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Review

The Prevalence of ESKAPE Pathogens and Their Drug Resistance Profiles in Aquatic Environments Around the World

Tunde Olarinde Olaniyan [1], Ana Verónica Martínez-Vázquez [1], Cesar Marcial Escobedo-Bonilla [2], Cristina López-Rodríguez [3], Patricia Huerta-Luévano [3], Oziel Castrejón-Sánchez [3], Wendy Lizeth de la Cruz-Flores [4], Manuel J. Cedeño-Castillo [4], Erick de Jesús de Luna-Santillana [1], Maria Antonia Cruz-Hernández [1], Gildardo Rivera [1] and Virgilio Bocanegra-García [1,*]

[1] Centro de Biotecnología Genómica, Instituto Politécnico Nacional, Reynosa 88710, Mexico; tolaniyan2200@alumno.ipn.mx (T.O.O.); avmartinez@ipn.mx (A.V.M.-V.); edeluna@ipn.mx (E.d.J.d.L.-S.); macruzh@ipn.mx (M.A.C.-H.); gildardors@hotmail.com (G.R.)
[2] CIIDIR Sinaloa, Instituto Politécnico Nacional, Guasave 81101, Mexico; cescobe@ipn.mx
[3] Campus Reynosa, Universidad del Valle de México, Reynosa 88769, Mexico; cristinalopezrdz@gmail.com (C.L.-R.); pattyhuerta10@hotmail.com (P.H.-L.); oziel_castre@hotmail.com (O.C.-S.)
[4] Campus de Medicina, Universidad México Americana del Norte, Reynosa 88640, Mexico; wendyliz@outlook.es (W.L.d.l.C.-F.); drcedeno26@gmail.com (M.J.C.-C.)
* Correspondence: vbocanegra@ipn.mx

Abstract

Antimicrobial-resistant bacteria (ARB) in the ESKAPE group include *Enterococcus faecium*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Acinetobacter baumannii*, *Pseudomonas aeruginosa*, and *Enterobacter* spp. These pathogens continue to pose a global threat to human health. Urban and non-urban rivers affected by anthropogenic activities such as farming can act as reservoirs for ARB. The influx of wastewater from animal farms and irrigation processes can affect the normal microbiota in surrounding waterbodies. New bacteria, such as those in the ESKAPE family, may be introduced into these waterbodies, since most ESKAPE pathogens are domiciled in humans and animals. There is a dearth of information on the persistence of ESKAPE isolates and their associated health hazards in non-nosocomial settings. Therefore, this review aimed to collect data on the global distribution of ESKAPE pathogens in aquatic systems. PubMed and Google Scholar were searched for articles published from 2009 to 2025. A total of 76 studies published in peer-reviewed journals were included. Data were collected from 21 papers for *E. faecium/faecalis*, 12 for *S. aureus*, 15 for *K. pneumoniae*, 11 for *A. baumannii*, 8 for *P. aeruginosa*, and 9 for *Enterobacter* spp. The findings in this review will increase public health awareness on the significance of ESKAPE pathogens in aquatic systems.

Keywords: antimicrobial resistant; ESKAPE pathogens; anthropogenic activities; bacterial community; waterbodies

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1. Introduction

Antibiotic-resistant bacterial (ARB) pathogens are considered a serious global health hazard by the World Health Organization [1] and the U.S. Centers for Disease Control and Prevention [2]. This is based on widespread patterns of resistance, impacts on animal and plant health, and the cost of treatment [1]. A major contributor to antimicrobial resistance (AMR) is the intensive and widespread use of antibiotics globally, leading to an increasing

public health hazard [3,4]. To minimize risk and address the challenges associated with AMR, the WHO has identified certain bacteria as key contributors to resistance and classified them into three priority groups: critical, high, and medium. The critical priority group includes carbapenem-resistant *Acinetobacter baumannii*, as well as carbapenem-resistant and third-generation cephalosporin-resistant *Klebsiella pneumoniae* and *Enterobacter* spp., while the high-priority group includes carbapenem-resistant *Pseudomonas aeruginosa* and *Enterococcus faecium* and *Staphylococcus aureus,* resistant to vancomycin and methicillin, respectively [5,6].

AMR poses a significant challenge globally, affecting nations irrespective of their geographic location or economic status [1]. Antibiotic-resistant microbes are thought to be the cause of over 2 million infections and at least 29,000 fatalities per year in the United States [7,8]. In Thailand and the United States, the anticipated total cost of combating resistance to five pathogenic bacteria (*S. aureus*, *Escherichia coli*, *K. pneumoniae*, *A. baumannii*, and *P. aeruginosa*) was approximately USD 0.5 billion and USD 2.9 billion, respectively [9]. AMR infections are responsible for more than 33,000 annual deaths and 874,000 disability-adjusted life years (DALYs) in countries in the European Union (EU) and European Economic Area (EEA) in Europe, and result in USD 1.5 billion in direct and indirect costs [10,11]. Switzerland (a non-EEA member) estimated 276 associated deaths and 7400 DALYs from over 7000 infections [12]. Compared with individual EU and EEA countries, Switzerland's 2015 estimate (87.8 DALYs per 100,000) was higher than that of Austria (77.2), Germany (64.3), and economically comparable countries, such as Luxembourg (70.9) and Denmark (52.3), but lower than that of Italy (448.4) and France (220.7) [10,12]. The deaths caused by AMR in sub-Saharan Africa were estimated at 23.5 deaths per 100,000 people in 2019, the highest in the region [13]. It is predicted that AMR will cause approximately 10 million deaths annually worldwide by 2050 if no preventative measures are implemented to control it [14].

According to standardized international terminology developed by the European Centre for Disease Prevention and Control (ECDC) and the U.S. CDC, multidrug-resistant (MDR) bacteria are characterized by non-susceptibility to at least one agent in three or more antimicrobial categories, and extensively drug-resistant (XDR) bacteria are defined as bacterial isolates susceptible to only one or two categories of antimicrobial agents [15,16].

The high incidence of resistance among the ESKAPE pathogens is associated with the diversity of the resistance mechanisms that these bacteria utilize [17]. These mechanisms include drug inactivation, modification of the target site, antibiotic elimination mediated by efflux pumps, reduced absorption of drugs, and the development of biofilms [17]. A significant number of resistance genes are located on mobile genetic elements (plasmids, transposons, and integrative conjugative elements), which enable the swift spread of resistance characteristics among ESKAPE pathogens. This hastens the dissemination of resistance mechanisms both within and among species through horizontal gene transfer (HGT) [18,19]. Recent studies have found that *A. baumannii* is becoming the hardest ESKAPE pathogen to treat with regular antibiotics because it quickly picks up and spreads resistance genes, mainly through mobile genetic elements such as plasmids, resistance islands, and insertion sequences [20]. Biofilms generated by *A. baumannii* help it develop resistance to several drugs [20]. Another study reported carbapenem-resistant *A. baumannii* resistance to most antibiotics except colistin[6].

