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Thème

Étude phénotypique et moléculaire de la résistance aux antibiotiques du dernier recours (Carbapénèmes et Colistine) chez les bacilles à Gram négatif isolés à partir de l'eau : cas de la région de Batna

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بِسْمِ ٱللَّهِ ٱلرَّحْمَٰنِ ٱلرَّحِيمِ

قَالُوا۟ سُبْحَٰنَكَ لَا عِلْمَ لَنَآ إِلَّا مَا عَلَّمْتَنَآ ۖ إِنَّكَ
أَنتَ ٱلْعَلِيمُ ٱلْحَكِيمُ ﴿٣٢﴾

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Sommaire

Introduction générale

Pendant quelques décennies après leur introduction, les antibiotiques semblent avoir résolu le problème des maladies infectieuses bactériennes pour toujours **(Berglund, 2015)**. Néanmoins, le miracle de ces médicaments a été de plus en plus menacé par l'apparition, la dissémination et la persistance des souches résistantes. L'émergence des bactéries résistantes a conduit à une utilisation parfois anarchique et/ou abusive des antibiotiques de dernier recours à savoir les carbapénèmes et la colistine concernant les bactéries à Gram négatif. Par conséquent, cette utilisation inappropriée a conduit à l'émergence des souches résistantes aux antibiotiques de dernier recours **(Maltezou, 2009)**.

La résistance aux antibiotiques constitue aujourd'hui l'une des plus graves menaces pesant sur la santé mondiale, la sécurité alimentaire et le développement **(Zhang *et al.*, 2019)**. La commission européenne a affirmé que les coûts liés aux infections bactériennes résistantes s'élevaient à 1,5 milliards d'euros par an. Ainsi, les systèmes de santé aux États-Unis estiment que le coût supplémentaire des infections résistantes aux antimicrobiens s'élèverait à 20 milliards de dollar Américain par an et que les pertes de productivité s'élèveraient à 35 milliards de dollar Américain par an. D'autre part, une étude réalisée au Royaume-Uni sur la résistance aux antimicrobiens, a estimé que 10 millions de personnes décéderaient chaque année (au niveau mondial) d'infections résistantes aux antimicrobiens d'ici à 2050, dont les coûts totaux s'élèveraient à 100 trillions de dollars Américain **(Maestre-Carballa *et al.*, 2019)**. La discussion sur ce phénomène de résistance aux antimicrobiens est souvent axée sur les résultats pour la santé humaine. Cependant, une prise en compte plus large des impacts sur la santé animale et l'environnement est essentielle.

Bien que la résistance aux antibiotiques soit l'une des plus grandes menaces de santé publique, sa surveillance a longtemps été focalisée sur les milieux cliniques **(Manaia *et al.*, 2016)**. Cependant, depuis que **Pruden *et al.* (2006)** considéraient les gènes de résistance aux antibiotiques comme des polluants biologiques émergents, la contamination de l'environnement par les gènes de résistance et les bactéries résistantes aux antibiotiques a suscité une prise de conscience considérable.

La préservation de l'environnement est devenue une préoccupation mondiale majeure. Elle consiste à prendre des mesures strictes pour réduire voire supprimer l'impact négatif des activités humaines polluantes. Cette action est avant tout une action scientifique car elle nécessite d'identifier d'abord les différents types de polluants qui peuvent être d'origine chimique, physique ou biologique **(Hernando-Amado *et al.*, 2019)**. Parmi les polluants

biologiques on trouve les microorganismes y compris les bactéries pathogènes présentant différents niveaux de virulence et de résistance aux antibiotiques. Ces dernières peuvent présenter un vrai danger surtout lorsqu'elles disposent de mécanismes leur conférant un niveau élevé de résistance aux antibiotiques particulièrement ceux de dernier recours. Ces mécanismes peuvent être portés par des éléments génétiques mobiles facilitant leur large dissémination **(Diene et Rolain, 2014)**.

En effet, les milieux aquatiques représentent l'un des habitats microbiens les plus importants sur notre planète jouant ainsi le rôle de réservoirs et de vecteurs à travers lesquels les microorganismes y compris ceux présentant des niveaux élevés de résistance aux antibiotiques sont largement disséminés entre l'environnement naturel, les humains et les animaux **(Manaia *et al.*, 2016; Vaz-Moreira *et al.*, 2014; Zhang *et al.*, 2009)**. Ce phénomène est amplifié suite aux déversements dans l'environnement des eaux usées de différentes provenances, à savoir celles liées aux milieux cliniques où l'utilisation des antibiotiques est parfois abusive et au domaine agrovétérinaire connu par l'utilisation massive de ces molécules. D'autre part, l'un des grands défis auxquels l'humanité est confrontée est la pénurie d'eau. Ce problème affecte particulièrement les régions arides et semi-arides dans de nombreuses régions du monde telles que le Moyen-Orient, l'Afrique, l'Asie du Sud et l'Europe du Sud. Malheureusement, l'eau douce dans ces régions est insuffisante pour l'irrigation et la réutilisation des eaux usées traitées ou même non traitées reste la seule solution **(Gatica et Cytryn, 2013)**, contribuant ainsi à la dissémination des bactéries résistantes.

De plus, ces milieux représentent un environnement idéal pour les échanges génétiques horizontaux des mécanismes responsables de l'antibiorésistance **(Manaia *et al.*, 2016)**. Ce phénomène est aujourd'hui considéré comme un problème écologique et un sujet extrêmement complexe à la croisée des chemins entre la santé humaine, la santé des animaux, la préservation des végétaux, la sécurité alimentaire et la protection de l'environnement **(Huijbers *et al.*, 2019)**.

Étant donné que les efforts d'un seul secteur ne peuvent pas contenir ce problème, récemment en Septembre 2017 l'organisation mondiale de la santé a annoncé l'approche « un monde, une santé » dans laquelle la lutte contre la résistance aux antimicrobiens est l'un de ses domaines les plus pertinents. Dans cette approche, de nombreux professionnels aux compétences multiples de différents secteurs tels que la santé et l'environnement devraient

mettre en œuvre des interventions conjointes pour répondre à cette menace qui pèse sur la santé mondiale **(One Health Commission, 2019)**.

Dans le contexte de cette approche, et au vu de la rareté d'informations concernant la dissémination de tels organismes dans l'environnement en Algérie en général, et particulièrement dans la région de Batna, nous nous sommes intéressés par l'étude de la résistance aux antibiotiques de dernier recours chez les bacilles à Gram négatif dans l'eau de différentes sources. Ce travail a pour objectif de définir la vraie situation de l'antibiorésistance ainsi qu'identifier ses principaux mécanismes en question au niveau de certains milieux aquatiques de la ville de Batna (eau souterraine, eau de robinet, eaux usées hospitalières et eaux usées déchargées dans l'environnement).

Ainsi ce manuscrit s'articule sur trois chapitres présentés comme suit :

Chapitre I : consacré à une revue de littérature **(Article 1)** sur les mécanismes et l'épidémiologie mondiale de la résistance aux carbapénèmes *via* la production de carbapénémases chez les bactéries à Gram négatif dans les milieux aquatiques. Cette revue résume les données en question de tous les travaux scientifiques réalisés dans ce contexte et publiés jusqu'au 30 Avril 2020.

Chapitre II : consacré à une deuxième revue de littérature **(Article 2)** sur le principe et l'épidémiologie mondiale de la résistance à la colistine à médiation plasmidique (*mcr*) chez les bactéries à Gram négatif dans les milieux aquatiques. Cette revue rassemble les données en question de toutes les publications scientifiques réalisées dans ce contexte et parues avant le 28 Février 2021.

Chapitre III : consacré à la présentation de notre étude expérimentale portée sur la recherche des bactéries à Gram négatif résistantes aux carbapénèmes et à la colistine dans l'eau de différentes provenances au niveau de la ville de Batna. Cette étude a fait l'objet de trois publications (**Article 3, Article 4 et Article 5)**.

Références bibliographiques

Berglund, B. 2015. Environmental dissemination of antibiotic resistance genes and correlation to anthropogenic contamination with antibiotics. *Infection ecology & epidemiology* **5**: 28564. doi:10.3402/iee.v5.28564.

Diene, S.M. and Rolain, J.M. 2014. Carbapenemase genes and genetic platforms in Gram-negative bacilli: *Enterobacteriaceae*, *Pseudomonas* and *Acinetobacter* species. *Clinical microbiology and infection : the official publication of the European Society of Clinical Microbiology and Infectious Diseases* **20**(9): 831-838. doi:10.1111/1469-0691.12655.

Gatica, J. and Cytryn, E. 2013. Impact of treated wastewater irrigation on antibiotic resistance in the soil microbiome. *Environmental science and pollution research international* **20**(6): 3529-3538. doi:10.1007/s11356-013-1505-4.

Hernando-Amado, S., Coque, T.M., Baquero, F., and Martínez, J.L. 2019. Defining and combating antibiotic resistance from One Health and Global Health perspectives. *Nature microbiology* **4**(9): 1432-1442. doi:10.1038/s41564-019-0503-9.

Huijbers, P.M.C., Flach, C.-F., and Larsson, D.G.J. 2019. A conceptual framework for the environmental surveillance of antibiotics and antibiotic resistance. *Environment international* **130**: 104880. doi:https://doi.org/10.1016/j.envint.2019.05.074.

Maestre-Carballa, L., Lluesma Gomez, M., Angla Navarro, A., Garcia-Heredia, I., Martinez-Hernandez, F., and Martinez-Garcia, M. 2019. Insights into the antibiotic resistance dissemination in a wastewater effluent microbiome: bacteria, viruses and vesicles matter. *Environ Microbiol* **21**(12): 4582-4596. doi:10.1111/1462-2920.14758.

Maltezou, H.C. 2009. Metallo-beta-lactamases in Gram-negative bacteria: introducing the era of pan-resistance? *International journal of antimicrobial agents* **33**(5): 405 e401-407. doi:10.1016/j.ijantimicag.2008.09.003.

Manaia, C.M., Macedo, G., Fatta-Kassinos, D., and Nunes, O.C. 2016. Antibiotic resistance in urban aquatic environments: can it be controlled? *Applied microbiology and biotechnology* **100**(4): 1543-1557. doi:10.1007/s00253-015-7202-0.

One Health Commission, 2019. What is one health? Disponible on : https://www.onehealthcommission.org/en/why_one_health/what_is_one_health/, Consulté le 10 Juin 2021.

Pruden, A., Pei, R., Storteboom, H., and Carlson, K.H. 2006. Antibiotic resistance genes as emerging contaminants: studies in northern Colorado. *Environmental science & technology* **40**(23): 7445-7450.

Vaz-Moreira, I., Nunes, O.C., and Manaia, C.M. 2014. Bacterial diversity and antibiotic resistance in water habitats: searching the links with the human microbiome. *FEMS microbiology reviews* **38**(4): 761-778. doi:10.1111/1574-6976.12062.

Zhang, H., Wei, W., Huang, M., Umar, Z., and Feng, Y. 2019. Definition of a Family of Nonmobile Colistin Resistance (NMCR-1) Determinants Suggests Aquatic Reservoirs for MCR-4. *Advanced science (Weinheim, Baden-Wurttemberg, Germany)* **6**(11): 1900038. doi:10.1002/advs.201900038.

Zhang, X.X., Zhang, T., and Fang, H.H. 2009. Antibiotic resistance genes in water environment. *Applied microbiology and biotechnology* **82**(3): 397-414. doi:10.1007/s00253-008-1829-z.

Partie bibliographique

Chapitre I

Les bactéries à Gram négatif productrices des carbapénémases dans les milieux aquatiques

Suite au développement inquiétant du phénomène de la résistance aux antibiotiques, en 2016, l'Organisation Mondiale de la Santé (OMS) a été appelée par ses États membres à classer par ordre de priorité les bactéries résistantes aux antibiotiques nécessitant la recherche de nouvelles molécules efficaces. Ce qui est intéressant de noter, est que la priorité critique était réservée aux *Acinetobacter baumannii*, *Pseudomonas aeruginosa* et *Enterobacteriaceae* résistants aux carbapénèmes, en plus des *Enterobacteriaceae* résistantes aux céphalosporines de troisième génération **(Tacconelli *et al.*, 2018)**. Les carbapénèmes sont les β-lactamines présentant le spectre d'activité le plus large. L'imipénème, commercialisé pour la première fois en 1985, a été le premier carbapénème disponible pour le traitement des infections bactériennes **(Papp-Wallace *et al.*, 2011)**. Par la suite, plusieurs carbapénèmes ont été développés au cours des deux décennies suivantes. De nos jours, les plus utilisés cliniquement sont l'ertapénème, le méropénème, le doripénème et l'imipénème **(Potter *et al.*, 2016)**. Cependant, en moins d'une décennie après leur utilisation, les bactéries à Gram négatif (BGN) résistantes aux carbapénèmes sont apparues **(Maltezou, 2009)**. Du fait que les carbapénèmes sont les antibiotiques de choix et, dans de nombreux cas, le traitement de dernier recours de plusieurs infections bactériennes, l'émergence et la propagation de BGN résistantes aux carbapénèmes constituent une crise majeure de santé publique **(Diene et Rolain, 2014)**. La résistance aux carbapénèmes chez les BGN peut être conférée par divers mécanismes, notamment des changements quantitatifs et/ou qualitatifs de la perméabilité membranaire ou la modification de l'expression et/ou la fonction des porines *via* des mutations chromosomiques dans les gènes codant pour les pompes à efflux, l'association d'imperméabilité avec la production des β-lactamases à spectre élargie ou une surexpression des β-lactamases de classe C, ou *via* la production d'enzymes hydrolysant les carbapénèmes « carbapénémases », ce dernier représente le mécanisme le plus préoccupant de résistance aux carbapénèmes. Ces enzymes possèdent une capacité hydrolytique polyvalente et confèrent une résistance à la plupart des β-lactamines **(Bakthavatchalam *et al.*, 2016; Jean *et al.*, 2015; Nordmann *et al.*, 2012; Papp-Wallace *et al.*, 2011; Potter *et al.*, 2016)**.

Dans l'objectif de faciliter la mise en œuvre des stratégies de contrôle et de prévention contre la propagation dans l'environnement et à grande échelle des BGN productrices des carbapénémases *via* les milieux aquatiques, ce chapitre a été consacré à la réalisation d'une revue de littérature présentant les connaissances actuelles concernant les mécanismes responsables de la production des carbapénémases chez les BGN et leur épidémiologie mondiale dans les différents environnements aquatiques.

Références bibliographiques

Bakthavatchalam, Y.D., Anandan, S., and Veeraraghavan, B. 2016. Laboratory Detection and Clinical Implication of Oxacillinase-48 like Carbapenemase: The Hidden Threat. *Journal of global infectious diseases* **8**(1): 41-50. doi:10.4103/0974-777X.176149.

Diene, S.M. and Rolain, J.M. 2014. Carbapenemase genes and genetic platforms in Gram-negative bacilli: *Enterobacteriaceae, Pseudomonas* and *Acinetobacter* species. *Clinical microbiology and infection : the official publication of the European Society of Clinical Microbiology and Infectious Diseases* **20**(9): 831-838. doi:10.1111/1469-0691.12655.

Jean, S.S., Lee, W.S., Lam, C., Hsu, C.W., Chen, R.J., and Hsueh, P.R. 2015. Carbapenemase-producing Gram-negative bacteria: current epidemics, antimicrobial susceptibility and treatment options. *Future microbiology* **10**(3): 407-425. doi:10.2217/fmb.14.135.

Maltezou, H.C. 2009. Metallo-beta-lactamases in Gram-negative bacteria: introducing the era of pan-resistance? *International journal of antimicrobial agents* **33**(5): 405 e401-407. doi:10.1016/j.ijantimicag.2008.09.003.

Nordmann, P., Dortet, L., and Poirel, L. 2012. Carbapenem resistance in *Enterobacteriaceae*: here is the storm! *Trends in molecular medicine* **18**(5): 263-272. doi:10.1016/j.molmed.2012.03.003.

Papp-Wallace, K.M., Endimiani, A., Taracila, M.A., and Bonomo, R.A. 2011. Carbapenems: past, present, and future. *Antimicrobial agents and chemotherapy* **55**(11): 4943-4960. doi:10.1128/AAC.00296-11.

Potter, R.F., D'Souza, A.W., and Dantas, G. 2016. The rapid spread of carbapenem-resistant *Enterobacteriaceae*. *Drug resistance updates : reviews and commentaries in antimicrobial and anticancer chemotherapy* **29**: 30-46. doi:10.1016/j.drup.2016.09.002.

Tacconelli, E., Carrara, E., Savoldi, A., Harbarth, S., Mendelson, M., Monnet, D.L., Pulcini, C., Kahlmeter, G., Kluytmans, J., Carmeli, Y., Ouellette, M., Outterson, K., Patel, J., Cavaleri, M., Cox, E.M., Houchens, C.R., Grayson, M.L., Hansen, P., Singh, N., Theuretzbacher, U., Magrini, N., Aboderin, A.O., Al-Abri, S.S., Awang

Jalil, N., Benzonana, N., Bhattacharya, S., Brink, A.J., Burkert, F.R., Cars, O., Cornaglia, G., Dyar, O.J., Friedrich, A.W., Gales, A.C., Gandra, S., Giske, C.G., Goff, D.A., Goossens, H., Gottlieb, T., Guzman Blanco, M., Hryniewicz, W., Kattula, D., Jinks, T., Kanj, S.S., Kerr, L., Kieny, M.-P., Kim, Y.S., Kozlov, R.S., Labarca, J., Laxminarayan, R., Leder, K., Leibovici, L., Levy-Hara, G., Littman, J., Malhotra-Kumar, S., Manchanda, V., Moja, L., Ndoye, B., Pan, A., Paterson, D.L., Paul, M., Qiu, H., Ramon-Pardo, P., Rodríguez-Baño, J., Sanguinetti, M., Sengupta, S., Sharland, M., Si-Mehand, M., Silver, L.L., Song, W., Steinbakk, M., Thomsen, J., Thwaites, G.E., van der Meer, J.W.M., Van Kinh, N., Vega, S., Villegas, M.V., Wechsler-Fördös, A., Wertheim, H.F.L., Wesangula, E., Woodford, N., Yilmaz, F.O., and Zorzet, A. 2018. Discovery, research, and development of new antibiotics: the WHO priority list of antibiotic-resistant bacteria and tuberculosis. *The Lancet Infectious Diseases* **18**(3): 318-327. doi:10.1016/s1473-3099(17)30753-3.

Article 1

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

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

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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 giving 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.

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

(*continued on next page*)

Table 1 (*continued*)

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

(*continued on next page*)

Table 1 (*continued*)

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

(*continued on next page*)

Table 1 (*continued*)

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

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

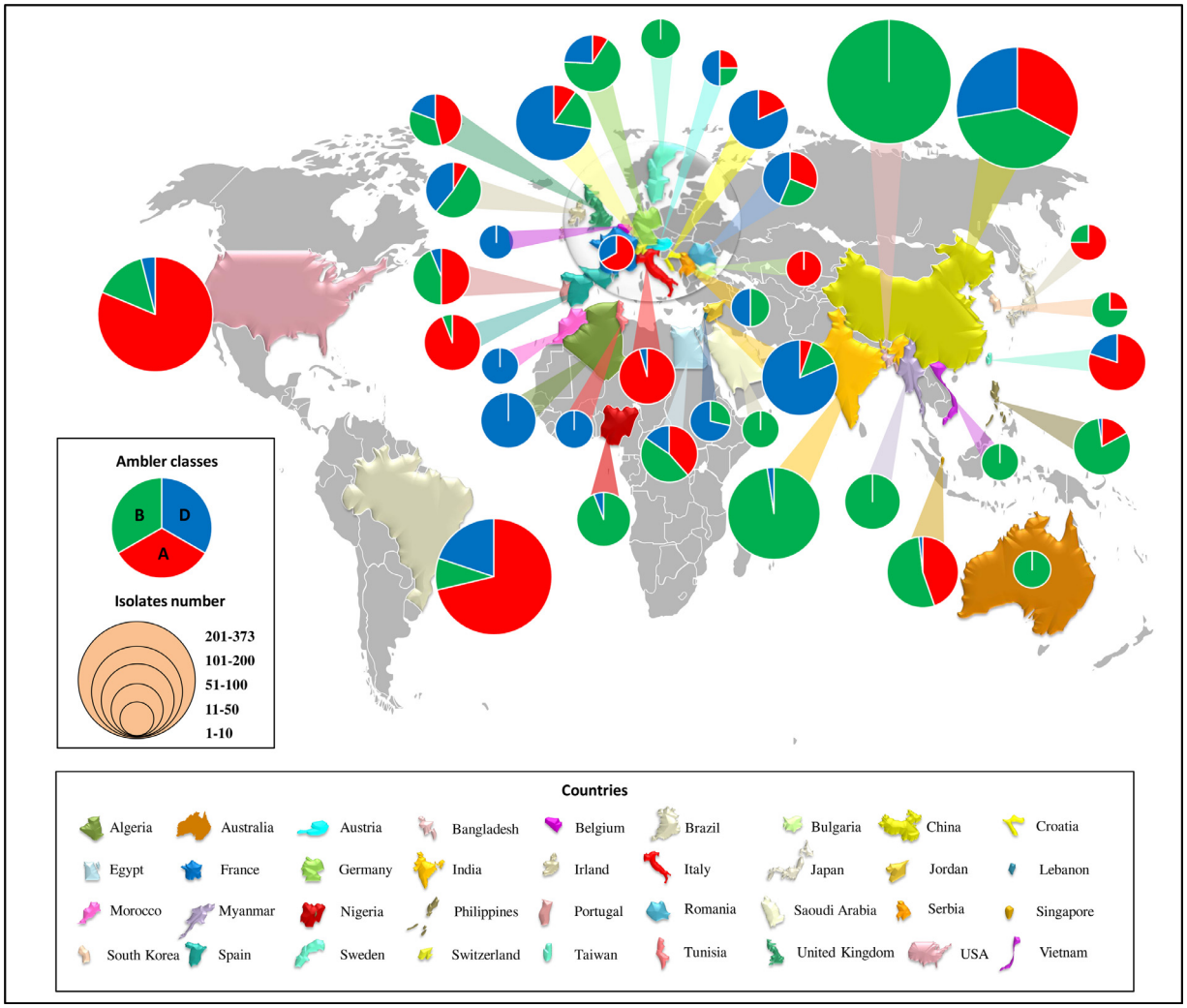

Fig. 1. Worldwide distribution of carbapenemase-producing Gram-negative bacteria in aquatic environments.

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. hydrophilia* 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 activity, 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-

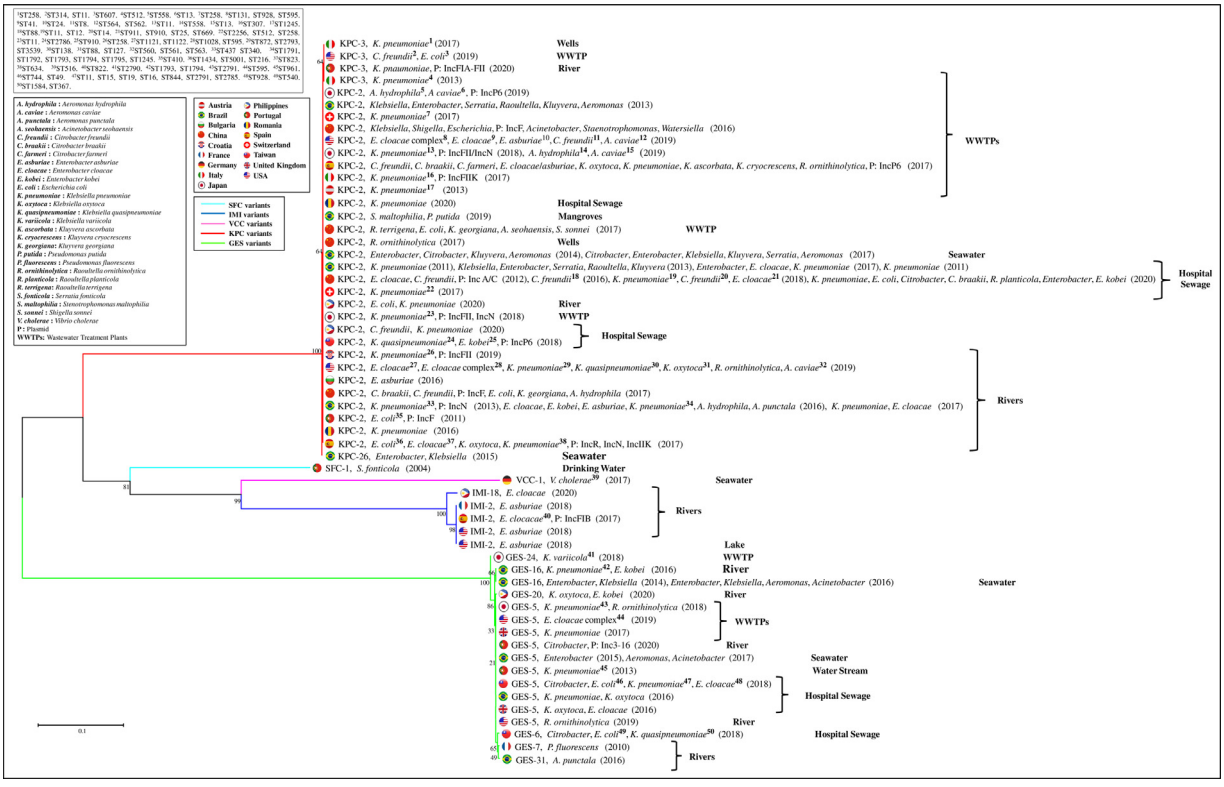

Fig. 2. Phylogenetic tree of class A carbapenemase variants detected in different aquatic environments. The evolutionary history was inferred using the neighbour-joining method. Evolutionary distances were computed using the Kimura 2-parameter method. The number above the nodes is the level of bootstrap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

