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► To cite this version:

Zineb Cherak, Lotfi Loucif, Abdelhamid Moussi, Jean-Marc Rolain. Carbapenemase-producing Gram-negative bacteria in aquatic environments: a review. *Journal of Global Antimicrobial Resistance*, 2021, 25, pp.287-309. 10.1016/j.jgar.2021.03.024 . hal-03333771

HAL Id: hal-03333771

<https://amu.hal.science/hal-03333771>

Submitted on 10 Sep 2021

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Carbapenemase-producing Gram-negative bacteria in aquatic environments: a review

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ARTICLE INFO

Article history:

Received 10 November 2020

Revised 4 March 2021

Accepted 20 March 2021

Available online 23 April 2021

Editor: Dr Jon Hobman

Keywords:

Carbapenemase

Gram-negative bacilli

Aquatic environment

Epidemiology

ABSTRACT

Antibiotic resistance is one of the greatest public-health challenges worldwide, especially with regard to Gram-negative bacteria (GNB). Carbapenems are the β -lactam antibiotics of choice with the broadest spectrum of activity and, in many cases, are the last-resort treatment for several bacterial infections. Carbapenemase-encoding genes, mainly carried by mobile genetic elements, are the main mechanism of resistance against carbapenems in GNB. These enzymes exhibit a versatile hydrolytic capacity and confer resistance to most β -lactam antibiotics. After being considered a clinical issue, increasing attention is being given to the dissemination of such resistance mechanisms in the environment and especially through water. Aquatic environments are among the most significant microbial habitats on our planet, known as a favourable medium for antibiotic gene transfer, and they play a crucial role in the huge spread of drug resistance in the environment and the community. In this review, we present current knowledge regarding the spread of carbapenemase-producing isolates in different aquatic environments, which may help the implementation of control and prevention strategies against the spread of such dangerous resistant agents in the environment.

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1. Introduction

Antibiotic resistance is one of the greatest public-health challenges worldwide, especially with regard to Gram-negative bacteria (GNB). In 2016, the World Health Organization (WHO) was called on by its member states to name a priority list of drug-resistant bacteria that require the development of new effective medicines. Interestingly, the critical priority level was reserved for carbapenem-resistant *Acinetobacter baumannii*, *Pseudomonas aeruginosa* and Enterobacteriaceae as well as third-generation cephalosporin-resistant Enterobacteriaceae [1]. Carbapenems are the β -lactam antibiotics with the broadest spectrum of activity. Imipenem, first marketed in 1985, was the first carbapenem available for the treatment of bacterial infections [2]. Thereafter, several carbapenems were developed in the subsequent two decades.

Nowadays, the most clinically used carbapenems are ertapenem, meropenem, doripenem and imipenem [3]. However, in less than a decade after their use, carbapenem-resistant GNB have emerged [4]. Owing to the fact that carbapenems are the antibiotics of choice and, in many cases, the last-resort treatment for several bacterial infections, the emergence and spread of carbapenem-resistant GNB are currently a major global public-health crisis [5]. Carbapenem resistance in GNB may be conferred by various mechanisms, including quantitative and/or qualitative changes in membrane permeability owing to chromosomal mutations in efflux pump-encoding genes or alterations in the expression and/or function of porins, the association of impermeability with extended-spectrum β -lactamases (ESBLs) or overexpression of AmpC β -lactamases or by the production of carbapenem-hydrolysing enzymes ('carbapenemases'), which represent the most worrying mechanism of carbapenem resistance. These enzymes exhibit a versatile hydrolytic capacity and confer resistance to most β -lactam antibiotics [2,3,6–8].

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Classically, antibiotic resistance has been known to be restricted to clinical settings [9]. However, several studies have demonstrated the dissemination of resistant organisms in the environment, particularly in water. Indeed, water is one of the most significant microbial habitats on our planet and it has been proven that antibiotic resistance genes are common in different water ecosystems, which may play a crucial role in the propagation of antibiotic resistance between the natural environment and humans and other animals [10–12].

In this review, we present current knowledge regarding the spread of carbapenemase-producing isolates in different aquatic environments, which may help the implementation of control and prevention strategies against the spread of such dangerous resistant agents in the environment.

For this purpose, we carried out a comprehensive literature search on PubMed and Google Scholar websites. We included papers published in English language up to April 2020 using the following search terms and/or phrases: 'Gram negative bacilli', 'Enterobacteriaceae', '*Pseudomonas aeruginosa*', '*Acinetobacter baumannii*', and 'carbapenemases', 'metallo- β -lactamases', 'KPC', 'GES', 'IMI', 'VIM', 'NDM', 'IMP', 'OXA-48', and 'water environments', 'aquatic environments', 'water', 'wastewater', 'sewage', 'hospital wastewater', 'hospital sewage', 'wastewater treatment plants', 'surface water', 'ground water'. Search terms were separated by the 'AND' Boolean operator.

2. Carbapenemases in Gram-negative bacteria

Carbapenemases constitute a large variety of enzymes that are categorised either functionally (Bush classification) or genetically (Ambler molecular classification) [5]. However, the most common classification is the molecular one based on the Ambler classification scheme [13] in which carbapenemases are assigned to three of the four Ambler classes (A, B and D). According to the functional classification, they fall under the functional groups 2df, 2f, 3a and 3b [14]. Despite the great number of carbapenemase enzymes identified in GNB, the five major and most prevalent carbapenemases are KPC (*Klebsiella pneumoniae* carbapenemase), NDM (New Delhi metallo- β -lactamase), IMP (imipenem-resistant *Pseudomonas*), VIM (Verona integron-encoded metallo- β -lactamase) and OXA-48 (oxacillinase) [15,16]. Carbapenemases are either chromosomally encoded, such as SME (*Serratia marcescens* enzyme) and NMC-A (non-metallo-carbapenemase-A), which are usually reported on the chromosome of some Enterobacterales species including *S. marcescens* and *Enterobacter cloacae*, or more frequently are encoded by mobile genetic elements including plasmids, integrons and transposons [5,17].

3. Epidemiology of carbapenemase-producing Gram-negative bacteria in aquatic environments

Carbapenemases of the three Ambler classes have been detected in aquatic environments whether using culture or culture-independent methods. The worldwide epidemiology of carbapenemase-producers isolated from various aquatic environments is summarised in Fig. 1 and presented below by Ambler class.

3.1. Class A carbapenemases

The first reported class A carbapenem-hydrolysing β -lactamase (SME) was from the UK in a *S. marcescens* isolate in 1990 [3]. Enzymes in this β -lactamase class are serine proteins (using a serine residue for their activity) and are inhibited by clavulanic acid, tazobactam and boronic acid compounds [6,18]. They have a broad

spectrum of activity including penicillins, cephalosporins, aztreonam and carbapenems [18]. The most prevalent and clinically significant enzyme among the class A carbapenemases is KPC, which is commonly identified in Enterobacterales species but also occasionally in *P. aeruginosa* and *A. baumannii* [13,19].

3.1.1. Enterobacterales

Class A carbapenemase-producing Enterobacterales have been widely isolated from aquatic environments worldwide (Table 1; Fig. 2). In 2004, Henriques et al. characterised a new class A carbapenemase-encoding gene designated as SFC-1 (for *Serratia fonticola* carbapenemase) on the chromosome of a *S. fonticola* isolate obtained from untreated drinking water in Portugal [20]. Furthermore, IMI-2-producing *Enterobacter asburiae* isolates were recovered from rivers and lake water in the USA and France [21–23]. In addition, Piedra-Carrasco et al. have reported the isolation of IMI-2-producing *E. cloacae* from river water in Spain [24]. Another Ambler class A enzyme that is commonly reported among members of the Enterobacterales is KPC, whose variants are the most detected class A carbapenemases in Enterobacterales isolated from aquatic environments. KPC-producing *Enterobacter* spp., *Klebsiella* spp., *Citrobacter* spp., *Kluyvera* spp., *Escherichia coli*, *E. cloacae*, *Enterobacter kobei*, *E. asburiae*, *K. pneumoniae*, *Klebsiella oxytoca* and *Citrobacter freundii* complex have been isolated from river water, seawater, hospital sewage, wastewater treatment plants (WWTPs), drinking water and a hospital water dispenser [25–35]. KPC-2-producing *Citrobacter* spp., *Enterobacter* spp., *Klebsiella* spp., *Serratia* spp., *Raoultella* spp., *Kluyvera* spp., *Shigella* spp., *Escherichia* spp., *E. coli*, *K. pneumoniae*, *Klebsiella quasipneumoniae*, *K. oxytoca*, *E. cloacae* complex, *E. cloacae*, *E. kobei*, *E. asburiae*, *C. freundii*, *Citrobacter braakii*, *Citrobacter farmeri*, *Kluyvera georgiana*, *Kluyvera ascorbata*, *Kluyvera cryocrescens*, *Raoultella ornithinolytica*, *Raoultella planticola* and *Raoultella terrigena* have been isolated from rivers, hospital sewage, WWTPs, seawater and wells [24,36–57]. Another KPC variant, namely KPC-3, has been detected in *K. pneumoniae* isolated from wells and WWTPs in Italy [45,58], in *K. pneumoniae* cultivated from a Portuguese river [59], and in *E. coli* and *C. freundii* isolates obtained from WWTPs in the USA [57]. In addition, the new variant KPC-26 was first identified in *Klebsiella* spp. and *Enterobacter* spp. isolates obtained from seawater in Brazil [48]. Other Ambler class A enzymes that have been shown to possess carbapenemase activity are some GES variants (for Guiana extended spectrum). The GES-5 enzyme has been detected in *K. pneumoniae* isolated from stream water [60], in *R. ornithinolytica* and *Citrobacter* sp. recovered from river water [57,59], in *Enterobacter* spp. isolates obtained from seawater [25,48], in *Citrobacter* spp., *E. coli*, *K. pneumoniae*, *K. oxytoca* and *E. cloacae* obtained from hospital sewage [53,61,62], in *E. cloacae* complex, in *K. pneumoniae* and in *R. ornithinolytica* isolated from WWTPs [53,57,63]. GES-6-producing *Citrobacter* spp., *E. coli* and *K. quasipneumoniae* have been isolated from hospital sewage in Taiwan [53]. In addition, the GES-16 enzyme has been detected in *Enterobacter* spp. and *Klebsiella* spp. isolates recovered from seawater [25,48] and in *K. pneumoniae* obtained from river water [42]. GES-20-producing *K. oxytoca* and *E. kobei* were isolated from river water in the Philippines [64]. Finally, a GES-24-producing *Klebsiella variicola* was isolated from a WWTP in Japan [53].