Healthcare-associated infections are predominantly caused by ESKAPE pathogens [17]. These organisms have contributed to the increasing incidence of treatment failures and high death rates, and increased the cost of healthcare [18,21]. As important as the ESKAPE bacteria seem to be, most studies have focused on hospital or clinically associated strains, whereas environmentally associated pressures may also be responsible for the development

of resistant behaviors in ESKAPE pathogens. Rivers, being critical sources of water for drinking, agriculture (livestock production and irrigation), and recreational activities, can become reservoirs for these ESKAPE pathogens, posing risks to public health and the environment at large. Some studies have reported the presence of ESKAPE pathogens in the environment, with 576 research studies published in PubMed reporting on individual ESKAPE members in surface water between 2000 and 2025 (Figure 1).

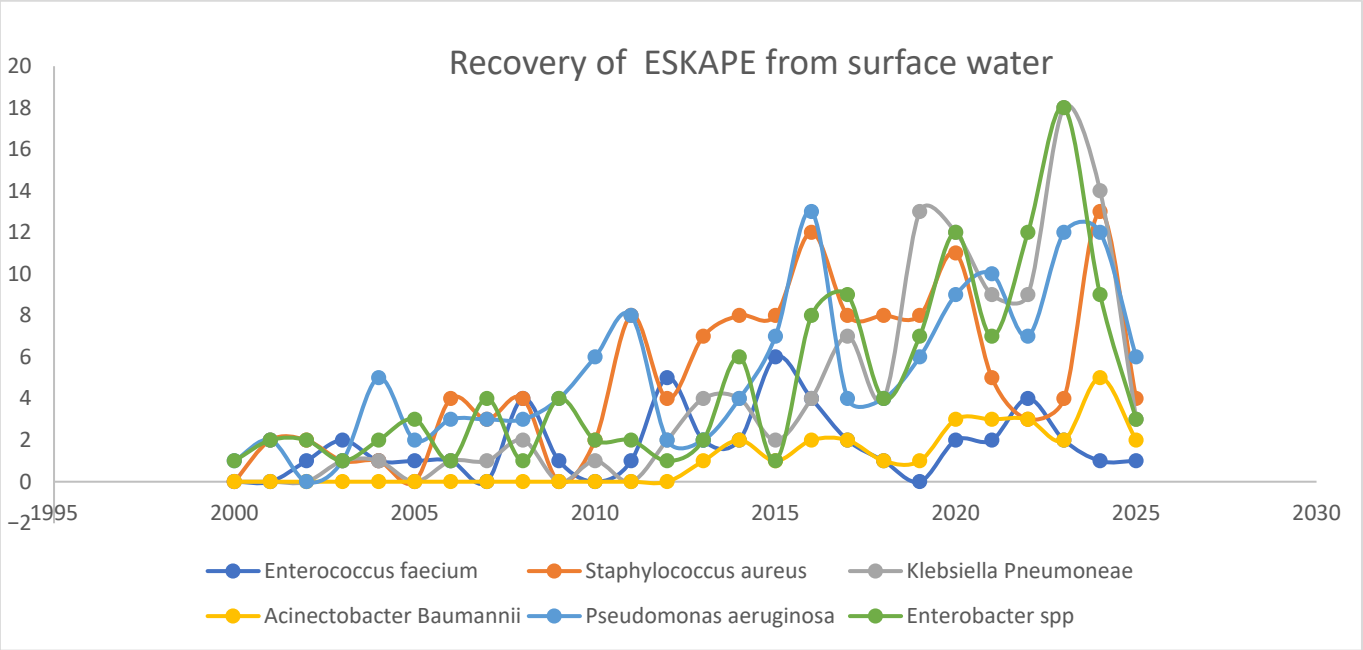


Figure 1. Research publications on individual ESKAPE pathogens in surface water between 2000 and 2025.

According to Figure 1, *Pseudomonas aeruginosa* was the most commonly identified ESKAPE pathogen in surface water, as reported in articles on PubMed between 2000 and 2025. It was followed closely by *S. aureus* and *Enterobacter species.* Articles on *K. pneumoniae* also showed that it was common in the environment, while articles on *E. faecium* and *A. baumannii* were published less often. The graph shows an increase in PubMed publications on ESKAPE bacteria in surface waters from 2010, with the trend continuing to increase after 2020. The years with the highest publication activity were 2023 and 2024, during which more research articles were published on *K. pneumoniae* and *Enterobacter* spp. Reports on *P. aeruginosa* and *S. aureus* were also found. The increase in reports of these ESKAPE pathogens in surface water, particularly from 2020 to 2024, may be attributed to the European Union's implementation of the "One Health" approach in 2017 to address AMR [22]. The discovery of widely distributed ESKAPE bacteria within the environment by Savin et al. (2020) is also a possibility [23]. Even though articles on *A. baumannii* were rare, they did appear from time to time, with a notable increase in 2024. The increase could be due to new outbreaks of drug-resistant *A. baumannii* infections or health alerts around the world. The data in Figure 1 indicate an increasing environmental burden of ARB in surface waterbodies, which may pose potential risks to public health through environmental exposure, recreational activities, and the water–food–animal interface. Therefore, this review evaluated the prevalence of ESKAPE pathogens and analyzed their current AMR and virulence profiles, with a focus on recent global trends in significant aquatic environments.

2. Materials and Methods

2.1. Study Design

The Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines were followed to ensure a systematic and transparent approach to identification, screening, eligibility, and inclusion of studies focused on individual ESKAPE bacteria and those examining multiple bacterial species, including ESKAPE members, in rivers.

2.2. Search Strategy

PubMed and Google Scholar articles with complete information on investigated rivers, sampling sites, sample collection, targeted antibiotic resistance genes, individual ESKAPE pathogens, and the effects on the environment and public health were searched to collect relevant information. The search was restricted to articles published between 2000 and 2025, with a focus on those from 2009 to 2025. to collect relevant information.

2.3. Study Selection

To achieve a comprehensive coverage in this review, both general and specific keywords and phrases were applied using the Boolean research method on PubMed and Google Scholar databases [24]. The search strategy used keywords and Boolean operators such as ("Prevalence" OR "Occurrence" OR "Diversity") AND ("ESKAPE pathogens" OR "*E. faecium*" OR "*S. aureus*" OR "*K. pneumoniae*" OR "*A. baumannii* " OR "*P. aeruginosa*" OR "*Enterobacter* spp.") AND ("river water" OR "dams" OR "streams" OR "springs" OR "lake water" OR "beach") AND ("antibiotic resistance" OR "contamination" OR "environmental impact") AND ("Africa" OR "Asia" OR "Europe" OR "North America" OR "South America" OR "Australia"). This Boolean combination is designed to retrieve scholarly articles that are of high quality, credible, use scientifically valid methods, are peer-reviewed, data-driven, and suitable for informed scientific analysis.