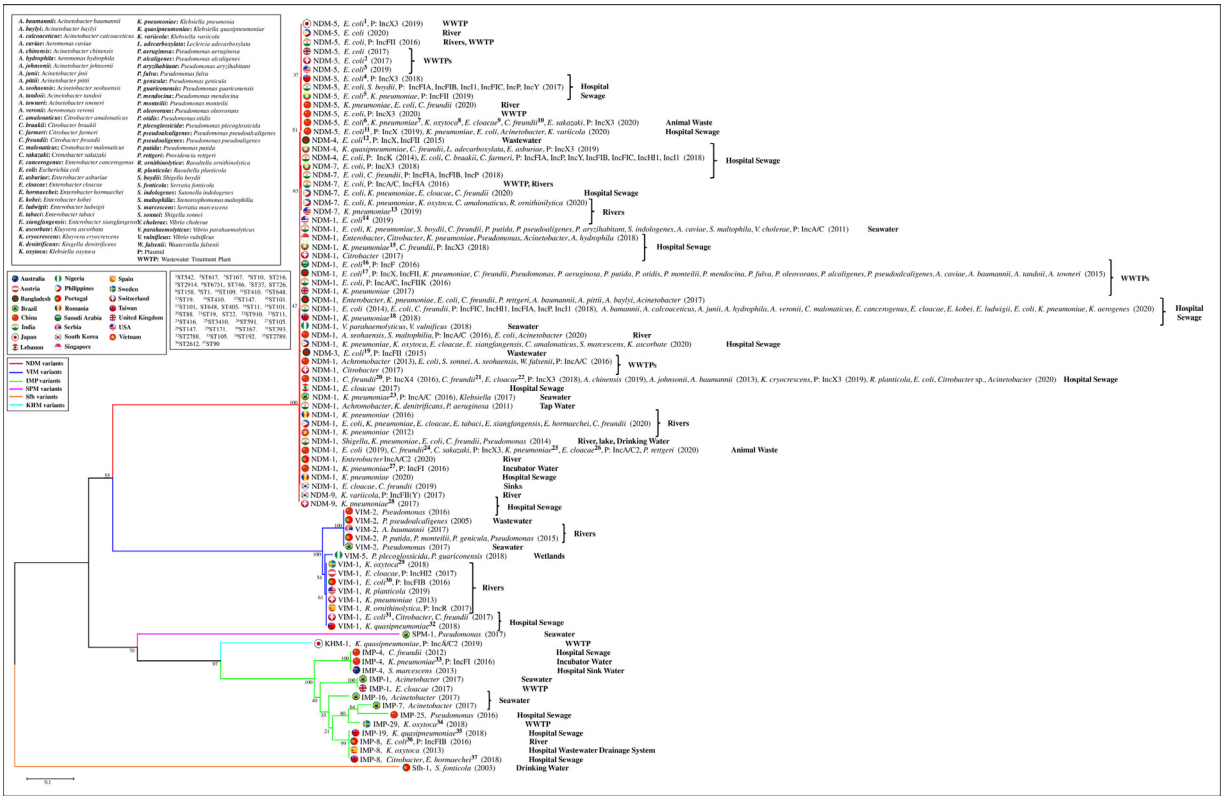

Fig. 3. Phylogenetic tree of class B carbapenemase variants detected in different aquatic environments. The evolutionary history was inferred using the neighbour-joining method. Evolutionary distances were computed using the Kimura 2-parameter method. The number above the nodes is the level of bootstrap 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	*Enterobacter cloacae* complex (1)	–	–	[76]
IMP	Ireland	Hospital sewage	*Klebsiella pneumoniae* (2)	ST3146	–	[32]
			Enterobacter cloacae complex (3)	–	–	[76]
	Singapore	Hospital sewage	*Aeromonas caviae* (1)	–	–	[74]
	USA	WWTP	*Escherichia coli* (1)	–	–	[30]
IMP-1	Brazil	Seawater	*Acinetobacter* (3)	–	–	[48]
	UK	WWTP	*Enterobacter cloacae* (1)	–	–	[63]
IMP-4	Australia	Hospital sink water	*Serratia marcescens* (4)	–	–	[104]
	China	Incubator water	*Klebsiella pneumoniae* (1)	ST105	IncFI	[83]
		Hospital sewage	*Citrobacter freundii* (1)	–	–	[38]
IMP-7	Brazil	Seawater	*Acinetobacter* (1)	–	–	[48]
IMP-8	Portugal	River	*Escherichia coli* (2)	ST2612	IncFIB	[106]
	Spain	Hospital WW drainage system	*Klebsiella oxytoca* (1)	–	–	[105]
	Taiwan	Hospital sewage	*Enterobacter hormaechei* (2)	ST90	–	[53]
			Citrobacter (1)	–	–	
IMP-16	Brazil	Seawater	*Acinetobacter* (4)	–	–	[48]
IMP-19	Taiwan	Hospital sewage	*Klebsiella quasipneumoniae* (1)	ST2789	–	[53]
IMP-25	China	Hospital sewage	*Pseudomonas* (1)	–	–	[116]
IMP-29	Sweden	WWTP	*Klebsiella oxytoca* (1)	ST192	–	[107]
KHM-1	Japan	WWTP	*Klebsiella quasipneumoniae* (1)	–	IncA/C2	[111]
NDM	Egypt	DW	*Klebsiella pneumoniae* (6)	–	–	[27]
	Germany	Clinical WW	*Enterobacter cloacae* complex (2), *Klebsiella oxytoca* (1)	–	–	[76]
		Clinical and urban WW	*Klebsiella quasivariicola* (1), *Klebsiella pneumoniae* (3)	–	–	
	Ireland	Hospital sewage	*Escherichia coli* (1)	ST617	–	[32]
		FSW, WW	*Escherichia coli* (5)	–	–	[77]
		Seawater	*Klebsiella pneumoniae* (11)	–	–	
	Jordan	DW	*Escherichia coli* (12)	–	–	[34]
	Singapore	Hospital sewage	*Enterobacter cloacae* (1), *Escherichia coli* (1), *Citrobacter freundii* (1)	–	–	[74]
	UK	Hospital sewage	*Enterobacter cloacae* complex (2), *Citrobacter freundii* (4)	–	–	[61]
NDM-1	Bangladesh	Hospital sewage	*Klebsiella pneumoniae* (46), *Escherichia coli* (30), *Citrobacter freundii* (2), *Providencia rettgeri* (1), *Enterobacter* (9), *Acinetobacter baumannii* (8), *Acinetobacter pittii* (4), *Acinetobacter baylyi* (1), *Acinetobacter* spp. (3)	–	–	[89]

(*continued on next page*)

Table 2 (*continued*)

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

(*continued on next page*)

Table 2 (*continued*)

Carbapenemase	Country	Source	Host(s) (*n*)	ST	Plasmid group	Reference
		WWTP	*Achromobacter* sp. (1)	–	–	[122]
			Escherichia coli (3), *Shigella sonnei* (2), *Acinetobacter seohaensis* (4), *Wautersiella falsenii* (1)	–	IncA/C	[43]
	India	Hospital sewage	*Escherichia coli* (1)	–	–	[80]
			Escherichia coli (1), *Citrobacter freundii* (7)	–	IncFIC, IncHI1, IncFIA, IncP, IncI1	[75]
			Acinetobacter baumannii (11), *Acinetobacter calcoaceticus* (1), *Acinetobacter junii* (1), *Aeromonas hydrophila* (2)	–	–	[95]
	India	Hospital sewage	*Aeromonas veronii* (2), *Cronobacter malonaticus* (1), *Enterobacter cancerogenus* (1), *Enterobacter cloacae* (1), *Enterobacter kobei* (1), *Enterobacter ludwigii* (1), *Escherichia coli* (14), *Klebsiella pneumoniae* (10), *Klebsiella aerogenes* (1)	–	–	[95]
		River, lake, DW	*Klebsiella pneumoniae* (3), *Shigella* (2), *Escherichia coli* (1), *Citrobacter freundii* (1), *Pseudomonas* (3)	–	–	[79]
		Seawater	*Pseudomonas putida* (2), *Pseudomonas pseudoalcaligenes* (2), *Pseudomonas oryzihabitans* (1), *Suttonella indologenes* (1), *Aeromonas caviae* (1), *Stenotrophomonas maltophilia* (1), *Vibrio cholerae* (1)	–	IncA/C	[78]
		Tap water	*Achromobacter* spp. (2), *Kingella denitrificans* (1), *Pseudomonas aeruginosa* (1)	–	–	
		WWTP	*Escherichia coli* (2)	–	IncA/C, IncFIIK	[87]
	Lebanon	Hospital sewage	*Enterobacter cloacae* (2)	–	–	[90]
	Myanmar	Hospital sewage	*Citrobacter freundii* (1)	–	IncX3	[91]
			Klebsiella pneumoniae (1)	ST147	IncX3	
	Nigeria	Seawater	*Vibrio parahaemolyticus* (1), *Vibrio vulnificus* (5)	–	–	[123]

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Table 2 (*continued*)

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

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Table 2 (*continued*)

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

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Table 2 (*continued*)

Carbapenemase	Country	Source	Host(s) (*n*)	ST	Plasmid group	Reference
	Germany	WWTP	*Pseudomonas aeruginosa* (1), *Escherichia coli* (3)	–	–	[108]
	Ireland	Hospital sewage	*Klebsiella oxytoca* (1)	ST202	–	[32]
			Enterobacter cloacae complex (1)	–	–	
	Jordan	DW	*Escherichia coli* (75)	–	–	[34]
	Switzerland	WWTP	*Enterobacter aerogenes* (1)	–	–	[46]
	USA	WWTP	*Escherichia coli* (36)	–	–	[30]
VIM-1	Austria	River	*Enterobacter cloacae* (1)	–	IncHI2	[88]
	Portugal	River	*Escherichia coli* (1)	ST167	IncFIB	[106]
	Spain	River	*Raoultella ornithinolytica* (1)	–	IncR	[24]
	Sweden	River	*Klebsiella oxytoca* (1)	ST172	–	[107]
	Switzerland	Hospital sewage	*Citrobacter* (1), *Citrobacter freundii* (1)	–	–	[46]
			Escherichia coli (1)	ST393	–	
		River	*Klebsiella pneumoniae* (1)	–	–	[109]
	Taiwan	Hospital sewage	*Klebsiella quasipneumoniae* (1)	ST2788	–	[53]
	USA	River	*Raoultella planticola* (1)	–	–	[57]
VIM-2	Brazil	Seawater	*Pseudomonas* (2)	–	–	[48]
	China	Hospital sewage	*Pseudomonas* (1)	–	–	[116]
	Portugal	River	*Pseudomonas putida* (1), *Pseudomonas geniculata* (1), *Pseudomonas monteilii* (1) *Pseudomonas* sp. (2)	–	–	[119]
		Wastewater	*Pseudomonas pseudoalcaligenes* (1)	–	–	[118]
	Serbia	River	*Acinetobacter baumannii* (2)	–	–	[120]
VIM-5	Nigeria	Wetlands	*Pseudomonas plecoglossicida* (6), *Pseudomonas guariconensis* (4)	–	–	[121]
VIM-34	Portugal	River	*Escherichia coli* (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 oryzihabitans* 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	*Acinetobacter baumannii* (2)	–	–	[131]
	Brazil	Hospital sewage	*Acinetobacter baumannii* (3)	–	–	[138]
		Seawater	*Acinetobacter* spp. (11)	–	–	[48]
	Croatia	Hospital sewage	*Acinetobacter baumannii* (2)	–	–	[139]
		Liquid manure	*Acinetobacter baumannii* (2)	ST195	–	[147]
		WWTP	*Acinetobacter baumannii* (10)	–	–	[141]
			Acinetobacter baumannii (1)	–	–	[143]
			Acinetobacter baumannii (10)	ST2	–	[145]
	France	River	*Acinetobacter baumannii* (1)	–	–	[137]
	Romania	River	*Acinetobacter baumannii* (2)	–	–	[120]
	Serbia	River	*Acinetobacter baumannii* (2)	–	–	[119]
OXA-24/40	Brazil	Seawater	*Acinetobacter* spp. (6)	–	–	[48]
	Croatia	WWTP	*Acinetobacter baumannii* (3)	–	–	[141]
	Nigeria	Surface water	*Acinetobacter baumannii* (1)	–	–	[146]
	Serbia	River	*Acinetobacter baumannii* (1)	–	–	[119]
OXA-48	Algeria	Fountain water	*Raoultella ornithinolytica* (1)	–	IncL	[129]
		Irrigation water	*Klebsiella pneumoniae* (1)	ST1393		
	Algeria	River	*Klebsiella pneumoniae* (3)	ST133, ST2055, ST2192	–	[126]
			Raoultella ornithinolytica (3), *Citrobacter braakii* (1), *Citrobacter freundii* (1)	–		
			Escherichia coli (9)	ST559, ST38, ST212, ST1972, ST2142		
			Klebsiella pneumoniae (1)	ST13	IncL	[129]
			Escherichia coli (2)	ST5, ST19		
			Raoultella ornithinolytica (1)	–		
		Seawater	*Cronobacter malonaticus* (1)	–		
		Spring water	*Cronobacter malonaticus* (1)	–		
		Dam water	*Klebsiella pneumoniae* (1)	ST1878		
		WW	*Klebsiella pneumoniae* (2)	ST1393, ST13		
			Enterobacter cloacae (1)	ST527		
		WWTP	*Klebsiella pneumoniae* (1)	ST35		
	Austria	WWTP	*Escherichia coli* (1)	ST38	–	[39]
			Klebsiella pneumoniae (1)	ST15	–	
	Belgium	Hospital WW (sinks)	*Citrobacter freundii* (5)	–	–	[130]
	Egypt	DW	*Klebsiella pneumoniae* (2)	–	–	[27]
	Germany	Clinical and urban WW	*Klebsiella pneumoniae* (3)	ST253, ST11	–	[76]
		WWTP	*Escherichia coli* (3)	–	–	[108]
	India	Hospital sewage	*Citrobacter braakii* (2), *Citrobacter farmeri* (1)	–	IncFIC, IncP, IncHI1	[75]
	Ireland	Hospital sewage	*Enterobacter cloacae* complex (6), *Citrobacter freundii* (3)	–	–	[32]
			Klebsiella pneumoniae (2)	ST3145, ST323	–	
			Klebsiella oxytoca (1)	ST95	–	
	Lebanon	Estuaries	*Escherichia coli* (1)	ST354	IncL	[127]
			Klebsiella pneumoniae (1)	ST16	IncL	
	Morocco	Puddles	*Serratia marcescens* (2)	–	–	[125]
	Philippines	River	*Escherichia coli* (1)	–	–	[64]
	Portugal	River	*Shewanella xiamenensis* (1)	–	–	[140]
	Romania	Hospital sewage	*Klebsiella pneumoniae* (12)	–	–	[97]

(*continued on next page*)

Table 3 (*continued*)

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

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

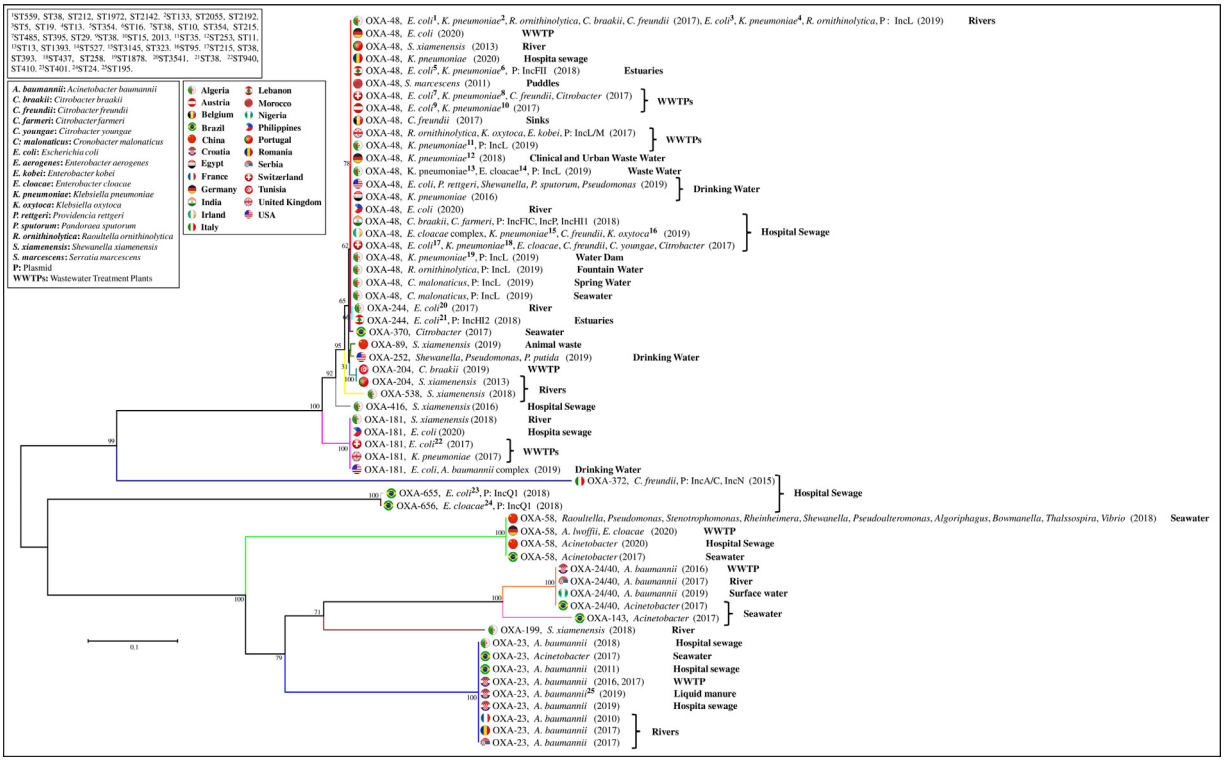

Fig. 4. Phylogenetic tree of class D carbapenemase variants detected in different aquatic environments. The evolutionary history was inferred using the neighbour-joining method. Evolutionary distances were computed using the Kimura 2-parameter method. The number above the nodes is the level of bootstrap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

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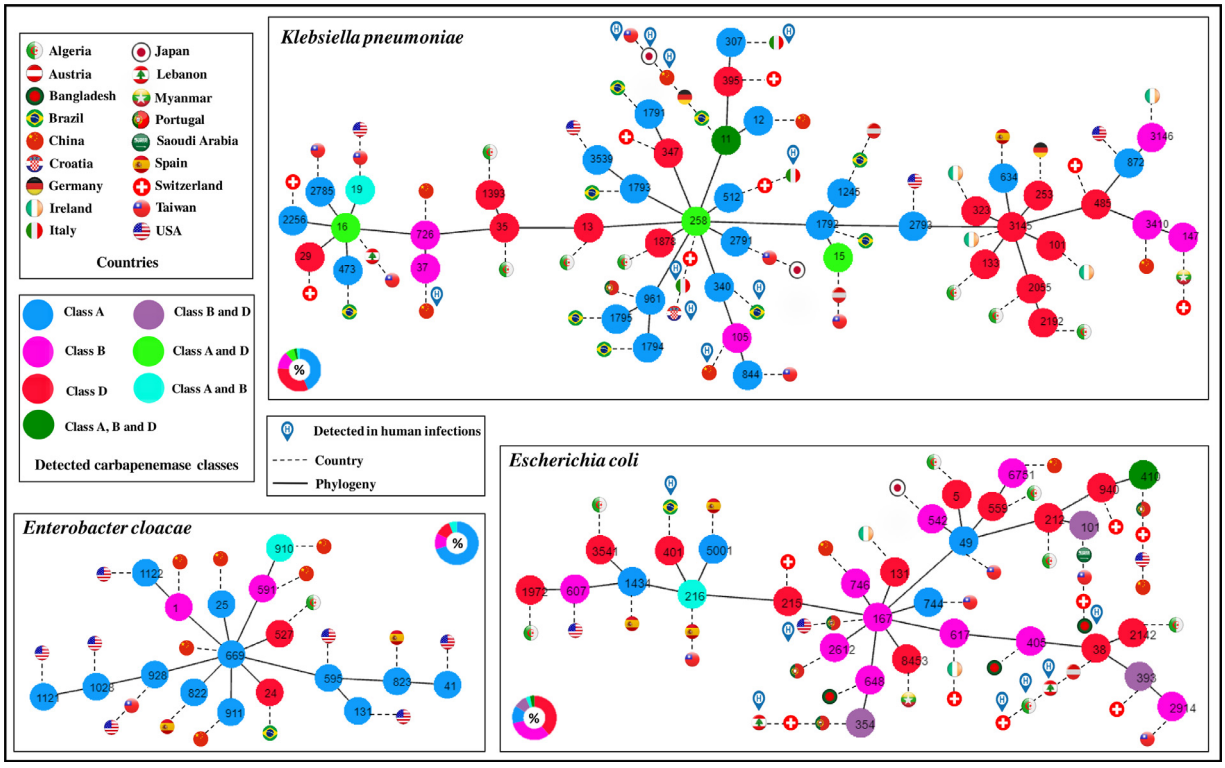

Fig. 5. Multilocus sequence typing (MLST) data set generated using PHYLOViZ Online, indicating the sequence types (STs) of carbapenemase-producing *Klebsiella pneumoniae*, *Escherichia coli* and *Enterobacter cloacae* detected in aquatic environments with the respective carbapenemase class and geographical area.

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Ethical approval: Not required.

References

[1] Tacconelli E, Carrara E, Savoldi A, et al. Discovery, research, and development of new antibiotics: the WHO priority list of antibiotic-resistant bacteria and tuberculosis. Lancet Infect Dis 2018;18:318–27.
[2] Papp-Wallace KM, Endimiani A, Taracila MA, et al. Carbapenems: past, present, and future. Antimicrob Agents Chemother 2011;55:4943–60.
[3] Potter RF, D'Souza AW, Dantas G. The rapid spread of carbapenem-resistant Enterobacteriaceae. Drug Resist Updat 2016;29:30–46.
[4] Maltezou HC. Metallo-β-lactamases in Gram-negative bacteria: introducing the era of pan-resistance? Int J Antimicrob Agents 2009;33 405.e1–7.
[5] Diene SM, Rolain JM. Carbapenemase genes and genetic platforms in Gram-negative bacilli: Enterobacteriaceae, *Pseudomonas* and *Acinetobacter* species. Clin Microbiol Infect 2014;20:831–8.
[6] Nordmann P, Dortet L, Poirel L. Carbapenem resistance in Enterobacteriaceae: here is the storm!. Trends Mol Med 2012;18:263–72.
[7] Bakthavatchalam YD, Anandan S, Veeraraghavan B. Laboratory detection and clinical implication of oxacillinase-48 like carbapenemase: the hidden threat. J Glob Infect Dis 2016;8:41–50.
[8] Jean SS, Lee WS, Lam C, et al. Carbapenemase-producing Gram-negative bacteria: current epidemics, antimicrobial susceptibility and treatment options. Future Microbiol 2015;10:407–25.
[9] Berglund B. Environmental dissemination of antibiotic resistance genes and correlation to anthropogenic contamination with antibiotics. Infect Ecol Epidemiol 2015;5:28564.
[10] Vaz-Moreira I, Nunes OC, Manaia CM. Bacterial diversity and antibiotic resistance in water habitats: searching the links with the human microbiome. FEMS Microbiol Rev 2014;38:761–78.
[11] Zhang XX, Zhang T, Fang HH. Antibiotic resistance genes in water environment. Appl Microbiol Biotechnol 2009;82:397–414.
[12] Manaia CM, Macedo G, Fatta-Kassinos D, et al. Antibiotic resistance in urban aquatic environments: can it be controlled? Appl Microbiol Biotechnol 2016;100:1543–57.
[13] Tamma PD, Simner PJ. Phenotypic detection of carbapenemase-producing organisms from clinical isolates. J Clin Microbiol 2018;56:e011470 -18.
[14] Bush K, Jacoby GA. Updated functional classification of β-lactamases. Antimicrob Agents Chemother 2010;54:969–76.
[15] Yoo JH. The Infinity War: how to cope with carbapenem-resistant Enterobacteriaceae. J Korean Med Sci 2018;33:e255.
[16] Oueslati S, Girlich D, Dortet L, et al. Evaluation of the Amplidiag CarbaR+VRE Kit for accurate detection of carbapenemase-producing bacteria. J Clin Microbiol 2018;56:e01092 -17.
[17] Walther-Rasmussen J, Hoiby N. Class A carbapenemases. J Antimicrob Chemother 2007;60:470–82.
[18] Iovleva A, Doi Y. Carbapenem-resistant Enterobacteriaceae. Clin Lab Med 2017;37:303–15.
[19] Nordmann P, Poirel L. The difficult-to-control spread of carbapenemase producers among Enterobacteriaceae worldwide. Clin Microbiol Infect 2014;20:821–30.
[20] Henriques I, Moura A, Alves A, et al. Molecular characterization of a carbapenem-hydrolyzing class A β-lactamase, SFC-1, from *Serratia fonticola* UTAD54. Antimicrob Agents Chemother 2004;48:2321–4.
[21] Aubron C, Poirel L, Ash RJ, et al. Carbapenemase-producing Enterobacteriaceae, U.S. rivers. Emerg Infect Dis 2005;11:260–4.
[22] Harmon DE, Miranda OA, McCarley A, et al. Prevalence and characterization of carbapenem-resistant bacteria in water bodies in the Los Angeles–Southern California area. Microbiologyopen 2018;8:e00692.
[23] Laurens C, Jean-Pierre H, Licznar-Fajardo P, et al. Transmission of IMI-2 carbapenemase-producing Enterobacteriaceae from river water to human. J Glob Antimicrob Resist 2018;15:88–92.
[24] Piedra-Carrasco N, Fabrega A, Calero-Caceres W, et al. Carbapenemase-producing Enterobacteriaceae recovered from a Spanish river ecosystem. PLoS One 2017;12:e0175246.
[25] Montezzi LF, Campana EH, Correa LL, et al. Occurrence of carbapenemase-producing bacteria in coastal recreational waters. Int J Antimicrob Agents 2015;45:174–7.
[26] Sanchez DG, de Melo FM, Savazzi EA, et al. Detection of different β-lactamases encoding genes, including bla_{NDM}, and plasmid-mediated quinolone resistance genes in different water sources from Brazil. Environ Monit Assess 2018;190:407.
[27] Hamza E, Dorgham SM, Hamza DA. Carbapenemase-producing *Klebsiella pneumoniae* in broiler poultry farming in Egypt. J Glob Antimicrob Resist 2016;7:8–10.
[28] Haller L, Chen H, Ng C, et al. Occurrence and characteristics of extended-spectrum β-lactamase- and carbapenemase-producing bacteria from hospital effluents in Singapore. Sci Total Environ 2018;615:1119–25.
[29] Weingarten RA, Johnson RC, Conlan S, et al. Genomic analysis of hospital plumbing reveals diverse reservoir of bacterial plasmids conferring carbapenem resistance. mBio 2018;9:e02011 -17.
[30] Hoelle J, Johnson JR, Johnston B, et al. Survey of US wastewater for carbapenem-resistant Enterobacteriaceae. J Water Health 2019;17:219–26.
[31] Haberecht HB, Nealon NJ, Gilliland JR, et al. Antimicrobial-resistant *Escherichia*