3.1.2. Other Gram-negative bacilli

Compared with Enterobacterales, few published reports have documented the isolation of class A carbapenemase-producing glucose-non-fermenting GNB from aqueous ecosystems. KPC-, KPC-2-, GES-5- and GES-16-producing *Acinetobacter* spp. and *Aeromonas* spp. isolates were recovered from WWTPs, hospital sewage, and river and seawater samples in the USA, Brazil and China [25,29,40,43,48,65]. In addition, the GES-31 carbapenemase was first described in an *Aeromonas punctata* isolate recovered from a

Table 1
Epidemiology of class A carbapenemase-producers detected in aquatic environments

Carbapenemase	Country	Source	Host(s) (n)	ST	Plasmid group	Reference
GES-5	Brazil	Hospital sewage	<i>Klebsiella pneumoniae</i> (1) <i>Klebsiella oxytoca</i> (1)	–	–	[62]
		Seawater	<i>Enterobacter</i> (2) <i>Aeromonas</i> (1), <i>Acinetobacter</i> (1)	– –	– –	[25] [48]
	Japan	WWTP	<i>Raoultella ornithinolytica</i> (1) <i>Klebsiella pneumoniae</i> (1)	– ST2791	– –	[53]
			<i>Citrobacter</i> sp. (1) <i>Klebsiella pneumoniae</i> (4)	– ST961	Inc3-16 –	[59] [60]
	Taiwan	Hospital sewage	<i>Klebsiella pneumoniae</i> (11) <i>Enterobacter cloacae</i> (1)	ST11, ST15, ST19, ST16, ST844, ST2791, ST2785 ST928	– –	[53]
			<i>Escherichia coli</i> (6) <i>Citrobacter</i> (1)	ST744, ST49 –	– –	
	UK	Hospital sewage	<i>Klebsiella oxytoca</i> (6) <i>Enterobacter cloacae</i> complex (5)	–	–	[61]
		WWTP	<i>Klebsiella pneumoniae</i> (1)	–	–	[63]
	USA	River	<i>Raoultella ornithinolytica</i> (1)	–	–	[57]
		WWTP	<i>Enterobacter cloacae</i> complex (1)	ST595	–	
GES-6	Taiwan	Hospital sewage	<i>Escherichia coli</i> (1) <i>Klebsiella quasipneumoniae</i> (5)	ST540 ST1584, ST367	– –	[53]
			<i>Citrobacter</i> (1)	–	–	
GES-7 (BIC-1)	France	River	<i>Pseudomonas fluorescens</i> (1)	–	–	[67]
GES-16	Brazil	River	<i>Enterobacter kobei</i> (1), <i>Aeromonas</i> (1), <i>Acinetobacter</i> (1) <i>Klebsiella pneumoniae</i> (2)	– ST1793, ST1794	– –	[42]
		Seawater	<i>Enterobacter</i> (2), <i>Klebsiella</i> (1)	–	–	[25]
GES-20	Philippines	River	<i>Klebsiella oxytoca</i> (1), <i>Enterobacter kobei</i> (1)	–	–	[64]
GES-24	Japan	WWTP	<i>Klebsiella variicola</i> (1)	ST2790	–	[53]
GES-31	Brazil	River	<i>Aeromonas punctata</i> (1)	–	–	[42]
IMI-2	France	River	<i>Enterobacter asburiae</i> (1)	–	–	[23]
	Spain	River	<i>Enterobacter cloacae</i> (1)	ST822	IncFIB	[24]
	USA	River	<i>Enterobacter asburiae</i> (1)	–	–	[22]
IMI-18	Philippines	River	<i>Enterobacter asburiae</i> (7) <i>Enterobacter cloacae</i> (1)	– –	– –	[64]
			<i>Pseudomonas aeruginosa</i> (14)	–	–	[117]
KPC	Brazil	Hospital sewage	<i>Klebsiella pneumoniae</i> (5)	–	–	[27]
	Egypt	Drinking water	<i>Citrobacter freundii</i> (4)	–	–	[32]
	Ireland	Hospital sewage	<i>Escherichia coli</i> (5)	–	–	[34]
	Jordan	Drinking water	<i>Escherichia coli</i> (1)	–	–	[35]
	Korea	Water dispenser	<i>Enterobacter cloacae</i> (1), <i>Enterobacter kobei</i> (1), <i>Enterobacter asburiae</i> (1)	–	–	[74]

(continued on next page)

Table 1 (continued)

Carbapenemase	Country	Source	Host(s) (n)	ST	Plasmid group	Reference
			<i>Klebsiella pneumoniae</i> (8), <i>Citrobacter</i> (1), <i>Enterobacter</i> (9), <i>Pseudomonas</i> (4)	–	–	[28]
	USA	Hospital sewage	<i>Citrobacter freundii</i> , <i>Escherichia coli</i> , <i>Klebsiella pneumoniae</i> , <i>Klebsiella oxytoca</i> , <i>Aeromonas</i> , <i>Acinetobacter</i> (total, 40)	–	IncN	[92]
<i>Aeromonas</i> (23), <i>Serratia marcescens</i> (70), <i>Klebsiella pneumoniae</i> (1), <i>Klebsiella oxytoca</i> (6), <i>Enterobacter cloacae</i> complex (15), <i>Kluyvera intermedia</i> (1), <i>Pantoea</i> (3), <i>Citrobacter freundii</i> (12), <i>Raoultella</i> (4), other Enterobacteriaceae (6)			–	–	[25]	
WWTP		<i>Escherichia coli</i> (21)	–	–	[31]	
		<i>Escherichia coli</i> (2)	–	–	[30]	
KPC-2	Austria	WWTP	<i>Klebsiella pneumoniae</i> (1)	ST1245	–	[39]
	Brazil	Hospital sewage	<i>Klebsiella pneumoniae</i> (2)	–	–	[37]
			<i>Enterobacter</i> (1), <i>Enterobacter cloacae</i> (1), <i>Klebsiella pneumoniae</i> (11)	–	–	[47]
		Hospital sewage, WWTP	<i>Klebsiella</i> (25), <i>Enterobacter</i> (26), <i>Serratia</i> (3), <i>Raoultella</i> (4), <i>Kluyvera</i> (5)	–	–	[40]
		Mangroves	<i>Pseudomonas putida</i> (2), <i>Stenotrophomonas maltophilia</i> (4)	–	–	[68]
	Brazil	River	<i>Klebsiella pneumoniae</i> (3)	ST437, ST340	IncN	[41]
			<i>Aeromonas hydrophila</i> (1), <i>Aeromonas punctata</i> (2)	–	–	[42]
			<i>Klebsiella pneumoniae</i> (7)	ST1792, ST1791, ST1245, ST1793, ST1794, ST1795	–	
			<i>Enterobacter cloacae</i> (3), <i>Enterobacter kobei</i> (12) <i>Enterobacter asburiae</i> (6)	–	–	
			<i>Klebsiella pneumoniae</i> (1), <i>Enterobacter cloacae</i> (1)	–	–	[47]
			<i>Enterobacter</i> (9), <i>Citrobacter</i> (1), <i>Kluyvera</i> (2)	–	–	
		Seawater	<i>Aeromonas</i> (1), <i>Enterobacter</i> (9), <i>Citrobacter</i> (1), <i>Kluyvera</i> (2)	–	–	[25]

(continued on next page)

Table 1 (continued)

Carbapenemase	Country	Source	Host(s) (n)	ST	Plasmid group	Reference
			<i>Citrobacter</i> (1), <i>Enterobacter</i> (65), <i>Klebsiella</i> (17), <i>Kluyvera</i> (2), <i>Serratia</i> (7), <i>Aeromonas</i> sp. (5)	–	–	[48]
	Bulgaria	WWTP	<i>Aeromonas</i> (4)	–	–	[40]
		River	<i>Enterobacter asburiae</i> (1)	–	–	[44]
	China	Hospital sewage	<i>Enterobacter cloacae</i> (3), <i>Citrobacter freundii</i> (5)	–	IncA/C	[38]
			<i>Citrobacter freundii</i> (1)	ST88	–	[85]
			<i>Citrobacter freundii</i> (1)	ST14	–	[52]
	China	Hospital sewage	<i>Enterobacter cloacae</i> (4) <i>Klebsiella pneumoniae</i> (4) <i>Klebsiella pneumoniae</i> (3), <i>Escherichia coli</i> (1), <i>Citrobacter</i> sp. (1), <i>Citrobacter braakii</i> (1), <i>Raoultella planticola</i> (2), <i>Enterobacter</i> sp. (1), <i>Enterobacter kobei</i> (1)	ST911, ST910, ST25, ST669 ST11, ST12	–	[52]
			<i>Aeromonas hydrophila</i> (3) <i>Citrobacter braakii</i> (2), <i>Citrobacter freundii</i> (2) <i>Escherichia coli</i> (4), <i>Kluyvera georgiana</i> (1)	–	–	[96]
		River	<i>Raoultella ornithinolytica</i> (1) <i>Klebsiella</i> (10), <i>Shigella</i> (17), <i>Escherichia</i> (12) <i>Acinetobacter</i> (19), <i>Stenotrophomonas</i> (10), <i>Wautersiella</i> (9)	–	–	[49]
		Wells	<i>Raoultella ornithinolytica</i> (1) <i>Klebsiella</i> (10), <i>Shigella</i> (17), <i>Escherichia</i> (12) <i>Acinetobacter</i> (19), <i>Stenotrophomonas</i> (10), <i>Wautersiella</i> (9)	–	IncF	[50]
		WWTP	<i>Raoultella terrigena</i> (1), <i>Escherichia coli</i> (8), <i>Kluyvera georgiana</i> (2), <i>Acinetobacter seohaensis</i> (3), <i>Shigella sonnei</i> (1)	–	–	[51]
	Croatia	River	<i>Klebsiella pneumoniae</i> (4)	ST258	IncFII	[43]
	Italy	WWTP	<i>Klebsiella pneumoniae</i> (1)	ST307	IncFIIK	[56]
	Japan	WWTP	<i>Klebsiella pneumoniae</i> (1) <i>Aeromonas caviae</i> (1) <i>Aeromonas hydrophila</i> (1)	ST11 ST13 ST 558	IncFII, IncN IncP6 IncP6	[45] [54] [66]
	Philippines	Hospital sewage	<i>Citrobacter freundii</i> (1), <i>Klebsiella pneumoniae</i> (2)	–	–	[64]
		River	<i>Escherichia coli</i> (2), <i>Klebsiella pneumoniae</i> (2)	–	–	
	Portugal	River	<i>Escherichia coli</i> (1)	ST410	IncF	[36]
	Romania	Hospital sewage	<i>Klebsiella pneumoniae</i> (9)	–	–	[97]

(continued on next page)

Table 1 (continued)

Carbapenemase	Country	Source	Host(s) (n)	ST	Plasmid group	Reference
	Spain	River	<i>Klebsiella pneumoniae</i> (1)	–	–	[44]
		River	<i>Enterobacter cloacae</i> (1)	ST823	IncN, IncR, IncFIIK	[24]
			<i>Klebsiella pneumoniae</i> (1)	ST634	IncN, IncR, IncFIIK	
			<i>Klebsiella oxytoca</i> (1)	–	IncN, IncR, IncFIIK	
			<i>Escherichia coli</i> (3)	ST1434, ST5001, ST216	IncN, IncR, IncFIIK	
	Switzerland	WWTP	<i>Citrobacter freundii</i> (5), <i>Citrobacter braakii</i> (1), <i>Citrobacter farmeri</i> (1), <i>Enterobacter cloacae/asburiae</i> (5), <i>Klebsiella oxytoca</i> (6), <i>Klebsiella pneumoniae</i> (1), <i>Kluyvera ascorbata</i> (1), <i>Kluyvera cryocrescens</i> (2), <i>Raoultella ornithinolytica</i> (3)	–	IncP/6	[55]
		WWTP	<i>Klebsiella pneumoniae</i> (1)	ST258	–	[46]
		Hospital sewage	<i>Klebsiella pneumoniae</i> (4)	ST2256, ST512, ST258	–	
	Taiwan	Hospital sewage	<i>Enterobacter kobei</i> (1)	ST910	IncP6	[53]
			<i>Klebsiella quasipneumoniae</i> (1)	ST2786	IncP6	
	USA	River	<i>Enterobacter cloacae</i> (2)	ST1121, ST1122	–	[57]
			<i>Enterobacter cloacae</i> complex (2)	ST595, ST1028	–	
			<i>Klebsiella pneumoniae</i> (3)	ST3539, ST872, ST2793	–	
			<i>Klebsiella quasipneumoniae</i> (1)	ST138	–	
			<i>Klebsiella oxytoca</i> (2)	ST88, ST127	–	
		WWTP	<i>Aeromonas caviae</i> (2)	ST560, ST561, ST563	–	
			<i>Raoultella ornithinolytica</i> (1)	–	–	
			<i>Aeromonas caviae</i> (2)	ST564, ST562	–	
			<i>Enterobacter cloacae</i> complex (3)	ST131, ST928, ST595	–	
			<i>Enterobacter cloacae</i> (1)	ST41	–	
KPC-3	Italy	Wells	<i>Enterobacter asburiae</i> (1)	ST24	–	
			<i>Citrobacter freundii</i> (1)	ST8	–	
	Portugal	Wells	<i>Klebsiella pneumoniae</i> (1)	ST258	IncFIIK	[45]
		WWTP	<i>Klebsiella pneumoniae</i> (20)	ST512	–	[58]
		River	<i>Klebsiella pneumoniae</i> (9)	–	IncFIA-FII	[59]
KPC-26	USA	WWTP	<i>Citrobacter freundii</i> (2)	ST413, ST11	–	[57]
	Brazil	Seawater	<i>Escherichia coli</i> (1)	ST607	–	
			<i>Enterobacter</i> (2), <i>Klebsiella</i> (1)	–	–	[25]
SFC-1	Portugal	Drinking water	<i>Serratia fonticola</i> (1)	–	–	[20]
VCC-1	Germany	Seawater	<i>Vibrio cholerae</i> (3)	ST516	–	[69]