2.4. Quality Assessment of the Studies

Titles were initially reviewed to assess the relevance of the selected articles. Next, all article abstracts were screened, and full-text articles were retrieved and evaluated to identify those relevant to this review. Studies on bacterial diversity; occurrence or prevalence of antimicrobial resistance and/or virulence; multidrug or environmental surveillance; and molecular characterization, whole-genome sequencing (WGS), comparative genomics, and quantitative risk assessment of bacteria in aquatic systems were included. Articles in the selection that reported on all or individual ESKAPE pathogens were considered relevant. All articles reviewed for this study are listed in the Scopus and JCR databases.

2.5. Data Collection and Analysis

Data on individual ESKAPE pathogens in aquatic systems were compiled using Microsoft Excel® (Microsoft Corporation, Redmond, WA, USA). We extracted relevant information from the articles; this included the year of study, the country of origin, the type of aquatic environment, the presence of antibiotic resistance genes (ARGs), virulence genes (VGs), multidrug resistance (MDR), genetic lineages (ST/CC), and the genomic analysis conducted. The variables were descriptively compared using tables.

3. Results

3.1. Eligibility of Assessment Outcomes

A total of 76 articles that reported the prevalence of individual ESKAPE pathogens in aquatic systems, and/or ARGs, VGs, MDR, ST/CC, and genomic analysis were used in

this review. Studies reporting on more than one ESKAPE pathogen were consolidated and presented as a single article in the analysis.

3.2. Characteristics and Quality of the Study

The geographical distribution of ESKAPE pathogens in waterbodies in different continents and the total number of articles used are presented in Table 1. From a continental perspective, America has the highest number of studies on individual ESKAPE pathogens (23), followed by Asia (20), Europe (19), Africa (13), and Oceania (1). The most studied organism is *E. faecium/faecalis* (n = 21), followed by *K. pneumoniae* (n = 15), *S. aureus* (n = 12), *A. baumannii* (n = 11), *Enterobacter* spp. (n = 9), and *P. aeruginosa* (n = 8).

Table 1. Geographical distribution of ESKAPE pathogens in waterbodies in different continents.

ESKAPE Pathogen	**Continent**					
	Africa	**Americas**	**Asia**	**Europe**	**Oceania**	**Total no. of Each ESKAPE Pathogen per Continent**
Enterococcus faecium/faecalis	3	7	4	6	1	21
Staphylococcus aureus	3	4	2	3	-	12
Klebsiella pneumoniae	2	5	4	4	-	15
Acinetobacter baumannii	2	2	4	3	-	11
Pseudomonas aeruginosa	1	2	4	1	-	8
Enterobacter spp.	2	3	2	2	-	9
Total number of used articles	13	23	20	19	1	76

Five studies were duplicated.

According to data gathered from the studies included, the ESKAPE bacterial isolates identified across various studies included *E. faecium* (682), *E. faecalis* (1397), *S. aureus* (373), *K. pneumoniae* (89), *A. baumannii* (628), *P. aeruginosa* (719), and *Enterobacter* spp. (39) from one hundred and nine (109) aquatic systems, including rivers, springs, dams, beaches, streams, and lakes across 29 countries worldwide. Dams and beaches were studied because they are important components of water environments used for recreational activities such as fishing and swimming. The countries with the largest number of studies are South Africa (n = 10), followed by Brazil (n = 9), India (n = 7), USA (n = 6), Spain (n = 5), Mexico (n = 3), China (4), Japan (n = 3), Portugal (n = 3), Austria (n = 3), Italy (n = 2), Germany (n = 2), Switzerland (n = 2), Canada (n = 2), Bangladesh (n = 2), Australia (n = 1), Chile (n = 1), Bolivia (n = 1), Ecuador (n = 1), Philippines (n = 1), France (n = 1), Poland (n = 1), Croatia (n = 1), Iran (n = 1), Iraq (n = 1), Taiwan (n = 1), Algeria (n = 1), Tunisia (n = 1), and Isreal (n = 1). The global prevalence and indicators of pathogenicity of individual ESKAPE pathogens in different waterbodies, based on data from the 76 studies, are presented in Tables 2–7. In each table, the articles are arranged chronologically, starting with the most recent.

Table 2. Studies describing the prevalence, ARGs, VGs, MDR, and ST/CC of *E. faecium/faecalis* in waterbodies worldwide.

Year/Country	Aquatic System	Number of Isolates	ARG	VG	MDR *E. faecium /faecalis*	ST/CC	Genomic Analysis	Reference
2025 South Africa	Palmiet River stream	*E. faecium* (11)	*aac*(6′)-Ii *ant*(6)-Ia *dfr*G *erm*B *msr*C *tet*L *tet*M	n. d.	4	ST94 ST361 ST2013 ST2042 ST2431	WGS	[25]
2024 Mexico	River in Veracruz	*E. faecalis* (2)	n. d.	*eda1* *ccf*	0	n. d.	No	[26]
2023 India	Ganga, Yamuna, and Sangam rivers	*E. faecium* (19) *E. faecalis* (7)	n. d.	n. d.	n. d.	n. d.	No	[27]
2022 Italy	Salinello Estuary River	*E. faecium* (2)	*aph*(3′)-III *aac*(6′)-*Ii* *ant*(6)-Ia *aac*(6′)-*aph*(2″) *erm*B *fex*B *msr*C *tet*M *tet*L *poxt*A	n. d.	1	n. d.	WGS	[28]
2021 Brazil	Rivers and beaches in the southeastern region	*E. faecium* (16)	*erm*B	*gel*E *esp* *ace*	6	n. d.	No	[29]