coli from environmental waters in Northern Colorado. J Environ Public Health 2019;2019:3862949.
[32] Cahill N, O'Connor L, Mahon B, et al. Hospital effluent: a reservoir for carbapenemase-producing Enterobacterales? Sci Total Environ 2019;672:618–24.
[33] Sakkas H, Bozidis P. Antimicrobial resistance in bacterial pathogens and detection of carbapenemases in *Klebsiella pneumoniae* isolates from hospital wastewater. Antibiotics (Basel) 2019;8:85.
[34] Swedan S, Abu Alrub H. Antimicrobial resistance, virulence factors, and pathotypes of *Escherichia coli* isolated from drinking water sources in Jordan. Pathogens 2019;8:86.
[35] Jung J, Choi HS, Lee JY, et al. Outbreak of carbapenemase-producing Enterobacteriaceae associated with a contaminated water dispenser and sink drains in the cardiology units of a Korean hospital. J Hosp Infect 2020;104:476–83.
[36] Poirel L, Barbosa-Vasconcelos A, Simoes RR, et al. Environmental KPC-producing *Escherichia coli* isolates in Portugal. Antimicrob Agents Chemother 2012;56:1662–3.
[37] Chagas TP, Seki LM, da Silva DM, et al. Occurrence of KPC-2-producing *Klebsiella pneumoniae* strains in hospital wastewater. J Hosp Infect 2011;77:281.
[38] Zhang X, Lu X, Zong Z. Enterobacteriaceae producing the KPC-2 carbapenemase from hospital sewage. Diagn Microbiol Infect Dis 2012;73:204–6.
[39] Galler H, Feierl G, Petternel C, et al. KPC-2 and OXA-48 carbapenemase-harbouring Enterobacteriaceae detected in an Austrian wastewater treatment plant. Clin Microbiol Infect 2014;20:O132–4.
[40] Picao RC, Cardoso JP, Campana EH, et al. The route of antimicrobial resistance from the hospital effluent to the environment: focus on the occurrence of KPC-producing *Aeromonas* spp. and Enterobacteriaceae in sewage. Diagn Microbiol Infect Dis 2013;76:80–5.
[41] Oliveira S, Moura RA, Silva KC, et al. Isolation of KPC-2-producing *Klebsiella pneumoniae* strains belonging to the high-risk multiresistant clonal complex 11 (ST437 and ST340) in urban rivers. J Antimicrob Chemother 2014;69:849–52.
[42] de Araujo CF, Silva DM, Carneiro MT, et al. Detection of carbapenemase genes in aquatic environments in Rio de Janeiro, Brazil. Antimicrob Agents Chemother 2016;60:4380–3.
[43] Yang F, Mao D, Zhou H, et al. Prevalence and fate of carbapenemase genes in a wastewater treatment plant in Northern China. PLoS One 2016;11:e0156383.
[44] Kittinger C, Lipp M, Folli B, et al. Enterobacteriaceae isolated from the River Danube: antibiotic resistances, with a focus on the presence of ESBL and carbapenemases. PLoS One 2016;11:e0165820.
[45] Caltagirone M, Nucleo E, Spalla M, et al. Occurrence of extended spectrum β-lactamases, KPC-type, and MCR-1.2-producing Enterobacteriaceae from wells, river water, and wastewater treatment plants in Oltrepo Pavese Area. Northern Italy. Front Microbiol 2017;8:2232.
[46] Zurfluh K, Bagutti C, Brodmann P, et al. Wastewater is a reservoir for clinically relevant carbapenemase- and 16S rRNA methylase-producing Enterobacteriaceae. Int J Antimicrob Agents 2017;50:436–40.
[47] de Oliveira DV, Nunes LS, Barth AL, et al. Genetic background of β-lactamases in Enterobacteriaceae isolates from environmental samples. Microb Ecol 2017;74:599–607.
[48] Paschoal RP, Campana EH, Correa LL, et al. Concentration and variety of carbapenemase producers in recreational coastal waters showing distinct levels of pollution. Antimicrob Agents Chemother 2017;61:e01963 -17.
[49] Xu H, Wang X, Yu X, et al. First detection and genomics analysis of KPC-2-producing Citrobacter isolates from river sediments. Environ Pollut 2018;235:931–7.
[50] Yang F, Huang L, Li L, et al. Discharge of KPC-2 genes from the WWTPs contributed to their enriched abundance in the receiving river. Sci Total Environ 2017;581–582:136–43.
[51] Sun P, Bi Z, Nilsson M, et al. Occurrence of bla_{KPC-2}, bla_{CTX-M}, and *mcr-1* in Enterobacteriaceae from well water in rural China. Antimicrob Agents Chemother 2017;61:e02569 -16.
[52] Jin L, Wang R, Wang X, et al. Emergence of *mcr-1* and carbapenemase genes in hospital sewage water in Beijing. China. J Antimicrob Chemother 2018;73:84–7.
[53] Gomi R, Matsuda T, Yamamoto M, et al. Characteristics of carbapenemase-producing Enterobacteriaceae in wastewater revealed by genomic analysis. Antimicrob Agents Chemother 2018;62:e02501 -17.
[54] Sekizuka T, Yatsu K, Inamine Y, et al. Complete genome sequence of a bla_{KPC-2}-positive *Klebsiella pneumoniae* strain isolated from the effluent of an urban sewage treatment plant in. Japan. mSphere 2018;3:e00314 -18.
[55] Yao Y, Lazaro-Perona F, Falgenhauer L, et al. Insights into a novel bla_{KPC-2}-encoding IncP-6 plasmid reveal carbapenem-resistance circulation in several Enterobacteriaceae species from wastewater and a hospital source in Spain. Front Microbiol 2017;8:1143.
[56] Jelic M, Hrenovic J, Dekic S, et al. First evidence of KPC-producing ST258 *Klebsiella pneumoniae* in river water. J Hosp Infect 2019;103:147–50.
[57] Mathys DA, Mollenkopf DF, Feicht SM, et al. Carbapenemase-producing Enterobacteriaceae and *Aeromonas* spp. present in wastewater treatment plant effluent and nearby surface waters in the US. PLoS One 2019;14:e0218650.
[58] Perilli M, Bottoni C, Pontieri E, et al. Emergence of bla_{KPC-3}–*Tn4401a* in *Klebsiella pneumoniae* ST512 in the municipal wastewater treatment plant and in the university hospital of a town in central Italy. J Glob Antimicrob Resist 2013;1:217–20.
[59] Teixeira P, Tacao M, Pureza L, et al. Occurrence of carbapenemase-producing Enterobacteriaceae in a Portuguese river: bla_{NDM}, bla_{KPC} and bla_{GES} among the detected genes. Environ Pollut 2020;260:113913.
[60] Manageiro V, Ferreira E, Canica M, et al. GES-5 among the β-lactamases detected in ubiquitous bacteria isolated from aquatic environment samples. FEMS Microbiol Lett 2014;351:64–9.
[61] White L, Hopkins KL, Meunier D, et al. Carbapenemase-producing Enterobacteriaceae in hospital wastewater: a reservoir that may be unrelated to clinical isolates. J Hosp Infect 2016;93:145–51.
[62] Conte D, Palmeiro JK, da Silva Nogueira K, et al. Characterization of CTX-M enzymes, quinolone resistance determinants, and antimicrobial residues from hospital sewage, wastewater treatment plant, and river water. Ecotoxicol Environ Saf 2017;136:62–9.
[63] Ludden C, Reuter S, Judge K, et al. Sharing of carbapenemase-encoding plasmids between Enterobacteriaceae in UK sewage uncovered by MinION sequencing. Microb Genom 2017;3:e000114.
[64] Suzuki Y, Nazareno PJ, Nakano R, et al. Environmental presence and genetic characteristics of carbapenemase-producing Enterobacteriaceae from hospital sewage and river water in the Philippines. Appl Environ Microbiol 2020;86:e01906 -19.
[65] Mathers AJ, Vegesana K, German Mesner I, et al. Intensive care unit wastewater interventions to prevent transmission of multispecies *Klebsiella pneumoniae* carbapenemase-producing organisms. Clin Infect Dis 2018;67:171–8.
[66] Sekizuka T, Inamine Y, Segawa T, et al. Potential KPC-2 carbapenemase reservoir of environmental *Aeromonas* hydrophila and *Aeromonas* caviae isolates from the effluent of an urban wastewater treatment plant in Japan. Environ Microbiol Rep 2019;11:589–97.
[67] Girlich D, Poirel L, Nordmann P. Novel Ambler class A carbapenem-hydrolyzing β-lactamase from a *Pseudomonas* fluorescens isolate from the Seine River, Paris, France. Antimicrob Agents Chemother 2010;54:328–32.
[68] Neto WRN, Araujo JMM, da Silva DF, et al. Detection of Gram-negative bacteria carrying the bla_{KPC-2} gene from mangrove sediments. J Pharm Pharmacol 2019;7:485–92.
[69] Hammerl JA, Jackel C, Bortolaia V, et al. Carbapenemase VCC-1-producing Vibrio cholerae in coastal waters of Germany. Emerg Infect Dis 2017;23:1735–7.
[70] Sabath LD, Abraham EP. Zinc as a cofactor for cephalosporinase from Bacillus cereus 569. Biochem J 1966;98 11C–3C.
[71] Ju LC, Cheng Z, Fast W, et al. The continuing challenge of metallo-β-lactamase inhibition: mechanism matters. Trends Pharmacol Sci 2018;39:635–47.
[72] Zahedi Bialvaei A, Samadi Kafil H, Ebrahimzadeh Leylabadlo H, et al. Dissemination of carbapenemases producing Gram negative bacteria in the Middle East. Iran J Microbiol 2015;7:226–46.
[73] Potron A, Poirel L, Nordmann P. Emerging broad-spectrum resistance in *Pseudomonas* aeruginosa and *Acinetobacter* baumannii: mechanisms and epidemiology. Int J Antimicrob Agents 2015;45:568–85.
[74] Koh TH, Ko K, Jureen R, et al. High counts of carbapenemase-producing Enterobacteriaceae in hospital sewage. Infect Control Hosp Epidemiol 2015;36:619–21.
[75] Parvez S, Khan AU. Hospital sewage water: a reservoir for variants of New Delhi metallo-β-lactamase (NDM)- and extended-spectrum β-lactamase (ESBL)-producing Enterobacteriaceae. Int J Antimicrob Agents 2018;51:82–8.
[76] Muller H, Sib E, Gajdiss M, et al. Dissemination of multi-resistant Gram-negative bacteria into German wastewater and surface waters. FEMS Microbiol Ecol 2018;94.
[77] Mahon BM, Brehony C, McGrath E, et al. Indistinguishable NDM-producing *Escherichia coli* isolated from recreational waters, sewage, and a clinical specimen in Ireland, 2016 to 2017. Euro Surveill 2017;22:30513.
[78] Walsh TR, Weeks J, Livermore DM, et al. Dissemination of NDM-1 positive bacteria in the New Delhi environment and its implications for human health: an environmental point prevalence study. Lancet Infect Dis 2011;11:355–62.
[79] Shah TA, Zahra R. Screening of environment water for the presence of bla_{NDM-1} gene containing microorganisms. J Coll Physicians Surg Pak 2014;24:695–7.
[80] Chandran SP, Diwan V, Tamhankar AJ, et al. Detection of carbapenem resistance genes and cephalosporin, and quinolone resistance genes along with *oqx*AB gene in *Escherichia coli* in hospital wastewater: a matter of concern. J Appl Microbiol 2014;117:984–95.
[81] Toleman MA, Bugert JJ, Nizam SA. Extensively drug-resistant New Delhi metallo-β-lactamase-encoding bacteria in the environment, Dhaka, Bangladesh, 2012. Emerg Infect Dis 2015;21:1027–30.
[82] Mantilla-Calderon D, Jumat MR, Wang T, et al. Isolation and characterization of NDM-positive *Escherichia coli* from municipal wastewater in Jeddah, Saudi Arabia. Antimicrob Agents Chemother 2016;60:5223–31.
[83] Zheng R, Zhang Q, Guo Y, et al. Outbreak of plasmid-mediated NDM-1-producing *Klebsiella pneumoniae* ST105 among neonatal patients in Yunnan, China. Ann Clin Microbiol Antimicrob 2016;15:10.
[84] Campana EH, Montezzi LF, Paschoal RP, et al. NDM-producing *Klebsiella pneumoniae* ST11 goes to the beach. Int J Antimicrob Agents 2017;49:119–21.
[85] Wu W, Espedido B, Feng Y, et al. Citrobacter freundii carrying bla_{KPC-2} and bla_{NDM-1}: characterization by whole genome sequencing. Sci Rep 2016;6:30670.
[86] Yang F, Mao D, Zhou H, et al. Propagation of New Delhi metallo-β-lactamase genes (bla_{NDM-1}) from a wastewater treatment plant to its receiving river. Environ Sci Technol Lett 2016;3:138–43.

[87] Akiba M, Sekizuka T, Yamashita A, et al. Distribution and relationships of antimicrobial resistance determinants among extended-spectrum–cephalosporin-resistant or carbapenem-resistant *Escherichia coli* isolates from rivers and sewage treatment plants in India. Antimicrob Agents Chemother 2016;60:2972–80.
[88] Zarfel G, Lipp M, Gurtl E, et al. Troubled water under the bridge: screening of River Mur water reveals dominance of CTX-M harboring *Escherichia coli* and for the first time an environmental VIM-1 producer in Austria. Sci Total Environ 2017;593–594:399–405.
[89] Islam MA, Islam M, Hasan R, et al. Environmental spread of New Delhi metallo-β-lactamase-1-producing multidrug-resistant bacteria in Dhaka, Bangladesh. Appl Environ Microbiol 2017;83:e00793 -17.
[90] Daoud Z, Farah J, Sokhn ES, et al. Multidrug-resistant Enterobacteriaceae in Lebanese hospital wastewater: implication in the One Health concept. Microb Drug Resist 2018;24:166–74.
[91] Sugawara Y, Akeda Y, Hagiya H, et al. Spreading patterns of NDM-producing Enterobacteriaceae in clinical and environmental settings in Yangon, Myanmar. Antimicrob Agents Chemother 2019;63:e01924 -18.
[92] Hmede Z, Sulaiman AAA, Jaafar H, et al. Emergence of plasmid-borne colistin resistance gene *mcr-1* in multidrug-resistant *Escherichia coli* isolated from irrigation water in Lebanon. Int J Antimicrob Agents 2019;54:102–4.
[93] Yang F, Gu Y, Zhou J, et al. Swine waste: a reservoir of high-risk bla_{NDM} and *mcr-1*. Sci Total Environ 2019;683:308–16.
[94] Li Y, Luo L, Xiao Z, et al. Characterization of a carbapenem-resistant Kluyvera cryocrescens isolate carrying bla_{NDM-1} from hospital sewage. Antibiotics (Basel) 2019;8:149.
[95] Bardhan T, Chakraborty M, Bhattacharjee B. Prevalence of colistin-resistant, carbapenem-hydrolyzing Proteobacteria in hospital water bodies and out-falls of West Bengal, India. Int J Environ Res Public Health 2020;17:1007.
[96] Zhang L, Ma X, Luo L, et al. The prevalence and characterization of extended-spectrum β-lactamase- and carbapenemase-producing bacteria from hospital sewage, treated effluents and receiving rivers. Int J Environ Res Public Health 2020;17:1182.
[97] Surleac M, Czobor Barbu I, Paraschiv S, et al. Whole genome sequencing snapshot of multi-drug resistant *Klebsiella pneumoniae* strains from hospitals and receiving wastewater treatment plants in Southern Romania. PLoS One 2020;15:e0228079.
[98] Zhai R, Fu B, Shi X, et al. Contaminated in-house environment contributes to the persistence and transmission of NDM-producing bacteria in a Chinese poultry farm. Environ Int 2020;139:105715.
[99] Isozumi R, Yoshimatsu K, Yamashiro T, et al. bla_{NDM-1}-positive *Klebsiella pneumoniae* from environment. Vietnam. Emerg Infect Dis 2012;18:1383–5.
[100] Khan AU, Parvez S. Detection of bla_{NDM}-4 in *Escherichia coli* from hospital sewage. J Med Microbiol 2014;63:1404–6.
[101] Long H, Feng Y, Ma K, et al. The co-transfer of plasmid-borne colistin-resistant genes *mcr-1* and mcr-3.5, the carbapenemase gene bla_{NDM}-5 and the 16S methylase gene rmtB from *Escherichia coli*. Sci Rep 2019;9:696.
[102] Sekizuka T, Inamine Y, Segawa T, et al. Characterization of NDM-5- and CTX-M-55-coproducing *Escherichia coli* GSH8M-2 isolated from the effluent of a wastewater treatment plant in Tokyo Bay. Infect Drug Resist 2019;12:2243–9.
[103] Di DY, Jang J, Unno T, et al. Emergence of Klebsiella variicola positive for NDM-9, a variant of New Delhi metallo-β-lactamase, in an urban river in South Korea. J Antimicrob Chemother 2017;72:1063–7.
[104] Kotsanas D, Wijesooriya WR, Korman TM, et al. Down the drain': carbapenem-resistant bacteria in intensive care unit patients and handwashing sinks. Med J Aust 2013;198:267–9.
[105] Vergara-Lopez S, Dominguez MC, Conejo MC, et al. Wastewater drainage system as an occult reservoir in a protracted clonal outbreak due to metallo-β-lactamase-producing Klebsiella oxytoca. Clin Microbiol Infect 2013;19:E490–8.
[106] Kieffer N, Poirel L, Bessa LJ, et al. VIM-1, VIM-34, and IMP-8 carbapenemase-producing *Escherichia coli* strains recovered from a Portuguese river. Antimicrob Agents Chemother 2016;60:2585–6.
[107] Khan FA, Hellmark B, Ehricht R, et al. Related carbapenemase-producing Klebsiella isolates detected in both a hospital and associated aquatic environment in Sweden. Eur J Clin Microbiol Infect Dis 2018;37:2241–51.
[108] Schages L, Wichern F, Kalscheuer R, et al. Winter is coming—impact of temperature on the variation of β-lactamase and mcr genes in a wastewater treatment plant. Sci Total Environ 2020;712:136499.
[109] Zurfluh K, Hachler H, Nuesch-Inderbinen M, et al. Characteristics of extended-spectrum β-lactamase- and carbapenemase-producing Enterobacteriaceae isolates from rivers and lakes in Switzerland. Appl Environ Microbiol 2013;79:3021–6.
[110] Saavedra MJ, Peixe L, Sousa JC, et al. Sfh-I, a subclass B2 metallo-β-lactamase from a *Serratia fonticola* environmental isolate. Antimicrob Agents Chemother 2003;47:2330–3.
[111] Suzuki Y, Ida M, Kubota H, et al. Multiple β-lactam resistance gene-carrying plasmid harbored by Klebsiella quasipneumoniae isolated from urban sewage in Japan. mSphere 2019;4:e00391 -19.
[112] Sekiguchi J-i, Morita K, Kitao T, et al. KHM-1, a novel plasmid-mediated metallo-β-lactamase from a Citrobacter freundii clinical isolate. Antimicrob Agents Chemother 2008;52:4194.
[113] Zong Z, Zhang X. bla_{NDM-1}-carrying *Acinetobacter* johnsonii detected in hospital sewage. J Antimicrob Chemother 2013;68:1007–10.
[114] Zhang C, Qiu S, Wang Y, et al. Higher isolation of NDM-1 producing *Acinetobacter* baumannii from the sewage of the hospitals in Beijing. PLoS One 2014;8:e64857.
[115] Hu Y, Feng Y, Qin J, et al. *Acinetobacter* chinensis, a novel *Acinetobacter* species, carrying bla_{NDM-1}, recovered from hospital sewage. J Microbiol 2019;57:350–5.
[116] Hu Y, Wu W, Feng Y, et al. Draft genome sequence of a *Pseudomonas* sp. strain carrying bla_{IMP-25} and bla_{VIM-2} carbapenemase genes from hospital sewage. Genome Announc 2016;4:e01027 -16.
[117] Miranda CC, de Filippis I, Pinto LH, et al. Genotypic characteristics of multidrug-resistant *Pseudomonas* aeruginosa from hospital wastewater treatment plant in Rio de Janeiro, Brazil. J Appl Microbiol 2015;118:1276–86.
[118] Quinteira S, Ferreira H, Peixe L. First isolation of bla_{VIM-2} in an environmental isolate of *Pseudomonas* pseudoalcaligenes. Antimicrob Agents Chemother 2005;49:2140–1.
[119] Tacao M, Correia A, Henriques IS. Low prevalence of carbapenem-resistant bacteria in river water: resistance is mostly related to intrinsic mechanisms. Microb Drug Resist 2015;21:497–506.
[120] Kittinger C, Kirschner A, Lipp M, et al. Antibiotic resistance of *Acinetobacter* spp. isolates from the River Danube: susceptibility stays high. Int J Environ Res Public Health 2017;15:52.
[121] Adelowo OO, Vollmers J, Mausezahl I, et al. Detection of the carbapenemase gene bla_{VIM-5} in members of the *Pseudomonas* putida group isolated from polluted Nigerian wetlands. Sci Rep 2018;8:15116.
[122] Luo Y, Yang F, Mathieu J, et al. Proliferation of multidrug-resistant New Delhi metallo-β-lactamase genes in municipal wastewater treatment plants in Northern China. Environ Sci Technol Lett 2013;1:26–30.
[123] Oyelade AA, Adelowo OO, Fagade OE. bla_{NDM-1}-producing Vibrio parahaemolyticus and V. vulnificus isolated from recreational beaches in Lagos, Nigeria. Environ Sci Pollut Res Int 2018;25:33538–47.
[124] Poirel L, Potron A, Nordmann P. OXA-48-like carbapenemases: the phantom menace. J Antimicrob Chemother 2012;67:1597–606.
[125] Potron A, Poirel L, Bussy F, et al. Occurrence of the carbapenem-hydrolyzing β-lactamase gene bla_{OXA-48} in the environment in Morocco. Antimicrob Agents Chemother 2011;55:5413–14.
[126] Tafoukt R, Touati A, Leangapichart T, et al. Characterization of OXA-48-like-producing Enterobacteriaceae isolated from river water in Algeria. Water Res 2017;120:185–9.
[127] Diab M, Hamze M, Bonnet R, et al. Extended-spectrum β-lactamase (ESBL)- and carbapenemase-producing Enterobacteriaceae in water sources in Lebanon. Vet Microbiol 2018;217:97–103.
[128] Tanner WD, VanDerslice JA, Goel RK, et al. Multi-state study of Enterobacteriaceae harboring extended-spectrum β-lactamase and carbapenemase genes in U.S. drinking water. Sci Rep 2019;9:3938.
[129] Mairi A, Pantel A, Ousalem F, et al. OXA-48-producing Enterobacterales in different ecological niches in Algeria: clonal expansion, plasmid characteristics and virulence traits. J Antimicrob Chemother 2019;74:1848–55.
[130] De Geyter D, Blommaert L, Verbraeken N, et al. The sink as a potential source of transmission of carbapenemase-producing Enterobacteriaceae in the intensive care unit. Antimicrob Resist Infect Control 2017;6:24.
[131] Yousfi K, Touati A, Lefebvre B, et al. Characterization of multidrug-resistant Gram-negative bacilli isolated from hospitals effluents: first report of a bla_{OXA-48}-like in Klebsiella oxytoca, Algeria. Braz J Microbiol 2019;50:175–83.
[132] Mahon BM, Brehony C, Cahill N, et al. Detection of OXA-48-like-producing Enterobacterales in Irish recreational water. Sci Total Environ 2019;690:1–6.
[133] Antonelli A, D'Andrea MM, Vaggelli G, et al. OXA-372, a novel carbapenem-hydrolysing class D β-lactamase from a Citrobacter freundii isolated from a hospital wastewater plant. J Antimicrob Chemother 2015;70:2749–56.
[134] Sghaier S, Abbassi MS, Pascual A, et al. Extended-spectrum β-lactamase-producing Enterobacteriaceae from animal origin and wastewater in Tunisia: first detection of O25b-B23-CTX-M-27-ST131 *Escherichia coli* and CTX-M-15/OXA-204-producing Citrobacter freundii from wastewater. J Glob Antimicrob Resist 2019;17:189–94.
[135] Xin R, Zhang K, Wu N, et al. The pollution level of the bla_{OXA-58} carbapenemase gene in coastal water and its host bacteria characteristics. Environ Pollut 2019;244:66–71.
[136] Kotsakis SD, Flach CF, Razavi M, et al. Characterization of the first OXA-10 natural variant with increased carbapenemase activity. Antimicrob Agents Chemother 2018;63:e01817–18.
[137] Girlich D, Poirel L, Nordmann P. First isolation of the bla_{OXA-23} carbapenemase gene from an environmental *Acinetobacter* baumannii isolate. Antimicrob Agents Chemother 2010;54:578–9.
[138] Ferreira AE, Marchetti DP, De Oliveira LM, et al. Presence of OXA-23-producing isolates of *Acinetobacter* baumannii in wastewater from hospitals in southern Brazil. Microb Drug Resist 2011;17:221–7.
[139] Bedenic B, Siroglavic M, Slade M, et al. Comparison of clinical and sewage isolates of *Acinetobacter* baumannii from two long-term care facilities in Zagreb; mechanisms and routes of spread. Arch Microbiol 2020;202:361–8.
[140] Tacão M, Correia A, Henriques I. Environmental Shewanella xiamenensis strains that carry bla_{OXA-48} or $bla_{OXA-204}$ genes: additional proof for bla_{OXA-48}-like gene origin. Antimicrob Agents Chemother 2013;57:6399–400.
[141] Goic-Barisic I, Hrenovic J, Kovacic A, et al. Emergence of oxacillinases in environmental carbapenem-resistant *Acinetobacter* baumannii associated with clinical isolates. Microb Drug Resist 2016;22:559–63.
[142] Yousfi K, Touati A, Lefebvre B, et al. A novel plasmid, pSx1, harboring a new Tn1696 derivative from extensively drug-resistant Shewanella xiamenensis encoding OXA-416. Microb Drug Resist 2017;23:429–36.

[143] Goic-Barisic I, Seruga Music M, Kovacic A, et al. Pan drug-resistant environmental isolate of *Acinetobacter* baumannii from Croatia. Microb Drug Resist 2017;23:494–6.

[144] Tafoukt R, Leangapichart T, Hadjadj L, et al. Characterisation of $bla_{OXA-538}$, a new variant of bla_{OXA-48}, in Shewanella xiamenensis isolated from river water in Algeria. J Glob Antimicrob Resist 2018;13:70–3.

[145] Higgins PG, Hrenovic J, Seifert H, et al. Characterization of *Acinetobacter* baumannii from water and sludge line of secondary wastewater treatment plant. Water Res 2018;140:261–7.

[146] Le Terrier C, Masseron A, Uwaezuoke NS, et al. Wide spread of carbapenemase-producing bacterial isolates in a Nigerian environment. J Glob Antimicrob Resist 2020;21:321–3.

[147] Hrenovic J, Seruga Music M, Durn G, et al. Carbapenem-resistant *Acinetobacter* baumannii recovered from swine manure. Microb Drug Resist 2019;25:725–30.

[148] Zou H, Zhou Z, Xia H, et al. Characterization of chromosome-mediated $bla_{OXA-894}$ in Shewanella xiamenensis isolated from pig wastewater. Int J Environ Res Public Health 2019;16:3768.

[149] Qiao M, Ying GG, Singer AC, et al. Review of antibiotic resistance in China and its environment. Environ Int 2018;110:160–72.

[150] Subirats J, Royo E, Balcazar JL, et al. Real-time PCR assays for the detection and quantification of carbapenemase genes (bla_{KPC}, bla_{NDM}, and bla_{OXA-48}) in environmental samples. Environ Sci Pollut Res Int 2017;24:6710–14.

[151] Nasri E, Subirats J, Sanchez-Melsio A, et al. Abundance of carbapenemase genes (bla_{KPC}, bla_{NDM} and bla_{OXA-48}) in wastewater effluents from Tunisian hospitals. Environ Pollut 2017;229:371–4.

[152] Proia L, Anzil A, Borrego C, et al. Occurrence and persistence of carbapenemases genes in hospital and wastewater treatment plants and propagation in the receiving river. J Hazard Mater 2018;358:33–43.

[153] Marathe NP, Berglund F, Razavi M, et al. Sewage effluent from an Indian hospital harbors novel carbapenemases and integron-borne antibiotic resistance genes. Microbiome 2019;7:97.

[154] Lubbert C, Baars C, Dayakar A, et al. Environmental pollution with antimicrobial agents from bulk drug manufacturing industries in Hyderabad, South India, is associated with dissemination of extended-spectrum β-lactamase and carbapenemase-producing pathogens. Infection 2017;45:479–91.

[155] Dias MF, da Rocha Fernandes G, Cristina de Paiva M, et al. Exploring the resistome, virulome and microbiome of drinking water in environmental and clinical settings. Water Res 2020;174:115630.

[156] Tuo H, Yang Y, Tao X, et al. The prevalence of colistin resistant strains and antibiotic resistance gene profiles in Funan River. China. Front Microbiol 2018;9:3094.

[157] Bondarczuk K, Piotrowska-Seget Z. Microbial diversity and antibiotic resistance in a final effluent-receiving lake. Sci Total Environ 2019;650:2951–61.

[158] Bartley PS, Domitrovic TN, Moretto VT, et al. Antibiotic resistance in Enterobacteriaceae from surface waters in urban Brazil highlights the risks of poor sanitation. Am J Trop Med Hyg 2019;100:1369–77.

[159] Klase G, Lee S, Liang S, et al. The microbiome and antibiotic resistance in integrated fishfarm water: implications of environmental public health. Sci Total Environ 2019;649:1491–501.

[160] Yang F, Zhang K, Zhi S, et al. High prevalence and dissemination of β-lactamase genes in swine farms in Northern China. Sci Total Environ 2019;651:2507–13.

[161] Girlich D, Poirel L, Szczepanowski R, et al. Carbapenem-hydrolyzing GES-5-encoding gene on different plasmid types recovered from a bacterial community in a sewage treatment plant. Appl Environ Microbiol 2012;78:1292–5.

[162] Mi R, Patidar R, Farenhorst A, et al. Detection of fecal bacteria and antibiotic resistance genes in drinking water collected from three First Nations communities in Manitoba, Canada. FEMS Microbiol Lett 2019;366 fnz067.

[163] Dang B, Mao D, Luo Y. Complete nucleotide sequence of pGA45, a 140,698-bp IncFIIY plasmid encoding bla_{IMI-3}-mediated carbapenem resistance, from river sediment. Front Microbiol 2016;7:188.

[164] Khan FA, Soderquist B, Jass J. Prevalence and diversity of antibiotic resistance genes in Swedish aquatic environments impacted by household and hospital wastewater. Front Microbiol 2019;10:688.

[165] Wang Y, Zhang R, Li J, et al. Comprehensive resistome analysis reveals the prevalence of NDM and MCR-1 in Chinese poultry production. Nat Microbiol 2017;2:16260.

[166] Wang RN, Zhang Y, Cao ZH, et al. Occurrence of super antibiotic resistance genes in the downstream of the Yangtze River in China: prevalence and antibiotic resistance profiles. Sci Total Environ 2019;651:1946–57.

[167] Stange C, Yin D, Xu T, et al. Distribution of clinically relevant antibiotic resistance genes in Lake Tai. China. Sci Total Environ 2018;655:337–46.

[168] Zhou ZC, Feng WQ, Han Y, et al. Prevalence and transmission of antibiotic resistance and microbiota between humans and water environments. Environ Int 2018;121:1155–61.

[169] Rathinasabapathi P, Hiremath DS, Arunraj R, et al. Molecular detection of New Delhi metallo-β-lactamase-1 (NDM-1) positive bacteria from environmental and drinking water samples by loop mediated isothermal amplification of bla_{NDM-1}. Indian J Microbiol 2015;55:400–5.

[170] Ahammad ZS, Sreekrishnan TR, Hands CL, et al. Increased waterborne bla_{NDM-1} resistance gene abundances associated with seasonal human pilgrimages to the Upper Ganges River. Environ Sci Technol 2014;48:3014–20.

[171] Gatica J, Tripathi V, Green S, et al. High throughput analysis of integron gene cassettes in wastewater environments. Environ Sci Technol 2016;50:11825–36.

[172] Fernando DM, Tun HM, Poole J, et al. Detection of antibiotic resistance genes in source and drinking water samples from a First Nations Community in Canada. Appl Environ Microbiol 2016;82:4767–75.

[173] Devarajan N, Laffite A, Graham ND, et al. Accumulation of clinically relevant antibiotic-resistance genes, bacterial load, and metals in freshwater lake sediments in Central Europe. Environ Sci Technol 2015;49:6528–37.

[174] Le TH, Ng C, Tran NH, et al. Removal of antibiotic residues, antibiotic resistant bacteria and antibiotic resistance genes in municipal wastewater by membrane bioreactor systems. Water Res 2018;145:498–508.

[175] Makowska N, Philips A, Dabert M, et al. Metagenomic analysis of β-lactamase and carbapenemase genes in the wastewater resistome. Water Res 2020;170:115277.

[176] Voigt AM, Zacharias N, Timm C, et al. Association between antibiotic residues, antibiotic resistant bacteria and antibiotic resistance genes in anthropogenic wastewater—an evaluation of clinical influences. Chemosphere 2020;241:125032.

[177] Tacao M, Silva I, Henriques I. Culture-independent methods reveal high diversity of OXA-48-like genes in water environments. J Water Health 2017;15:519–25.

[178] Bengtsson-Palme J, Hammaren R, Pal C, et al. Elucidating selection processes for antibiotic resistance in sewage treatment plants using metagenomics. Sci Total Environ 2016;572:697–712.

[179] Voigt AM, Ciorba P, Dohla M, et al. The investigation of antibiotic residues, antibiotic resistance genes and antibiotic-resistant organisms in a drinking water reservoir system in Germany. Int J Hyg Environ Health 2020;224:113449.

[180] Maiden MC. Multilocus sequence typing of bacteria. Annu Rev Microbiol 2006;60:561–88.