n, number of strains, ST, sequence type; WWTP, wastewater treatment plant.

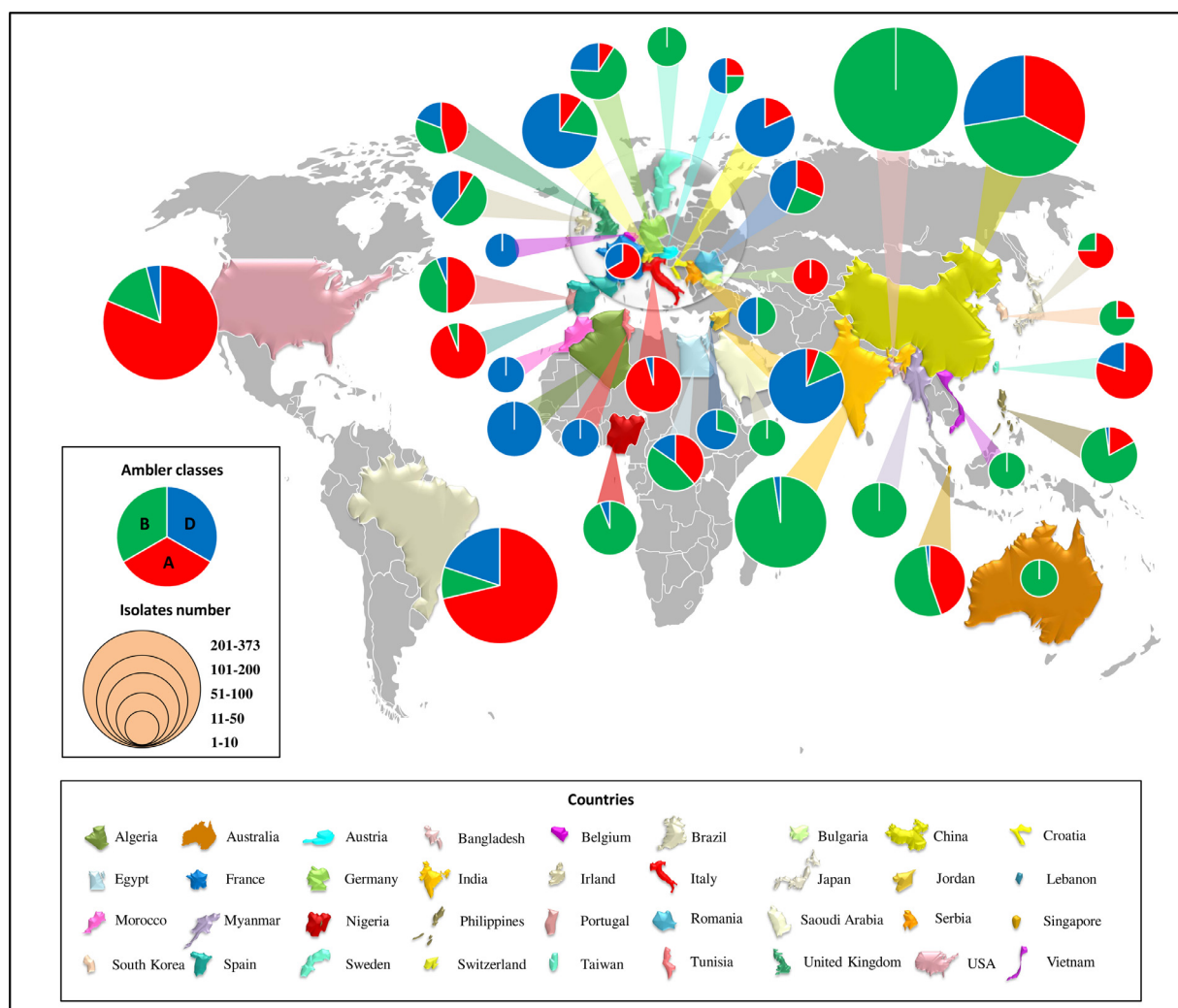


Fig. 1. Worldwide distribution of carbapenemase-producing Gram-negative bacteria in aquatic environments.

Brazilian river [42]. de Araujo et al. and Xu et al. have reported the isolation of KPC-2-producing *Aeromonas hydrophila* from rivers in Brazil and China, respectively [42,49]. Recently, KPC-2-producing *A. hydrophila* and *Aeromonas caviae* were recovered from river water and WWTP effluents [57,66].

Although rarely identified, Haller et al. have reported the isolation of KPC-producing *Pseudomonas* spp. from hospital sewage in Singapore [28]. Furthermore, BIC-1 (GES-7)-producing *Pseudomonas fluorescens* was obtained from a river in France [67]. More recently, Neto et al. have detected KPC-2-producing *Pseudomonas putida* in mangroves in Brazil [68].

In addition, KPC-2-producing *Stenotrophomonas* spp. and *Wautersiella* spp. isolates were recovered from a WWTP in China [43] and KPC-2-producing *Stenotrophomonas maltophilia* was recovered from mangroves in Brazil [68]. Finally, Hammerl et al. have documented the isolation of VCC-1 (for *Vibrio cholerae* carbapenemase)-producing *V. cholerae* from seawater in Germany [69].

3.2. Class B carbapenemases

Unlike class A carbapenemases, which were first reported in Enterobacterales, the first acquired class B β -lactamase (BCII) was detected in a *Bacillus cereus* isolate in 1966 [70]. Owing to the fact that they use one or two zinc (Zn^{2+}) ions for their activ-

ity, these enzymes are also called metallo- β -lactamases (MBLs) [71] and this property makes them susceptible to inhibition by metallic ion chelators such as ethylene diamine tetra-acetic acid (EDTA) [72]. However, they are resistant to the commercially available β -lactamase inhibitors (clavulanic acid, tazobactam and sulbactam) [42]. MBLs breakdown all β -lactams except monobactams (aztreonam), with VIM, IMP and NDM groups being the most commonly identified acquired MBLs [15]. The global epidemiology of class B carbapenemases in aquatic environments is presented in Table 2 and Fig. 3.

3.2.1. Enterobacterales

NDM enzymes are among the most newly characterised MBLs, which are widely distributed in Enterobacterales species [73]. The *bla*_{NDM} genes have been detected in *E. cloacae*, *E. coli*, *C. freundii*, *C. braakii*, *C. farmeri*, *K. pneumoniae*, *K. oxytoca* and *Shigella boydii* isolated from municipal and hospital sewage [32,33,61,74–76]. Mahon et al. have reported the isolation of NDM-producing *E. coli* from recreational freshwater and wastewater [77]. Furthermore, NDM-producing *K. pneumoniae* and *K. quasipneumoniae* could also be isolated from sewage and seawater [76,77]. More worryingly, two studies have reported the isolation of NDM-producing *K. pneumoniae* and *E. coli* from drinking water in Egypt and Jordan, respectively [27,34]. Several NDM variants have been detected in Enterobacterales species isolated from aqueous ecosys-

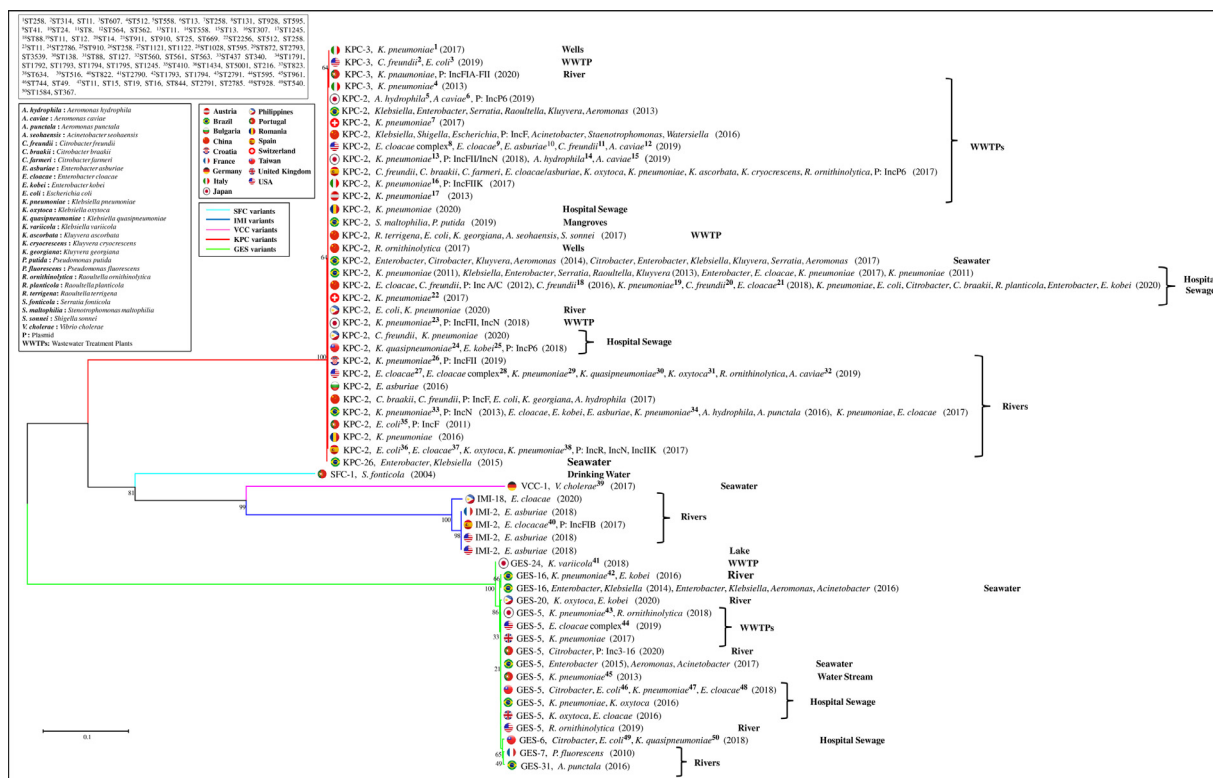


Fig. 2. Phylogenetic tree of class A carbapenemase variants detected in different aquatic environments. The evolutionary history was inferred using the neighbour-joining method. Evolutionary distances were computed using the Kimura 2-parameter method. The number above the nodes is the level of bootstrap from 1000 replicates.