Table 2. *Cont.*

Year/Country	Aquatic System	Number of Isolates	ARG	VG	MDR *E. faecium* /*faecalis*	ST/CC	Genomic Analysis	Reference
2021 Switzerland	Aare, Rhein, and Rhone rivers Streams	*E. faecium* (6)	*aac(6′)-Ii* *cat*A *efr*A *msr*C *tet*M *van*A	n. d.	n. d.	ST133	WGS	[30]
2020 USA	Oconee River	*E. faecium* (33) *E. faecalis* (169)	n. d.	n. d.	7/8	n. d.	No	[31]
2020 Spain	Alhama River	*E. faecium* (1)	*van*B	n. d.	1	n. d.	No	[32]
2020 South Africa	Apies River	*E. faecium* (13) *E. faecalis* (5)	*aac(6′)-Ii* *van*A/C/N/L/G *isa*A *tet*M	*Acm* *esp* *hyl*E	n. d.	ST80, ST203,	WGS, comparative genomic analysis	[33]
2019 South Africa	Rivers and dam in the Northwest Province	*E. faecium* (11) *E. faecalis* (9)	*van*A *van*B	*asa*1 *esp* *gel* *hyl*a	11/9	n. d.	No	[34]
2018 Poland	Vistula River	*E. faecium* (75) *E. faecalis* (39)	*erm*C *tet*A *tet*O *tet*W		43/6	n. d.	No	[35]
2016 Brazil	Tiete ˆand Pinheiros rivers	*E. faecium* (5) *E. faecalis* (1)	*van*A	n. d.	5/1	ST17, ST18, ST78, ST117, ST192, ST280, ST412 and ST478	Comparative genomic analysis	[36]

Table 2. *Cont.*

Year/Country	Aquatic System	Number of Isolates	ARG	VG	MDR *E. faecium* /*faecalis*	ST/CC	Genomic Analysis	Reference
2016 India	River Gomti	*E. faecalis* (33)	*van*B	*gel*E *ace* *efa*A *esp*	33	n. d.	No	[37]
2015 Japan	Yae River	*E. faecium* (38) *E. faecalis* (20)	*van*C1 *van*C2/3	n. d.	2/3	n. d.	No	[38]
2014 Portugal	Ave River	*E. faecium* (18) *E. faecalis* (15)	*van*A	n. d.	16/10	n. d.	No	[39]
2014 Iran	Babolrud River	*E. faecium* (7) *E. faecalis* (20)	n. d.	n. d.	n. d.	n. d.	No	[40]
2013 Italy	Monte Cotugno Lake	*E. faecium* (132) *E. faecalis* (21)	n. d.	Agg *efa*A *gel*E	n. d.	n. d.	No	[41]
2013 USA	Passaic River	*E. faecium* (103) *E. faecalis* (396)	n. d.	*gel*E β hemolysis *cyl*A/B/M bacteriocin	33/10	n. d.	No	[42]
2012 Australia	Coomera River	*E. faecium* (47) *E. faecalis* (55)	*van*A *van*B	*gel*E	0/0	n. d.	No	[43]
2011 Canada	South Nation River	*E. faecium* (145) *E. faecalis* (567)	n. d.	*ccf* *cpd* *esp* *enl*A *gel*E	n. d.	n. d.	No	[44]
2011 Mexico	Mololoa River	*E. faecalis* (38)	n. d.	n. d.	n. d.	n. d.	No	[45]

ARG = Antimicrobial-resistant gene; VG = Virulence gene; STC/CC = Sequence Type; n. d.= not determined; WGS = Whole-Genome Sequencing.

Table 3. Studies describing the prevalence, ARGs, VGs, MDR, and ST/CC of *S. aureus* in waterbodies worldwide.

Country	Aquatic System	Number of Isolates	ARG	VG	MDR	Molecular Typing		Genomic Analysis	Reference
						spa-Type	ST/CC		
2023 Brazil	Extrema River Spring	MRSA (20)	n.d.	n. d.	14	n. d.	n. d.	No	[46]
2021 USA	Lehia, Richardson's, Honoli'i, Pohoiki, Kealoha, Onekahakaha beach parks, Wailuku River Estuary	*mec*A-MRSA (9)	*aph*(3′)-III *bla*Z *erm*C *mec*A, *mph*C *msr*A	*hlg*A *hlg*B *hlg*C *luk*D *luk*E *luk*F-PV *luk*S-PV sek seq	9	n. d.	ST8 (CC8).	WGS assembly and analysis	[47]
		MSSA (27)	*bla*Z *erm*T *aph(3′)*-III *ant(6)*-I*a* *mph*C *tet*K	*hlg*A/B/C hemolysin genes *luk*E *seg*	4	n. d.	ST5 ST398 ST72 ST15 (CC15) ST508 (CC45) ST97 (CC97) ST518 ST6 ST1155 ST3269 ST1181		
2021 Portugal	Rivers from the Douro River Basin	*mec*C-MRSA (3)	*bla*Z-*SCCmec*IX	*Hld*	0	t742	ST425	No	[48]
		MSSA (9)	*bla*Z *erm*T *vga*A	*hla* *hlb* *hld*	0	t008 t571 t742 t208 t098 t4735 t8083	ST398 ST8 (CC8) ST49 (CC49) ST3223 ST49 (CC49) ST6835 ST133 ST6836		

Table 3. *Cont.*

Country	Aquatic System	Number of Isolates	ARG	VG	MDR	Molecular Typing		Genomic Analysis	Reference
						***spa*-Type**	**ST/CC**		
	Rivers from Bon Accord Dam	*mec*A-MRSA (6)	*bla*Z *erm*A	*sec* and *seq*.	0	n. d.	ST80 (CC80) ST728	No	[49]
2021 South Africa 2019 South Africa	Durban area Rivers	*mec*A-MRSA (80)	*aac*(6′) *aph*(2) *bla*Z *erm*C *msr*A *tet*K	*hlg*A *hlg*D lukS/FPV sea	80	n. d.	n. d.	No	[50]
2017 Austria	River Mur	*mec*A-MRSA (1)	n. d.	*hlg*A/B/C *agr type* *arc*A/B/C *aur* *cap*5H	1	t008	ST8 (CC8)	WGS	[51]
2017 South Africa	Beaches in Eastern Cape Province	*mecA*-MRSA (5) MSSA (25)	*bla*Z *mec*A *rpo*B *erm*B *tet*M	n. d.	30	n. d.	n. d.	No	[52]
2015 India	River Cauvery	MRSA (42)	n. d.	n. d.	42	n. d.	n. d.	No	[53]
2014 Spain	Game estate Rivers	*mec*C-MRSA (3)	$bla Z_{LGA251}$	n. d.	n. d.	t11212	ST425	WGS	[54]
2014 India	Ganga River	MRSA (16)	n. d.	n. d.	n. d.	n. d.	n. d.	No	[55]
		MSSA (72)	n. d.	n. d.	n. d.	n. d.	n. d.		