[181] Smith JM, Feil EJ, Smith NH. Population structure and evolutionary dynamics of pathogenic bacteria. Bioessays 2000;22:1115–22.

[182] Ribeiro-Gonçalves B, Francisco AP, Vaz C, et al. PHYLOViZ Online: web-based tool for visualization, phylogenetic inference, analysis and sharing of minimum spanning trees. Nucleic Acids Res 2016;44:W246–51.

[183] Brink AJ. Epidemiology of carbapenem-resistant Gram-negative infections globally. Curr Opin Infect Dis 2019;32:609–16.

[184] Pitout JDD, Peirano G, Kock MM, et al. The global ascendency of OXA-48-type carbapenemases. Clin Microbiol Rev 2019;33:e00102 -19.

[185] Uchida H, Tada T, Tohya M, et al. Emergence in Japan of an isolate of *Klebsiella pneumoniae* co-harbouring bla_{KPC-2} and rmtB. J Glob Antimicrob Resist 2019;17:157–9.

[186] Lu MC, Tang HL, Chiou CS, et al. Clonal dissemination of carbapenemase-producing *Klebsiella pneumoniae*: two distinct sub-lineages of sequence type 11 carrying bla_{KPC-2} and bla_{OXA-48}. Int J Antimicrob Agents 2018;52:658–62.

[187] Fu P, Tang Y, Li G, et al. Pandemic spread of bla_{KPC-2} among *Klebsiella pneumoniae* ST11 in China is associated with horizontal transfer mediated by IncFII-like plasmids. Int J Antimicrob Agents 2019;54:117–24.

[188] Jelic M, Butic I, Plecko V, et al. KPC-producing *Klebsiella pneumoniae* isolates in Croatia: a nationwide survey. Microb Drug Resist 2016;22:662–7.

[189] García-Fernández A, Villa L, Carta C, et al. *Klebsiella pneumoniae* ST258 producing KPC-3 identified in Italy carries novel plasmids and OmpK36/OmpK35 porin variants. Antimicrob Agents Chemother 2012;56:2143–5.

[190] Loconsole D, Accogli M, De Robertis AL, et al. Emerging high-risk ST101 and ST307 carbapenem-resistant *Klebsiella pneumoniae* clones from bloodstream infections in Southern Italy. Ann Clin Microbiol Antimicrob 2020;19:24.

[191] Pereira PS, de Araujo CF, Seki LM, et al. Update of the molecular epidemiology of KPC-2-producing *Klebsiella pneumoniae* in Brazil: spread of clonal complex 11 (ST11, ST437 and ST340). J Antimicrob Chemother 2013;68:312–16.

[192] Zhu J, Sun L, Ding B, et al. Outbreak of NDM-1-producing *Klebsiella pneumoniae* ST76 and ST37 isolates in neonates. Eur J Clin Microbiol Infect Dis 2016;35:611–18.

[193] Bonura C, Giuffrè M, Aleo A, et al. An update of the evolving epidemic of bla_{KPC} carrying *Klebsiella pneumoniae* in Sicily, Italy, 2014: emergence of multiple non-ST258 clones. PLoS One 2015;10:e0132936.

[194] Khan ER, Aung MS, Paul SK, et al. Prevalence and molecular epidemiology of clinical isolates of *Escherichia coli* and *Klebsiella pneumoniae* harboring extended-spectrum β-lactamase and carbapenemase genes in Bangladesh. Microb Drug Resist 2018;24:1568–79.

[195] Flerlage T, Brazelton de Cardenas JN, Garner CD, et al. Multiple NDM-5-expressing *Escherichia coli* isolates from an immunocompromised pediatric host. Open Forum Infect Dis 2020;7 ofaa018.

[196] Dagher C, Salloum T, Alousi S, et al. Molecular characterization of carbapenem resistant *Escherichia coli* recovered from a tertiary hospital in Lebanon. PLoS One 2018;13:e0203323.

[197] Falgenhauer L, Nordmann P, Imirzalioglu C, et al. Cross-border emergence of clonal lineages of ST38 *Escherichia coli* producing the OXA-48-like carbapenemase OXA-244 in Germany and Switzerland. Int J Antimicrob Agents 2020;56:106157.

[198] Qin S, Zhou M, Zhang Q, et al. First identification of NDM-4-producing *Escherichia coli* ST410 in China. Emerg Microbes Infect 2016;5:e118.

[199] Yagoubat M, Ould El-Hadj-Khelil A, Malki A, et al. Genetic characterisation of carbapenem-resistant Gram-negative bacteria isolated from the University Hospital Mohamed Boudiaf in Ouargla, southern Algeria. J Glob Antimicrob Resist 2017;8:55–9.

[200] Sanderson H, Fricker C, Brown RS, et al. Antibiotic resistance genes as an emerging environmental contaminant. Environ Rev 2016;24:205–18.

Chapitre II

Epidémiologie de la résistance à la colistine à médiation plasmidique par les gènes *mcr* dans les milieux aquatiques

La dissémination rapide des BGN résistantes aux antibiotiques particulièrement aux carbapénèmes, ainsi que le ralentissement du développement de nouveaux antibiotiques efficaces ont conduit au retour obligatoire à l'utilisation de la polymyxine E ou colistine face à ces agents **(Dhariwal et Tullu, 2013)**. La colistine a été introduite pour la première fois en thérapeutique en 1959, mais en raison de sa toxicité et de l'introduction d'autres antibiotiques efficaces et potentiellement moins toxiques, l'utilisation de la colistine a été restreinte du début des années 70 jusqu'au début du 21^{ième} siècle **(Biswas *et al.*, 2012)**. Malgré sa toxicité, la colistine est aujourd'hui considérée comme le traitement de dernier recours des infections causées par les BGN résistantes aux carbapénèmes **(Caniaux *et al.*, 2017)**. Néanmoins, outre les mécanismes classiques connus de la résistance à la colistine résultant des mutations chromosomiques, un mécanisme plasmidique de résistance à cet antibiotique a été rapporté chez *Escherichia coli* initialement en Chine fin 2015 **(Liu *et al.*, 2016)**, par la suite il a été identifié dans de nombreuses espèces de différentes sources et pays de cinq continents, porté par des plasmides diversifiés et des environnements génétiques complexes **(Baron *et al.*, 2016; Feng, 2018)**. L'émergence et la propagation d'un tel mécanisme de résistance à la colistine représentent une nouvelle étape vers une résistance pratiquement totale aux antibiotiques chez les BGN, mettant ainsi notre arsenal thérapeutique en risque **(Caniaux *et al.*, 2017)**.

Ce nouveau mécanisme codé par un ensemble de gènes appelés *mcr*, est une phosphoéthanolamine transférase qui agisse en diminuant l'affinité de la colistine pour le lipopolysaccharide (LPS) bactérien par l'ajout de groupements phosphoéthanolamine au lipide A du LPS **(Nordmann et Poirel, 2016)**.

En effet, des preuves récentes ont considéré le sol et l'eau comme des sources, des réservoirs et des récepteurs de niveaux de résistance aux antibiotiques cliniquement pertinents **(Pruden *et al.*, 2013)**. Récemment, dans une étude publiée dans la revue *Scientific Reports*, suite à l'analyse de plus de 60 000 génomes bactériens dans l'objectif de mieux comprendre les origines et les réservoirs des gènes *mcr*, les auteurs ont signalé que presque tous les gènes *mcr* décrits semblaient provenir de bactéries environnementales, en particulier d'origine aquatique. Par conséquent, il a été suggéré que les environnements aquatiques représentent le principal réservoir et source des gènes du type *mcr* **(Khedher *et al.*, 2020)**. De plus, des études visant à déterminer les origines des gènes de résistance à médiation plasmidique à la colistine récemment identifiés ont proposé des espèces environnementales des genres *Moraxella* et *Shewanella* comme origines de ce mécanisme **(Kieffer *et al.*, 2017; Snesrud *et al.*, 2018; Zhang *et al.*, 2019a; Zhang *et al.*, 2019b)**.

Dans ce chapitre, une revue de littérature a été réalisée pour présenter le rôle des différents milieux aquatiques (y compris les eaux usées, l'eau d'aquaculture, les eaux de surface, les eaux souterraines et l'eau potable) en tant que réservoirs et/ou voies de propagation de la résistance aux antibiotiques. Ainsi que pour donner un aperçu sur l'occurrence et la distribution mondiales des gènes de résistance à la colistine à médiation plasmidique (*mcr*) dans les différents environnements aquatiques.

Références bibliographiques

Baron, S., Hadjadj, L., Rolain, J.M., and Olaitan, A.O. 2016. Molecular mechanisms of polymyxin resistance: knowns and unknowns. *International journal of antimicrobial agents* **48**(6): 583-591. doi:10.1016/j.ijantimicag.2016.06.023.

Biswas, S., Brunel, J.M., Dubus, J.C., Reynaud-Gaubert, M., and Rolain, J.M. 2012. Colistin: an update on the antibiotic of the 21st century. *Expert review of anti-infective therapy* **10**(8): 917-934. doi:10.1586/eri.12.78.

Caniaux, I., van Belkum, A., Zambardi, G., Poirel, L., and Gros, M.F. 2017. MCR: modern colistin resistance. *European journal of clinical microbiology & infectious diseases : official publication of the European Society of Clinical Microbiology* **36**(3): 415-420. doi:10.1007/s10096-016-2846-y.

Dhariwal, A.K. and Tullu, M.S. 2013. Colistin: re-emergence of the 'forgotten' antimicrobial agent. *Journal of postgraduate medicine* **59**(3): 208-215. doi:10.4103/0022-3859.118040.

Feng, Y. 2018. Transferability of MCR-1/2 Polymyxin Resistance: Complex Dissemination and Genetic Mechanism. *ACS infectious diseases* **4**(3): 291-300. doi:10.1021/acsinfecdis.7b00201.

Khedher, M.B., Baron, S.A., Riziki, T., Ruimy, R., Raoult, D., Diene, S.M., and Rolain, J.-M. 2020. Massive analysis of 64,628 bacterial genomes to decipher water reservoir and origin of mobile colistin resistance genes: is there another role for these enzymes? *Scientific reports* **10**(1): 5970. doi:10.1038/s41598-020-63167-5.

Kieffer, N., Nordmann, P., and Poirel, L. 2017. *Moraxella* Species as Potential Sources of MCR-Like Polymyxin Resistance Determinants. *Antimicrobial agents and chemotherapy* **61**(6). doi:10.1128/AAC.00129-17.

Liu, Y.Y., Wang, Y., Walsh, T.R., Yi, L.X., Zhang, R., Spencer, J., Doi, Y., Tian, G., Dong, B., Huang, X., Yu, L.F., Gu, D., Ren, H., Chen, X., Lv, L., He, D., Zhou, H., Liang, Z., Liu, J.H., and Shen, J. 2016. Emergence of plasmid-mediated colistin resistance mechanism MCR-1 in animals and human beings in China: a microbiological and

molecular biological study. *The Lancet. Infectious diseases* **16**(2): 161-168. doi:10.1016/s1473-3099(15)00424-7.

Nordmann, P. and Poirel, L. 2016. Plasmid-mediated colistin resistance: an additional antibiotic resistance menace. *Clinical microbiology and infection : the official publication of the European Society of Clinical Microbiology and Infectious Diseases* **22**(5): 398-400. doi:10.1016/j.cmi.2016.03.009.

Pruden, A., Larsson, D.G., Amezquita, A., Collignon, P., Brandt, K.K., Graham, D.W., Lazorchak, J.M., Suzuki, S., Silley, P., Snape, J.R., Topp, E., Zhang, T., and Zhu, Y.G. 2013. Management options for reducing the release of antibiotics and antibiotic resistance genes to the environment. *Environmental health perspectives* **121**(8): 878-885. doi:10.1289/ehp.1206446.

Snesrud, E., McGann, P., and Chandler, M. 2018. The Birth and Demise of the *ISApl1-mcr-1-ISApl1* Composite Transposon: the Vehicle for Transferable Colistin Resistance. *mBio* **9**(1). doi:10.1128/mBio.02381-17.

Zhang, H., Wei, W., Huang, M., Umar, Z., and Feng, Y. 2019a. Definition of a Family of Nonmobile Colistin Resistance (NMCR-1) Determinants Suggests Aquatic Reservoirs for MCR-4. *Advanced science (Weinheim, Baden-Wurttemberg, Germany)* **6**(11): 1900038. doi:10.1002/advs.201900038.

Zhang, H., Hou, M., Xu, Y., Srinivas, S., Huang, M., Liu, L., and Feng, Y. 2019b. Action and mechanism of the colistin resistance enzyme MCR-4. *Communications Biology* **2**(1): 36. doi:10.1038/s42003-018-0278-1.

Article 2

Epidemiology of mobile colistin resistance (*mcr*) genes in aquatic environments

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Review

Epidemiology of mobile colistin resistance (*mcr*) genes in aquatic environments

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ABSTRACT

Colistin is one of the last-line therapies against multidrug-resistant Gram-negative pathogens, especially carbapenemase-producing isolates, making resistance to this compound a major global public-health crisis. Until recently, colistin resistance in Gram-negative bacteria was known to arise only by chromosomal mutations. However, a plasmid-mediated colistin resistance mechanism was described in late 2015. This mechanism is encoded by different mobile colistin resistance (*mcr*) genes that encode phosphoethanolamine (pEtN) transferases. These enzymes catalyse the addition of a pEtN moiety to lipid A in the bacterial outer membrane leading to colistin resistance. MCR-producing Gram-negative bacteria have been largely disseminated worldwide. However, their environmental dissemination has been underestimated. Indeed, water environments act as a connecting medium between different environments, allowing them to play a crucial role in the spread of antibiotic resistance between the natural environment and humans and other animals. For a better understanding of the role of such environments as reservoirs and/or dissemination routes of *mcr* genes, this review discusses primarily the various water habitats contributing to the spread of antibiotic resistance. Thereafter, we provide an overview of existing knowledge regarding the global epidemiology of *mcr* genes in water environments. This review confirms the global distribution of *mcr* genes in several water environments, including wastewater from different origins, surface water and tap water, making these environments reservoirs and dissemination routes of concern for this resistance mechanism.

1. Introduction

One of the greatest achievements of the 20th century was the discovery of antibacterial drugs [1]. Antibiotics are undoubtedly lifesaving compounds that have significantly reduced mortality rates due to bacterial infections [2]. For several years after their therapeutic use, these compounds appeared to have tackled the challenge of bacterial infections [3], thus preserving their efficacy remained a top priority [4]. However, bacterial resistance to these compounds nowadays represents an international fear since it is estimated that the number of deaths from infections caused by resistant organisms may reach 10 million by 2050 [5,6]. Antibiotic resistance represents an inevitable consequence of the escalating use of antibacterial drugs [6]. This phenomenon was predicted early in 1945 by Sir Alexander Fleming when he said 'There is the danger that the ignorant man may easily underdose himself and by exposing his microbes to nonlethal quantities of the drug make them resistant' [7]. The emergence and massive spread of antibiotic-resistant organisms has jeopardised the capability of these drugs and reversed advances in antibacterial therapy, limiting our treatment options and bringing us into a post-antibiotic era [7,8]. Nowadays, antibiotic resistance represents one of the most worrying health challenges at a global level [9], with multidrug-resistant Gram-negative bacteria being the most relevant actors. The rapid dissemination of such pathogens along with the slow development of newer effective antibiotics have led to the re-

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emergence of the old 'forgotten' drug polymyxin E (colistin) [10]. Colistin was first introduced into therapeutic use in 1959; however, due to its toxicity and the introduction of other potentially less toxic antibiotics, use of colistin was restricted from the beginning of the 1970s until the early 21st century [11]. Despite its toxicity, colistin is nowadays considered the last-resort treatment option for infections caused by carbapenem-resistant Gram-negative bacteria [12]. Nevertheless, besides the known classical colistin resistance mechanisms resulting from chromosomal mutations, a transferable plasmid-mediated colistin resistance mechanism has been reported, initially in *Escherichia coli* from China in late 2015 [13]. It has subsequently been identified in many species from different sources and countries in five continents and is harboured by diverse plasmid types and with complex genetic environments [14,15]. The emergence and spread of such a colistin resistance mechanism represent a new step towards virtual pan-drug resistance among Gram-negative bacteria, thus jeopardising our therapeutic arsenal [12].

Historically, antibiotic resistance has been considered a clinical problem and has therefore been studied in nosocomial settings [3]. However, the large application of antibiotics in human medicine as well as in veterinary settings for treatment and prophylactic purposes or as growth promoters in animal farming has led to a huge dissemination of drug-resistant organisms worldwide [16]. Therefore, bacteria resistant to even last-resort antibiotics have been isolated from different sources, including water environments [17–19]. Indeed, water acts as a connecting medium between different environments, enabling it to play a key role in the widespread dissemination of antibiotic resistance [20]. Furthermore, direct transmission of clinically relevant bacteria presenting a high antibiotic resistance level to humans by exposure to river water has been reported [21]. On the one hand, water represents an important means for the introduction and dissemination of drug-resistant organisms and antibiotic resistance genes (ARGs) into the natural environment and, on the other hand, is an ideal environment for the horizontal exchange of ARGs [1,3,22]. In this regard, and in order to contain the problem of antibiotic resistance and to reduce the risk to public health presented by the widespread dissemination of multidrug-resistant organisms, much more attention is currently directed at determining their potential reservoirs and dissemination routes and growing interest is given to water contamination since it can lead to an unlimited diffusion of drug resistance mechanisms. This review provides an overview on the occurrence and distribution of mobile colistin resistance (*mcr*) genes carried by Gram-negative bacteria in various aquatic environments, which may help to gain insights on the worldwide epidemiology of these organisms and elucidate the role of aqueous ecosystems as a reservoir of such a mechanism of drug resistance, thus highlighting the link between the environment and clinics.

For this purpose, we performed a comprehensive literature search of the PubMed database using the following search terms and/or phrases: '*mcr* genes' and 'water', 'wastewater', 'sewage', 'hospital sewage', 'aquatic environments'. Only research articles published in the English language until February 2021 were included. Search terms were separated by the 'AND' Boolean operator.

2. Aquatic ecosystems and antibiotic resistance: reservoirs and dissemination routes

The worldwide dissemination of antibiotic resistance is a grave issue both for human and animal health, where the main drivers of its spread are both the excessive and inappropriate use of antibiotics [23]. Indeed, recent evidence has considered soil and water environments as sources, reservoirs and recipients of clinically relevant antibiotic resistance [24]. A recent study analysed more than 60 000 bacterial genomes in order to better understand the origins and reservoirs of *mcr* genes [25]. Interestingly, almost all described *mcr* genes appeared to originate from environmental bacteria, especially from water sources. The authors suggested water environments as the main reservoir and source of *mcr*-like genes [25]. Moreover, studies to determine the origins of recently identified plasmid-mediated colistin resistance genes have proposed *Moraxella* and *Shewanella* species as the origin of this mechanism [26–29].

In this section, the role of different water environments (i.e. wastewater, aquaculture, surface water, groundwater and drinking water) as reservoirs and/or dissemination pathways of antibiotic resistance will be discussed.

2.1. Hospital effluent

Hospitals and healthcare units are considered hotspots for antibiotic resistance where the most dramatic selection pressure is expected due to the intensive use of antibiotic therapy [30]. Colonised patients and wastewater are the main pathways by which antibiotic-resistant bacteria (ARB) and ARGs exit hospitals [23]. Several studies have demonstrated the role of hospital effluent as a reservoir of relevant ARB carrying ARGs encoding resistance even to last-resort antibiotics [31,32]. Additionally, hospital waste contains large amounts of antibiotics and disinfectants that are known to exert a selection pressure contributing to the evolution of antibiotic resistance. These contaminants favour ARB compared with susceptible isolates and, on the other hand, positively enhance the horizontal exchange of antibiotic resistance determinants between resistant and susceptible bacteria [33]. In fact, only a few countries recommend pre-treatment of hospital waste before its discharge into treatment plants since it is categorised as domestic effluent [23,30]. Therefore, effluents from hospitals may represent an important reservoir of clinically relevant ARB and ARGs and a privileged pathway for their entry into the environment. However, it seems that they are not the main source of resistant bacteria release in municipal effluents [30,33].

2.2. Animal waste

Antibiotic consumption is not restricted to the human population; these compounds are largely used in animals for the treatment and prevention of bacterial diseases or as growth promoters. For the two latter purposes, they are used at subinhibitory concentrations [34,35]. In fact, the relative link between the use of antibacterial drugs on farms and antibiotic resistance is still disputed [36]. Whatever its cause, the spread of antibiotic resistance in animals is today a fact that poses a serious threat to human health. Several studies have reported the detection of highly-resistant bacteria in faecal samples from farm animals [37], which eventually end up in the environment. This has become more important as liquid manure from farms is frequently used as an organic fertiliser after stabilisation [35]. Among the proposed strategies that have been shown to be effective in reducing resistance to colistin owing to *mcr* genes is banning the use of colistin in animal feed. This strategy has been applied in China and Japan and it has shown its effectiveness with a rapid and significant decrease in the prevalence of the *mcr-1* gene and colistin-resistant *E. coli* from animals as well as human colonisation and infections [38–41]. This strategy is recommended to be implemented in all countries in order to reduce colistin resistance and thus conserve the effectiveness of this crucial antibiotic.

2.3. Wastewater treatment plants (WWTPs)

As mentioned above, almost all hospital wastewater worldwide is released into receiving WWTPs without any pre-treatment aimed at reducing the levels of antibiotic resistance. Therefore, hospitals are expected to be the most important source of antibiotic resistance release into the environment [33]. However, taking into consideration (i) the dilution factor due to the very low percentage of hospital waste in WWTP effluent, (ii) the emergence and spread of ARB in the community, (iii) the large-scale use of antibiotics in households since the majority of antibiotics prescribed to humans are used at home and (iv) finally, the detection of ARB and ARGs in WWTPs not receiving hospital waste, we can conclude that the community is probably the main source responsible for antibiotic resistance release into WWTPs [1,42].

Urban wastewater and WWTP effluent are among the most important sources of ARB and ARG dissemination [43,44]. WWTPs are man-made environments that receive bacteria from different sources and environments [45,46]. They receive wastewater from hospitals, animal husbandry and urban citizens [47]. To the best of our knowledge, all known kinds of mechanisms involved in bacterial resistance to antibiotics have been detected in WWTPs, highlighting the important role of these facilities as reservoirs and dissemination pathways of antibiotic resistance. In addition, several studies have suggested that treatment processes may positively affect the selection and spread of ARB and ARGs [43]. Several factors make WWTPs an ideal environment for bacterial proliferation and horizontal exchange of ARGs, including the abundance of nutrient sources, the stable pH and temperature, the high bacterial density, biofilm formation and the stress exposure due to the different pollutants present including antibiotics, disinfectants and heavy metals [30,45,48]. Furthermore, some resistance types have been found to be more prevalent in treated effluent than in the raw influent [30]. Thus, WWTPs can be considered as critical control points for the emergence and spread of drug resistance [24].

2.4. Aquaculture

As with farming, aquaculture is the rearing of aquatic animals in a controlled environment such as the sea, a lake or river [49]. This industry is growing worldwide and antibiotic use in this field is rising, mainly for prophylactic purposes, where they are added directly to the water body [46,50]. Although the introduction of new vaccines has significantly reduced the use of antibiotics in developed countries, aquaculture management systems are still contributing to the development of antibiotic resistance in developing countries [24]. Several studies have defined these systems as hotspots for antibiotic resistance [50]. Indeed, the emergence and spread of ARB and ARGs in aquaculture results in contamination of the human food chain [46]. However, the greatest threat to human health is the risk that aquaculture turns into a reservoir of ARB, where transferable ARGs can be easily disseminated between aquatic bacteria and ultimately transferred to human pathogens [24]. Concerningly, a study by Cabello et al. reported that the emergence of the recently described plasmid-mediated *mcr* genes is the result of aquaculture activities that still contribute to its spread and evolution [28,51,52].

2.5. Surface water

Antibiotics as well as bacteria presenting different antibiotic resistance levels are present in surface water. Surface water receives ARB and ARG input from several sources, including runoff water, aquaculture, WWTPs, agriculture and animal waste [42,47]. Several studies have reported the detection of ARB in rivers [53], lakes [54], estuaries [55] and seawater [56], suggesting that waterways could be a source of ARB and ARGs. However, unlike in the water habitats discussed above, antibiotic concentrations in such water systems are very low to alter bacterial populations [33].

2.6. Groundwater

Antibiotics are rarely detected in groundwater and, if they exist, they are found at very low concentrations [33]. However, a number of studies have detected the presence of ARB and ARGs in groundwater [57]. ARB and ARGs can reach groundwater via several means, including infiltration from surface water or soil after improving farm land with animal manure or the use WWTP effluent and sludge as fertilisers [47,58]. In addition, runoff from farms and broken sewage pipes are also possible routes for the input of antibiotic resistance into groundwater [42].

2.7. Drinking water

Drinking water commonly originates from surface water and groundwater. Because ARB and ARGs could be present in these two sources, they could therefore pass upon drinking water treatment processes and end up in water distribution systems [58]. Despite the scare information regarding antibiotic resistance in these types of water habitats, bacteria presenting relevant antibiotic resistance levels have been detected in drinking water [59,60] and there are suggestions that water treatment and its subsequent distribution could play an important role in antibiotic resistance selection [33]. Indeed, it seems difficult to assess the origin of antibiotic-resistant organisms found in drinking water as well as to evaluate the risk to human health associated with their presence. However, the detection of these organisms in drinking water is of great importance because drinking water is one of the potential routes of transmission of antibiotic resistance to humans [30].

3. Horizontal gene transfer (HGT)

Bacteria can influence the effect of an antibiotic via the acquisition of foreign resistance genes through different mechanisms involving bacterial transformation, transduction or conjugation [61]. Horizontal transfer of ARGs is one of the key factors in the spread of antibiotic resistance [62]; interestingly, all of these mechanisms have been proven to occur in aquatic environments.

3.1. Transformation

Bacterial transformation is the process by which a recipient bacterium takes up a foreign naked DNA molecule from its environment and integrates it into its chromosome by homologous recombination or converts it into an autonomously replicating element [63]. Considering the vulnerability of DNA to degradation, the dilution effect in water environments and limited naturally competent bacteria, transformation may be considered a rare event [3,58]. However, Baur et al. have reported that *E. coli* is able to develop natural competence in freshwater habitats (river water, spring water and bottled mineral water) in the presence of different Ca^{2+} concentrations [64].

3.2. Transduction

Bacteriophages (viruses that infect bacteria) can act as natural vectors of genes between bacteria [65]. In transduction, a DNA fragment is transferred from an infected host and delivered into a new bacterium via phage particles [63]. Unlike in transformation where naked DNA is very susceptible to alteration, phage particles are more resistant in the environment [3]. Although transduction is less frequently associated with horizontal transfer of resistance

genes than other HGT mechanisms [66], several factors, such as the stability of viral particles in the environment, their high number in marine and fresh water estimated to be approximately 10-fold higher than bacterial counts, and the broad host range of some bacteriophages, make transduction ideal for horizontal gene exchange in the water environment between even spatially distant bacteria [3,63]. In a study investigating the occurrence of ARGs in bacteriophages in hospital wastewater, the authors demonstrated using a metagenomic approach that the abundance of ARGs in the viral DNA fraction was higher than in the bacterial DNA fraction [67]. In addition, it has been reported that antibiotics (commonly found in aquatic ecosystems) enhance phage production from lysogens [68]. These findings highlight the important role of phages as reservoirs and vehicles of resistance genes in the environment.

3.3. Conjugation

The most important and most prevalent mechanism of resistance gene transmission in bacteria is conjugation, which is also considered as the main facilitator of resistance sharing between bacteria [3,69]. Conjugation involves the transfer of a plasmid or conjugative transposon from a donor bacterium to a recipient strain via sexual pili that are present only in the donor cell [63]. This phenomenon has been observed in several environments and requires tight cell-to-cell contact [3,65]. Several studies have reported the presence of conjugative plasmids carrying significant ARGs in bacterial isolates obtained from different aquatic ecosystems [70–72].

It is worth noting that several experiments have suggested that conjugative transmission frequencies in nature are probably more important than those in the laboratory [68]. In aquatic environments, several factors may play a significant role as drivers of ARG transfer. First, as mentioned in the previous section, except drinking water, aquatic ecosystems receive antibiotic contamination from various sources. The presence of antibiotics in water exerts a high selective pressure that increases bacterial fitness, selects for resistant bacteria and facilitates ARG acquisition as an adaptation response [58,62,73]. Additionally, it has been proven that subinhibitory antibiotic concentrations, commonly detected in water environments, promote the horizontal exchange of resistance genes [45]. Moreover, other contaminants present in water ecosystems, such as metals and heavy metals, can also increase gene exchange between bacteria, and several studies have demonstrated that HGT appears to be easier in metal-contaminated environments [58,74].

In the case of WWTPs, the situation gets more complicated, where the high bacterial density may provide an ideal environment for resistance gene transfer [45]. Several studies have suggested that wastewater treatment processes may positively affect HGT [43]. In addition, some studies have reported that chlorination (the most widely used disinfection process) does not affect plasmid DNA [1].