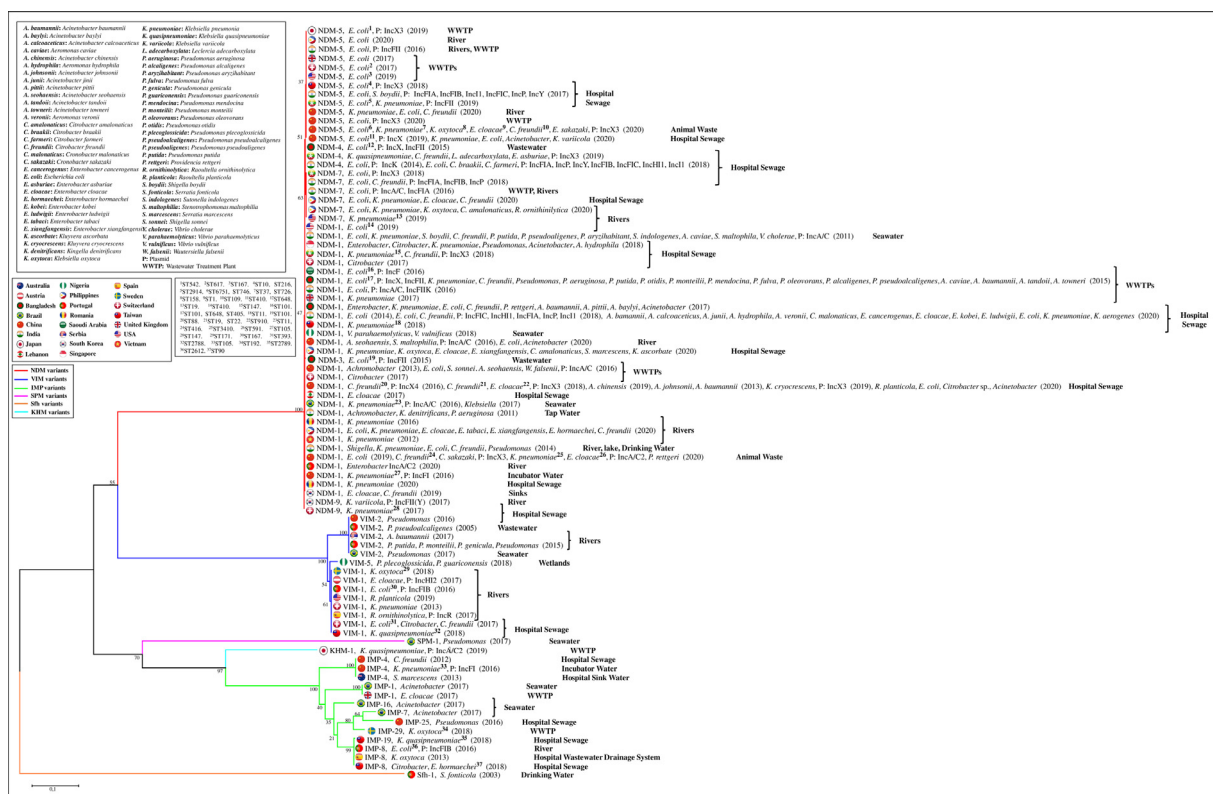


Fig. 3. Phylogenetic tree of class B carbapenemase variants detected in different aquatic environments. The evolutionary history was inferred using the neighbour-joining method. Evolutionary distances were computed using the Kimura 2-parameter method. The number above the nodes is the level of bootstrap from 1000 replicates.

Table 2
Epidemiology of class B carbapenemase-producers detected in aquatic environments

Carbapenemase	Country	Source	Host(s) (n)	ST	Plasmid group	Reference
GIM	Germany	Clinical and urban WW	<i>Enterobacter cloacae</i> complex (1)	–	–	[76]
IMP	Ireland	Hospital sewage	<i>Klebsiella pneumoniae</i> (2)	ST3146	–	[32]
			<i>Enterobacter cloacae</i> complex (3)	–	–	[76]
	Singapore	Hospital sewage	<i>Aeromonas caviae</i> (1)	–	–	[74]
IMP-1	USA	WWTP	<i>Escherichia coli</i> (1)	–	–	[30]
	Brazil	Seawater	<i>Acinetobacter</i> (3)	–	–	[48]
	UK	WWTP	<i>Enterobacter cloacae</i> (1)	–	–	[63]
IMP-4	Australia	Hospital sink water	<i>Serratia marcescens</i> (4)	–	–	[104]
	China	Incubator water	<i>Klebsiella pneumoniae</i> (1)	ST105	IncFI	[83]
		Hospital sewage	<i>Citrobacter freundii</i> (1)	–	–	[38]
IMP-7	Brazil	Seawater	<i>Acinetobacter</i> (1)	–	–	[48]
IMP-8	Portugal	River	<i>Escherichia coli</i> (2)	ST2612	IncFIB	[106]
	Spain	Hospital WW drainage system	<i>Klebsiella oxytoca</i> (1)	–	–	[105]
	Taiwan	Hospital sewage	<i>Enterobacter hormaechei</i> (2)	ST90	–	[53]
			<i>Citrobacter</i> (1)	–	–	
IMP-16	Brazil	Seawater	<i>Acinetobacter</i> (4)	–	–	[48]
IMP-19	Taiwan	Hospital sewage	<i>Klebsiella quasipneumoniae</i> (1)	ST2789	–	[53]
IMP-25	China	Hospital sewage	<i>Pseudomonas</i> (1)	–	–	[116]
IMP-29	Sweden	WWTP	<i>Klebsiella oxytoca</i> (1)	ST192	–	[107]
KHM-1	Japan	WWTP	<i>Klebsiella quasipneumoniae</i> (1)	–	IncA/C2	[111]
NDM	Egypt	DW	<i>Klebsiella pneumoniae</i> (6)	–	–	[27]
	Germany	Clinical WW	<i>Enterobacter cloacae</i> complex (2), <i>Klebsiella oxytoca</i> (1)	–	–	[76]
		Clinical and urban WW	<i>Klebsiella quasivariicola</i> (1), <i>Klebsiella pneumoniae</i> (3)	–	–	
	Ireland	Hospital sewage	<i>Escherichia coli</i> (1)	ST617	–	[32]
		FSW, WW	<i>Escherichia coli</i> (5)	–	–	[77]
		Seawater	<i>Klebsiella pneumoniae</i> (11)	–	–	
	Jordan	DW	<i>Escherichia coli</i> (12)	–	–	[34]
	Singapore	Hospital sewage	<i>Enterobacter cloacae</i> (1), <i>Escherichia coli</i> (1), <i>Citrobacter freundii</i> (1)	–	–	[74]
	UK	Hospital sewage	<i>Enterobacter cloacae</i> complex (2), <i>Citrobacter freundii</i> (4)	–	–	[61]
NDM-1	Bangladesh	Hospital sewage	<i>Klebsiella pneumoniae</i> (46), <i>Escherichia coli</i> (30), <i>Citrobacter freundii</i> (2), <i>Providencia rettgeri</i> (1), <i>Enterobacter</i> (9), <i>Acinetobacter baumannii</i> (8), <i>Acinetobacter pittii</i> (4), <i>Acinetobacter baylyi</i> (1), <i>Acinetobacter</i> spp. (3)	–	–	[89]

(continued on next page)

Table 2 (continued)

Carbapenemase	Country	Source	Host(s) (n)	ST	Plasmid group	Reference
	Bangladesh	Wastewater	<i>Escherichia coli</i> (49)	ST101, ST648, ST405	IncX, IncFII	[81]
			<i>Klebsiella pneumoniae</i> (76), <i>Citrobacter freundii</i> (10)	–	–	
			<i>Pseudomonas</i> spp. (2), <i>Pseudomonas aeruginosa</i> (4), <i>Pseudomonas putida</i> (24), <i>Pseudomonas otitidis</i> (10), <i>Pseudomonas monteilii</i> (3), <i>Pseudomonas mendocina</i> (8), <i>Pseudomonas fulva</i> (3), <i>Pseudomonas oleovorans</i> (7), <i>Pseudomonas alcaligenes</i> (1), <i>Pseudomonas pseudoalcaligenes</i> (3), <i>Aeromonas caviae</i> (1), <i>Acinetobacter baumannii</i> (2), <i>Acinetobacter tandonii</i> (4), <i>Acinetobacter townneri</i> (5), unidentified (15)	–	–	
	Brazil	Seawater	<i>Klebsiella pneumoniae</i> (1)	ST11	IncA/C	[84]
			<i>Klebsiella</i> (1)	–	–	[48]
	China	Animal waste	<i>Escherichia coli</i> (1)	–	–	[93]
			<i>Citrobacter freundii</i> (2)	ST416	–	[98]
			<i>Cronobacter sakazakii</i> (1)	–	IncX3	
			<i>Klebsiella pneumoniae</i> (1)	ST3410	IncA/C	
			<i>Providencia rettgeri</i> (1)	–	–	
			<i>Enterobacter cloacae</i> (1)	ST591	IncA/C	
		Hospital sewage	<i>Acinetobacter johnsonii</i> (2)	–	–	[113]
	China	Hospital sewage	<i>Acinetobacter baumannii</i> (10)	–	–	[114]
			<i>Citrobacter freundii</i> (1)	ST88	IncX4	[85]
			<i>Enterobacter cloacae</i> (2)	ST910	IncX3	[52]
			<i>Citrobacter freundii</i> (2)	ST19, ST22		
			<i>Acinetobacter chinensis</i> (2)	–	–	[115]
			<i>Kluyvera cryocrescens</i> (1)	–	–	[94]
			<i>Raoultella planticola</i> (1), <i>Escherichia coli</i> (3), <i>Citrobacter</i> sp. (1), <i>Acinetobacter</i> (11)	–	–	[96]
		Incubator water	<i>Klebsiella pneumoniae</i> (1)	ST105	IncFI	[83]
		River	<i>Acinetobacter seohaensis</i> (2), <i>Stenotrophomonas maltophilia</i> (1)	–	IncA/C	[86]
			<i>Escherichia coli</i> (1), <i>Acinetobacter</i> (1)	–	–	[96]

(continued on next page)

Table 2 (continued)