Table 3. *Cont.*

Country	Aquatic System	Number of Isolates	ARG	VG	MDR	Molecular Typing		Genomic Analysis	Reference
						spa-Type	ST/CC		
2012 USA	Beaches in the Marine, Freshwater, and Lake Washington	*mec*A-MRSA (31),	*tet*K *erm*C *msr*A *aad*D	n. d.	26	n. d.	ST8, ST30, ST45, ST88, ST15, ST88, ST97 ST1875 ST133 ST1956 ST2049	No	[56]
		MSSA (14)	n. d.	n. d.	n. d.	n. d.	n. d.		
2009 USA	Washington State beaches	MRSA (6)	*ccr*B *erm*A *tet*K *tet*M	n. d.	5	n. d.	ST145 ST45 ST59 ST30	No	[57]
		MSSA (4)	None	n. d.	0	n. d.	ST15 ST59 ST30		

ARG = Antimicrobial-resistant gene; VG = Virulence gene; STC/CC = Sequence Type Cluster/Clonal Complex; n. d. = not determined; WGS = Whole-Genome Sequencing.

Table 4. Studies describing the prevalence, ARGs, VGs, MDR, and ST/CC of *K. pneumoniae* waterbodies worldwide.

Country	Aquatic System	Number of Isolates	ARG	VG	MDR	ST/CC	Genomic Analysis	Reference
2024 Portugal	Rivers and dam along the Douro River Basin	9	*aad*A1 *bla*AmpC *bla*CTX-U *bla*CTX-M9 *bla*SHV *str*A *sul2*	*bfp* *pap*C,	4	n. d.	No	[58]

Table 4. *Cont.*

Country	Aquatic System	Number of Isolates	ARG	VG	MDR	ST/CC	Genomic Analysis	Reference
2024 China	Qinhuai River	5	*Acr*B *aph(3′)-Ib* *aph(6)-Id* *bac*A *mdt*B *oqx*B *rsm*A *bla*SHV-1 *ugd*	n. d.	n. d.	n. d.	No	[59]
2023 India	Kathajodi River	1	*bla*CTX-M *bla*NDM-5 *bla*SHV *bla*TEM	*Fim*H *ent*B *irp*-1 *mrk*D *ybt*S	1	ST437	No	[60]
2023 China	Dongluo, Quanfu, and Shunhe rivers	4	*aadA* *bla*CTX-M *bla*KPC-2 *bla*NDM-1 *bla*TEM *fos*A *rmt*B	*ent*A/B/C/D/E/F *ent*D *fep*A/B/C/D/G *iut*A	4	ST17 ST11 ST730	WGS	[61]

Table 4. *Cont.*

Country	Aquatic System	Number of Isolates	ARG	VG	MDR	ST/CC	Genomic Analysis	Reference
2022 Mexico	Rivers and dam along the Lerma River basin	7	*aac(3)-IIa* *aac(6′)Ib-cr* *bla*CTX-M-15 *bla*OXA-1 *bla*OXA-232 *bla*SHV-11 *bla*TEM-1B *cat*B4 *dfr*A14 *fos*A *oqx*A/B *Qnr*S1 *Qnr*B66 *str*A/B *sul*2 *tet*A	*Dam*, *fim*H *ppd*D	6	n. d.	WGS	[62]
2022 Brazil	Guajará Bay River	1	*bla*BKC-*1* *bla*CTX-M-15 *bla*SHV-11 *aph(3′)*-VIa *aac(6′)-Ib-cr* *aac(3)-Iia* *qnrE1* *qnrB19* *rsm*A *emr*R *crp* *fos*A6 *cat*B3 *rsm*A	*iut*A *acr*A/B *mrk*A/C/D/F/H/I *fim*A/B/C/D/E/F/G/H/I/K	1	ST11 CC258	WGS	[63]

Table 4. *Cont.*

Country	Aquatic System	Number of Isolates	ARG	VG	MDR	ST/CC	Genomic Analysis	Reference
2020 Tunisia	Rouriche River	2	*aad*A2 *bla*CTX-M-15 *dfr*A12 *bla*SHV *qac*EΔ1 *sul*1 *sul*2 *tet*A		2	ST1540 ST661.	No	[64]
2020 Brazil	Sertãozinho Stream, Euclides Moreli Lake, and Monjolinho River	3	*bla*SHV-26 *bla*SHV-27 *bla*SHV-81 *fos*A *sul*1 *tet*A	*iut*A *ent*B *ybt*PQ *irp*1 *fim*A/B/C/D/E/F/G/H/I/K, *mrk*A/B/C/D/F *ecp*A/B/C/D/E7R, *pul*B/C/D/E/F/G/H/I/J/K/L/M/f*ep*CG *iro*CN *mce*G *kfu*BC,	3	ST661 (CC661) ST4415 (CC515) ST4416 (CC2654)	WGS, genomic islands, phage-related sequences	[65]
2019 Croatia	Krapina River	4	*bla*KPC-2 *bla*SHV-1 *aac (3′)-II* *aac(6′)-Ib* *aph(3′)-Ia*	n. d.	4	ST258.	No	[66]

Table 4. *Cont.*

Country	Aquatic System	Number of Isolates	ARG	VG	MDR	ST/CC	Genomic Analysis	Reference
2019 Austria	Inn, Drau, Glan, Traun, and Danube Rivers	5	*aac*(3)-Iic *aac(6′)-Ib* *aph(3′)-Ia* *aad*A *bae*R *bla*CTX-M-15 *bla*OXA-1 *bla*SHV-1 *bla*VIM-1 *erm*B/R *fos*A6 *Qnr*B1 *mph*A	*iut*A *mrk*A/*mrk*B/C/D/F/H/I/J *ybt*S/tX	5	ST11 ST985 ST405 ST3400 ST323	WGS	[67]
2017 Brazil	Ibirapuera Lake	2	*bla*KPC-2 *bla*SHV-11 *oqx*A/B *dfr*A30 *tet*A *fos*A	n. d.	2	ST11/CC258	WGS	[68]
2017 Austria	River Mur	2	*Ram*R	n. d.	0	ST2392 ST2394	No	[69]
2017 Spain	Llobregat River	1	*bla*KPC-2	*fim*H *mrk*D *wab*G *uge* *mag*A *rmp*A *ure*A *all*S *kfu*BC	1	ST634	No	[70]

Table 4. *Cont.*

Country	Aquatic System	Number of Isolates	ARG	VG	MDR	ST/CC	Genomic Analysis	Reference
2017 Algeria	Soummam River	3	*bla*OXA 48 *bla*SHV	n. d.	3	ST133 ST2192 ST2055	No	[71]
2015 Iraq	Tigris River	40	*bla*CTX-M	n. d.	n. d.	n. d.	No	[72]

ARG = Antimicrobial-resistant gene; VG = Virulence gene; STC/CC = Sequence Type Cluster/Clonal Complex; n. d. = not determined; WGS = Whole-Genome Sequencing.

Table 5. Studies describing the prevalence, ARGs, VGs, MDR, and ST/CC of *A. baumannii* in waterbodies worldwide.