4. Colistin and colistin resistance

Colistin (polymyxin E) is a polycationic antimicrobial peptide belonging to the polymyxin family [14]. Produced by the Gram-positive bacterium *Paenibacillus polymyxa* subspecies *colistinus*, colistin was discovered in 1949 and was introduced to our antibacterial armamentarium in 1959; it was among the earliest antimicrobials exhibiting significant activity against Gram-negative pathogens [75,76]. Colistin has a narrow spectrum, which includes most Gram-negative rods except for *Campylobacter, Legionella, Chromobacterium, Neisseria, Proteus, Serratia, Providencia, Vibrio, Brucella, Burkholderia* and *Edwardsiella* species, *Morganella morganii, Aeromonas jandaei* and *Pseudomonas mallei*. Polymyxins are inactive against Gram-positive bacteria, all cocci and anaerobes [75–77]. Colistin is a concentration-dependent antibiotic that acts on the bacterial outer cell membrane [10]. Due to its high positive charge and hydrophobic acyl chain, colistin interacts electrostatically with lipopolysaccharide (LPS) molecules and displaces their divalent cations, resulting in cell membrane rupture. This process leads to an increase in cell permeability and leakage of cell content resulting in cell death [75].

Acquired colistin resistance was classically related to chromosomal mutations leading to LPS modification with 4-amino-4-deoxy-L-arabinose (L-Ara4N) and/or phosphoethanolamine (pEtN), efflux pump regulation or the complete loss of LPS [14]. However, unfortunately, in late 2015 bad news was reported from China when Liu et al. described the first mobile colistin resistance (*mcr*) mechanism in *E. coli* [13]. Nowadays, ten *mcr* genes have been described belonging to multiple distinct clades (Fig. 1) and conferring moderate levels of colistin resistance [78]. This recent colistin resistance mechanism encoded by plasmid-borne *mcr* genes is a pEtN transferase decreasing the affinity of colistin for LPS by the addition of a pEtN group to lipid A [79].

5. Epidemiology of plasmid-mediated colistin resistance gene-carrying Gram-negative bacteria in aquatic environments

In the following, we will present the worldwide epidemiology of *mcr* genes detected in aqueous environments (Tables 1 and 2). Nowadays, all described *mcr* genes with several variants have been detected in aquatic environments by culture and/or culture-independent methods (Fig. 1).

5.1. Enterobacterales

In a relatively short period after its first description, the *mcr-1* gene has successfully disseminated worldwide and this dissemination was accompanied by the emergence of new variants. Although the *mcr* genes were primarily found in *E. coli*, these genes are now prevalent in multiple bacterial species. Several studies have reported the isolation of *mcr*-harbouring Enterobacterales from non-clinical sources, including different water habitats (Fig. 2). In 2016, two studies detected the occurrence of *mcr-1* carrying *E. coli* in river and pond water in Switzerland and Malaysia, respectively [80,81]. The Swiss isolate belonged to sequence type 359 (ST359) and co-produced the SHV-12 (for sulfhydryl variable) extended-spectrum β-lactamase (ESBL) [80]. However, the pond water isolate obtained from Malaysia belonged to ST410 [81,82]. In the same year, an *mcr-1*-harbouring *Kluyvera ascorbata* isolate was recovered from hospital sewage in China [83]. Interestingly, the *mcr-1* gene was located on an IncI2 conjugative plasmid and the isolate co-produced CTX-M-185 (for cefotaximase-Munich) ESBL.

In 2017, several studies were published describing the emergence of *mcr-1*-harbouring enterobacteria in water environments in different countries and it is noteworthy that 6 of the 12 studies were from China. *mcr-1*-harbouring *E. coli* belonging to different STs were recovered from wastewater from canals in Thailand (ST5951 and ST6624) [84], from urban sludge in Bangladesh [85], from stream water in Italy (ST10) [57] and from beaches in Brazil (ST10, ST46 and ST1638) [71] and Norway (ST10) [19]. In Spain, Ovejero et al. reported the isolation of MCR-1-producing *E. coli* ST1196 and ST224 and *Klebsiella pneumoniae* ST526 from WWTPs [86]. The colistin resistance genes were located on IncI2 plasmids and the worrying association of an ESBL and MCR was also detected as the isolates were CTX-M-55-producers [86].

In the case of China, six reports were published in 2017 reporting the detection of MCR-1 (including MCR-1.4 and MCR-1.7)-producing Enterobacterales in water environments. Chen et al. reported the isolation of MCR-1-producing *E. coli, K. pneumoniae* and

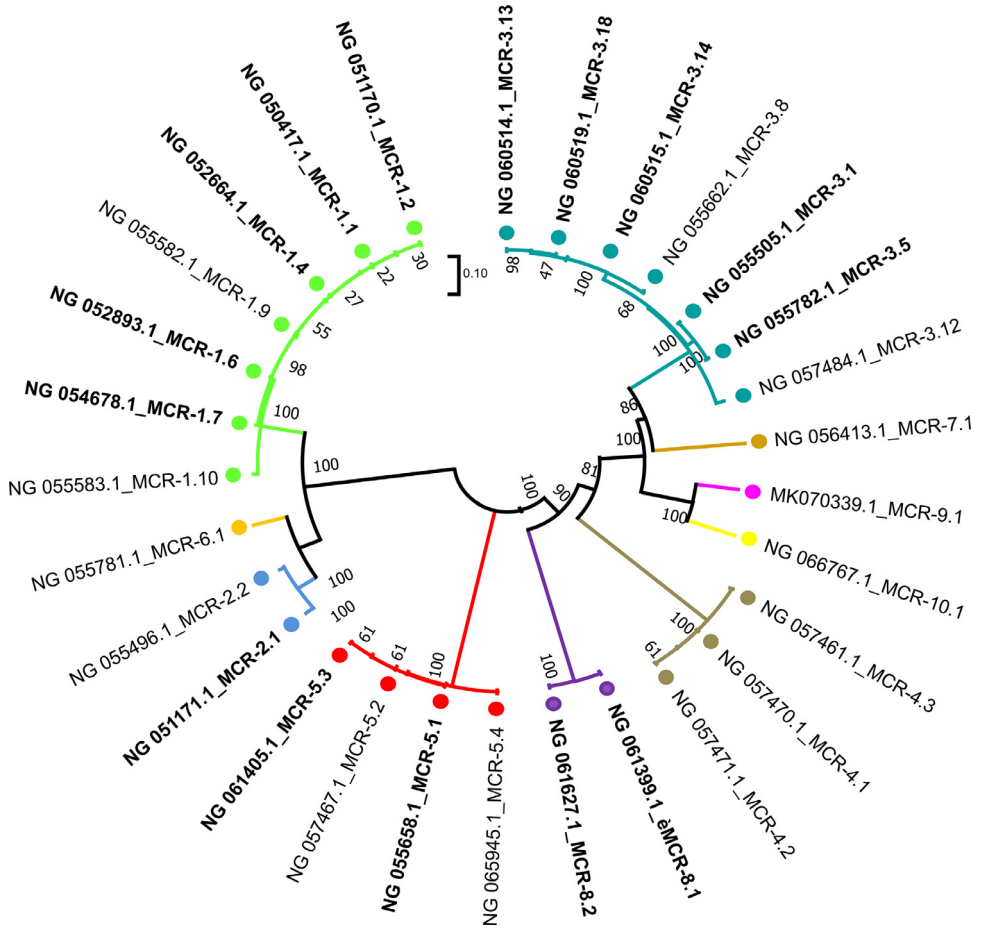

Fig. 1. Phylogeny of *mcr* variants detected in aquatic environments. The evolutionary history was inferred using the neighbour-joining method. Evolutionary distances were computed using the Kimura two-parameter method. The number above the nodes is the level of bootstrap from 1000 replicates. Variants in bold were detected by culture techniques.

Table 1
Mobile colistin resistance (*mcr*) genes detected in different aquatic environments

	Aquatic habitat																					
mcr	AW		DW		DWTP		FSW		GW		HS		IW		SW		TW		WW		WWTPs	
gene	C	CIM	C	CIM	C	CIM	C	CIM	C	CIM	C	CIM	C	CIM	C	CIM	C	CIM	C	CIM	C	CIM
mcr-1	x	x	x	x		x	x	x	x	x	x	x	x		x				x	x	x	x
mcr-2						x	x	x														
mcr-3		x				x	x	x			x	x					x				x	x
mcr-4						x		x														x
mcr-5	x					x		x			x	x						x				x
mcr-6						x		x														
mcr-7						x		x				x										
mcr-8	x					x																
mcr-9						x						x										
mcr-10											x											

AW, animal waste; DW, drinking water; DWTP, drinking water treatment plant; FSW, fresh surface water; GW, groundwater; HS, hospital sewage; IW, irrigation water; SW, seawater; TW, tap water; WW, wastewater; WWTP, wastewater treatment plant; C, culture; CIM, culture-independent method.

Klebsiella variicola from WWTPs as well as *mcr-1*-harbouring *E. coli* from seawater [87]. The *mcr-1* gene in *E. coli* isolates was encoded on conjugative IncI2 and IncX4 plasmids. Fresh surface water ecosystems are also reservoirs for MCR-1-producers. In a study conducted to isolate colistin-resistant bacteria from environmental water samples, *mcr-1*-harbouring Enterobacterales were obtained from rivers, creeks, lakes, canals and wetlands [88]. MCR-1-producing *E. coli* were isolated from all water types. However, *Citrobacter freundii, Citrobacter braakii* and *Klebsiella oxytoca* were isolated from lakes only. In addition, *Enterobacter cloacae* was obtained only from canals. *mcr-1* was chromosomally encoded in several *E. coli* isolates and the association of an ESBL and MCR-1 was also observed in this study.

Hospital sewage was also proposed as a reservoir and dissemination pathway of *mcr-1*-harbouring bacteria to the environment. Three studies from China reported the isolation of MCR-1-producing *E. coli* ST10, ST349, ST2016, ST410, ST6756, ST7122, ST101, ST7087, ST7086, ST1196, ST34 and ST48 [31,89] and *K. pneumoniae* ST313 [90] from hospital sewage. Worryingly, one of the *E. coli* isolates belonging to ST7087 was found to co-harbour *mcr-1*

Table 2
mcr-harbouring isolates from aquatic environments

mcr gene	Species (n)	Sequence type (ST)	Country	Water source	Gene location (P/Ch)	Reference
mcr-1	*Aeromonas dhakensis* (1)	–	India	WWTP	P	[106]
mcr-1	*Aeromonas veronii* (1)	–	India	WWTP	P	[106]
mcr-1	*Citrobacter braakii* (2)	–	China	Lake	P	[88]
mcr-1	*Citrobacter freundii* (2)	–	China	Lake	P	[88]
mcr-1	*Enterobacter cloacae* (1)	–	China	Canal	–	[88]
mcr-1	*E. cloacae* (1)	–	China	Animal waste	–	[101]
mcr-1	*E. cloacae* (1)	–	China	River	–	[92]
mcr-1	*Escherichia coli* (1)	ST345	Algeria	Irrigation water	–	[121]
mcr-1	*E. coli* (2)	ST23, ST115	Algeria	Seawater	P	[56]
mcr-1	*E. coli* (2)	–	Bangladesh	Hand rinse	–	[107]
mcr-1	*E. coli* (4)	–	Bangladesh	Surface water	–	[107]
mcr-1	*E. coli* (1)	–	Bangladesh	Urban wastewater	–	[85]
mcr-1	*E. coli* (1)	ST58	Brazil	Mangrove	P (IncX4)	[93]
mcr-1	*E. coli* (3)	ST1638, ST46, ST10	Brazil	Seawater	P (IncX4)	[71]
mcr-1	*E. coli* (3)	ST10, ST1011, ST165	China	Aquaculture	P (IncI2, IncX4)	[114]
mcr-1	*E. coli* (1)	ST101	China	Canal	P	[88]
mcr-1	*E. coli* (1)	ST181	China	Creek	P	[88]
mcr-1	*E. coli* (1)	ST48	China	Drinking water	P	[102]
mcr-1	*E. coli* (9)	ST10, ST349, ST2016, ST410, ST101, ST6756, ST7122	China	Hospital sewage	P (IncX4)	[89]
mcr-1	*E. coli* (7)	ST10, ST48, ST34, ST7086, ST7087	China	Hospital sewage	P (IncHI2, IncN, IncX4, IncP)	[31]
mcr-1	*E. coli* (4)	ST10, ST101	China	Lake	P	[88]
mcr-1	*E. coli* (1)	ST744	China	Pharmaceutical wastewater	P (IncY)	[97]
mcr-1	*E. coli* (84)	–	China	River	P	[105]
mcr-1	*E. coli* (6)	ST43, ST181, ST10, ST1638	China	River	P	[88]
mcr-1	*E. coli* (2)	–	China	River	–	[91]
mcr-1	*E. coli* (17)	–	China	River	–	[92]
mcr-1	*E. coli* (25)	–	China	Seawater	P	[87]
mcr-1	*E. coli* (4)	ST10, ST515	China	Wastewater	Ch/P (IncI2)	[102]
mcr-1	*E. coli* (2)	ST10, ST48	China	Well water	–	[72]
mcr-1	*E. coli* (4)	ST206, ST1638	China	Wetland	P	[88]
mcr-1	*E. coli* (52)	–	China	WWTP	P	[87]
mcr-1	*E. coli* (2)	–	Egypt	Surface water	–	[109]
mcr-1	*E. coli* (18)	–	Germany	Slaughterhouse	–	[110]
mcr-1	*E. coli* (31)	–	Germany	Slaughterhouses	P (IncF, IncX4, IncI1, IncHI2, IncI2)	[112]
mcr-1	*E. coli* (8)	–	Germany	Slaughterhouses	–	[111]
mcr-1	*E. coli* (2)	ST10, ST155	Germany	Surface water	–	[96]
mcr-1	*E. coli* (3)	–	India	WWTP	P	[106]
mcr-1	*E. coli* (7)	ST131, ST871, ST767, ST10, ST135, ST457, ST453	Japan	WWTP	P (IncX4, IncI2)	[98]
mcr-1	*E. coli* (1)	ST2936	Lebanon	Drinking water	–	[120]
mcr-1	*E. coli* (22)	–	Lebanon	Irrigation water	P	[118]
mcr-1	*E. coli* (1)	ST1638	Lebanon	Well water	–	[120]
mcr-1	*E. coli* (1)	ST410	Malaysia	Water pond	P (IncI2)	[81,82]
mcr-1	*E. coli* (2)	ST10	Norway	Seawater	P	[19]
mcr-1	*E. coli* (1)	ST10	Singapore	Lake	P	[108]
mcr-1	*E. coli* (31)	–	South Africa	WWTP	–	[99]
mcr-1	*E. coli* (1)	–	Spain	Slaughterhouse	–	[113]
mcr-1	*E. coli* (29)	ST1196, ST224	Spain	WWTP	P (IncI2)	[86]
mcr-1	*E. coli* (1)	ST359	Switzerland	River	–	[80]
mcr-1	*E. coli* (2)	ST5951, ST6624	Thailand	Canals	P	[84]
mcr-1	*E. coli* (1)	ST8900	Tunisia	WWTP	–	[100]
mcr-1	*Kluyvera ascorbata* (1)	–	China	Animal waste	–	[101]
mcr-1	*K. ascorbata* (1)	–	China	Hospital sewage	IncI2	[83]
mcr-1	*Klebsiella oxytoca* (2)	–	China	Lake	–	[88]
mcr-1	*Klebsiella pneumoniae* (1)	ST313	China	Hospital sewage	P (IncP)	[90]
mcr-1	*K. pneumoniae* (4)	–	China	WWTP	–	[87]
mcr-1	*K. pneumoniae* (1)	–	Egypt	Surface water	–	[109]
mcr-1	*K. pneumoniae* (3)	–	Germany	Slaughterhouse	–	[110]
mcr-1	*K. pneumoniae* (7)	–	Germany	Slaughterhouse	P (IncI1, IncX4)	[112]
mcr-1	*K. pneumoniae* (1)	ST526	Spain	WWTP	P (IncI2)	[86]
mcr-1	*Klebsiella variicola* (3)	–	China	WWTP	–	[87]
mcr-1	*Proteus mirabilis* (3)	–	Lebanon	Drinking water	P	[119]
mcr-1	*P. mirabilis* (2)	–	Lebanon	Sewage	P	[119]

(*continued on next page*)

Table 2 (*continued*)

mcr gene	Species (n)	Sequence type (ST)	Country	Water source	Gene location (P/Ch)	Reference
mcr-1	*P. mirabilis* (3)	–	Lebanon	Well water	P	[119]
mcr-1.1 and ***mcr-1.7***	*E. coli* (1)	ST1196	China	Hospital sewage	P (IncX4, IncI2)	[31]
mcr-1.1 and ***mcr-3.5***	*E. coli* (1)	ST410	China	Hospital sewage	P (IncHI2(ST3)/IncN, IncP)	[94]
mcr-1.2	*E. coli* (1)	ST10	Italy	Stream	P (IncX4)	[57]
mcr-1.4	*E. coli* (1)	ST7087	China	Hospital sewage	P (IncX4)	[31]
mcr-1.6	*E. coli* (1)	ST10	China	Drinking water	P (IncX4)	[102]
mcr-1.6	*E. coli* (2)	ST10, ST1434	China	River	P	[102]
mcr-2	*E. coli* (1)	–	Egypt	Surface water	–	[109]
mcr-2	*K. pneumoniae* (1)	–	Egypt	Surface water	–	[109]
mcr-3	*Aeromonas hydrophila* (4)	–	China	River	–	[92]
mcr-3	*Aeromonas salmonicida* (1)	ST601	China	Tap water	–	[124]
mcr-3	*Aeromonas veronii* (2)	–	China	River	–	[92]
mcr-3	*E. coli* (1)	ST1730	Singapore	Lake	–	[108]
mcr-3.1	*E. coli* (1)	ST393	Japan	Wastewater	P (IncF)	[115]
mcr-3.13 and ***mcr-3.18***	*Aeromonas caviae* (2)	–	China	River	–	[122]
mcr-3.14	*Aeromonas bivalvium* (2)	–	China	River	–	[122]
mcr-5	*Enterobacter* sp. (1)	–	China	Hospital sewage	P	[116]
mcr-5.3 and ***mcr-8.2***	*Stenotrophomonas* sp. (1)	–	China	Animal waste	Ch	[123]
mcr-8	*K. pneumoniae* (1)	ST3410	China	Animal waste	P (IncA/C2)	[104]
mcr-10	*Enterobacter roggenkampii* (3)	ST595, ST1237, ST1059	China	Hospital sewage	P (IncFIB–FII)	[117]

WWTP, wastewater treatment plant; P, plasmid; Ch, Chromosome; (n), isolates number.

and *mcr-1.4* on the same conjugative IncX4 plasmid. In addition, another *E. coli* isolate belonging to ST1169 was found to co-express MCR-1.7 on an additional plasmid (IncI2) [31].

MCR-1 detection is not confined to wastewater and surface water but is also observed from groundwater. Sun et al. have documented the isolation of *mcr-1*-harbouring *E. coli* ST10 co-producing CTX-M-65 and ST48 co-producing CTX-M-14 from well water [72].

In 2018, four studies were published describing the detection of MCR-1-producing Enterobacterales in water, two from China, one from Algeria and one from Brazil. Wu et al. reported the isolation of *mcr-1*-harbouring *E. coli* from the Jin River [91]. In addition, Tuo et al. described the isolation of MCR-1-producing *E. coli* and *E. cloacae* from the Funan River [92]. The first African MCR-1-producing environmental *E. coli* isolates, belonging to ST23 and ST115, were obtained from seawater in Algeria [56]. Furthermore, an *mcr-1*-positive, ESBL-producing *E. coli* ST58 isolate was recovered from a mangrove in Brazil [93].

More recently, a Chinese study reported the isolation of *E. coli* co-harbouring *mcr-1* and *mcr-3.5* genes on IncHI2/IncN and IncP plasmids, respectively [94]. The isolate was obtained from hospital sewage and belonged to ST410. Interestingly, the abovementioned strain co-produced NDM-5 carbapenemase, CTX-M-65 ESBL and the 16S rRNA methylase gene *rmtB*. In addition, the authors highlighted that although their encoding genes were located on different plasmids, the *mcr-1, mcr-3.5,* bla_{NDM-5} and *rmtB* genes were horizontally transferred together. In addition, *mcr-1*-positive *E. coli* isolates were also obtained from WWTPs and surface water (ST10) in Germany [95,96], China [97], Japan [98], South Africa [99] and Tunisia [100], from sewage of poultry and pig farms [101–104], from drinking water (ST10 and ST48) and river water (ST10 and ST1434) in China [102,105], from urban sewage in India [106], from surface water in Bangladesh [107], Singapore [108] and Egypt [109], from slaughterhouses in Germany [110–112] and Spain [113], and from aquaculture water in China [114]. In addition, *mcr-1*-harbouring *K. pneumoniae* isolates were detected in surface water in Egypt and animal waste in Germany [109,110]. Furthermore, *mcr-2*-harbouring *E. coli* and *K. pneumoniae* as well as *mcr-3.1*-harbouring *E. coli* and *mcr-8*-positive *K. pneumoniae* were detected in surface water in Egypt, in WWTP in Japan and in animal waste in China, respectively [104,109,115]. *mcr-3*- and *mcr-5*-positive *E. coli* and *Enterobacter* sp. were isolated from surface water in Singapore and hospital sewage in China, respectively [108,116]. The latest *mcr* gene described (*mcr-10*) was recently detected in *Enterobacter roggenkampii* isolates obtained from hospital sewage water in China. The *mcr-10* genes were located on self-transferable IncFIB plasmids [117].

In Lebanon, Hmede et al. reported the isolation of *mcr-1*-harbouring multidrug-resistant *E. coli* isolates from irrigation water from the two major Lebanese agricultural areas [118]. Interestingly, 2 of the 22 obtained isolates co-harboured a carbapenemase-encoding gene (NDM-1 or OXA-48) [118]. In recently published studies, *mcr-1*-positive *Proteus mirabilis* and *E. coli* isolates were cultured from well water, drinking water and sewage collected in Syrian refugee camps [119,120].

The Lebanese study was not the only study reporting the detection of mobile colistin resistance genes in irrigation water. *mcr-1*-harbouring *E. coli* ST345 was detected in irrigation water in Algeria [121]. The occurrence of such resistance mechanisms in irrigation water is of great concern since this can affect a variety of matrices causing widespread dissemination of resistance mechanisms.

5.2. Other Gram-negative bacilli

Although *mcr* genes are mostly found in Enterobacterales, *Aeromonas* spp. are suggested to be the origin and a potential reservoir for the *mcr-3* variant [122]. The *mcr-1* gene has been

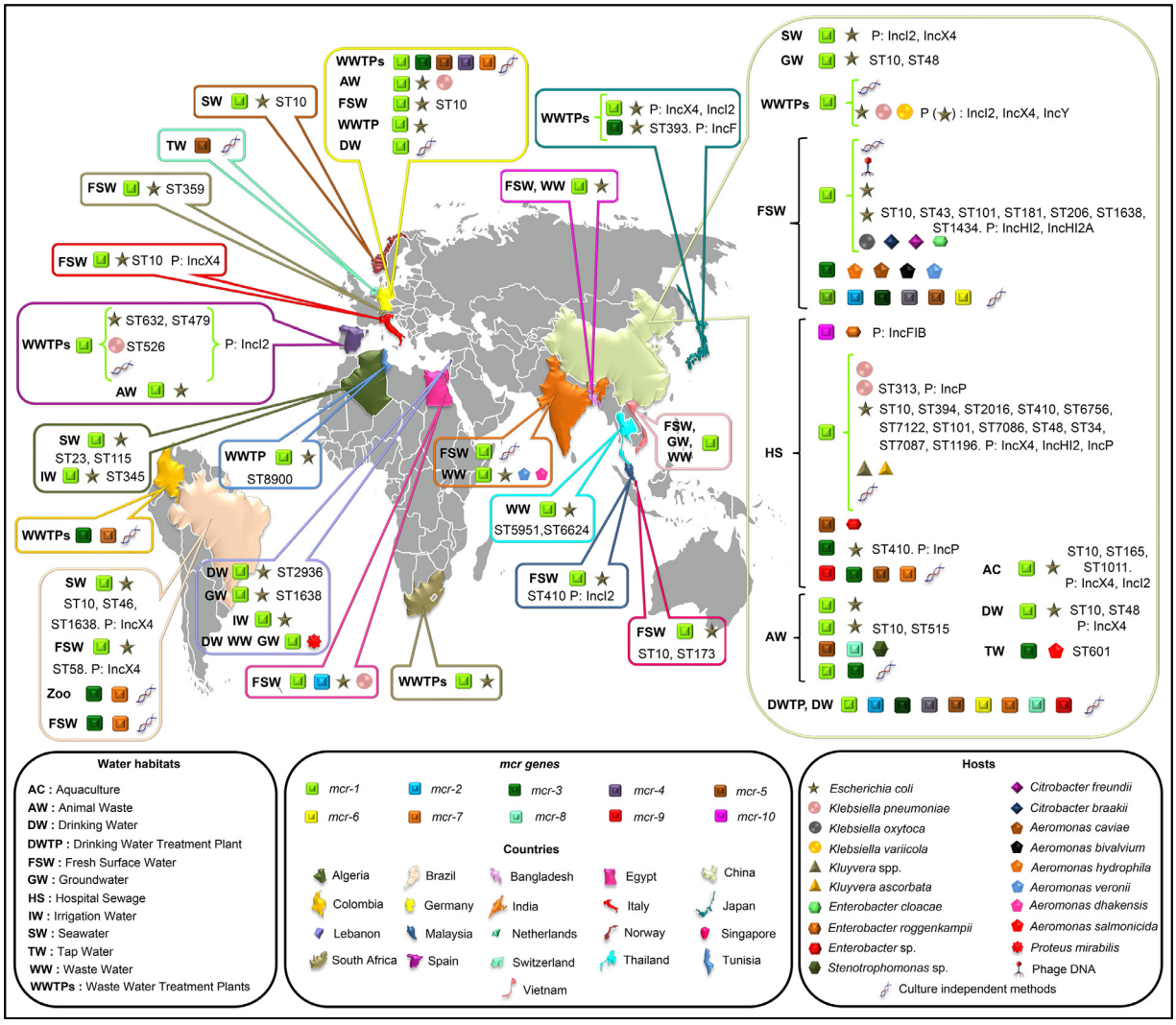

Fig. 2. Worldwide epidemiology of plasmid-mediated mobile colistin resistance (*mcr*) genes in aquatic environments. P, plasmid, ST, sequence type.

detected in *Aeromonas veronii* and *Aeromonas dhakensis* isolated from urban sewage in India [106]. In addition, the occurrence of *mcr-3* in *Aeromonas hydrophila* and *A. veronii*, of *mcr-3.13* and *mcr-3.18* in *Aeromonas caviae* and of *mcr-3.14* in *Aeromonas bivalvium* in river water has been reported from China [92,122]. A study recent reported the detection of two *mcr* genes in a colistin-resistant *Stenotrophomonas* strain isolated from sewage water of a Chinese poultry farm. The aforementioned strain was subjected to whole-genome sequencing, which revealed the chromosomal occurrence of the *mcr-5.3* and *mcr-8.2* genes. Interestingly, the colistin resistance was transferable by conjugation [123]. More recently, an *mcr-3*-positive *Aeromonas salmonicida* ST601 isolate has been recovered from a tap water system in China [124].

5.3. mcr *genes detected by culture-independent methods*

DNA-based techniques are also used to detect *mcr* genes in water bodies. *mcr-1* genes were detected in WWTPs in Germany [125,126], Spain [127] and China [128]. Furthermore, *mcr-3* and *mcr-5* genes were detected in WWTPs in Colombia [129]. In addition, *mcr* genes including *mcr-1, mcr-3, mcr-4, mcr-5* and *mcr-7* were detected using a metagenomics approach in German municipal wastewater [130]. Unlike the aforementioned studies, a high relative abundance of *mcr-3, mcr-4, mcr-5* and *mcr-7* genes was observed compared with the *mcr-1* gene, which was detected in only 1 of the 14 analysed samples [130]. In addition, rivers were also investigated using culture-independent methods for the occurrence of *mcr-1* and positive results were obtained from China [128,131] and India [132]. Recent studies conducted in Brazil have reported the detection of *mcr-7.1* and *mcr-3* in surface water and in water from a zoo [133–135]. Regarding hospital wastewater, *mcr-1, mcr-3, mcr-5, mcr-7* and *mcr-9* genes were detected in wastewater of a Chinese hospital [117]. In a study aimed at assessing the prevalence of the *mcr-1* gene in water sources in Vietnam, this gene was detected with different abundance rates in urban drainage, river, lake and groundwater [136]. Furthermore, *mcr-1* to *mcr-6* genes were detected in a lake in China [137].