Carbapenemase	Country	Source	Host(s) (n)	ST	Plasmid group	Reference
		WWTP	<i>Achromobacter</i> sp. (1)	–	–	[122]
			<i>Escherichia coli</i> (3), <i>Shigella sonnei</i> (2), <i>Acinetobacter seohaensis</i> (4), <i>Wautersiella falsenii</i> (1)	–	IncA/C	[43]
	India	Hospital sewage	<i>Escherichia coli</i> (1)	–	–	[80]
			<i>Escherichia coli</i> (1), <i>Citrobacter freundii</i> (7)	–	IncFIC, IncHI1, IncFIA, IncP, InkI1	[75]
			<i>Acinetobacter baumannii</i> (11), <i>Acinetobacter calcoaceticus</i> (1), <i>Acinetobacter junii</i> (1), <i>Aeromonas hydrophila</i> (2)	–	–	[95]
	India	Hospital sewage	<i>Aeromonas veronii</i> (2), <i>Cronobacter malonaticus</i> (1), <i>Enterobacter cancerogenus</i> (1), <i>Enterobacter cloacae</i> (1), <i>Enterobacter kobei</i> (1), <i>Enterobacter ludwigii</i> (1), <i>Escherichia coli</i> (14), <i>Klebsiella pneumoniae</i> (10), <i>Klebsiella aerogenes</i> (1)	–	–	[95]
		River, lake, DW	<i>Klebsiella pneumoniae</i> (3), <i>Shigella</i> (2), <i>Escherichia coli</i> (1), <i>Citrobacter freundii</i> (1), <i>Pseudomonas</i> (3)	–	–	[79]
		Seawater	<i>Pseudomonas putida</i> (2), <i>Pseudomonas pseudoalcaligenes</i> (2), <i>Pseudomonas oryzae</i> (1), <i>Suttonella indologenes</i> (1), <i>Aeromonas caviae</i> (1), <i>Stenotrophomonas maltophilia</i> (1), <i>Vibrio cholerae</i> (1)	–	IncA/C	[78]
		Tap water	<i>Achromobacter</i> spp. (2), <i>Kingella denitrificans</i> (1), <i>Pseudomonas aeruginosa</i> (1)	–	–	
	Lebanon	WWTP	<i>Escherichia coli</i> (2)	–	IncA/C, IncFIIK	[87]
		Hospital sewage	<i>Enterobacter cloacae</i> (2)	–	–	[90]
	Myanmar	Hospital sewage	<i>Citrobacter freundii</i> (1)	–	IncX3	[91]
			<i>Klebsiella pneumoniae</i> (1)	ST147	IncX3	
	Nigeria	Seawater	<i>Vibrio parahaemolyticus</i> (1), <i>Vibrio vulnificus</i> (5)	–	–	[123]

(continued on next page)

Table 2 (continued)

Carbapenemase	Country	Source	Host(s) (n)	ST	Plasmid group	Reference
NDM-3 NDM-4	Philippines	Hospital sewage	<i>Klebsiella pneumoniae</i> (4), <i>Klebsiella oxytoca</i> (1), <i>Enterobacter cloacae</i> (4), <i>Enterobacter xiangfangensis</i> (1), <i>Citrobacter amalonaticus</i> (1), <i>Serratia marcescens</i> (1), <i>Kluyvera ascorbata</i> (1)	–	–	[64]
		River	<i>Escherichia coli</i> (1), <i>Klebsiella pneumoniae</i> (1), <i>Enterobacter cloacae</i> (1), <i>Enterobacter tabaci</i> (1), <i>Enterobacter xiangfangensis</i> (1), <i>Enterobacter hormaechei</i> (1), <i>Citrobacter freundii</i> (1)	–	–	
	Portugal	River	<i>Enterobacter</i> spp. (3)	–	IncA/C2	[59]
	Romania	Hospital sewage	<i>Klebsiella pneumoniae</i> (7)	–	–	[97]
		River	<i>Klebsiella pneumoniae</i> (1)	–	–	[44]
	Saudi Arabia	WWTP	<i>Escherichia coli</i> (1)	ST101	IncF	[82]
	Singapore	Hospital sewage	<i>Klebsiella pneumoniae</i> (3), <i>Enterobacter</i> (3), <i>Citrobacter</i> (6), <i>Pseudomonas</i> (9), <i>Acinetobacter</i> (4), <i>Aeromonas hydrophila</i> (1)	–	–	[28]
	South Korea	Hospital sinks	<i>Enterobacter cloacae</i> (1), <i>Citrobacter freundii</i> (1)	–	–	[35]
	Switzerland	Hospital sewage	<i>Citrobacter</i> sp. (1)	–	–	[46]
		WWTP	<i>Citrobacter</i> sp. (1)	–	–	
	Taiwan	Hospital sewage	<i>Klebsiella pneumoniae</i> (1)	ST11	–	[53]
	USA	River	<i>Escherichia coli</i> (1)	ST410	–	[57]
	Vietnam	River	<i>Klebsiella pneumoniae</i> (3)	–	–	[99]
	UK	WWTP	<i>Klebsiella pneumoniae</i> (1)	–	–	[63]
	Bangladesh	Wastewater	<i>Escherichia coli</i> (3)	ST101	IncFII	[81]
			<i>Escherichia coli</i> (1)	ST648	IncX, IncFII	
	India	Hospital sewage	<i>Escherichia coli</i> (1)	–	IncK	[100]
			<i>Escherichia coli</i> (8), <i>Citrobacter braakii</i> (2), <i>Citrobacter farmeri</i> (1)	–	IncFIA, IncP, IncY, IncFIB, IncFIC, IncHI1, IncI1	[75]
NDM-5	Myanmar	Hospital sewage	<i>Enterobacter asburiae</i> (1), <i>Leclercia adecarboxylata</i> (1), <i>Citrobacter freundii</i> (2), <i>Klebsiella quasipneumoniae</i> (1)	–	IncX3	[91]
	China	Animal waste	<i>Klebsiella pneumoniae</i> (3)	ST37, ST726	IncX3	[98]

(continued on next page)

Table 2 (continued)

Carbapenemase	Country	Source	Host(s) (n)	ST	Plasmid group	Reference
NDM-7	China	Hospital sewage	<i>Klebsiella oxytoca</i> (1)	ST158		
			<i>Enterobacter cloacae</i> (4)	ST1		
			<i>Cronobacter sakazakii</i> (1)	–		
			<i>Citrobacter freundii</i> (9)	ST109		
			<i>Escherichia coli</i> (6)	ST6751, ST746		
		Hospital sewage	<i>Escherichia coli</i> (1)	ST410	IncX3	[101]
			<i>Klebsiella pneumoniae</i> (3),	–	–	[96]
			<i>Escherichia coli</i> (9),			
			<i>Acinetobacter</i> (1),			
			<i>Klebsiella variicola</i> (1)			
	India	Hospital sewage	<i>Klebsiella pneumoniae</i> (1),	–	–	
			<i>Escherichia coli</i> (1),			
			<i>Citrobacter freundii</i> (1)			
	Japan	WWTP, rivers	<i>Escherichia coli</i> (6),	–	IncFIA, IncFIB, IncI1, IncFIC, IncP, IncY	[75]
			<i>Shigella boydii</i> (4)			
	Myanmar	Hospital sewage	<i>Escherichia coli</i> (7)	–	IncFII	[87]
			<i>Escherichia coli</i> (1)	ST542	IncX3	[102]
	Philippines	River	<i>Klebsiella pneumoniae</i> (1)	–	IncFII	[91]
			<i>Escherichia coli</i> (4)	ST8453		
	Switzerland	WWTP	<i>Escherichia coli</i> (1)	–	–	[64]
			<i>Escherichia coli</i> (1)	ST617	–	[46]
	Taiwan	Hospital sewage	<i>Escherichia coli</i> (1)	ST10, ST216, ST2914	IncX3	[53]
	UK	WWTP	<i>Escherichia coli</i> (1)	–	–	[63]
			<i>Escherichia coli</i> (1)	ST167	–	[57]
	India	Hospital sewage	<i>Escherichia coli</i> (1),	–	IncFIA, IncFIB, IncP	[75]
			<i>Citrobacter freundii</i> (1)			
NDM-9	Myanmar	Hospital sewage	<i>Escherichia coli</i> (5)	–	IncA/C	[87]
			<i>Escherichia coli</i> (1)	–	IncX3	[91]
			<i>Escherichia coli</i> (2),	–	–	[64]
			<i>Klebsiella pneumoniae</i> (1),			
			<i>Enterobacter cloacae</i> (3),			
	Philippines	Hospital sewage	<i>Citrobacter freundii</i> (4)			
			<i>Escherichia coli</i> (3),	–	–	
			<i>Klebsiella pneumoniae</i> (1),			
			<i>Klebsiella oxytoca</i> (1),			
			<i>Citrobacter amalonaticus</i> (1),			
Sfh-1	USA	River	<i>Raoultella ornithinolytica</i> (1)			
			<i>Klebsiella pneumoniae</i> (1)	ST19	–	[57]
			<i>Klebsiella variicola</i> (3)	–	IncFII(Y)	[103]
	South Korea	Hospital sewage	<i>Klebsiella pneumoniae</i> (1)	ST147	–	[46]
			<i>Serratia fonticola</i> (1)	–	–	[110]
SPM	Brazil	Hospital sewage	<i>Pseudomonas aeruginosa</i> (6)	–	–	[117]
			<i>Pseudomonas aeruginosa</i> (1)	–	–	[48]
			<i>Pseudomonas aeruginosa</i> (14)	–	–	[117]
			<i>Enterobacter cloacae</i> complex (1),	–	–	[76]
			<i>Enterobacter</i> (1),			
	Germany	Hospital sewage	<i>Pseudomonas aeruginosa</i> (8)			

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Table 2 (continued)

Carbapenemase	Country	Source	Host(s) (n)	ST	Plasmid group	Reference
VIM-1	Germany	WWTP	<i>Pseudomonas aeruginosa</i> (1), <i>Escherichia coli</i> (3)	–	–	[108]
	Ireland	Hospital sewage	<i>Klebsiella oxytoca</i> (1) <i>Enterobacter cloacae</i> complex (1)	ST202 –	– –	[32]
	Jordan	DW	<i>Escherichia coli</i> (75)	–	–	[34]
	Switzerland	WWTP	<i>Enterobacter aerogenes</i> (1)	–	–	[46]
	USA	WWTP	<i>Escherichia coli</i> (36)	–	–	[30]
	Austria	River	<i>Enterobacter cloacae</i> (1)	–	IncHI2	[88]
	Portugal	River	<i>Escherichia coli</i> (1)	ST167	IncFIB	[106]
	Spain	River	<i>Raoultella ornithinolytica</i> (1)	–	IncR	[24]
	Sweden	River	<i>Klebsiella oxytoca</i> (1)	ST172	–	[107]
	Switzerland	Hospital sewage	<i>Citrobacter</i> (1), <i>Citrobacter freundii</i> (1)	–	–	[46]
		River	<i>Escherichia coli</i> (1) <i>Klebsiella pneumoniae</i> (1)	ST393 –	– –	[109]
	Taiwan	Hospital sewage	<i>Klebsiella quasipneumoniae</i> (1)	ST2788	–	[53]
	USA	River	<i>Raoultella planticola</i> (1)	–	–	[57]
VIM-2	Brazil	Seawater	<i>Pseudomonas</i> (2)	–	–	[48]
	China	Hospital sewage	<i>Pseudomonas</i> (1)	–	–	[116]
	Portugal	River	<i>Pseudomonas putida</i> (1), <i>Pseudomonas geniculata</i> (1), <i>Pseudomonas monteilii</i> (1) <i>Pseudomonas</i> sp. (2)	–	–	[119]
		Wastewater	<i>Pseudomonas pseudoalcaligenes</i> (1)	–	–	[118]
VIM-5	Serbia	River	<i>Acinetobacter baumannii</i> (2)	–	–	[120]
	Nigeria	Wetlands	<i>Pseudomonas plecoglossicida</i> (6), <i>Pseudomonas guariconensis</i> (4)	–	–	[121]
VIM-34	Portugal	River	<i>Escherichia coli</i> (1)	ST354	–	[106]

n, number of strains; ST, sequence type; WW, wastewater; WWTP, wastewater treatment plant; DW, drinking water; FSW, fresh surface water.