Country	Aquatic System	Number of Isolates	ARG	VG	MDR/ XDR	ST/CC	Genomic Analysis	Reference
2024 Ecuador	Machángara and Monjas rivers	10	*bla*OXA-51	n. d.	3	n. d.	No	[73]
2024 China	Qinhuai River	2	*msr*E mphE	n. d.	n. d.	n. d.	No	[59]
2022 France	Seine River	1	*bla*AmpC *bla*OXA-*23* *par*C *str*AB *tet*A	*blc* *omp*A *smp*A *csu*A, *csu*C *pga*A *bmf*R/S *gig*A *gac*A/S	1	ST2	WGS	[74]
2022 India	Kathajodi River	42	*bla*NDM *bla*OXA-48 *bla*TEM	Type 1 fimbriae Biofilm, Hemolytic activity, Gelatinase	23/19	n. d.	No	[75]

Table 5. *Cont.*

Country	Aquatic System	Number of Isolates	ARG	VG	MDR/ XDR	ST/CC	Genomic Analysis	Reference
2022 South Africa	Plankenbrug River	7	0	n. d.	3/1	ST945, ST2520.	No	[76]
2021 China	Xiangjiang River	1	*bla*OXA-2	n. d.	0	n. d.	No	[77]
2021 South Africa	Great Fish, Keiskamma, and Tyhume rivers	410	*aph*A1 *aph*A2 *bla*CTX-M *bla*CTX-M*1/2* *blaCTX*-M-*9* *bla*VEB *bla*GES *bla*IMP *bla*KPC *bla*PER, *blaOXA-48-like* *blaOXA-51* *bla*SHV *bla*TEM *bla*VIM *qnr*B *qnr*D *tet*A *tet*B *tet*L *tet*C *tet*M	n. d.	n. d.	n. d.	No	[78]
2021 USA	Kokosing River	5	*aad*A4 *cfx*A6 *msr*E *mph*E *tet* (39)	n. d.	5	n. d.	Shotgun sequencing	[79]

Table 5. *Cont.*

Country	Aquatic System	Number of Isolates	ARG	VG	MDR/ XDR	ST/CC	Genomic Analysis	Reference
2018 Taiwan	Puzi River	11	n. d.	n. d.	0	n. d.	No	[80]
2019 China	Tarim River	1	*bla*OXA-*69* *bla*ADC-2	n. d.	1	n. d.	WGS	[81]
2017 Germany to Romania	Danube River	135	*blaOXA*-23 *bla*OXA-24 *bla*OXA-51 *bla*TEM-1 *bla*VIM-2	n. d.	16	n. d.	No	[82]
2016 Brazil	Tietê Pinheiros Rivers	3	*bla*OXA-23 *bla*OXA-51	n. d.	3	ST79/CC79	No	[83]

ARG = Antimicrobial-resistant gene; VG = Virulence gene; STC/CC = Sequence Type Cluster/Clonal Complex; n. d. = not determined; WGS = Whole-Genome Sequencing.

Table 6. Studies describing the prevalence, ARGs, VGs, MDR, and ST/CC of *P. aeruginosa* in waterbodies worldwide.

Country	Aquatic System	Number of Isolates	ARG	VG	MDR	ST/CC	Genomic Analysis	Reference
2024 China	Qinhuai River	4	*bla*LCR-1 *mph*F *bla*OXA-101 *bla*OXA-827 *bla*TEM-1	n. d.	n. d.	n. d.	Metagenomic sequencing	[59]
2024 Spain	Iregua River	52	n. d.	*exo*U/S/Y/T/A *las*I/R *apr*A *rhl*AB/I/R *exl*A	0	ST136 ST274 ST679 ST782 ST2540	No	[84]

Table 6. *Cont.*

Country	Aquatic System	Number of Isolates	ARG	VG	MDR	ST/CC	Genomic Analysis	Reference
2024 Bangladesh	Buriganga River	70	n. d.	n. d.	3	n. d.	No	[85]
2024 India	Mahananda River	1	aph (3′)-IIb *cat*B/B7 *fos*A *emr*E *gyr*A *mex*A/B/C/D/E/F/L/G/H *opr*D/M/N *opm*D/E/H *bla*OXA-50 *pmr*A	*alg*W *exo*T/Y *flh*B *hfq* *mod*A *muc*A *nuo*D PA0082 *pil*D *phz*S *pur*D/H *pyr*F *rel*A	1	n. d.	NGS, Comparative genomic analysis	[86]
2021 South Africa	Mthatha Dam Mthatha River	36	*bla*SHV *bla*CTX-M *bla*TEM *bla*VIM	n. d.	20	n. d.	No	[87]
2016 Brazil	Tietê and Pinheiros Rivers	3	*blaSPM*-1	n. d.	3	ST277	No	[83]
2015 Bangladesh.	Lake and River in Dhaka City	28	n. d.	n. d.	28	n. d.	No	[88]
2016 Brazil	Mindu stream	8	n. d.	n. d.	0	n. d.	No	[89]
2013 Japan	Kiyotake River Yae River	516	n. d.	n. d.	n. d.	n. d.	No	[90]

Table 6. *Cont.*

Country	Aquatic System	Number of Isolates	ARG	VG	MDR	ST/CC	Genomic Analysis	Reference
2011 Brazil	Tietê River	1	*bla*SPM-1 *bla*OXA-56 *rmt*D1 *aac*A4 *aad*A7 *sul*1 *dhfr*	n. d.	1	n. d.	No	[91]

ARG = Antimicrobial-resistant gene; VG = Virulence gene; STC/CC = Sequence Type Cluster/Clonal Complex; n. d. = not determined; WGS = Whole-Genome Sequencing.

Table 7. Studies describing the prevalence, ARGs, VGs, MDR, and ST/CC of *Enterobacte*r spp. in waterbodies worldwide.

Country	Aquatic System	Number of Isolates	ARG	VG	MDR	ST/CC	Genomic Analysis	Reference
2024 China	Qinhuai River	*E. cloacae* (2) *E. hormaechei* (1)	DHA-7 *mex*B ugd	n. d.	n. d.	n. d.	Metagenomic sequencing	[59]
2023 Chile	Cachapoal River and Villarrica Lake	*E. cloacae* (2)	*bla*TEM *bla*CTX-M	n. d	2	n. d.	No.	[92]
2021 Japan	River in Osaka	*E. asburiae* (1), *E.* spp. (1)	*bla*FRI-4 *bla*FRI-8	n. d.	2	n. d.	WGS	[93]
2020 Philippines	Metro Manila River	*E. cloacae* (1) *E. hormaechei* (1) *E. kobei* (1) *E. tabaci* (1) *E. xiangfangensis* (1)	*bla*GES-20 *bla*IMI18	n. d.	n. d.	n. d.	WGS	[94]