Animal waste has been largely recognised as a reservoir of ARGs. Recently, a metagenomics approach was used to examine the types of ARGs harboured by bacteria and bacteriophages in swine feedlot wastewater [138]. Importantly, the authors reported the detection of *mcr-1* in the phage population, which confirms that phages may play an important role as a driving force for the horizontal transfer of such worrisome ARGs in the environment. More recently, Wang et al. reported the detection of *mcr-1* and *mcr-3* genes in sewage samples collected from a pig farm in China [101]. Importantly, despite that 85.7% of samples were positive for the *mcr-3* gene, no *mcr-3*-positive isolate was obtained, thus confirming the importance of culture-independent methods for the detection of ARGs. In addition, in a recent study, Xia et al. reported that

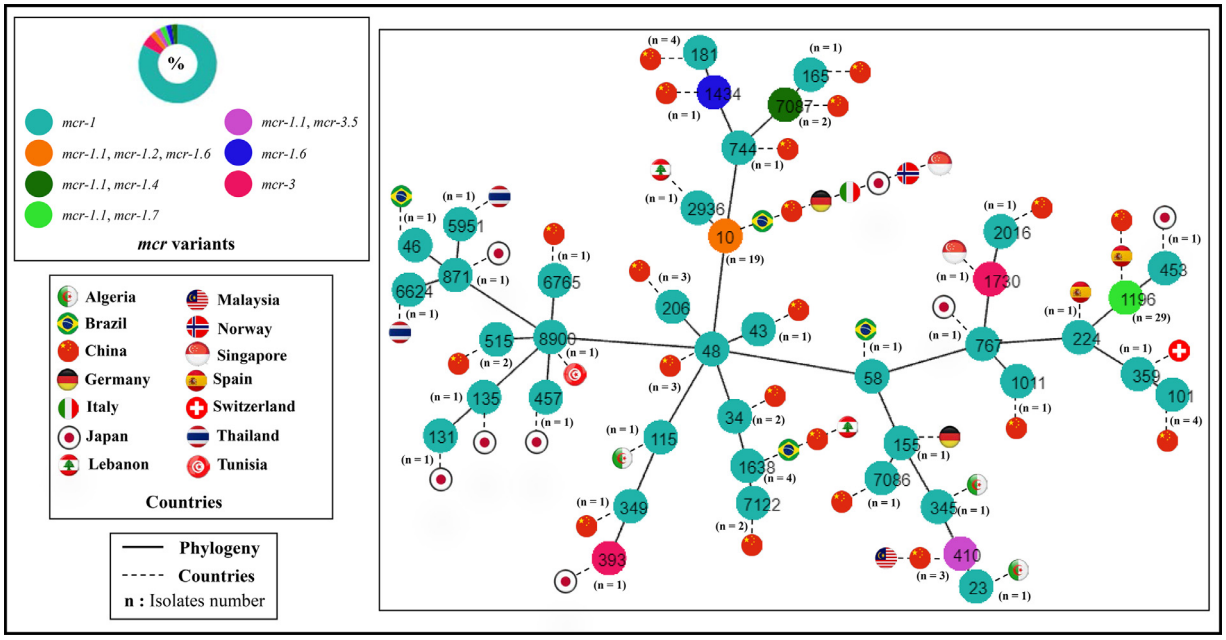

Fig. 3. Population snapshot of *mcr*-harbouring *Escherichia coli* sequence types (STs) detected in aquatic environments with the respective geographical area, generated using PHYLOViZ Online [144].

colistin residues have a direct impact on *mcr-1* accumulation in manure [139].

More worryingly was the detection of the *mcr-1* gene in drinking water, which was reported from China in 2018 when Wang et al. detected the *mcr-1* gene in a drinking water treatment plant [128]. In addition, metagenomic shotgun sequencing allowed the first report of the *mcr-5.4* variant through its detection in hospital tap water in the Netherlands [140]. Recently, the *mcr-1* gene was detected in drinking water in Germany [141] and China [142]. In addition, 17 *mcr* variants (*mcr-1.4, mcr-1.9, mcr-1.10, mcr-2.2, mcr-3.5, mcr-3.8, mcr-3.12, mcr-4, mcr-4.2, mcr-4.3, mcr-4.4, mcr-5, mcr-5.2, mcr-6.1, mcr-7.1, mcr-8* and *mcr-9*) were detected by metagenomic analysis in drinking water treatment plants in China [143].

6. Multilocus sequence typing (MLST)

Among *mcr*-harbouring species detected in aquatic environments, *E. coli* was the most isolated and the most characterised by MLST. MLST analysis was available for 105 *E. coli* isolates where 41 STs were reported. Phylogenetic analysis of the detected STs, conducted using PHYLOViZ Online [144], revealed a high genotypic diversity (Fig. 3). ST1196 was the most dominant with 29 isolates, followed by ST10 with 19 isolates. However, ST10 was the most geographically distributed, with *mcr-1*-positive *E. coli* ST10 isolates detected in seven countries from three continents.

7. Conclusions

In response to the storm of carbapenem-resistant Gram-negative bacteria, colistin has re-emerged as a drug of last resort [145] for the treatment of life-threatening Gram-negative bacterial infections. However, this last line of defence against these deadly infections is significantly threatened by the emergence and rapid spread of colistin resistance, especially by *mcr* genes. It is now clearer than ever that water environments represent a worrisome reservoir of antibiotic resistance that significantly promotes the horizontal exchange of ARGs between bacteria from different origins, contributing to a difficult-to-control evolution and spread of antibiotic resistance. In addition, the transmission of ARB from water bodies to humans causing grave infections has been proven. Consequently, an urgent need exists to assess the scale of antibiotic resistance, especially in water habits, in order to curtail its occurrence and dissemination.

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Competing interests

None declared.

Ethical approval

Not required.

References

[1] Marti E, Variatza E, Balcazar JL. The role of aquatic ecosystems as reservoirs of antibiotic resistance. Trends Microbiol 2014;22:36–41.
[2] Shlaes DM, Bradford PA. Antibiotics—from there to where? Pathog Immun 2018;3:19–43.
[3] Berglund B. Environmental dissemination of antibiotic resistance genes and correlation to anthropogenic contamination with antibiotics. Infect Ecol Epidemiol 2015;5:28564.
[4] Allen HK, Donato J, Wang HH, Cloud-Hansen KA, Davies J, Handelsman J. Call of the wild: antibiotic resistance genes in natural environments. Nat Rev Microbiol 2010;8:251–9.
[5] Abat C, Raoult D, Rolain JM. Are we living in an antibiotic resistance nightmare? Clin Microbiol Infect 2018;24:568–9.
[6] Nicolaou KC, Rigol S. A brief history of antibiotics and select advances in their synthesis. J Antibiot (Tokyo) 2018;71:153–84.
[7] Kuenzli E. Antibiotic resistance and international travel: causes and consequences. Travel Med Infect Dis 2016;14:595–8.
[8] Carvalho IT, Santos L. Antibiotics in the aquatic environments: a review of the European scenario. Environ Int 2016;94:736–57.
[9] Doi Y, Iovleva A, Bonomo RA. The ecology of extended-spectrum β-lactamases (ESBLs) in the developed world. J Travel Med 2017;24(Suppl 1):S44–51.

[10] Dhariwal AK, Tullu MS. Colistin: re-emergence of the 'forgotten' antimicrobial agent. J Postgrad Med 2013;59:208–15.
[11] Biswas S, Brunel JM, Dubus JC, Reynaud-Gaubert M, Rolain JM. Colistin: an update on the antibiotic of the 21st century. Expert Rev Anti Infect Ther 2012;10:917–34.
[12] Caniaux I, van Belkum A, Zambardi G, Poirel L, Gros MF. MCR: modern colistin resistance. Eur J Clin Microbiol Infect Dis 2017;36:415–20.
[13] Liu YY, Wang Y, Walsh TR, Yi LX, Zhang R, Spencer J, et al. Emergence of plasmid-mediated colistin resistance mechanism MCR-1 in animals and human beings in China: a microbiological and molecular biological study. Lancet Infect Dis 2016;16:161–8.
[14] Baron S, Hadjadj L, Rolain JM, Olaitan AO. Molecular mechanisms of polymyxin resistance: knowns and unknowns. Int J Antimicrob Agents 2016;48:583–91.
[15] Feng Y. Transferability of MCR-1/2 polymyxin resistance: complex dissemination and genetic mechanism. ACS Infect Dis 2018;4:291–300.
[16] Laxminarayan R, Matsoso P, Pant S, Brower C, Rottingen JA, Klugman K, et al. Access to effective antimicrobials: a worldwide challenge. Lancet 2016;387:168–75.
[17] Paschoal RP, Campana EH, Correa LL, Montezzi LF, Barrueto LRL, da Silva IR, et al. Concentration and variety of carbapenemase producers in recreational coastal waters showing distinct levels of pollution. Antimicrob Agents Chemother 2017;61 e01963-17.
[18] Toleman MA, Bugert JJ, Nizam SA. Extensively drug-resistant New Delhi metallo-β-lactamase-encoding bacteria in the environment, Dhaka, Bangladesh, 2012. Emerg Infect Dis 2015;21:1027–30.
[19] Jorgensen SB, Soraas A, Arnesen LS, Leegaard T, Sundsfjord A, Jenum PA. First environmental sample containing plasmid-mediated colistin-resistant ESBL-producing *Escherichia coli* detected in Norway. APMIS 2017;125:822–5.
[20] Sanderson H, Fricker C, Brown RS, Majury A, Liss SN. Antibiotic resistance genes as an emerging environmental contaminant. Environmental Reviews 2016;24:205–18.
[21] Laurens C, Jean-Pierre H, Licznar-Fajardo P, Hantova S, Godreuil S, Martinez O, et al. Transmission of IMI-2 carbapenemase-producing Enterobacteriaceae from river water to human. J Glob Antimicrob Resist 2018;15:88–92.
[22] Baquero F, Martínez JL, Cantón R. Antibiotics and antibiotic resistance in water environments. Curr Opin Biotechnol 2008;19:260–5.
[23] Hocquet D, Muller A, Bertrand X. What happens in hospitals does not stay in hospitals: antibiotic-resistant bacteria in hospital wastewater systems. J Hosp Infect 2016;93:395–402.
[24] Pruden A, Larsson DG, Amezquita A, Collignon P, Brandt KK, Graham DW, et al. Management options for reducing the release of antibiotics and antibiotic resistance genes to the environment. Environ Health Perspect 2013;121:878–85.
[25] Khedher MB, Baron SA, Riziki T, Ruimy R, Raoult D, Diene SM, et al. Massive analysis of 64,628 bacterial genomes to decipher water reservoir and origin of mobile colistin resistance genes: is there another role for these enzymes? Sci Rep 2020;10:5970.
[26] Kieffer N, Nordmann P, Poirel L. *Moraxella* species as potential sources of MCR-like polymyxin resistance determinants. Antimicrob Agents Chemother 2017;61 e00129-17.
[27] Snesrud E, McGann P, Chandler M. The birth and demise of the *ISApl1–mcr-1–ISApl1* composite transposon: the vehicle for transferable colistin resistance. mBio 2018;9 e02381-17.
[28] Zhang H, Wei W, Huang M, Umar Z, Feng Y. Definition of a family of nonmobile colistin resistance (NMCR-1) determinants suggests aquatic reservoirs for MCR-4. Adv Sci (Weinh) 2019;6:1900038.
[29] Zhang H, Hou M, Xu Y, Srinivas S, Huang M, Liu L, et al. Action and mechanism of the colistin resistance enzyme MCR-4. Commun Biol 2019;2:36.
[30] Manaia CM, Macedo G, Fatta-Kassinos D, Nunes OC. Antibiotic resistance in urban aquatic environments: can it be controlled? Appl Microbiol Biotechnol 2016;100:1543–57.
[31] Zhao F, Feng Y, Lu X, McNally A, Zong Z. Remarkable diversity of *Escherichia coli* carrying *mcr-1* from hospital sewage with the identification of two new *mcr-1* variants. Front Microbiol 2017;8:2094.
[32] Haller L, Chen H, Ng C, Le TH, Koh TH, Barkham T, et al. Occurrence and characteristics of extended-spectrum β-lactamase- and carbapenemase-producing bacteria from hospital effluents in Singapore. Sci Total Environ 2018;615:1119–25.
[33] Kümmerer K. Resistance in the environment. J Antimicrob Chemother 2004;54:311–20.
[34] Broom LJ. The sub-inhibitory theory for antibiotic growth promoters. Poult Sci 2017;96:3104–8.
[35] Hong PY, Al-Jassim N, Ansari MI, Mackie RI. Environmental and public health implications of water reuse: antibiotics, antibiotic resistant bacteria, and antibiotic resistance genes. Antibiotics (Basel) 2013;2:367–99.
[36] Hoelzer K, Wong N, Thomas J, Talkington K, Jungman E, Coukell A. Antimicrobial drug use in food-producing animals and associated human health risks: what, and how strong, is the evidence? BMC Vet Res 2017;13:211.
[37] Madec JY, Haenni M, Nordmann P, Poirel L. Extended-spectrum β-lactamase/AmpC- and carbapenemase-producing Enterobacteriaceae in animals: a threat for humans? Clin Microbiol Infect 2017;23:826–33.
[38] Olaitan AO, Dandachi I, Baron SA, Daoud Z, Morand S, Rolain JM. Banning colistin in feed additives: a small step in the right direction. Lancet Infect Dis 2021;21:29–30.
[39] Shen C, Zhong L-L, Yang Y, Doi Y, Paterson DL, Stoesser N, et al. Dynamics of *mcr-1* prevalence and *mcr-1*-positive *Escherichia coli* after the cessation of colistin use as a feed additive for animals in China: a prospective cross-sectional and whole genome sequencing-based molecular epidemiological study. Lancet Microbe 2020;1:e34–43.
[40] Usui M, Nozawa Y, Fukuda A, Sato T, Yamada M, Makita K, et al. Decreased colistin resistance and *mcr-1* prevalence in pig-derived *Escherichia coli* in Japan after banning colistin as a feed additive. J Glob Antimicrob Resist 2021;24:383–6.
[41] Wang Y, Xu C, Zhang R, Chen Y, Shen Y, Hu F, et al. Changes in colistin resistance and *mcr-1* abundance in *Escherichia coli* of animal and human origins following the ban of colistin-positive additives in China: an epidemiological comparative study. Lancet Infect Dis 2020;20:1161–71.
[42] Kümmerer K. Antibiotics in the aquatic environment—a review—part II. Chemosphere 2009;75:435–41.
[43] Rizzo L, Manaia C, Merlin C, Schwartz T, Dagot C, Ploy MC, et al. Urban wastewater treatment plants as hotspots for antibiotic resistant bacteria and genes spread into the environment: a review. Sci Total Environ 2013;447:345–60.
[44] Michael-Kordatou I, Karaolia P, Fatta-Kassinos D. The role of operating parameters and oxidative damage mechanisms of advanced chemical oxidation processes in the combat against antibiotic-resistant bacteria and resistance genes present in urban wastewater. Water Res 2018;129:208–30.
[45] Karkman A, Do TT, Walsh F, Virta MPJ. Antibiotic-resistance genes in waste water. Trends Microbiol 2018;26:220–8.
[46] Vaz-Moreira I, Nunes OC, Manaia CM. Bacterial diversity and antibiotic resistance in water habitats: searching the links with the human microbiome. FEMS Microbiol Rev 2014;38:761–78.
[47] Gao H, Zhang L, Lu Z, He C, Li Q, Na G. Complex migration of antibiotic resistance in natural aquatic environments. Environ Pollut 2018;232:1–9.
[48] Manaia CM, Rocha J, Scaccia N, Marano R, Radu E, Biancullo F, et al. Antibiotic resistance in wastewater treatment plants: tackling the black box. Environ Int 2018;115:312–24.
[49] Barcelos D, Knidel C, Fernandes C. Emergence and dispersion of resistance genes by the aquatic environment: a review. Pollution 2018;4:305–15.
[50] Santos L, Ramos F. Antimicrobial resistance in aquaculture: current knowledge and alternatives to tackle the problem. Int J Antimicrob Agents 2018;52:135–43.
[51] Cabello FC, Tomova A, Ivanova L, Godfrey HP. Aquaculture and *mcr* colistin resistance determinants. mBio 2017;8 e01229-7.
[52] Shen Y, Zhang R, Schwarz S, Wu C, Shen J, Walsh TR, et al. Farm animals and aquaculture: significant reservoirs of mobile colistin resistance genes. Environ Microbiol 2020;22:2469–84.
[53] Zarfel G, Lipp M, Gurtl E, Folli B, Baumert R, Kittinger C. Troubled water under the bridge: screening of River Mur water reveals dominance of CTX-M harboring *Escherichia coli* and for the first time an environmental VIM-1 producer in Austria. Sci Total Environ 2017;593–594:399–405.
[54] Zurfluh K, Hachler H, Nuesch-Inderbinen M, Stephan R. Characteristics of extended-spectrum β-lactamase- and carbapenemase-producing Enterobacteriaceae isolates from rivers and lakes in Switzerland. Appl Environ Microbiol 2013;79:3021–6.
[55] Diab M, Hamze M, Bonnet R, Saras E, Madec JY, Haenni M. Extended-spectrum β-lactamase (ESBL)- and carbapenemase-producing Enterobacteriaceae in water sources in Lebanon. Vet Microbiol 2018;217:97–103.
[56] Drali R, Berrazeg M, Zidouni LL, Hamitouche F, Abbas AA, Deriet A, et al. Emergence of *mcr-1* plasmid-mediated colistin-resistant *Escherichia coli* isolates from seawater. Sci Total Environ 2018;642:90–4.
[57] Caltagirone M, Nucleo E, Spalla M, Zara F, Novazzi F, Marchetti VM, et al. Occurrence of extended spectrum β-lactamases, KPC-type, and MCR-1.2-producing Enterobacteriaceae from wells, river water, and wastewater treatment plants in Oltrepo Pavese Area, Northern Italy. Front Microbiol 2017;8:2232.
[58] Zhang XX, Zhang T, Fang HH. Antibiotic resistance genes in water environment. Appl Microbiol Biotechnol 2009;82:397–414.
[59] Rathinasabapathi P, Hiremath DS, Arunraj R, Parani M. Molecular detection of New Delhi metallo-β-lactamase-1 (NDM-1) positive bacteria from environmental and drinking water samples by loop mediated isothermal amplification of bla_{NDM-1}. Indian J Microbiol 2015;55:400–5.
[60] Walsh TR, Weeks J, Livermore DM, Toleman MA. Dissemination of NDM-1 positive bacteria in the New Delhi environment and its implications for human health: an environmental point prevalence study. Lancet Infect Dis 2011;11:355–62.
[61] Waglechner N, Wright GD. Antibiotic resistance: it's bad, but why isn't it worse? BMC Biol 2017;15:84.
[62] Fletcher S. Understanding the contribution of environmental factors in the spread of antimicrobial resistance. Environ Health Prev Med 2015;20:243–52.
[63] Heuer H, Smalla K. Horizontal gene transfer between bacteria. Environ Biosafety Res 2007;6:3–13.
[64] Baur B, Hanselmann K, Schlimme W, Jenni B. Genetic transformation in freshwater: *Escherichia coli* is able to develop natural competence. Appl Environ Microbiol 1996;62:3673–8.
[65] Arber W. Horizontal gene transfer among bacteria and its role in biological evolution. Life (Basel) 2014;4:217–24.
[66] Kumar S, Singh BR. An overview of mechanisms and emergence of antimicrobials drug resistance. Adv Anim Vet Sci 2013;1(2S):7–14.

[67] Subirats J, Sanchez-Melsio A, Borrego CM, Balcazar JL, Simonet P. Metagenomic analysis reveals that bacteriophages are reservoirs of antibiotic resistance genes. Int J Antimicrob Agents 2016;48:163–7.
[68] Davies J, Davies D. Origins and evolution of antibiotic resistance. Microbiol Mol Biol Rev 2010;74:417–33.
[69] Alanis AJ. Resistance to antibiotics: are we in the post-antibiotic era? Arch Med Res 2005;36:697–705.
[70] Schwarz S, Johnson AP. Transferable resistance to colistin: a new but old threat. J Antimicrob Chemother 2016;71:2066–70.
[71] Fernandes MR, Sellera FP, Esposito F, Sabino CP, Cerdeira L, Lincopan N. Colistin-resistant *mcr-1*-positive *Escherichia coli* on public beaches, an infectious threat emerging in recreational waters. Antimicrob Agents Chemother 2017;61 e00234-17.
[72] Sun P, Bi Z, Nilsson M, Zheng B, Berglund B, Stalsby Lundborg C, et al. Occurrence of bla_{KPC-2}, bla_{CTX-M}, and *mcr-1* in Enterobacteriaceae from well water in rural China. Antimicrob Agents Chemother 2017;61 e02569-16.
[73] Lupo A, Coyne S, Berendonk TU. Origin and evolution of antibiotic resistance: the common mechanisms of emergence and spread in water bodies. Front Microbiol 2012;3:18.
[74] Martínez JL. Antibiotics and antibiotic resistance genes in natural environments. Science 2008;321:365–7.
[75] Yahav D, Farbman L, Leibovici L, Paul M. Colistin: new lessons on an old antibiotic. Clin Microbiol Infect 2012;18:18–29.
[76] Li J, Nation RL, Milne RW, Turnidge JD, Coulthard K. Evaluation of colistin as an agent against multi-resistant Gram-negative bacteria. Int J Antimicrob Agents 2005;25:11–25.
[77] El-Sayed Ahmed MAE-G, Zhong L-L, Shen C, Yang Y, Doi Y, Tian G-B. Colistin and its role in the era of antibiotic resistance: an extended review (2000–2019). Emerg Microbes Infect 2020;9:868–85.
[78] Zhang H, Srinivas S, Xu Y, Wei W, Feng Y. Genetic and biochemical mechanisms for bacterial lipid A modifiers associated with polymyxin resistance. Trends Biochem Sci 2019;44:973–88.
[79] Nordmann P, Poirel L. Plasmid-mediated colistin resistance: an additional antibiotic resistance menace. Clin Microbiol Infect 2016;22:398–400.
[80] Zurfuh K, Poirel L, Nordmann P, Nuesch-Inderbinen M, Hachler H, Stephan R. Occurrence of the plasmid-borne *mcr-1* colistin resistance gene in extended-spectrum-β-lactamase-producing Enterobacteriaceae in river water and imported vegetable samples in Switzerland. Antimicrob Agents Chemother 2016;60:2594–5.
[81] Yu CY, Ang GY, Chin PS, Ngeow YF, Yin WF, Chan KG. Emergence of *mcr-1*-mediated colistin resistance in *Escherichia coli* in Malaysia. Int J Antimicrob Agents 2016;47:504–5.
[82] Yu CY, Ang GY, Chong TM, Chin PS, Ngeow YF, Yin WF, et al. Complete genome sequencing revealed novel genetic contexts of the *mcr-1* gene in *Escherichia coli* strains. J Antimicrob Chemother 2017;72:1253–5.
[83] Zhao F, Zong Z. *Kluyvera ascorbata* strain from hospital sewage carrying the *mcr-1* colistin resistance gene. Antimicrob Agents Chemother 2016;60:7498–501.
[84] Runcharoen C, Raven KE, Reuter S, Kallonen T, Paksanont S, Thammachote J, et al. Whole genome sequencing of ESBL-producing *Escherichia coli* isolated from patients, farm waste and canals in Thailand. Genome Med 2017;9:81.
[85] Islam A, Rahman Z, Monira S, Rahman MA, Camilli A, George CM, et al. Colistin resistant *Escherichia coli* carrying *mcr-1* in urban sludge samples: Dhaka, Bangladesh. Gut Pathog 2017;9:77.
[86] Ovejero CM, Delgado-Blas JF, Calero-Caceres W, Muniesa M, Gonzalez-Zorn B. Spread of *mcr-1*-carrying Enterobacteriaceae in sewage water from Spain. J Antimicrob Chemother 2017;72:1050–3.
[87] Chen K, Chan EW, Xie M, Ye L, Dong N, Chen S. Widespread distribution of *mcr-1*-bearing bacteria in the ecosystem, 2015 to 2016. Euro Surveill 2017;22:17–00206.
[88] Zhou HW, Zhang T, Ma JH, Fang Y, Wang HY, Huang ZX, et al. Occurrence of plasmid- and chromosome-carried *mcr-1* in waterborne Enterobacteriaceae in China. Antimicrob Agents Chemother 2017;61 e00017-17.
[89] Jin L, Wang R, Wang X, Wang Q, Zhang Y, Yin Y, et al. Emergence of *mcr-1* and carbapenemase genes in hospital sewage water in Beijing. China. J Antimicrob Chemother 2018;73:84–7.
[90] Zhao F, Feng Y, Lu X, McNally A, Zong Z. IncP plasmid carrying colistin resistance gene *mcr-1* in *Klebsiella pneumoniae* from hospital sewage. Antimicrob Agents Chemother 2017;61 e02229-16.
[91] Wu J, Huang Y, Rao D, Zhang Y, Yang K. Evidence for environmental dissemination of antibiotic resistance mediated by wild birds. Front Microbiol 2018;9:745.
[92] Tuo H, Yang Y, Tao X, Liu D, Li Y, Xie X, et al. The prevalence of colistin resistant strains and antibiotic resistance gene profiles in Funan River. China. Front Microbiol 2018;9:3094.
[93] Sacramento AG, Fernandes MR, Sellera FP, Munoz ME, Vivas R, Dolabella SS, et al. Genomic analysis of MCR-1 and CTX-M-8 co-producing *Escherichia coli* ST58 isolated from a polluted mangrove ecosystem in Brazil. J Glob Antimicrob Resist 2018;15:288–9.
[94] Long H, Feng Y, Ma K, Liu L, McNally A, Zong Z. The co-transfer of plasmid-borne colistin-resistant genes *mcr-1* and *mcr-3.5*, the carbapenemase gene bla_{NDM-5} and the 16S methylase gene *rmtB* from *Escherichia coli*. Sci Rep 2019;9:696.
[95] Schages L, Wichern F, Kalscheuer R, Bockmuhl D. Winter is coming—impact of temperature on the variation of β-lactamase and *mcr* genes in a wastewater treatment plant. Sci Total Environ 2020;712:136499.
[96] Falgenhauer L, Schwengers O, Schmiedel J, Baars C, Lambrecht O, Heß S, et al. Multidrug-resistant and clinically relevant Gram-negative bacteria are present in German surface waters. Front Microbiol 2019;10:2779.
[97] Han H, Liu W, Cui X, Cheng X, Jiang X. Co-existence of *mcr-1* and bla_{NDM-5} in an *Escherichia coli* strain isolated from the pharmaceutical industry. WWTP. Infect Drug Resist 2020;13:851–4.
[98] Hayashi W, Tanaka H, Taniguchi Y, Iimura M, Soga E, Kubo R, et al. Acquisition of *mcr-1* and cocarriage of virulence genes in avian pathogenic *Escherichia coli* isolates from municipal wastewater influents in Japan. Appl Environ Microbiol 2019;85 e01661-19.
[99] Igwaran A, Iweriebor BC, Okoh AI. Molecular characterization and antimicrobial resistance pattern of *Escherichia coli* recovered from wastewater treatment plants in Eastern Cape South Africa. Int J Environ Res Public Health 2018;15:1237.
[100] Hassen B, Abbassi MS, Ruiz-Ripa L, Mama OM, Ibrahim C, Benlabidi S, et al. Genetic characterization of extended-spectrum β-lactamase-producing Enterobacteriaceae from a biological industrial wastewater treatment plant in Tunisia with detection of the colistin-resistance *mcr-1* gene. FEMS Microbiol Ecol 2021;97:fiaa231.
[101] Wang Z, Fu Y, Schwarz S, Yin W, Walsh TR, Zhou Y, et al. Genetic environment of colistin resistance genes *mcr-1* and *mcr-3* in *Escherichia coli* from one pig farm in China. Vet Microbiol 2019;230:56–61.
[102] Ji X, Zheng B, Berglund B, Zou H, Sun Q, Chi X, et al. Dissemination of extended-spectrum β-lactamase-producing *Escherichia coli* carrying *mcr-1* among multiple environmental sources in rural China and associated risk to human health. Environ Pollut 2019;251:619–27.
[103] Yang F, Gu Y, Zhou J, Zhang K. Swine waste: a reservoir of high-risk bla_{NDM} and *mcr-1*. Sci Total Environ 2019;683:308–16.
[104] Zhai R, Fu B, Shi X, Sun C, Liu Z, Wang S, et al. Contaminated in-house environment contributes to the persistence and transmission of NDM-producing bacteria in a Chinese poultry farm. Environ Int 2020;139:105715.
[105] Zhu L, Zhou Z, Liu Y, Lin Z, Shuai X, Xu L, et al. Comprehensive understanding of the plasmid-mediated colistin resistance gene *mcr-1* in aquatic environments. Environ Sci Technol 2020;54:1603–13.
[106] Gogry FA, Siddiqui MT, Haq QMR. Emergence of *mcr-1* conferred colistin resistance among bacterial isolates from urban sewage water in India. Environ Sci Pollut Res Int 2019;26:33715–17.
[107] Johura FT, Tasnim J, Barman I, Biswas SR, Jubyda FT, Sultana M, et al. Colistin-resistant *Escherichia coli* carrying *mcr-1* in food, water, hand rinse, and healthy human gut in Bangladesh. Gut Pathog 2020;12:5.
[108] Zhong Y, Guo S, Seow KLG, Ming GOH, Schlundt J. Characterization of extended-spectrum β-lactamase-producing *Escherichia coli* isolates from Jurong Lake, Singapore with whole-genome-sequencing. Int J Environ Res Public Health 2021;18:937.
[109] Ahmed ZS, Elshafiee EA, Khalefa HS, Kadry M, Hamza DA. Evidence of colistin resistance genes (*mcr-1* and *mcr-2*) in wild birds and its public health implication in Egypt. Antimicrob Resist Infect Control 2019;8:197.
[110] Savin M, Bierbaum G, Hammerl JA, Heinemann C, Parcina M, Sib E, et al. ESKAPE bacteria and extended-spectrum-β-lactamase-producing *Escherichia coli* isolated from wastewater and process water from German poultry slaughterhouses. Appl Environ Microbiol 2020;86 e02748-19.
[111] Savin M, Bierbaum G, Hammerl JA, Heinemann C, Parcina M, Sib E, et al. Antibiotic-resistant bacteria and antimicrobial residues in wastewater and process water from German pig slaughterhouses and their receiving municipal wastewater treatment plants. Science Total Environ 2020;727:138788.
[112] Savin M, Bierbaum G, Blau K, Parcina M, Sib E, Smalla K, et al. Colistin-resistant Enterobacteriaceae isolated from process waters and wastewater from German poultry and pig slaughterhouses. Front Microbiol 2020;11:575391.
[113] Pérez-Etayo L, González D, Leiva J, Vitas AIJM. Multidrug-resistant bacteria isolated from different aquatic environments in the north of Spain and south of France. Microorganisms 2020;8:1425.
[114] Shen Y, Lv Z, Yang L, Liu D, Ou Y, Xu C, et al. Integrated aquaculture contributes to the transfer of *mcr-1* between animals and humans via the aquaculture supply chain. Environ Int 2019;130:104708.
[115] Gomi R, Matsuda T, Yamamoto M, Tanaka M, Ichiyama S, Yoneda M, et al. Molecular characterization of a multidrug-resistant IncF plasmid carrying *mcr-3.1* in an *Escherichia coli* sequence type 393 strain of wastewater origin. Int J Antimicrob Agents 2019;54:524–6.
[116] Xu T, Ji Y, Song J, Huang J, Chen R, Qiu C, et al. A novel host of MCR-5 belonging to *Enterobacter* spp. isolated from hospital sewage water. Environ Microbiol Rep 2021;13:234–7.
[117] Xu T, Zhang C, Ji Y, Song J, Liu Y, Guo Y, et al. Identification of *mcr-10* carried by self-transmissible plasmids and chromosome in *Enterobacter roggenkampii* strains isolated from hospital sewage water. Environ Pollut 2021;268:115706.
[118] Hmede Z, Sulaiman AAA, Jaafar H, Kassem II. Emergence of plasmid-borne colistin resistance gene *mcr-1* in multidrug-resistant *Escherichia coli* isolated from irrigation water in Lebanon. Int J Antimicrob Agents 2019;54:102–4.
[119] Alhaj Sulaiman AA, Kassem II. First report of the plasmid-borne colistin resistance gene (*mcr-1*) in *Proteus mirabilis* isolated from domestic and sewer waters in Syrian refugee camps. Travel Med Infect Dis 2020;33:101482.
[120] Nasser NA, Alhaj Sulaiman A, Mann D, Li S, Deng X, Kassem II. Draft genome sequences of multidrug-resistant and *mcr-1.1*-harboring *Escherichia coli* isolated from drinking and well waters used in Syrian refugee camps. Microbiol Resour Accounc 2021;10 e01252-20.