tems. NDM-1-producing *Enterobacter* spp., *Klebsiella* spp., *Citrobacter* spp., *Shigella* spp., *E. coli*, *C. freundii*, *Citrobacter amalonaticus*, *Cronobacter malonaticus*, *Cronobacter sakazakii*, *S. boydii*, *K. pneumoniae*, *K. oxytoca*, *Klebsiella aerogenes*, *Kluyvera cryocrescens*, *K. ascorbata*, *Shigella sonnei*, *Providencia rettgeri*, *Enterobacter xiangfangensis*, *E. cloacae*, *Enterobacter tabaci*, *Enterobacter hormaechei*, *Enterobacter cancerogenus*, *Enterobacter ludwigii*, *S. marcescens* and *R. planticola* have been isolated from seawater, drinking water, rivers, lakes, hospital sewage, wastewater, WWTPs, irrigation water and animal sewage [28,35,44,46,48,52,53,57,59,63,64,75,78–99]. Only one study reported the isolation of an NDM-3-producer from an aquatic environment. The isolate was obtained from wastewater and was identified as *E. coli* sequence type 101 (ST101) [81]. In addition, an NDM-4 variant was detected in *E. coli*, *K. quasipneumoniae*, *C. freundii*, *C. braakii*, *C. farmeri*, *Leclercia adecarboxylata* and *E. asburiae* isolates obtained from hospital sewage and wastewater [75,81,91,100]. The NDM-5 enzyme was detected in *E. coli*, *K. pneumoniae*, *K. variicola*, *K. oxytoca*, *C. freundii*, *C. sakazakii*, *E. cloacae* and *S. boydii* isolates obtained from WWTPs, rivers, animal

waste and hospital sewage [28,46,57,63,64,87,91,96,98,101,102]. In addition, NDM-7-producers, namely *E. coli*, *C. freundii*, *C. amalonaticus*, *E. cloacae*, *K. pneumoniae*, *K. oxytoca* and *R. ornithinolytica*, have been recovered from WWTPs, rivers and hospital sewage [57,64,75,87,91]. Finally, NDM-9-producing *E. coli* and *K. variicola* have been isolated from WWTPs and river water, respectively [46,103].

Other important MBLs in GNB are the IMP enzymes. IMP-producing *E. coli*, *E. cloacae* complex and *K. pneumoniae* isolates have been recovered from US WWTPs and from Irish hospital sewage [30,32]. In addition, IMP-1-producing *E. cloacae* has been detected in WWTPs in the UK [63]. IMP-4-producing *C. freundii* and *S. marcescens* were isolated from hospital wastewater [38,104]. In China, an IMP-4-producing *K. pneumoniae* strain co-producing NDM-1 was isolated from incubator water in a neonatal intensive care unit (NICU). This strain was clonally related to NDM-1- and IMP-4-producing *K. pneumoniae* recovered from an outbreak in the same NICU, and the authors suggested that the incubator water may be a reservoir for the diffusion of such MBL-producing GNB

[83]. Furthermore, IMP-8-producing *K. oxytoca*, *Citrobacter* spp. and *E. hormaechei* and IMP-19-producing *K. quasipneumoniae* have been isolated from hospital wastewater [53,105]. In addition, Kieffer et al. have reported the isolation of IMP-8-producing *E. coli* from river water [106]. Recently, IMP-29-producing *K. oxytoca* ST192 was isolated from sewage of a WWTP in Sweden [107].

Further studies reported the detection of other MBLs among Enterobacterales isolated from water habitats. VIM-producing *E. coli*, *K. pneumoniae*, *K. oxytoca*, *Enterobacter aerogenes*, *E. cloacae* complex and *Enterobacter* spp. have been detected in drinking water, WWTPs and hospital sewage [30,32–34,46,76,108]. In addition, *bla*_{VIM-1}-harbouring *K. pneumoniae*, *K. variicola*, *E. coli*, *E. cloacae*, *R. planticola* and *R. ornithinolytica* and VIM-34-producing *E. coli* have been recovered from rivers [24,57,88,106,109]. Furthermore, VIM-1-producing *Citrobacter* spp., *E. coli*, *C. freundii* and *K. quasipneumoniae* have been isolated from hospital sewage [46,53]. In addition, a VIM-1-producing *K. oxytoca* ST172 isolate was recovered from a river in Sweden [107].

Other less common carbapenemases were also detected in water environments. This is the case with Sfh-1-producing *S. fonticola* isolated from drinking water in Portugal [110] and with GIM-producing *E. cloacae* complex (for Germany IMipenemase) isolated from urban and clinical wastewater in Germany [76]. In addition, the KHM-1 (Kyorin Health Science MBL-1) enzyme first described in a clinical isolate in Korea was detected in a *K. quasipneumoniae* isolated from a WWTP in Japan [111,112].

3.2.2. Other Gram-negative bacilli

Several studies have reported the detection of MBL-producing *Acinetobacter* and *Pseudomonas* species in environmental water habitats. NDM-1-producing *Acinetobacter* spp., *A. baumannii*, *Acinetobacter calcoaceticus*, *Acinetobacter junii*, *Acinetobacter johnsonii* and *Pseudomonas* spp. have been recovered from hospital sewage in Bangladesh, China, Singapore and the Philippines [28,64,89,96,113,114]. The occurrence of such superbugs in WWTPs was also described in some studies. NDM-1-producing *Pseudomonas* spp., *Acinetobacter* spp. and *Acinetobacter seohaensis* were detected in WWTPs in Bangladesh and China [81,86]. NDM-producing *Pseudomonas* and *Acinetobacter* species were also described in surface water. NDM-producing *Pseudomonas* spp. were isolated from rivers and lakes in India in 2013 [79]. In addition, NDM-1 producing *Acinetobacter* spp. and *A. seohaensis* were isolated from river water in China [86, 96] and NDM-1-producing *P. putida*, *Pseudomonas pseudoalcaligenes* and *Pseudomonas oryzae* were obtained from seawater in India [78]. Recently, Hu et al. have reported the description of NDM-1-producing *Acinetobacter chinensis*, a novel *Acinetobacter* species isolated from hospital sewage in China [115]. In addition, the NDM-5 variant was detected in *Acinetobacter* sp. isolated from hospital sewage in China [96]. Regarding IMP carbapenemases, four variants were detected in *Acinetobacter* and *Pseudomonas* species isolated from water. IMP-1, IMP-7 and IMP-16 variants were detected in *Acinetobacter* spp. isolates obtained from seawater in Brazil [48], and the IMP-25 variant was detected in *Pseudomonas* sp. recovered from hospital sewage in China [116]. VIM enzymes were also detected. Miranda et al. have reported the detection of VIM-producing *Pseudomonas* sp. in hospital sewage [117]. The VIM-2 carbapenemase was also detected in *Pseudomonas* sp., *P. pseudoalcaligenes* and *P. aeruginosa* from hospital sewage [76,116,118], in *Pseudomonas* spp., *P. putida*, *Pseudomonas monteilii* and *Pseudomonas geniculata* from river water [119], in *Pseudomonas* spp. from seawater [48] and in *A. baumannii* in river water [120]. Recently, VIM-5-producing *P. putida* group, namely *Pseudomonas plecoglossicida* and *Pseudomonas guariconensis*, were obtained from wetlands in Nigeria [121]. In addition, SPM (Sao Paulo metallo- β -lactamase)-producing *P. aeruginosa* and

SPM-1-producing *Pseudomonas* spp. were recovered from hospital sewage and seawater, respectively [48,117].

On the other hand, carbapenemase-producers belonging to several GNB genera, other than *Pseudomonas* and *Acinetobacter*, could also be detected in different aquatic habitats. NDM-1-producing *A. hydrophila* and *A. caviae* were recovered from hospital sewage in Singapore and the Philippines and from seawater in India [28,64,78]. In addition, *bla*_{IMP}-harbouring *A. caviae* was isolated from hospital wastewater in India [78]. Luo et al. have reported the isolation of NDM-1-producing *Achromobacter* sp. from a WWTP in China [122]. Isolates of *V. cholerae*, *Vibrio parahaemolyticus* and *Vibrio vulnificus* isolated from seawater in India and Nigeria were found to be NDM-1-producers [78,123]. NDM-1-producing *S. maltophilia* isolates were obtained from river and seawater in China and India, respectively [78,86]. Finally, NDM-1-producing *Suttonella indologenes* and *Wautersiella falsenii* were isolated from seawater and a WWTP, respectively [78,86].

3.3. Class D carbapenemases

Only some variants of the class D β -lactamases possess carbapenemase activity, the so-called carbapenem-hydrolysing class D β -lactamases (CHDLs) [19]. The first identified class D carbapenemase was the OXA-23 enzyme, which was detected in an *A. baumannii* isolate from the UK in 1985 [3]. Subsequently, several CHDLs have been reported, mostly in *Acinetobacter* spp. However, the most prevalent CHDL in Enterobacterales, OXA-48, was reported in 2001 in Turkey from a clinical *K. pneumoniae* isolate [3,13]. Class D carbapenemases are serine enzymes that are resistant to inhibition by the commercially available β -lactamase inhibitors (clavulanic acid, tazobactam and sulbactam), although they are inhibited in vitro by NaCl [124]. Notably, despite their significant activity, all class D carbapenemases do not confer a high level of carbapenem resistance owing to their weak carbapenem-hydrolysing activity [19,124].

3.3.1. Enterobacterales

The most reported CHDL among Enterobacterales isolated from different aqueous environments is the phantom menace, the OXA-48 enzyme (Table 3; Fig. 4). OXA-48-producers belonging to different Enterobacterales genera and species including *Citrobacter* spp., *E. coli*, *K. pneumoniae*, *K. oxytoca*, *C. freundii*, *C. braakii*, *Citrobacter youngae*, *C. farmeri*, *E. cloacae*, *E. aerogenes*, *E. kobei*, *S. marcescens*, *P. rettgeri*, *R. ornithinolytica* and *C. malonaticus* have been cultivated from wastewater and WWTPs, hospital sewage, puddles, rivers, estuaries, spring water, irrigation water, fountain water, seawater, water dam and drinking water in different parts of the world [27,32,39,46,63,75,92,97,108,125–130]. Although OXA-48-type is the most prevalent, other variants were reported among different Enterobacterales. OXA-48-like-producing *K. oxytoca* has been isolated from hospital sewage in Algeria [131]. More recently, the *bla*_{OXA-48-like} gene was detected in *K. pneumoniae* and *E. coli* strains isolated from seawater in Ireland [132]. In addition, Antonelli et al. have reported the isolation of OXA-372-producing *C. freundii* from hospital sewage in Italy [133]. Furthermore, OXA-370-producing *Citrobacter* sp. was recovered from seawater in Brazil. OXA-181-producing *E. coli* and *K. pneumoniae* were cultivated from hospital sewage in Switzerland and from a WWTP in the UK, respectively [46,63]. In addition, OXA-181-producing *E. coli* has been isolated from drinking water in the USA [128]. Another OXA-48-like variant, namely OXA-204, has been detected in a *C. braakii* isolate recovered from a WWTP in Tunisia [134]. Furthermore, OXA-244-producing *E. coli* isolates were obtained from river water in Algeria and from estuaries in Lebanon [126,127]. In China, Xin et al. reported the isolation of OXA-58-producing *Raoultella* from seawater [135]. More recently, OXA-655- and OXA-656-producing *E. coli*

Table 3
Epidemiology of class D carbapenemase-producers detected in aquatic environments

