard and slaughterhouse wastewater in Germany [36,37]. Detection of this gene in the respective area could indicate a potential contamination from wildlife or livestock.

In our study, all CPE isolates were resistant to ceftolozane/tazobactam as expected, while only the metallo-β-lactamase-producing isolates displayed resistance to ceftazidime/avibactam, which is in line with recently published data [38–40]. Interestingly, two *Citrobacter* isolates producing both KPC-2 and VIM-1 tested susceptible to imipenem/relebactam (MIC 2 mg/L). Both were resistant to imipenem (MIC >16 mg/L). The borderline susceptibility of these isolates can therefore likely be explained by the inhibition of KPC and AmpC by relebactam, while expression of VIM-1 was not high enough to confer full resistance to imipenem. Of note, imipenem and imipenem-relebactam MICs can be relatively low (≤2 mg/L) in VIM-producing *Citrobacter freundii* isolates, as previously reported in clinical isolates from Germany [40–43].

So far, at least five major carbapenemases have been identified in surface waters worldwide with varying prevalence according to the epidemiological trends in the area. [8,44–47]. There is only a limited number of studies that have analyzed CPE from surface waters and conducted genetic analyses of these organisms [13]. To date, only one study has reported the presence of CPE ($bla_{\text{OXA-51}}$ and $bla_{\text{VIM-1}}$) in surface waters of northern Germany [14]. In the current study, to the best of our knowledge the presence of $bla_{\text{KPC-2}}$, $bla_{\text{KPC-2}}$ and $bla_{\text{VIM-1}}$, and $bla_{\text{OXA-181}}$ was shown for the first time in a river in Germany. An unexpected result was that $bla_{\text{KPC-2}}$ accounted for the majority of the carbapenemase

genes, although Germany is not considered an area with a high prevalence of bla_{KPC-2} [48]. KPC-producing Enterobacterales are endemic in the United States, Colombia, Greece, and Italy [49]. Previously, several outbreaks of KPC-producing Enterobacterales have been reported in Germany [50–52]. In two of these outbreaks, the index patients were found to have been hospitalized in Greece, and the strain identified in the third outbreak was related to the strains identified in these two prior outbreaks. Our findings show that surface water in Lower Saxony can harbor CPE, with bla_{KPC-2} being the dominant gene and highlight the need for further surveillance and monitoring of environmental CPE.

Another concerning result of the study was the observation that the CPEs were also resistant to a significant number of other antibiotics. The detection of colistin resistance in two CPE isolates is particularly alarming as colistin is often used as a salvage therapy. Both *E. cloacae* isolates harbored the *erc* gene which mediates heteroresistance to colistin [35]. A third isolate was positive for *mcr-9* but was phenotypically susceptible to colistin [53]. The detection of *mcr*-harboring and colistin-resistant bacteria in the surface waters of Lower Saxony could potentially be related to the high density of agriculture in the region, as previous research has established a link between colistin resistance in Enterobacterales and the use of polymyxins in livestock [54]. Unlike many other countries that ban or limit its application, colistin is still regularly administered in veterinary medicine in Germany [55,56]. Despite the decline in sales of polymyxins for food producing animals over the years, the consumption in Germany for veterinary use still exceeds that of neighboring countries [55,57]. Notably, we identified a unique combination of two carbapenemase-encoding genes, bla_{KPC-2}, and bla_{VIM-1}, and the mobile colistin resistance gene *mcr-9* in *C. freundii* complex. According to previous reports, *mcr-9* is the second most prevalent mobile colistin resistance gene worldwide after *mcr-1* [58]. Unlike other identified *mcr* genes that typically confer resistance to colistin, *mcr-9* usually only reduces susceptibility which could lead to a silent dissemination of this gene [59]. Its prevalence is primarily attributed to the use of antibiotics in clinical settings; however, it is noteworthy that livestock production also plays a significant role in its prevalence [58]. In Germany, *mcr-9* has so far only been detected in *Salmonella* isolates in foods of animal origin [60].

Furthermore, of particular concern is the observation of resistance to the novel antibiotic cefiderocol in two *E. cloacae* isolates - one harboring $bla_{OXA-181}$ and the other bla_{KPC-2}. Cefiderocol, known for its potent activity against carbapenem-resistant Enterobacterales carrying class A, B, and D carbapenemases, has been extensively studied [61]. Recent studies have shown a variable resistance rate to cefiderocol among carbapenemase-producing Gram-negative bacteria originating from different countries [62–65]. Although observed at relatively low frequencies, the presence of cefiderocol resistance has been reported in Enterobacterales carrying OXA-181 and KPC-2 carbapenemases [64,65]. A recent study has demonstrated a low resistance rate in clinical isolates from Germany [66]. Resistance to cefiderocol in 2/8 isolates of environmental origin is therefore surprising.

Although WWTPs can reduce the number of ARB, studies have shown that achieving complete eradication is not possible; hence WWTPs could be hot spots for ARB and ARGs [67,68]. This situation becomes even more crucial when considering that WWTP's release points are surface waters, especially rivers [10,68]. Our study supports these observations. Out of the 18 MDR Enterobacterales isolates we detected in one river; half were located downstream from the point where the WWTP discharged water into the river.

There are some limitations of our study. It was conducted in a single season, making it difficult to ascertain the extent to which our results may have been influenced by seasonal weather and environmental factors. Additionally, each location was sampled only once. Nevertheless, our study is one of only few analyzing the presence of MDR Enterobacterales in German surface waters and provides WGS and phenotypic susceptibility data, including on the newest last resort antibiotics.

In conclusion, the present study provides evidence that 3rd generation cephalosporin resistant Enterobacterales and CPE are prevalent in surface waters in Lower Saxony, Germany. We identified a broad spectrum of ARGs, including different carbapenemase genes and the previously undiscovered $bla_{CTX-M-32}$ and *mcr-9* in an aquatic sample in Germany. The discovery of cefiderocol and colistin-resistant bacteria and the presence of mobile colistin resistance gene *mcr-9* adds further urgency to this issue, as both cefiderocol and colistin are often used as a last-resort therapy for patients infected with antibiotic-resistant bacteria. Overall, our study highlights the need for further research to fully comprehend the extent of antibiotic resistance and the impact of the various sources of ARB (e.g., livestock, hospital wastewater) on the aquatic environment.

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Ethical approval

Not required.

CRediT authorship contribution statement

Cansu Cimen: Investigation, Methodology, Data curation, Formal analysis, Writing – original draft. **Janina Noster:** Methodology, Resources, Writing – review & editing. **Yvonne Stelzer:** Methodology, Validation, Resources. **Andreas Rump:** Methodology, Data curation, Writing – review & editing. **Janko Sattler:** Data curation, Formal analysis, Investigation, Writing – review & editing. **Matthijs Berends:** Writing – review & editing. **Andreas Voss:** Writing – review & editing. **Axel Hamprecht:** Conceptualization, Project administration, Supervision, Funding acquisition, Resources, Writing – review & editing.

Declaration of Competing Interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

Axel Hamprecht reports financial support was provided by Lower Saxony State Ministry of Science and Culture.

Data availability

The data is hosted on a public repository. The Bioproject-ID is PRJNA958248.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.onehlt.2023.100606.

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