[121] Touati M, Hadjadj L, Berrazeg M, Baron S, Rolain JM. Emergence of *Escherichia coli* harbouring *mcr-1* and *mcr-3* gene in North West Algerian farmlands. J Glob Antimicrob Resist 2020;21:132–7.
[122] Shen Y, Xu C, Sun Q, Schwarz S, Ou Y, Yang L, et al. Prevalence and genetic analysis of *mcr-3*-positive *Aeromonas* species from humans, retail meat, and environmental water samples. Antimicrob Agents Chemother 2018;62 e00404-18.
[123] Li J, Liu S, Fu J, Yin J, Zhao J, Zhong C, et al. Co-occurrence of colistin and meropenem resistance determinants in a *Stenotrophomonas* strain isolated from sewage water. Microb Drug Resist 2019;25:317–25.
[124] Meng S, Wang YL, Liu CG, Yang J, Yuan M, Bai XN, et al. Genetic diversity, antimicrobial resistance, and virulence genes of *Aeromonas* isolates from clinical patients, tap water systems, and food. Biomed Environ Sci 2020;33:385–95.
[125] Hembach N, Schmid F, Alexander J, Hiller C, Rogall ET, Schwartz T. Occurrence of the *mcr-1* colistin resistance gene and other clinically relevant antibiotic resistance genes in microbial populations at different municipal wastewater treatment plants in Germany. Front Microbiol 2017;8:1282.
[126] Reichert G, Hilgert S, Alexander J, Rodrigues de Azevedo JC, Morck T, Fuchs S, et al. Determination of antibiotic resistance genes in a WWTP-impacted river in surface water, sediment, and biofilm: influence of seasonality and water quality. Sci Total Environ 2021;768:144526.
[127] Lekunberri I, Balcazar JL, Borrego CM. Detection and quantification of the plasmid-mediated *mcr-1* gene conferring colistin resistance in wastewater. Int J Antimicrob Agents 2017;50:734–6.
[128] Wang RN, Zhang Y, Cao ZH, Wang XY, Ma B, Wu WB, et al. Occurrence of super antibiotic resistance genes in the downstream of the Yangtze River in China: prevalence and antibiotic resistance profiles. Sci Total Environ 2019;651:1946–57.
[129] Rodríguez EA, Ramirez D, Balcázar JL, Jiménez JN. Metagenomic analysis of urban wastewater resistome and mobilome: a support for antimicrobial resistance surveillance in an endemic country. Environ Pollut 2021;276:116736.
[130] Kneis D, Berendonk TU, Heß S. High prevalence of colistin resistance genes in German municipal wastewater. Sci Total Environ 2019;694:133454.
[131] Yang D, Qiu Z, Shen Z, Zhao H, Jin M, Li H, et al. The occurrence of the colistin resistance gene *mcr-1* in the Haihe River (China). Int J Environ Res Public Health 2017;14:576.
[132] Marathe NP, Pal C, Gaikwad SS, Jonsson V, Kristiansson E, Larsson DGJ. Untreated urban waste contaminates Indian river sediments with resistance genes to last resort antibiotics. Water Res 2017;124:388–97.
[133] Dias MF, da Rocha Fernandes G, Cristina de Paiva M, Christina de Matos Salim A, Santos AB. Amaral Nascimento AM. Exploring the resistome, virulome and microbiome of drinking water in environmental and clinical settings. Water Res 2020;174:115630.
[134] Dos Santos LDR, Furlan JPR, Ramos MS, Gallo IFL, de Freitas LVP, Stehling EG. Co-occurrence of *mcr-1, mcr-3, mcr-7* and clinically relevant antimicrobial resistance genes in environmental and fecal samples. Arch Microbiol 2020;202:1795–800.
[135] Furlan JPR, Dos Santos LDR, Ramos MS, Gallo IFL, Moretto JAS, Stehling EG. Occurrence of clinically relevant antimicrobial resistance genes, including *mcr-3* and *mcr-7.1*, in soil and water from a recreation club. Int J Environ Health Res 2020 Jul 31 [Epub ahead of print]. doi:10.1080/09603123.2020.1799953.
[136] Nguyen NT, Liu M, Katayama H, Takemura T, Kasuga I. Association of the colistin resistance gene *mcr-1* with faecal pollution in water environments in Hanoi, Vietnam. Lett Appl Microbiol 2021;72:275–82.
[137] Chen H, Liu C, Teng Y, Zhang Z, Chen Y, Yang Y. Environmental risk characterization and ecological process determination of bacterial antibiotic resistome in lake sediments. Environ Int 2021;147:106345.
[138] Wang M, Xiong W, Liu P, Xie X, Zeng J, Sun Y, et al. Metagenomic insights into the contribution of phages to antibiotic resistance in water samples related to swine feedlot wastewater treatment. Front Microbiol 2018;9:2474.
[139] Xia X, Wang Z, Fu Y, Du XD, Gao B, Zhou Y, et al. Association of colistin residues and manure treatment with the abundance of *mcr-1* gene in swine feedlots. Environ Int 2019;127:361–70.
[140] Fleres G, Couto N, Schuele L, Chlebowicz MA, Mendes CI, van der Sluis LWM, et al. Detection of a novel *mcr-5.4* gene variant in hospital tap water by shotgun metagenomic sequencing. J Antimicrob Chemother 2019;74:3626–8.
[141] Voigt AM, Ciorba P, Dohla M, Exner M, Felder C, Lenz-Plet F, et al. The investigation of antibiotic residues, antibiotic resistance genes and antibiotic-resistant organisms in a drinking water reservoir system in Germany. Int J Hyg Environ Health 2020;224:113449.
[142] Khan H, Miao X, Liu M, Ahmad S, Bai X. Behavior of last resort antibiotic resistance genes (*mcr-1* and bla_{NDM-1}) in a drinking water supply system and their possible acquisition by the mouse gut flora. Environ Pollut 2020;259:113818.
[143] Khan H, Liu M, Kayani MUR, Ahmad S, Liang J, Bai X. DNA phosphorothioate modification facilitates the dissemination of *mcr-1* and bla_{NDM-1} in drinking water supply systems. Environ Pollut 2021;268:115799.
[144] Ribeiro-Gonçalves B, Francisco AP, Vaz C, Ramirez M, Carriço JA. PHYLOViZ Online: web-based tool for visualization, phylogenetic inference, analysis and sharing of minimum spanning trees. Nucleic Acids Res 2016;44:W246–51.
[145] Giamarellou H. Epidemiology of infections caused by polymyxin-resistant pathogens. Int J Antimicrob Agents 2016;48:614–21.

Partie expérimentale

Chapitre III

Partie expérimentale

En Algérie, les isolats de BGN résistantes aux carbapénèmes **(Touati et Mairi, 2019)** et à la colistine **(Drali *et al.*, 2018; Nabti *et al.*, 2020; Touati *et al.*, 2020)** sont de plus en plus détectés dans différents types d'échantillons de différentes sources, indiquant leur possible dissémination généralisée dans ce pays. En effet, malgré le peu d'études réalisées dans notre pays sur la détection des BGN résistantes aux carbapénèmes, ça fait presque dix ans que de grands chercheurs du domaine ont suggéré que les BGN productrices de carbapénémases et précisément celles de la classe D soient endémiques en Algérie **(Poirel *et al.*, 2012)**. De même, le plus ancien isolat algérien résistant à la colistine suite à l'expression du nouveau mécanisme codé par le gène *mcr-1* détecté en 2016 date du 2011 **(Berrazeg *et al.*, 2016)**. Cela suggère une ancienne propagation silencieuse de ces mécanismes de résistance dans notre pays comme résultat du manque d'études utilisant des technologies nouvelles de caractérisation moléculaire. D'où la nécessité de la réalisation d'études efficaces permettant l'identification des éventuels réservoirs et voies de diffusion de ces mécanismes.

Une étape importante dans notre quête pour évaluer le risque présenté par les milieux aquatiques à travers leur rôle comme réservoir et la possibilité de leur implication dans la dissémination des bactéries à Gram négatif résistantes aux antibiotiques de dernier recours à savoir les carbapénèmes et la colistine au niveau de la ville de Batna, une étude expérimentale a été menée entre Novembre 2018 et Octobre 2019. Cette partie avait comme objectif la recherche des bactéries citées précédemment et l'investigation de leurs mécanismes de résistance en question. Au cours de cette partie expérimentale, nous avons opté pour une démarche dont une partie propre à notre équipe de recherche basée sur l'isolement sélectif des bactéries recherchées suivie de leur caractérisation microbiologique, puis l'étude phénotypique et moléculaire des mécanismes de résistance aux antibiotiques ciblés. En parallèle la clonalité de certaines souches a été aussi étudiée. Ce protocole expérimental nous a permis la réalisation de trois articles de recherche.

- Le premier a été réalisé sur les eaux usées de l'établissement publique hospitalier de Batna en mois de Novembre 2018, où nous avons signalé la présence des BGN productrices de carbapénémases de la classe D (Oxacillinases) de type OXA-48 et de la classe B (métallo-β-lactamases) de types VIM et NDM. Cet article a été publié en 2021 dans la revue *Microbial Drug Resistance* éditée par *Mary Ann Liebert, Inc.* et indexée dans *Web Of Science* avec un facteur d'impact de 3,431 (année 2020).

- Dans le deuxième article, nous avons décrit la première détection du gène *mcr-5* responsable de la résistance à la colistine en Algérie, et la première description de ce gène dans l'espèce *Cupriavidus gilardii* dans le monde. Cette souche a été isolée à partir de l'eau de puit qui approvisionne la maternité de Batna en eau de robinet. L'article a été publié en 2021 dans la revue *mSphere* éditée par la Société Américaine de Microbiologie indexée dans *Web Of Science* avec un facteur d'impact de 4,282 (année 2020).
- Le troisième article qui est en cours de publication, rassemble l'investigation des autres milieux aquatiques traités au cours de cette thèse. Cette partie a été réalisée sur la période allant du mois de Janvier jusqu'au mois d'Octobre 2019 dans laquelle nous avons analysé 207 échantillons d'eau de différentes origines y compris l'eau des puits qui approvisionnent les quatre grands établissements hospitaliers de la ville de Batna en eau, l'eau de robinet et les eaux usées de ces mêmes établissements, en plus des eaux usées déversées dans Oued El Gourzi. Dans cet article, nous rapportons des résultats originaux d'une grande importance épidémiologique et sanitaire à savoir la détection des BGN productrices des carbapénémases dans les eaux souterraines, l'eau de robinet des hôpitaux ainsi que dans les eaux usées hospitalières et celles déversées dans l'environnement. En plus, nous avons détecté des souches d'*Escherichia coli* porteuses du gène *mcr-1* appartenant à différents clones dans les eaux usées hospitalières et celles rejetées dans l'environnement.

Références bibliographiques

Berrazeg, M., Hadjadj, L., Ayad, A., Drissi, M., and Rolain, J.M. 2016. First Detected Human Case in Algeria of *mcr-1* Plasmid-Mediated Colistin Resistance in a 2011 *Escherichia coli* Isolate. *Antimicrobial agents and chemotherapy* **60**(11): 6996-6997. doi:10.1128/aac.01117-16.

Drali, R., Berrazeg, M., Zidouni, L.L., Hamitouche, F., Abbas, A.A., Deriet, A., and Mouffok, F. 2018. Emergence of *mcr-1* plasmid-mediated colistin-resistant *Escherichia coli* isolates from seawater. *The Science of the total environment* **642**: 90-94. doi:10.1016/j.scitotenv.2018.05.387.

Nabti, L.Z., Sahli, F., Ngaiganam, E.P., Radji, N., Mezaghcha, W., Lupande-Mwenebitu, D., Baron, S.A., Rolain, J.-M., and Diene, S.M. 2020. Development of real-time PCR assay allowed describing the first clinical *Klebsiella pneumoniae* isolate harboring plasmid-mediated colistin resistance *mcr-8* gene in Algeria. *Journal of global antimicrobial resistance* **20**: 266-271. doi:https://doi.org/10.1016/j.jgar.2019.08.018.

Poirel, L., Potron, A., and Nordmann, P. 2012. OXA-48-like carbapenemases: the phantom menace. *The Journal of antimicrobial chemotherapy* **67**(7): 1597-1606. doi:10.1093/jac/dks121.

Touati, A. and Mairi, A. 2019. Carbapenemase-Producing *Enterobacterales* in Algeria: A Systematic Review. *Microbial drug resistance*. doi:10.1089/mdr.2019.0320.

Touati, M., Hadjadj, L., Berrazeg, M., Baron, S.A., and Rolain, J.M. 2020. Emergence of Escherichia coli harbouring *mcr-1* and *mcr-3* genes in North West Algerian farmlands. *Journal of global antimicrobial resistance* **21**: 132-137. doi:https://doi.org/10.1016/j.jgar.2019.10.001.

Article 3

Emergence of metallo-β-lactamases and OXA-48 carbapenemase producing Gram-negative bacteria in hospital wastewater in Algeria: a potential dissemination pathway into the environment

Publié dans « *Microbial Drug Resistance* »

Facteur d'impact : 3,431

Emergence of metallo-β-lactamases and OXA-48 carbapenemase producing Gram-negative bacteria in hospital wastewater in Algeria: a potential dissemination pathway into the environment

Running title : Carbapenemase producers in hospital wastewater

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Abstract

Antibiotic-resistant bacteria can leave hospitals and therefore contaminate the environment and, most likely, humans and animals, *via* different routes, among which wastewater discharge is of great importance. This study aims to assess the possible role of hospital sewage as reservoir and dissemination pathway of carbapenem resistant Gram-negative bacilli (GNB). Carbapenem resistant GNB were selectively isolated from wastewater collected from a public hospital in Batna, Algeria. Species identification was carried out using matrix-assisted laser desorption and ionization time-of-flight mass spectrometry and antibiotic susceptibility was evaluated by the disc diffusion method. β-lactamase production was investigated phenotypically using the double-disk synergy assay and the modified CarbaNP test, then the molecular mechanisms of β-lactam-resistance were studied by PCR and sequencing.

Ten *Enterobacteriaceae* and fourteen glucose-non-fermenting (Gnf) GNB isolates were obtained. All *Enterobacteriaceae* isolates were positive for OXA-48 and TEM-1D β-lactamases, where seven of them co-produced an extended-spectrum β-lactamase. VIM-2 carbapenemase was detected in six Gnf GNB isolates. However, three *Pseudomonas aeruginosa*, one *Comamonas jiangduensis* and one *Acinetobacter baumannii* isolates were positive for VIM-4 variant. In addition, NDM-1 enzyme was detected in four *Acinetobacter baumannii* isolates.

Our findings highlight the potential impact of hospital wastewater in the spread of drug resistance mechanisms outside of hospitals.

Keywords

Hospital sewage, Gram-negative bacteria, metallo-β-lactamases, OXA-48, Algeria.

Article 4

MCR-5-producing colistin resistant *Cupriavidus gilardii* strain from well water in Batna, Algeria

Publié dans « *mSphere* »

Facteur d'impact : 4,282

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OBSERVATION

MCR-5-Producing Colistin-Resistant *Cupriavidus gilardii* Strain from Well Water in Batna, Algeria

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ABSTRACT This paper presents the first description of the *mcr-5.1* gene in a colistin-resistant *Cupriavidus gilardii* isolate from well water that supplies a maternity hospital in Algeria. The whole-genome sequence of this strain showed the presence of putative β-lactamase, *aac(3)-IVa*, and multidrug efflux pump-encoding genes, which could explain the observed multidrug resistance phenotype. Our findings are of great interest, as we highlight a potential contamination route for the spread of *mcr* genes.

IMPORTANCE Colistin resistance mediated by *mcr* genes in Gram-negative bacteria has gained significant attention worldwide. This is due to the ability of these genes to be horizontally transferred between different bacterial genera and species. Aquatic environments have been suggested to play an important role in the emergence and spread of this resistance mechanism. Here, we describe the first report of an *mcr-5*-positive *Cupriavidus gilardii* aquatic isolate through its isolation from well water in Algeria. The significance of our study is in shedding the light on an important environmental reservoir of *mcr* genes.

KEYWORDS *Cupriavidus gilardii*, *mcr-5*, colistin resistance, groundwater, Algeria

Since the first detection of the plasmid-mediated colistin resistance mechanism in December 2015, 10 *mcr* genes and several variants have been identified worldwide from different sources (1, 2). Being transferable, this mechanism has received more attention than any of the colistin resistance mechanisms previously described. Indeed, the origin of this mechanism has long preoccupied researchers, and different studies have suggested an environmental origin, particularly an aquatic one (3–5), which could participate significantly in its dissemination to pathogenic bacteria. Likewise, aquatic environments can act as an important vehicle for the spread of such resistance mechanisms to humans either in the community or, more worryingly, in hospital settings.

In this paper, we present the first report of the *mcr-5* gene in an unusual bacterial isolate, *Cupriavidus gilardii*, recovered from well water that supplies a maternity hospital in the Batna province, Algeria.

During September and October 2019, 38 water samples were obtained from a maternity hospital in Batna city, Algeria. The hospital is located in an urban region where no agricultural activity is near the study site. One liter of water was collected in sterile glass bottles from the well that supplies the hospital with tap water, from water tanks, and from taps with the hospital's various wards. Each water sample was filtered through a cellulose

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membrane (0.45 μm pore size), and the filter was placed on a MacConkey agar plate (HiMedia, India). Plates were incubated overnight aerobically at 37°C. Cultures were purified and identified using matrix-assisted laser desorption ionization–time of flight mass spectrometry (6). Thereafter, isolates were screened by real-time PCR for the occurrence of *mcr-1*, *mcr-2*, *mcr-3*, *mcr-4*, *mcr-5*, and *mcr-8* genes as previously described (7, 8). The *mcr-5* gene was detected in one isolate (strain Q4897) which was identified as *Cupriavidus gilardii*. *Cupriavidus gilardii* is a glucose-nonfermenting Gram-negative bacterium (GNB) that belongs to the *Burkholderiaceae* family. It was previously classified as *Ralstonia gilardii* and *Wautersia gilardii* (9). The gene was fully amplified by standard PCR and sequenced using BigDye terminator chemistry on an ABI 3500xl automated sequencer (Applied Biosystems, Foster City, CA, USA). Sequence analysis confirmed an *mcr-5.1* variant.

The *mcr-5*-positive isolate was examined for its susceptibility to antibiotics using the disc diffusion method, and inhibition zone diameters were interpreted according to the antibiotic committee of the French Society for Microbiology (Société Française de Microbiologie) breakpoints (https://www.sfm-microbiologie.org/wp-content/uploads/2020/04/CASFM2020_Avril2020_V1.1.pdf). In addition, the colistin MIC was determined using the broth microdilution (BMD) method. Our isolate was resistant to ticarcillin, ticarcillin-clavulanate, aztreonam, ertapenem, meropenem, imipenem, gentamicin, fosfomycin, rifampin, and colistin (MIC = 8 μg/ml). The isolate was negative for carbapenemase production using the β-CARBA test (Bio-Rad, Marnes-la-Coquette, France). For whole-genome sequencing (WGS), genomic DNA was extracted using the EZ1 biorobot with the EZ1 DNA tissue kit (Qiagen, Hilden, Germany) and then sequenced on a MiSeq sequencer (Illumina Inc., San Diego, CA, USA) with the Nextera Mate Pair sample preparation kit and Nextera XT Paired End (Illumina). The assembly was performed using a Shovill pipeline (https://github.com/tseemann/shovill). Scaffolds of <800 bp and scaffolds with a depth value lower than 25% of the mean depth were removed. The assembly generated 66 contigs with a total length of 5,335,421 bp and a GC content of 67.3%. The occurrence of antibiotic resistance genes was investigated through the ABRicate function of the Galaxy web platform (https://usegalaxy.org.au/) using ARG-ANNOT, NCBI, CARD, and ResFinder as reference databases with minimum of 70% for identity and coverage. All detected hits are presented in Table 1. In addition to the *mcr-5.1* colistin resistance gene, we identified a class D β-lactamase which was highly similar (90.84% similarity with the reference sequence) to the OXA-837 enzyme and a putative aminoglycoside inactivation enzyme, "*aac(3)-IVa*." Interestingly, these two antibiotic resistance genes have been found to be well conserved in *C. gilardii* genomes (9). Furthermore, several conserved multidrug efflux pumps were detected, which could explain the multidrug resistance phenotype observed in our isolate.

In parallel, the *mcr-5* protein reference sequence (WP_053821788.1) from *Proteobacteria* was used to query its presence in all available complete and WGS genomes of *Cupriavidus* from the NCBI database. The *in silico* analysis showed that, of the 127 *Cupriavidus* genomes, five *mcr-5* chromosomic sequences (4% of analyzed genomes) exhibited an identity value at 100% and 100% alignment with the reference sequence. Indeed, it has been suggested that the *mcr-5* gene might have been transferred from environmental *C. gilardii* to *Salmonella enterica* (10); nevertheless, this gene was identified only in three out of the eight available *C. gilardii* genomes (Table 2) and in two genomes of *Cupriavidus* sp. However, we do not know the susceptibility of these strains to colistin, which could have provided us with more information on the resistance mechanism. In addition, Easyfig v2.2.5 software was used to investigate the genetic environment surrounding the *mcr-5* gene from the five selected genomes as well as from our isolate (Fig. 1).

Our *mcr-5*-positive isolate was recovered from the well supplying the hospital with tap water. Except for drinking, this water is used in all applications requiring water use in the hospital, including cooking, bathing of newborns, cleaning, and hand washing. It is worth mentioning that well water is directly used without any treatment.

The *mcr-5* gene was first described in *Salmonella enterica* subsp. *enterica* serovar Paratyphi B var. Java *d*Ta+ from Germany, where the authors confirmed that the *mcr-5* gene was located on a 7,337-bp Tn*3*-family transposon harbored by a ColE-type

TABLE 1 Antibiotic resistance determinants found in *C. gilardii* Q4897

Gene	% coverage	% identity	Product	Resistance
mcr-5.1	100.00	99.94	Phosphoethanolamine-lipid A transferase MCR-5.1	Colistin
aac(3)-IVa	97.81	70.73	Aminoglycoside *N*-acetyltransferase AAC(3)-IVa	Gentamicin
$bla_{OXA-837}$	100.00	90.84	Class D β-lactamase OXA-837	β-Lactams
Pseudomonas aeruginosa emrE	87.09	72.07	EmrE is a small multidrug transporter that functions as a homodimer and that couples the efflux of small polyaromatic cations from the cell with the import of protons down an electrochemical gradient. Confers resistance to tetraphenylphosphonium, methyl viologen, gentamicin, kanamycin, and neomycin.	Aminoglycoside
muxB	96.36	78.44	MuxB is one of the two necessary RND components in the *Pseudomonas aeruginosa* efflux pump system MuxABC-OpmB.	Aminocoumarin; macrolide; monobactam; tetracycline
muxC	72.52	72.05	MuxC is one of the two necessary RND components of the MuxABC-OpmB efflux pumps system in *Pseudomonas aeruginosa*.	Aminocoumarin; macrolide; monobactam; tetracycline
Pseudomonas aeruginosa soxR	89.17	70.24	SoxR is a redox-sensitive transcriptional activator that induces expression of a small regulon that includes the RND efflux pump-encoding operon *mexGHI-opmD*. SoxR was shown to be activated by pyocyanin.	Acridine dye; cephalosporin; fluoroquinolone; glycylcycline; penam; phenicol; rifamycin; tetracycline; triclosan
axyY	96.02	71.68	AxyY is the periplasmic adaptor protein of the AxyXY-OprZ efflux pump system in *Achromobacter* spp.	Aminoglycoside; cephalosporin; fluoroquinolone; macrolide
mexC	82.39	71.92	MexC is the membrane fusion protein of the MexCD-OprJ multidrug efflux complex.	Aminocoumarin; aminoglycoside; cephalosporin; diaminopyrimidine; fluoroquinolone; macrolide; penam; phenicol; tetracycline
mexD	97.35	74.52	MexD is the multidrug inner membrane transporter of the MexCD-OprJ complex.	Aminocoumarin; aminoglycoside; cephalosporin; diaminopyrimidine; fluoroquinolone; macrolide; penam; phenicol; tetracycline

TABLE 2 *mcr-5* detected in *Cupriavidus* genomes (100% of identity and coverage)

No.	Organism	Strain	Genome size (bp)	GC%	Total CDS[a]	Assembly level	Isolation source	Geographic location	Accession no.(s)
1	*C. gilardii*	CR3	5,578,743	67.55	4,988	Complete genome	Tar pits	Rancho La Brea, Los Angeles, CA, USA	NZ_CP010516.1; NZ_CP010517.1
2	*C. gilardii*	CCUG 38401	5,792,089	67.4	5,283	Contig	Whirlpool	Missing	NZ_VZOV00000000.1
3	*C. gilardii*	ATCC 700815	5,761,323	67.4	5,253	Contig	Whirlpool	Missing	NZ_JABEMD000000000
4	*C. gilardii*	Q4897	5,335,421	67.3	4,717	Contig	Well water	Batna, Algeria	JAGFTW000000000
5	*Cupriavidus* sp.	MKL-01	5,749,837	67.9	5,043	Scaffold	Blood	Seoul, South Korea	NZ_VWRN00000000
6	*Cupriavidus* sp.	ISTL7	5,578,573	66.75	4,655	Chromosome	Soil	Delhi, India	NZ_CP066227; NZ_CP066228

[a]CDS, coding DNA sequences.