Carbapenemase	Country	Source	Host(s) (n)	ST	Plasmid group	Reference
OXA-23	Algeria	Hospital sewage	<i>Acinetobacter baumannii</i> (2)	–	–	[131]
	Brazil	Hospital sewage	<i>Acinetobacter baumannii</i> (3)	–	–	[138]
	Croatia	Seawater	<i>Acinetobacter</i> spp. (11)	–	–	[48]
		Hospital sewage	<i>Acinetobacter baumannii</i> (2)	–	–	[139]
		Liquid manure	<i>Acinetobacter baumannii</i> (2)	ST195	–	[147]
		WWTP	<i>Acinetobacter baumannii</i> (10)	–	–	[141]
	France	River	<i>Acinetobacter baumannii</i> (1)	–	–	[143]
			<i>Acinetobacter baumannii</i> (10)	ST2	–	[145]
			<i>Acinetobacter baumannii</i> (1)	–	–	[137]
			<i>Acinetobacter baumannii</i> (2)	–	–	[120]
	Romania	River	<i>Acinetobacter baumannii</i> (2)	–	–	[119]
	Serbia	River	<i>Acinetobacter baumannii</i> (2)	–	–	[119]
	Brazil	Seawater	<i>Acinetobacter</i> spp. (6)	–	–	[48]
	Croatia	WWTP	<i>Acinetobacter baumannii</i> (3)	–	–	[141]
OXA-24/40	Nigeria	Surface water	<i>Acinetobacter baumannii</i> (1)	–	–	[146]
	Serbia	River	<i>Acinetobacter baumannii</i> (1)	–	–	[119]
OXA-48	Algeria	Fountain water	<i>Raoultella ornithinolytica</i> (1)	–	IncL	[129]
	Algeria	Irrigation water	<i>Klebsiella pneumoniae</i> (1)	ST1393	–	
		River	<i>Klebsiella pneumoniae</i> (3)	ST133, ST2055, ST2192	–	[126]
			<i>Raoultella ornithinolytica</i> (3), <i>Citrobacter braakii</i> (1), <i>Citrobacter freundii</i> (1) <i>Escherichia coli</i> (9)	ST559, ST38, ST212, ST1972, ST2142	–	
			<i>Klebsiella pneumoniae</i> (1) <i>Escherichia coli</i> (2)	ST13, ST5, ST19	IncL	[129]
			<i>Raoultella ornithinolytica</i> (1)	–	–	
		Seawater	<i>Cronobacter malonaticus</i> (1)	–	–	
		Spring water	<i>Cronobacter malonaticus</i> (1)	–	–	
		Dam water	<i>Klebsiella pneumoniae</i> (1)	ST1878	–	
		WW	<i>Klebsiella pneumoniae</i> (2)	ST1393, ST13	–	
			<i>Enterobacter cloacae</i> (1)	ST527	–	
	Austria	WWTP	<i>Klebsiella pneumoniae</i> (1)	ST35	–	
		WWTP	<i>Escherichia coli</i> (1)	ST38	–	[39]
			<i>Klebsiella pneumoniae</i> (1)	ST15	–	
	Belgium	Hospital WW (sinks)	<i>Citrobacter freundii</i> (5)	–	–	[130]
	Egypt	DW	<i>Klebsiella pneumoniae</i> (2)	–	–	[27]
	Germany	Clinical and urban WW	<i>Klebsiella pneumoniae</i> (3)	ST253, ST11	–	[76]
		WWTP	<i>Escherichia coli</i> (3)	–	–	[108]
	India	Hospital sewage	<i>Citrobacter braakii</i> (2), <i>Citrobacter farmeri</i> (1)	–	IncFIC, IncP, IncHI1	[75]
			<i>Enterobacter cloacae</i> complex (6), <i>Citrobacter freundii</i> (3)	–	–	[32]
	Ireland	Hospital sewage	<i>Klebsiella pneumoniae</i> (2)	ST3145, ST323	–	
			<i>Klebsiella oxytoca</i> (1)	ST95	–	
			<i>Escherichia coli</i> (1)	ST354	IncL	[127]
	Lebanon	Estuaries	<i>Klebsiella pneumoniae</i> (1)	ST16	IncL	
			<i>Serratia marcescens</i> (2)	–	–	[125]
	Morocco	Puddles	<i>Escherichia coli</i> (1)	–	–	[64]
	Philippines	River	<i>Shewanella xiamenensis</i> (1)	–	–	[140]
	Portugal	River	<i>Shewanella xiamenensis</i> (1)	–	–	[140]
	Romania	Hospital sewage	<i>Klebsiella pneumoniae</i> (12)	–	–	[97]

(continued on next page)

Table 3 (continued)

Carbapenemase	Country	Source	Host(s) (n)	ST	Plasmid group	Reference
OXA-48-like	Switzerland	Hospital sewage	<i>Escherichia coli</i> (5)	ST215, ST38, ST393	–	[46]
			<i>Klebsiella pneumoniae</i> (3)	ST437, ST258	–	
			<i>Enterobacter cloacae</i> (1), <i>Citrobacter freundii</i> (6), <i>Citrobacter youngae</i> (1), <i>Citrobacter</i> spp. (2)	–	–	
		WWTP	<i>Escherichia coli</i> (9)	ST38, ST10, ST354, ST215	–	
			<i>Klebsiella pneumoniae</i> (3)	ST485, ST395, ST29	–	
	UK	WWTP	<i>Citrobacter freundii</i> (3), <i>Citrobacter</i> sp. (1)	–	–	
			<i>Raoultella ornithinolytica</i> (2), <i>Klebsiella oxytoca</i> (1), <i>Enterobacter kobei</i> (1)	–	IncL/M	[63]
	USA	DW	<i>Escherichia coli</i> (1), <i>Providencia rettgeri</i> (1) <i>Shewanella</i> (2), <i>Pandoraea sputorum</i> (1), <i>Pseudomonas</i> (1)	–	–	[128]
	Algeria	Hospital sewage	<i>Klebsiella oxytoca</i> (2)	–	–	[131]
	Ireland	Seawater	<i>Escherichia coli</i> (1)	ST131	–	[132]
OXA-58	Singapore	Hospital sewage	<i>Klebsiella pneumoniae</i> (1)	ST101	–	
			<i>Aeromonas caviae</i> (1)	–	–	[74]
	Brazil	Seawater	<i>Acinetobacter</i> spp. (13)	–	–	[48]
	China	Hospital sewage	<i>Acinetobacter</i> spp. (7)	–	–	[96]
		Seawater	<i>Pseudomonas</i> (3), <i>Stenotrophomonas</i> (1), <i>Rheinheimera</i> (2), <i>Shewanella</i> (1), <i>Pseudoalteromonas</i> (1), <i>Algoriphagus</i> (1), <i>Bowmanella</i> (1), <i>Thalassospira</i> (1), <i>Raoultella</i> (1), <i>Vibrio</i> (1)	–	–	[135]
OXA-72 OXA-143 OXA-181	Germany	WWTP	<i>Acinetobacter lwoffii</i> (1), <i>Enterobacter cloacae</i> (1)	–	–	[108]
	Croatia	WWTP	<i>Acinetobacter baumannii</i> (2)	ST1	–	[145]
	Brazil	Seawater	<i>Acinetobacter</i> spp. (38)	–	–	[48]
	Algeria	River	<i>Shewanella xiamenensis</i> (1)	–	–	[144]
	Philippines	Hospital sewage	<i>Escherichia coli</i> (1)	–	–	[64]
OXA-199 OXA-204 OXA-244 OXA-252		WWTP	<i>Escherichia coli</i> (3)	ST940, ST410	–	[46]
	Switzerland					
	UK	WWTP	<i>Klebsiella pneumoniae</i> (1)	–	–	[63]
	USA	DW	<i>Escherichia coli</i> (1)	–	–	[128]
			<i>Acinetobacter baumannii</i> complex (1)	–	–	
OXA-370 OXA-372 OXA-416 OXA-538 OXA-655 OXA-656 OXA-894	Algeria	River	<i>Shewanella xiamenensis</i> (1)	–	–	[144]
	Tunisia	WWTP	<i>Citrobacter braakii</i> (1)	–	–	[134]
	Portugal	River	<i>Shewanella xiamenensis</i> (1)	–	–	[140]
	Algeria	River	<i>Escherichia coli</i> (3)	ST3541	–	[126]
	Lebanon	Estuaries	<i>Escherichia coli</i> (2)	ST38	IncHI2	[127]
	USA	DW	<i>Shewanella</i> (1), <i>Pseudomonas</i> (1), <i>Pseudomonas putida</i> (1)	–	–	[128]
	Brazil	Seawater	<i>Citrobacter</i> sp. (1)	–	–	[48]
	Italy	Hospital sewage	<i>Citrobacter freundii</i> (1)	–	IncA/C, IncN	[133]
	Algeria	Hospital sewage	<i>Shewanella xiamenensis</i> (1)	–	–	[141]
	Algeria	River	<i>Shewanella xiamenensis</i> (1)	–	–	[144]
	Brazil	Hospital waste	<i>Escherichia coli</i> (1)	ST401	IncQ1	[136]
			<i>Enterobacter cloacae</i> (1)	ST24	IncQ1	
	China	Animal waste	<i>Shewanella xiamenensis</i> (1)	–	–	[148]

n, number of strains; ST, sequence type; WWTP, wastewater treatment plant; WW, wastewater; DW, drinking water.

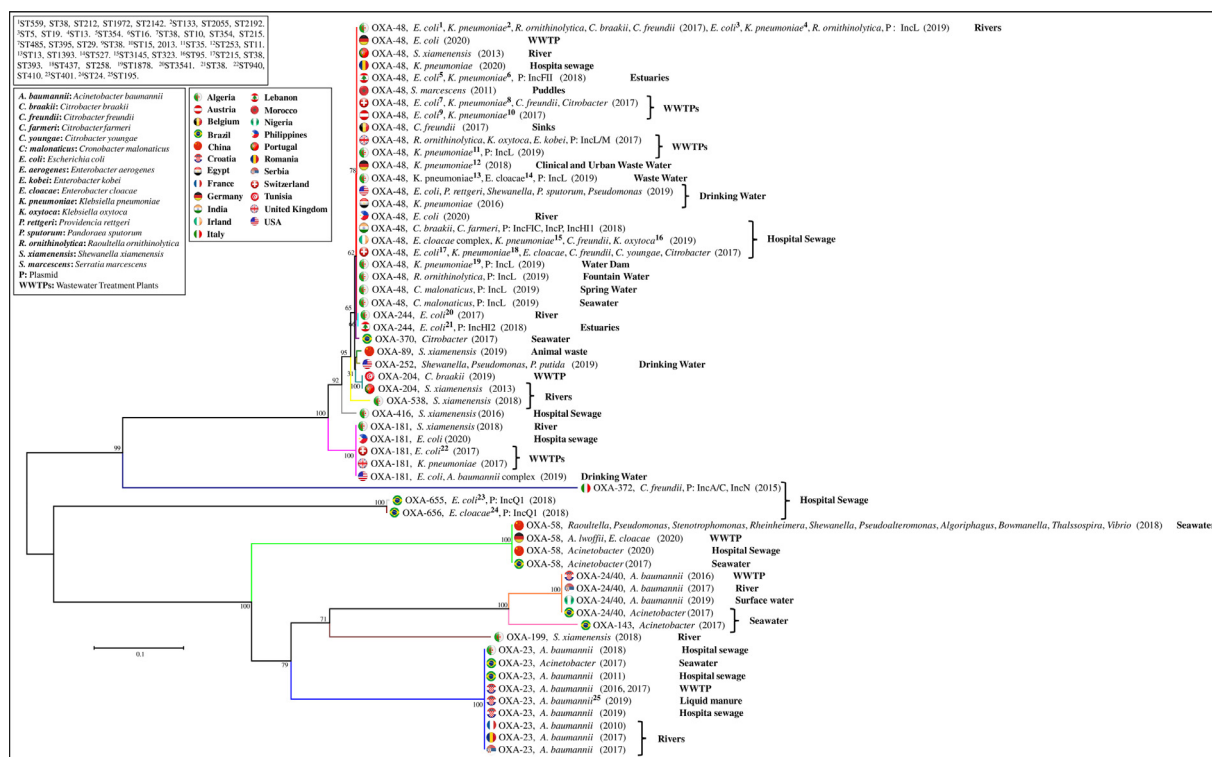


Fig. 4. Phylogenetic tree of class D carbapenemase variants detected in different aquatic environments. The evolutionary history was inferred using the neighbour-joining method. Evolutionary distances were computed using the Kimura 2-parameter method. The number above the nodes is the level of bootstrap from 1000 replicates.

and *E. cloacae* were isolated from hospital waste in Brazil [136]. Although mainly reported in *Acinetobacter* species, the OXA-58 carbapenemase was detected in an *E. cloacae* isolate recovered from a WWTP in Germany [108].