Table 7. *Cont.*

Country	Aquatic System	Number of Isolates	ARG	VG	MDR	ST/CC	Genomic Analysis	Reference
2020 Israel	Alexander River estuary	*E. asburiae* (1) *E. bugandensis* (1)	*bla*IMI, *Nmc*R *fos*A4 *mar*A *ram*A	n. d.	2	n. d.	Metagenomic sequencing	[95]
2020 South Africa	Tsomo and Tyhume rivers	*E. aerogenes* (7) *E. amnigenus* (1) *E. asburiae* (2) *E. cloacae* (4)	*bla*CTX-M *bla*SHV *bla*FOX, *cat*II *sul*1 *tet*A *tet*B *tet*D	n. d.	14	n. d.	No	[96]
2019 Bolivia	Choqueyapu River	*E. cloacae* (1)	*AAC(3)-Iic* *AAC(6′)-Ib-cr* *aad*A *APH(3′)-Ib* *APH(6)-Id* *bla*CTX-M-3 *blaOXA*-1 *Sul*2 *bla*TEM-1 *Tet*(A)	n. d.	n. d.	n. d.	WGS	[97]

Table 7. *Cont.*

Country	Aquatic System	Number of Isolates	ARG	VG	MDR	ST/CC	Genomic Analysis	Reference
2017 Spain	Llobregat River	*E. cloacae* (2)	*bla*IMI-2 *bla*KPC-2 *bla*OXA-1 *qnr*B6	n. d.	2	ST822 ST823	No	[70]
2016 Germany	Denube River	*E. cloacae (3)* *E. cancerogenus (1)* *E. asburiae (1)*	*bla*SHV-2 *bla*SHV-12 *bla*TEM-3 *bla*CTX-M-1	n. d.	5	ST145 ST159 ST505	No	[98]
2013 Switzerland	Landquart and Lorze rivers	*E. cloacae* (1) *E. amnigenus* (1)	*bla*TEM *bla*CTXM-15	n. d.	2	n. d.	No	[99]
2011 Canada	Salmon River	*E. cloacae* (1)	*fos*A2	n. d-	1	n. d.	No	[100]

ARG = Antimicrobial-resistant gene; VG = Virulence gene; STC/CC= Sequence Type Cluster/-Clonal Complex; n. d.= not determined; WGS = Whole-Genome Sequencing.

Table 2 presents a diverse range of *E. faecium/faecalis* isolates from studies published between 2011 and 2025, with the majority of studies originating from Europe. A total of 28 waterbodies, including rivers (n = 21), streams (n = 4), lakes (1), beaches (1), and dams (1), were sampled across the 21 selected papers. Thirteen of the studies reported the presence of important ARGss. Reports on *van*A (6/21), which confers resistance to vancomycin, were the most common, followed closely by its functional analog, *van*B (4/21). Some studies also found tetracycline {*tet*A (1), *tet*B, *tet*M (4), tetL (3)}, aminoglycosides {aac(6′)-Ii (4), *ant*(*6*)-Ia(2), *aph (3′)*-III (1), *aph(3′)*-III (1), and erythromycin {*erm*B (3) and *erm*C (1)}. Nine studies reported the recovery of virulence factors in *E. faecium/faecalis.* A total of 12 studies reported MDR *E. faecium*, while eight studies reported MDR *E. faecalis.*

Table 3 presents the diversity of *S. aureus* isolates from studies published between 2009 and 2023. The waterbodies in which *S. aureus* was identified included rivers (9), beaches (8), streams (1), dams (1), springs (1), and lakes (1). A total of 12 studies reported MRSA; among these, six studies reported *mec*A-MRSA. Additionally, six studies identified *bla*Z in either MRSA, MSSA, or both. Five studies reported VGs in this pathogen. Eight studies reported MDR in MRSA/MSSA. Seven studies also conducted molecular typing to determine the genetic relatedness of the identified *S. aureus* isolates.

The diversity of *K. pneumoniae* isolates identified in studies published between 2015 and 2024 is shown in Table 4. The waterbodies reporting the presence of *K. pneumoniae* were rivers (18), dams (2), streams (1), and lakes (2). ARGs were reported in all 15 studies in this review. Eight studies reported the co-occurrence of Extended-spectrum beta-lactamases (ESBLs) and carbapenemase, while five studies reported ESBLs alone, and only one study focused on carbapenemase alone. Furthermore, eight studies discovered VGs in some of their isolates. Of the 15 studies analyzed in this review, 12 reported the presence of MDR *K. pneumoniae*. The presence of *K. pneumoniae* ST11 was reported in Austria, China, and Brazil.

The distribution of *A. baumannii* in 14 rivers from 2016 to 2024 is presented in Table 5. The pathogen was most prevalent in Africa. The studies show similar resistance patterns to *K.* pneumoniae, with two studies showing the co-occurrence of ESBL and carbapenemases, and four studies reporting the presence of carbapenemases. Only two studies reported the detection of VGs. Additionally, eight studies reported the presence of MDR *A. baumannii*, while two studies isolated both MDR and XDR *A. baumannii*.

The distribution of *P. aeruginosa* in different aquatic systems from 2011 to 2024 is presented in Table 6. A total of 14 waterbodies—rivers (n = 11), streams (n = 1), lakes (1), and dams (1)—were reported in the 10 selected papers. Co-occurrence of ESBL and carbapenemases was reported in one study. Two studies also identified aminoglycosides in rivers, and two studies reported similar VGs such as *exo*T/Y.

Table 7 presents the diversity of *Enterobacter* spp. isolates based on studies published between 2011 and 2024. The waterbodies in which *Enterobacte*r spp. were identified included 13 rivers and a lake. Eleven studies reported different *Enterobacte*r spp. (*E. cloacae*, *E. hormaechei*, *E. asburiae*, *E. kobei*, *E. tabaci*, *E. xiangfangensis*, *E. aerogenes*, *E. cancerogenus*, and *E. amnigenus*). Five studies reported the presence of carbapenemases, including *bla*FRI^{-4} and *bla*FRI^{-8}. None of the studies identified VGs in *Enterobacte*r spp.; however, eight studies reported MDR in *Enterobacte*r spp.

4. Discussion

This study aimed to conduct a systematic review of data on the occurrence of ESKAPE pathogens in aquatic environments between 2009 and 2025. We focused on the presence of ARGs, VGs, MDR, genetic lineages, and geographic distribution (country and continent). This selection is crucial in understanding the presence and behavior of ESKAPE pathogens in water systems. ARGs, VGs, and MDR in ESKAPE pathogens are very significant because

they enable these bacteria to evade antibiotic treatment and cause severe infections, which leads to more illness, death, and healthcare costs around the world [18,101]. However, few studies have analyzed ESKAPE pathogens in water systems; therefore, examining data on each pathogen individually provides valuable insights into their environmental occurrence and pathogenicity.