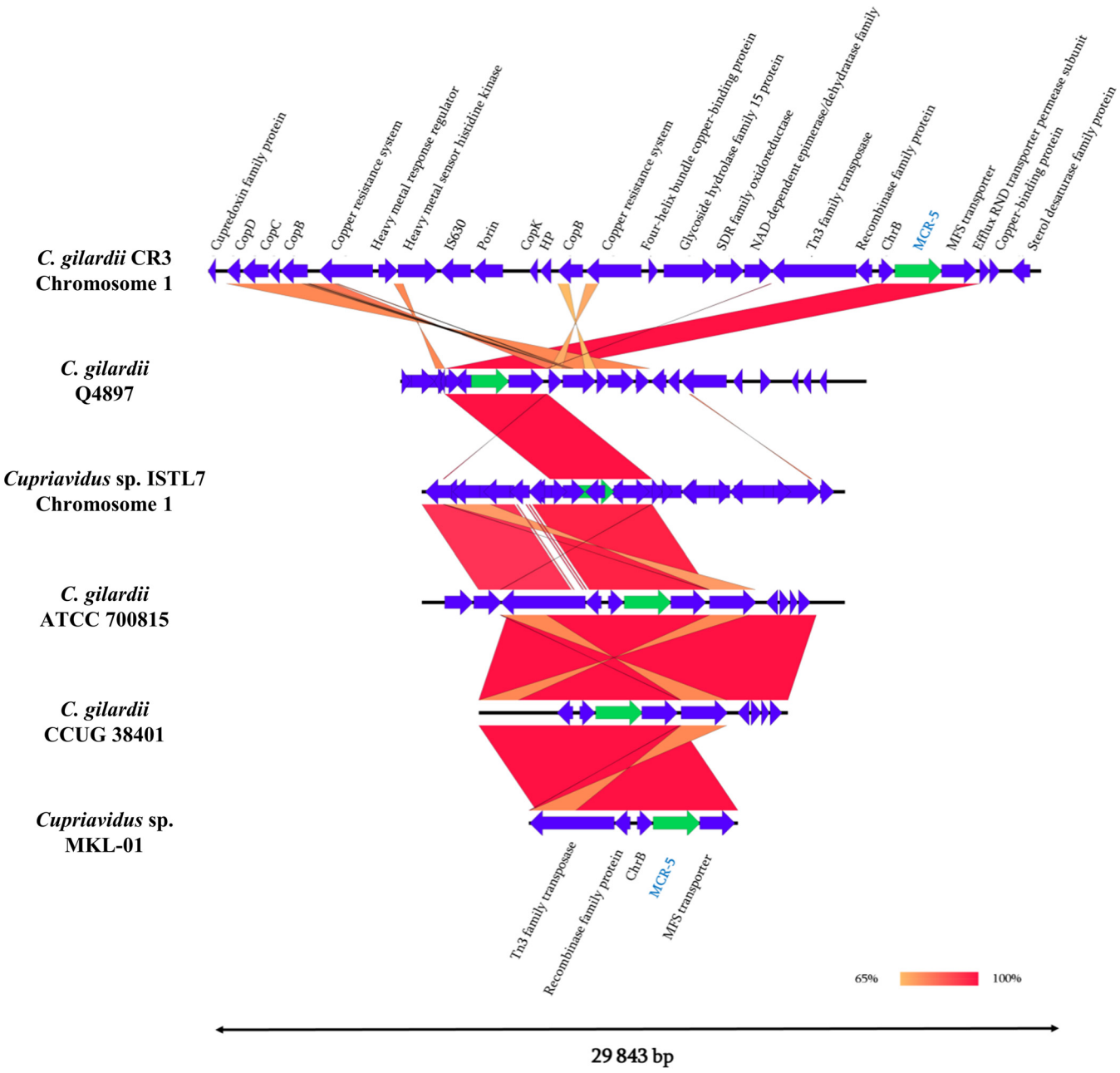

FIG 1 Genomic environment of *mcr-5* genes in *Cupriavidus* genomes. Linear comparison of the *mcr-5*-carrying chromosome fragments of *C. gilardii* strain CR3, *C. gilardii* strain CCUG 38401, *C. gilardii* strain ATCC 700815, *C. gilardii* strain Q4897, *Cupriavidus* sp. strain MKL-01, and *Cupriavidus* sp. strain ISTL7. Boxed arrows represent the position and transcriptional direction of open reading frames. Regions of >99% identity are marked by red shading. MFS, major facilitator superfamily.

plasmid (10). Interestingly, by using BLASTn search a Tn*3*-family transposon harboring the *mcr-5* gene was also detected in chromosome 1 of a *C. gilardii* strain (CR3) recovered in the United States (10).

mcr variants have been previously detected in aquatic environments. *mcr-5* and *mcr-5.4* have been detected by culture-independent methods in a wastewater treatment plant in Germany and in hospital tap water in the Netherlands, respectively (11, 12). In addition, the *mcr-5* gene has been detected in an *Enterobacter* sp. isolated from hospital sewage in China (13), and an MCR-5.3-producing *Stenotrophomonas* sp. has been isolated from animal waste in China (14). Recently, *mcr-5* has been detected in a *Cupriavidus* sp. closely related to *C. gilardii* isolated from the blood of an immunocompromised patient in South Korea (15).

Members of the *Cupriavidus* genius are known for their resistance to copper and other metals. This might be due to the presence of several metal resistance loci such as *cop* genes, as shown in Fig. 1.

C. gilardii is gaining increasing attention as an emerging pathogen, and several studies have reported its role in human infections, including perirectal inflammation, bloodstream infection, muscular abscess, and catheter sepsis (15). In terms of antibiotic resistance, it has been suggested that *C. gilardii* is intrinsically resistant to ertapenem, meropenem, ampicillin, amoxicillin-clavulanate, gentamicin, tobramycin, and streptomycin, while it is susceptible to imipenem and cefotaxime and intermediately resistant to spectinomycin (9). In a study carried out on 39 *Cupriavidus* clinical isolates, including six *C. gilardii* strains, the authors tested the MICs of these strains against 20 antibiotics by BMD, and the results showed that two *C. gilardii* strains were resistant to colistin and four were imipenem resistant. However, the resistance mechanisms were not characterized (16).

Our findings are of great interest, as we present here a potential route for the spread of such resistant organisms in the community, where further investigations and actions are required in order to contain this problem.

Data availability. This whole-genome sequence has been deposited at GenBank under accession no. JAGFTW000000000.

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There are no competing interests to declare.

REFERENCES

1. Wang C, Feng Y, Liu L, Wei L, Kang M, Zong Z. 2020. Identification of novel mobile colistin resistance gene *mcr-10*. Emerg Microbes Infect 9:508–516. https://doi.org/10.1080/22221751.2020.1732231.
2. Ling Z, Yin W, Shen Z, Wang Y, Shen J, Walsh TR. 2020. Epidemiology of mobile colistin resistance genes *mcr-1* to *mcr-9*. J Antimicrob Chemother 75:3087–3095. https://doi.org/10.1093/jac/dkaa205.
3. Khedher MB, Baron SA, Riziki T, Ruimy R, Raoult D, Diene SM, Rolain J-M. 2020. Massive analysis of 64,628 bacterial genomes to decipher water reservoir and origin of mobile colistin resistance genes: is there another role for these enzymes? Sci Rep 10:5970. https://doi.org/10.1038/s41598-020-63167-5.
4. Shen Y, Xu C, Sun Q, Schwarz S, Ou Y, Yang L, Huang Z, Eichhorn I, Walsh TR, Wang Y, Zhang R, Shen J. 2018. Prevalence and genetic analysis of *mcr-3*-positive *Aeromonas* species from humans, retail meat, and environmental water samples. Antimicrob Agents Chemother 62:e00404-18. https://doi.org/10.1128/AAC.00404-18.
5. Shen Y, Yin W, Liu D, Shen J, Wang Y. 2018. Reply to Cabello et al., "Aquaculture and *mcr* colistin resistance determinants." mBio 9:e01629-18. https://doi.org/10.1128/mBio.01629-18.
6. Seng P, Drancourt M, Gouriet F, La Scola B, Fournier PE, Rolain JM, Raoult D. 2009. Ongoing revolution in bacteriology: routine identification of bacteria by matrix-assisted laser desorption ionization time-of-flight mass spectrometry. Clin Infect Dis 49:543–551. https://doi.org/10.1086/600885.
7. Touati M, Hadjadj L, Berrazeg M, Baron SA, Rolain JM. 2020. Emergence of Escherichia coli harbouring *mcr-1* and *mcr-3* genes in North West Algerian farmlands. J Glob Antimicrob Resist 21:132–137. https://doi.org/10.1016/j.jgar.2019.10.001.
8. Nabti LZ, Sahli F, Ngaiganam EP, Radji N, Mezaghcha W, Lupande-Mwenebitu D, Baron SA, Rolain J-M, Diene SM. 2020. Development of real-time PCR assay allowed describing the first clinical *Klebsiella pneumoniae* isolate harboring plasmid-mediated colistin resistance *mcr-8* gene in Algeria. J Glob Antimicrob Resist 20:266–271. https://doi.org/10.1016/j.jgar.2019.08.018.
9. Ruiz C, McCarley A, Espejo ML, Cooper KK, Harmon DE. 2019. Comparative genomics reveals a well-conserved intrinsic resistome in the emerging multidrug-resistant pathogen *Cupriavidus gilardii*. mSphere 4:e00631-19. https://doi.org/10.1128/mSphere.00631-19.
10. Borowiak M, Fischer J, Hammerl JA, Hendriksen RS, Szabo I, Malorny B. 2017. Identification of a novel transposon-associated phosphoethanolamine transferase gene, *mcr-5*, conferring colistin resistance in d-tartrate fermenting *Salmonella enterica* subsp. *enterica* serovar Paratyphi B. J Antimicrob Chemother 72:3317–3324. https://doi.org/10.1093/jac/dkx327.
11. Kneis D, Berendonk TU, Heß S. 2019. High prevalence of colistin resistance genes in German municipal wastewater. Sci Total Environ 694:133454. https://doi.org/10.1016/j.scitotenv.2019.07.260.
12. Fleres G, Couto N, Schuele L, Chlebowicz MA, Mendes CI, van der Sluis LWM, Rossen JWA, Friedrich AW, García-Cobos S. 2019. Detection of a novel *mcr-5.4* gene variant in hospital tap water by shotgun metagenomic sequencing. J Antimicrob Chemother 74:3626–3628. https://doi.org/10.1093/jac/dkz363.
13. Xu T, Ji Y, Song J, Huang J, Chen R, Qiu C, Zhou K. 2021. A novel host of MCR-5 belonging to *Enterobacter* spp. isolated from hospital sewage water. Environ Microbiol Rep 13:234–237. https://doi.org/10.1111/1758-2229.12937.
14. Li J, Liu S, Fu J, Yin J, Zhao J, Zhong C, Cao G. 2019. Co-occurrence of colistin and meropenem resistance determinants in a *Stenotrophomonas* strain isolated from sewage water. Microb Drug Resist 25:317–325. https://doi.org/10.1089/mdr.2018.0418.
15. Kweon OJ, Lim YK, Kim HR, Kim T-H, Ha S-M, Lee M-K. 2020. Isolation of a novel species in the genus *Cupriavidus* from a patient with sepsis using whole genome sequencing. PLoS One 15:e0232850. https://doi.org/10.1371/journal.pone.0232850.
16. Massip C, Coullaud-Gamel M, Gaudru C, Amoureux L, Doleans-Jordheim A, Hery-Arnaud G, Marchandin H, Oswald E, Segonds C, Guet-Revillet H. 2020. In vitro activity of 20 antibiotics against *Cupriavidus* clinical strains. J Antimicrob Chemother 75:1654–1658. https://doi.org/10.1093/jac/dkaa066.

Article 5

Aquatic environments as reservoirs of carbapenemases and MCR-1 producing Gram-negative bacteria in Batna, Algeria

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Aquatic environments as reservoirs of carbapenemases and MCR-1 producing Gram-negative bacteria in Batna, Algeria

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Abstract

In recent years, the importance of the surrounding environment in the emergence and the dissemination of drug-resistant-bacteria has been emphasised, especially within the concept of the “One Health” approach, where water plays an important role. The aim of this study was to screen for carbapenem- and colistin-resistant Gram-negative bacteria (GNBs) in hospital tap water, hospital sewage, and environmental wastewater and to investigate their molecular determinants.

For this purpose, 172 tap water and 35 wastewater samples were collected in Batna city in Algeria. Carbapenem- and colistin-resistant GNBs were selectively isolated and then identified using matrix-assisted laser desorption and ionization time-of-flight mass spectrometry. Antibiotic susceptibility was assessed phenotypically using the disc diffusion, E-test, broth microdilution, the double-disk synergy test and the modified CarbaNP test. Subsequently, the molecular mechanisms of β-lactams and colistin resistance were investigated by PCR and sequencing. The clonal relatedness of *mcr-1* positive *E. coli* isolates was determined by multi-locus sequence typing.

We noticed high level of resistance in both tap water and wastewater. The most commonly found carbapenem-resistance mechanism was the OXA-48 enzyme. Other carbapenemases were also detected, including KPC, VIM and NDM variants. In addition, the *mcr-1* gene was detected in 18 *E. coli* isolates of different sequence types.

Our findings highlight the role of aquatic environments in the dissemination of resistant bacteria, especially considering that water is a connecting medium between different ecological systems and can easily transmit resistant bacteria and promote horizontal gene transfer. Thus, the development of effective treatment strategies for eliminating antibiotic-resistance is needed.

Conclusion générale et perspectives

Au terme de ce travail, nous avons constaté que les milieux aquatiques sont des réservoirs et peuvent véhiculer les BGN résistantes aux antibiotiques de dernier recours (carbapénèmes et colistine) permettant leur possible large dissémination dans l'environnement, d'où ces milieux aquatiques présentent aujourd'hui un problème majeur menaçant à la fois la santé publique, la santé animale, l'environnement, et la sécurité alimentaire.

L'identification des réservoirs environnementaux d'organismes résistants et des gènes de résistance est cruciale dans la quête mondiale pour contrôler leur dissémination. En se basant sur les données bibliographiques collectées au cours de la réalisation de cette thèse et particulièrement les deux revues de littérature, nous concluons que les BGN résistantes aux carbapénèmes et à la colistine par les gènes *mcr* ont connu une dissémination à grande échelle (mondiale) dans les différentes sources aquatiques, présentant ainsi une préoccupation majeure qui s'est imposée notamment au cours des dernières années.

Dans le présent travail, nous rapportons la première détection des BGN productrices d'OXA-48, des métallo-β-lactamases et de MCR-1 dans les eaux usées hospitalières et celles rejetées dans l'environnement en Algérie à travers leur description à la ville de Batna. Ces découvertes confirment le rôle potentiel de ces milieux dans la dissémination de tels mécanismes de résistance aux antibiotiques en dehors des hôpitaux et dans l'environnement. D'autre part, nous avons rapporté aussi la première détection des BGN productrices de carbapénémases dans l'eau de robinet et de puits assurant l'approvisionnement des hôpitaux en Algérie, signalant le danger présenté pour la santé des malades hospitalisés. Ce travail a permis aussi de détecter le premier isolat résistant à la colistine suite à l'expression du gène *mcr-5* en Algérie *via* son isolement à partir d'eau de puit d'un hôpital, confirmant l'utilité et l'importance de l'investigation de la contamination des environnements aquatiques par ces bactéries.

Les données présentées ici confirment la large diffusion des BGN productrices de carbapénémases et de MCR-1 dans l'environnement naturel et d'autres habitats aquatiques dans la ville de Batna, Algérie. Au cours de ce travail, nous avons utilisé des outils avancés d'identification et de caractérisation moléculaire des souches isolées à savoir le typage moléculaire et le séquençage du génome bactérien ce qui nous a permis de mieux comprendre les mécanismes responsables de ces niveaux de résistance, l'épidémiologie et la relation entre les souches isolées. Ceci est de grande valeur pour une surveillance efficace permettant de

lutter contre le phénomène de résistance aux antibiotiques quel que soit en milieu clinique ou dans l'environnement.

En effet, l'interdépendance entre l'environnement et la santé des humains, des animaux et des plantes rend la surveillance et le contrôle du phénomène de la résistance aux antibiotiques une tâche indispensable. D'où le besoin urgent d'une collaboration interdisciplinaire pour établir des stratégies efficaces de contrôle et de prévention contre la propagation de telles bactéries. De plus, il semble clair que les eaux usées, quelle que soit leur origine, sont le principal réservoir des bactéries résistantes parmi les autres milieux aquatiques. Par conséquent, elles devraient être une cible principale pour les efforts de contrôle et de prévention. Ainsi, le développement de méthodes efficaces de traitement des eaux usées pour éliminer ou au moins diminuer les bactéries résistantes aux antibiotiques et les gènes de résistance aux antibiotiques dans l'effluent final est fortement recommandé.

Les résultats obtenus au cours de ce travail ouvrent le champ pour des travaux futurs ciblant les perspectives suivantes :

- Poursuivre la surveillance de la résistance aux antibiotiques chez les BGN et l'élargir au groupe des bactéries à Gram positif à travers le lancement de plusieurs compagnies d'échantillonnage étalées dans le temps et à partir de différentes sources de plusieurs endroits.
- Etude de la clonalité des souches isolées d'échantillons de différents domaines pour comprendre le circuit de dissémination de ces gènes *via* l'eau.
- Evaluation du pouvoir de transfert des gènes de résistance en milieu aquatique à travers les éléments génétiques mobiles.
- Développement des techniques de *culturomics* pour la recherche de ces bactéries dans les différents types d'échantillons ce qui pourrait aider à isoler d'autres espèces non connues pouvant disséminer ou même être à l'origine des gènes de résistance aux antibiotiques.
- L'utilisation des techniques nouvelles de caractérisation moléculaire à savoir le séquençage à haut débit des génomes entiers des bactéries en question pour mieux comprendre ce phénomène d'antibiorésistance dans les milieux aquatiques et son impact sur la santé humaine et animale.

Résumés

Résumé

La résistance aux antibiotiques constitue aujourd'hui l'une des plus graves menaces pesant sur la santé publique, la sécurité alimentaire et le développement. Malheureusement, sa surveillance a longtemps été focalisée sur les milieux cliniques. Cependant, depuis que les bactéries résistantes aux antibiotiques sont considérées comme des polluants biologiques émergents, la contamination de l'environnement par les gènes de résistance et les bactéries résistantes aux antibiotiques a suscité une prise de conscience considérable.

Dans cette étude, nous avons analysé l'eau du robinet des hôpitaux, les eaux usées hospitalières et les eaux usées rejetées dans l'environnement pour la présence et la diversité de bactéries à Gram négatif (BGN) résistantes aux carbapénèmes et à la colistine ainsi que l'investigation des mécanismes moléculaires impliqués dans cette résistance. Au cours de ce travail, nous avons utilisé des protocoles d'isolement sélectif et non sélectif des bactéries cibles, ainsi que des outils avancés d'identification et de caractérisation moléculaire des souches isolées à savoir le typage moléculaire et le séquençage du génome entier.

Dans le présent travail, nous rapportons la première détection des BGN productrices de carbapénémases et des protéines MCR dans différents milieux aquatiques en Algérie, à travers leur isolement à partir des eaux usées hospitalières, eaux usées rejetées dans l'environnement, eau de robinet des hôpitaux ainsi que l'eau des puits à la ville de Batna. En ce qui concerne les carbapénémases, différentes enzymes ont été détectées à savoir KPC-2, NDM-5, VIM-2, VIM-4, OXA-23, OXA-48 et OXA-181, avec l'OXA-48 est la plus répondue. D'autre part, seulement deux gènes *mcr* ont été détectés dont le *mcr-1* chez des souches de l'espèce *Escherichia coli* appartenant à différentes séquences types et *mcr-5* chez une souche de *Cupriavidus gilardii*.

Les résultats de notre étude confirment que les milieux aquatiques sont des réservoirs et peuvent véhiculer les BGN résistantes aux antibiotiques de dernier recours (carbapénèmes et colistine) permettant leur possible large dissémination dans l'environnement, par conséquent ces milieux aquatiques présentent aujourd'hui un problème majeur menaçant à la fois la santé publique, la santé animale, l'environnement, et la sécurité alimentaire. D'où le besoin urgent d'une collaboration interdisciplinaire pour établir des stratégies efficaces de contrôle et de prévention contre la propagation de telles bactéries.

Mots clés : BGN, *mcr-1*, *mcr-5*, carbapénémases, milieux aquatiques, Algérie.

Summary

Antibiotic resistance is today one of the most serious threats to public health, food security and development. Unfortunately, its surveillance has long been focused on clinical settings. However, since antibiotic resistant bacteria have been seen as emerging biological pollutants, there has been considerable awareness of environmental contamination by resistance genes and antibiotic resistant bacteria.

In this study, we analyzed hospital tap water, hospital wastewater and wastewater discharged to the environment for the presence and diversity of Gram-negative bacteria (GNB) resistant to carbapenems and colistin as well as the investigation of the molecular mechanisms involved in this resistance. During this work, we used protocols for selective and non-selective isolation of target bacteria, as well as advanced tools for identification and molecular characterization of isolated strains, namely molecular typing and whole genome sequencing.

In the present work, we report the first detection of carbapenemases and MCR producing GNB in various aquatic environments in Algeria, *via* their isolation from hospital wastewater, wastewater discharged into the environment, hospital tap water as well as well water in Batna city. Regarding carbapenemases, different enzymes were detected namely KPC-2, NDM-5, VIM-2, VIM-4, OXA-23, OXA-48 and OXA-181, with OXA-48 being the most detected. On the other hand, only two *mcr* genes were detected, including *mcr-1* in *Escherichia coli* isolates belonging to different sequence types and *mcr-5* in a *Cupriavidus gilardii* isolate.

The results of our study confirm that aquatic environments are reservoirs and can transport GNB resistant to last-resort antibiotics (carbapenems and colistin) allowing their possible wide dissemination in the environment, consequently these aquatic environments today present a major problem threatening both public health, animal health, the environment and food safety. Hence the urgent need for interdisciplinary collaboration to establish effective strategies to control and prevent the spread of such bacteria.

Key words: GNB, *mcr-1*, *mcr-5*, carbapenemases, aquatic environments, Algeria.

ملخص

تعد مقاومة المضادات الحيوية اليوم أحد أخطر التهديدات للصحة العامة، الأمن الغذائي والتنمية. للأسف، دراسة هذه الظاهرة تركزت منذ فترة طويلة على الأوساط الاستشفائية. لكن، منذ أن أصبح ينظر إلى البكتيريا المقاومة للمضادات الحيوية على أنها ملوثات بيولوجية ناشئة، فقد أصبح هناك وعي كبير بالتلوث البيئي بجينات المقاومة والبكتيريا المقاومة للمضادات الحيوية.

في هذه الدراسة، قمنا بتحليل مياه الصنبور ومياه الصرف الصحي بالمستشفيات ومياه الصرف الصحي التي يتم تصريفها إلى البيئة من أجل الكشف عن وجود وتنوع البكتيريا سالبة الجرام المقاومة للكاربابينيمات والكوليستين وكذلك دراسة الآليات الجزيئية المسؤولة عن هذه المقاومة. خلال هذا العمل، استخدمنا بروتوكولات للعزل الانتقائي وغير الانتقائي للبكتيريا المستهدفة، فضلاً عن الأدوات المتطورة لتعريف السلالات المعزولة والدراسة الجزيئية لها.

في هذا العمل، قمنا لأول مرة بعزل بكتيريا سالبة الجرام مقاومة للكاربابينيمات والكوليستين في أوساط مائية مختلفة في الجزائر، وذلك في مياه الصرف الصحي بالمستشفيات، ومياه الصرف الصحي التي يتم تصريفها في البيئة، ومياه الحنفية بالمستشفيات وكذلك مياه الآبار في مدينة باتنة. كما تم تحديد آليات جزيئية متعددة لهذه المقاومة.

تؤكد نتائج دراستنا أن الأوساط المائية يمكن أن تلعب دورا مهما في نقل البكتيريا سالبة الجرام المقاومة للمضادات الحيوية (الكاربابينيمات والكوليستين) مما يسمح بانتشارها على نطاق واسع في البيئة، وبالتالي تمثل هذه البيئات المائية اليوم مشكلة كبيرة تهدد صحة الإنسان والحيوان، البيئة وسلامة الغذاء. ومن هنا تأتي الحاجة الماسة إلى تعاون متعدد التخصصات لوضع استراتيجيات فعالة للسيطرة على هذه البكتيريا ومنع انتشارها.

الكلمات المفتاحية: البكتيريا سالبة الجرام، الكاربابينيمات، الكوليستين، الأوساط المائية، الجزائر.

Résumé

La résistance aux antibiotiques constitue aujourd'hui l'une des plus graves menaces pesant sur la santé publique, la sécurité alimentaire et le développement. Malheureusement, sa surveillance a longtemps été focalisée sur les milieux cliniques. Cependant, depuis que les bactéries résistantes aux antibiotiques sont considérées comme des polluants biologiques émergents, la contamination de l'environnement par les gènes de résistance et les bactéries résistantes aux antibiotiques a suscité une prise de conscience considérable.

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Dans le présent travail, nous rapportons la première détection des BGN productrices de carbapénémases et des protéines MCR dans différents milieux aquatiques en Algérie, à travers leur isolement à partir des eaux usées hospitalières, eaux usées rejetées dans l'environnement, eau de robinet des hôpitaux ainsi que l'eau des puits à la ville de Batna. En ce qui concerne les carbapénémases, différentes enzymes ont été détectées à savoir KPC-2, NDM-5, VIM-2, VIM-4, OXA-23, OXA-48 et OXA-181, avec l'OXA-48 est la plus répondue. D'autre part, seulement deux gènes *mcr* ont été détectés dont le *mcr-1* chez des souches de l'espèce *Escherichia coli* appartenant à différentes séquences types et *mcr-5* chez une souche de *Cupriavidus gilardii*.

Les résultats de notre étude confirment que les milieux aquatiques sont des réservoirs et peuvent véhiculer les BGN résistantes aux antibiotiques de dernier recours (carbapénèmes et colistine) permettant leur possible large dissémination dans l'environnement, par conséquent ces milieux aquatiques présentent aujourd'hui un problème majeur menaçant à la fois la santé publique, la santé animale, l'environnement, et la sécurité alimentaire. D'où le besoin urgent d'une collaboration interdisciplinaire pour établir des stratégies efficaces de contrôle et de prévention contre la propagation de telles bactéries.

Mots clés : BGN, *mcr-1*, *mcr-5*, carbapénémases, milieux aquatiques, Algérie.

ملخص

تعد مقاومة المضادات الحيوية اليوم أحد أخطر التهديدات للصحة العامة، الأمن الغذائي والتنمية. للأسف، دراسة هذه الظاهرة تركزت منذ فترة طويلة على الأوساط الاستشفائية. لكن، منذ أن أصبح ينظر إلى البكتيريا المقاومة للمضادات الحيوية على أنها ملوثات بيولوجية ناشئة، فقد أصبح هناك وعي كبير بالتلوث البيئي بجينات المقاومة والبكتيريا المقاومة للمضادات الحيوية.

في هذه الدراسة، قمنا بتحليل مياه الصنبور ومياه الصرف الصحي بالمستشفيات ومياه الصرف الصحي التي يتم تصريفها إلى البيئة من أجل الكشف عن وجود وتنوع البكتيريا سالبة الجرام المقاومة للكاربابينيمات والكوليستين وكذلك دراسة الآليات الجزيئية المسؤولة عن هذه المقاومة. خلال هذا العمل، استخدمنا بروتوكولات للعزل الانتقائي وغير الانتقائي للبكتيريا المستهدفة، فضلاً عن الأدوات المتطورة لتعريف السلالات المعزولة والدراسة الجزيئية لها.

في هذا العمل، قمنا لأول مرة بعزل بكتيريا سالبة الجرام مقاومة للكاربابينيمات والكوليستين في أوساط مائية مختلفة في الجزائر، وذلك في مياه الصرف الصحي بالمستشفيات، ومياه الصرف الصحي التي يتم تصريفها في البيئة، ومياه الحنفية بالمستشفيات وكذلك مياه الآبار في مدينة باتنة. كما تم تحديد آليات جزيئية متعددة لهذه المقاومة.

تؤكد نتائج دراستنا أن الأوساط المائية يمكن أن تلعب دورا مهما في نقل البكتيريا سالبة الجرام المقاومة للمضادات الحيوية (الكاربابينيمات والكوليستين) مما يسمح بانتشارها على نطاق واسع في البيئة، وبالتالي تمثل هذه البيئات المائية اليوم مشكلة كبيرة تهدد صحة الإنسان والحيوان، البيئة وسلامة الغذاء. ومن هنا تأتي الحاجة الماسة إلى تعاون متعدد التخصصات لوضع استراتيجيات فعالة للسيطرة على هذه البكتيريا ومنع انتشارها.

الكلمات المفتاحية: البكتيريا سالبة الجرام، الكاربابينيمات، الكوليستين، الأوساط المائية، الجزائر.