3.3.2. Other Gram-negative bacilli

The first OXA-23-producing *A. baumannii* environmental isolate was obtained from the Seine river in downtown Paris. The *bla*_{OXA-23} gene was chromosomally encoded and pulse-field gel electrophoresis (PFGE) revealed that the isolate was clonally related to a previously identified human isolate obtained in New Caledonia in June 2004 [137]. Thereafter, OXA-23-producing *A. baumannii* isolates were recovered from hospital sewage in Brazil [138] and Croatia [139]. Subsequently, different studies have reported the detection of CHDLs in different water habitats. OXA-48- and OXA-204-producing *Shewanella xiamenensis* isolates were obtained from a Portuguese river in 2013 [140]. In 2015, Koh et al. reported the isolation of an OXA-48-type carbapenemase-producing *A. caviae* from hospital sewage in Singapore [74]. In addition, OXA-23- and OXA-40/24-producing *A. baumannii* isolates were obtained from a WWTP in Croatia in 2016 [141]. In the same year, Yousfi et al. reported the isolation of OXA-416-producing *S. xiamenensis* from hospital sewage in Algeria [142]. In 2017, OXA-23- and OXA-24/40-producing *A. baumannii* isolates were obtained from river water in Romania and Serbia [120]. In the same year, Goic-Barisic et al. described the detection of OXA-23-producing *A. baumannii* in a WWTP in Croatia [143]. In another study published in 2017, OXA-23-, OXA-24/40-, OXA-51-, OXA-58- and OXA-143-producing *Acinetobacter* spp. were isolated from seawater in Brazil [48].

In 2018, only three studies reported the detection of CHDL-producing glucose-non-fermenting GNB in a water environment. The first report described the isolation of *S. xiamenensis* isolates producing OXA-181, OXA-199 and a new variant, OXA-538, from

river water in Algeria [144]. The second report documented the isolation of OXA-23-producing *A. baumannii* from hospital sewage also in Algeria [131]. The third study was from Croatia where the authors have reported the isolation of OXA-23- and OXA-72-producing *A. baumannii* isolates from a WWTP [145].

Recently, OXA-58-producing isolates belonging to different genera, namely *Pseudomonas* spp., *Rheinheimera* spp., *Stenotrophomonas* spp., *Shewanella* spp., *Vibrio*, *Pseudoalteromonas*, *Algoriphagus*, *Bowmanella* and *Thalassospira*, were obtained from seawater in China [135]. In addition, Tacão et al. reported the isolation of OXA-48-producing *Shewanella* spp., *Pseudomonas* spp. and *Pandora sputorum*, OXA-181-producing *A. baumannii* complex and OXA-252-producing *Shewanella* sp. from drinking water in the USA [140]. Recently, OXA-40-producing *A. baumannii*, OXA-58-producing *Acinetobacter* spp. and *Acinetobacter lwoffii* were detected in surface water, hospital sewage and a WWTP, respectively [96,108,146].

Animal waste has also been reported as a reservoir of carbapenemase-producing isolates. Hrenovic et al. reported the isolation of OXA-23-producing *A. baumannii* ST195 from liquid manure in Croatia [147]. Furthermore, the new OXA-48-like variant OXA-894 was first described in a *S. xiamenensis* isolated from pig wastewater [148].

3.4. Carbapenemase-encoding genes detected by culture-independent methods

In addition to culture-based techniques, culture-independent methods such as quantitative real-time PCR and metagenomics are increasingly used in studying antibiotic resistance in the environment [149]. With regard to carbapenemase determinants, the *bla* genes encoding carbapenemases of Ambler classes A, B and D were detected in different water environments worldwide. Regarding

class A carbapenemases, *bla*_{KPC} was detected in hospital sewage in Spain [150], Tunisia [151], Germany [76], Belgium [152] and India [153]. The KPC-encoding gene was also detected in rivers in Spain [150], India [154], Brazil [26,155], China [156] and Belgium [152], streams and lagoon water in Brazil [26,42], lake water in Poland [157] and Brazil [158], a fishpond and pig wastewater in China [159,160], municipal wastewater in India [154], Germany [161] and Belgium [152], and drinking water from a first nation community in Canada [162] and Brazil [155]. The *bla*_{GES-5} and *bla*_{GES-16} carbapenemase-encoding genes were also detected by culture-independent methods in WWTP and lagoon water, respectively [42,161]. In addition, *bla*_{IMI} was detected in lake water in Poland [157] and in river sediments in China [163]. Finally, *bla*_{SFC-1} was detected in lake water in Sweden [164].

Among the class B carbapenemase determinants, *bla*_{GIM}, *bla*_{NDM}, *bla*_{IMP}, *bla*_{SPM} and *bla*_{VIM} genes were detected in different water habitats, including hospital sewage, wastewater and WWTPs, animal waste, rivers, lakes, rice field, drinking water and drinking water treatment plants. Detection of these MBL-encoding genes in the abovementioned environments was reported from many countries, namely Spain [150], Tunisia [151], China [43,156,165–168], India [153,154,169,170], Italy [171], Brazil [26,42,155,158], Canada [162,172], Switzerland [173], Belgium [152], Singapore [174], the USA [158], Poland [157,175], Germany [176] and Sweden [164].

Regarding class D carbapenemases, *bla*_{OXA-48} determinants were detected by culture-independent methods in hospital sewage, wastewater and WWTPs, rivers, creeks, lakes, rice field and drinking water from Spain [150], Germany [176], Tunisia [151], India [153,154], Brazil [26,158], Belgium [152], Canada [162,172], Portugal [177], Poland [175] and Sweden [164,178]. In addition, the OXA-23-encoding gene has been detected in hospital sewage in Sweden [164]. Recently, *bla*_{OXA-58} was detected in drinking water in Germany [179] and hospital sewage in India [153].

4. Multilocus sequence typing (MLST) analysis

MLST is a widely used method for typing bacterial strains. It was described in 1998 and consists of the examination of nucleotide sequences of seven housekeeping gene fragments of approximately 500 nucleotides. Since then, this method has been used for different purposes including epidemiological surveillance and population analysis [180,181].

Among carbapenemase-producing bacteria isolated from aquatic environments, *K. pneumoniae*, *E. coli* and *E. cloacae* are the most characterised species by MLST. PHYLOViZ Online [182] was used for conducting phylogenetic analysis of sequence types (STs) of *K. pneumoniae*, *E. coli* and *E. cloacae* detected in aquatic environments. Our analysis revealed a remarkable diversity of STs detected, especially regarding *K. pneumoniae* and *E. coli*. Phylogenetic analysis of *K. pneumoniae*, *E. coli* and *E. cloacae* STs with the harboured carbapenemase class and their geographical areas are presented in Fig. 5. Regarding *K. pneumoniae*, all of the major epidemic high-risk international clones, namely ST11, ST15, ST101, ST147 and ST258 [183], have been detected in different countries. Of note, carbapenemases of Ambler classes A and D have been detected in ST258, known to be the most globally disseminated. Regarding *E. coli*, several international high-risk clones such as ST38, ST131, ST410 and ST648 [184] have been detected in water environments. ST38 has been largely associated with Ambler class D carbapenemases (OXA-48). This was also the case in water isolates, where all *E. coli* ST38 isolates harboured the *bla*_{OXA-48} gene as shown in Fig. 5. In contrast, STs of *E. cloacae* are mostly associated with Ambler class A carbapenemases and, unlike *K. pneumoniae* and *E. coli*, none of the most prevalent and widespread *E. cloacae* clones were detected in aquatic environments.

In addition, we investigated the isolation of carbapenemase-producing *K. pneumoniae*, *E. coli* and *E. cloacae* clones detected in water environments from human infections in the respective countries and the results are also shown in Fig. 5. Several carbapenemase-producing clones of *K. pneumoniae* and *E. coli* have been reported to cause human infections in countries where they were detected in aquatic environments [83,185–199]. Indeed, detection of the same clones with the same resistance mechanism in the same geographical area both in clinical and water isolates might be of great importance. This foretells the danger that the presence of these organisms in water can cause and suggests the potential participation of aquatic environments in the dissemination of these bacteria.

5. Conclusion

Carbapenems are among our last-resort antibiotics against drug-resistant pathogens, making carbapenem resistance a great health concern, especially that due to carbapenemase production. Knowledge of environmental reservoirs of resistant organisms and resistance genes is crucial in our quest to control their dissemination. The data presented here confirm the wide dissemination of carbapenemase-producers and carbapenemase-encoding genes in the natural environment and other water habitats, presenting a serious problem for human and animal health. In this review, we aimed to give an overview of the state of the art regarding the spread of carbapenemase-producing bacteria in different aquatic environments, which may help in implementing prevention and control strategies. Indeed, the interconnectedness between the environment and the health of humans, animals and plants makes the surveillance and control of the antibiotic resistance phenomenon a very difficult task. Hence the urgent need for an interdisciplinary collaboration to establish effective control and prevention strategies against the spread of carbapenemase-producing bacteria. In this context, the US National Action Plan for Combating Antibiotic-Resistant Bacteria (CARB) was created and will be followed over 5 years (2020–2025) in order to change the course of antibiotic resistance [https://aspe.hhs.gov/system/files/pdf/264126/CARB-National-Action-Plan-2020-2025.pdf]. The main goals of CARB are (i) slowing the emergence of resistant bacteria and preventing the spread of resistant infections, (ii) strengthening 'One Health' surveillance efforts to combat bacterial resistance, (iii) development and use of rapid and innovative diagnostic tools, (iv) development of new antibiotics, other therapies and vaccines and (v) improving international collaboration for prevention, surveillance, control, research and development of antibiotics. This plan integrates in parallel a 'One Health' approach with special emphasis on understanding antibiotic resistance in the environment. The application of such plans in other countries could help in the control of spread of drug-resistant bacteria. On the other hand, the application of obligatory reporting of antibiotic resistance in veterinary and human clinical settings, and possibly in water treatment facilities, will enable countries that do not have such surveillance plans assess contemporary prevention measures and target the areas of greatest concern [200]. In addition, it seems clear that wastewater, whatever its origin, is the main reservoir of resistant bacteria among all aquatic environments. Consequently, it should be a primary target for control and prevention efforts. Thus, developing effective wastewater treatment methods for removing or at least decreasing antibiotic-resistant bacteria and antibiotic resistance genes in the final effluent is strongly recommended.

Acknowledgments: This work was supported by the French Government under the 'Investments for the Future' program man-

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