4.1. Enterococcus faecium/faecalis

E. faecium and *E. faecalis* are among the most common species of enterococci isolated from surface water [44,102,103]. Both are part of the human gut microbiome and are often regarded as indicators of fecal pollution in water samples [104–106]. Cho et al. (2020) [31] identified some MDR *E. faecium* harboring common genes (*tet*L, *tet*M, ermB, *erm*C), an intrinsic gene (*ant*(6)-Ia), and unusual genes (*tet*O, *lnu*B) in surface water in the Upper Oconee Watershed in Georgia, USA. The bacteria showed resistance to as many as five classes of antimicrobials; *E. faecalis* was also identified in the same waterbody. An important finding from their study is the resistance to some newly developed and rare antimicrobial agents, such as daptomycin and tigecycline, in these bacteria [31]. A related observation was reported, where several MDR *E. faecium* were recovered from rivers and beaches in Minas Gerais, Rio de Janeiro, and São Paulo, in the southeastern region of Brazil. Most *E. faecium* reported in their study harbored the *erm*B gene (known for conferring resistance to macrolides, lincosamides, and streptogramins) and three essential virulent genes: *gel*E, *esp*, and *ace* [29]. The *ermB* gene was also reported by Giebułtowicz et al. in MDR *E. faecium* and *E. faecalis* isolated from the sewage-impacted Vistula River in Poland, as well as by Mukwevho et al. in the Palmiet River stream near informal settlements in Durban and Pietermaritzburg, South Africa [25,35]. Another study found relatively low expression of multiple antibiotic resistance and virulence factors (including gelatinase, β-hemolysin, and bacteriocin) in *E. faecalis* and *E. faecium* in the upper Passaic River in Morris County, New Jersey. This observation suggests a potentially low public health risk [42].

In addition, vancomycin-resistant *E. faecium*/*faecalis* (VREs) have been isolated from rivers and other related waterbodies. In Brazil and Portugal, *E. faecalis* harboring the *van*A gene was recovered from the Tiete and Pinheiros Rivers in Brazil and the Ave River in Portugal. The detection of *van*A-containing enterococci indicates that rivers serve as an important reservoir of ARB and genes that can be disseminated across human pathogens [36,39]. Biggel and his colleagues detected *E. faecium* sequence type 133 (ST133) harboring a *van*A gene cluster on transposon Tn1546 in the Aare, Rhein, and Rhone Rivers in Switzerland. They concluded that the detection of the VRE clone ST133 in various water sampling sites across Switzerland suggests the local dominance of this lineage [30]. *Van*B-gene-harboring VREs have also been isolated from the Alhama River along the Ebro basin in the North of Spain and the South of France, showing the presence of extensive MDR and high-priority pathogens in different aquatic environments [32]. Similarly, Lata et al. identified *E. faecalis* carrying the *van*B gene in River Gomti in India. They also found that VR*E. faecalis* isolates in this river exhibited high resistance (22–100%) and carried multiple virulence markers, including gelatinase (*gelE*), collagen-binding protein (*ace*), and endocarditis-associated antigen (*efa*A) [37]. The presence of virulence factors such as *gelE*, *ace*, and *efaA* suggests an increased potential for these bacteria to cause severe infections, including endocarditis and biofilm-associated infections [37,107].

VRE strains harboring both *van*A and *van*B genes were identified in several studies. For example, Matlou et al. (2019) [34], and Rathnayake et al. (2012) [43] isolated *E. faecalis* and *E. faecium* isolates carrying *van*A and *van*B genes from diverse aquatic environments, including dams and rivers in Rustenburg, Potchefstroom, Taung, and Coligny in the Northwest Province of South Africa, and the Coomera River in Southeast Queensland,

Australia. Matlou et al. (2019) [34] highlighted the prevalence of VREs with antibiotic resistance and virulence traits in water sources used by humans; and Rathnayake et al. (2012) [43] showed that clinical isolates of *E. faecalis* and *E. faecium* have higher antibiotic resistance and virulence traits compared with environmental isolates. Ekwanzala et al. (2020) also isolated VRE harboring *van*A, *van*C, *van*N, *van*L, and *van*G gene clusters in the *E. faecalis* and *E. faecium* genomes in Apies River in South Africa [33]. This could lead to increased complexity of resistance mechanisms, resulting in more complications from the treatment of infections.

The presence of *E. faecalis* resistant to penicillin, gentamicin, fluoroquinolones, and kanamycin, has been reported in rivers in Mexico, India, and Iran [27,40,45]. *E. faecalis* isolated from the Mololoa River, in the municipality of Tepic, Nayarit, Mexico, also showed resistance to cadmium and chromium. The recovery of MDR and heavy-metal-resistant *E. faecalis* from the Mololoa River in Mexico indicates a high level of contamination in this waterbody. The authors concluded that the Mololoa River provides a model for studying the genetic diversity of bacteria affecting human health [45]. In addition to the antibiotics reported above, *E. faecium*/*faecalis* resistant to rifampicin were also identified in the Ganga, Yamuna, and Sangam rivers in India. The authors believe that detailed data on the resistance patterns of these pathogens would enhance understanding of treatment options for enterococcal infections in humans [27]. The resistance of these bacteria to commonly used antibiotics, such as tetracycline, and less frequently utilized antibiotics, such as chloramphenicol, was evaluated in some strains of *E. faecium*/*faecalis* from the Babolrud River and coastal waters of Babolsar in Iran. The presence of antibiotic-resistant *Enterococcus* spp. in coastal waters poses a potential health risk to swimmers [40]. Furthermore, unlike in India, Mexico, and Iran, the report from Monte Cotugno Lake, Italy, identified *E. faecium* as the dominant species exhibiting different virulence factors such as *gel*E, *agg*, *efaAfm*, *gelE+efaA*, and *agg+efaA* [41].

E. faecium with two copies of the *poxt*A gene was identified in the Salibnello estuary in the Abruzzo region of Italy. The *poxt*A gene is known to confer resistance toward linezolid in bacteria. Genes conferring resistance to phenicols (*fex*B), tetracyclines (*tet*(M) and *tet*(L)), macrolides, lincosamides, and quinupristin streptogramins group B (*msr*(C)) were also identified. Additionally, streptogramin group B (*erm*B), lincosamides (*lnu*B), and lincosamides and dalfopristin streptogramin group A (*lsa*E) were also found. Furthermore, genes that confer resistance toward aminoglycosides (*aph3′-III*, *aac6′-Ii*, *ant6-Ia*, and *aac6′-aph2″*) were also identified using whole genome analysis [28].

Table 2 summarizes the occurrence of *E. faecium* and *E. faecalis* in major waterbodies worldwide. We analyzed studies published between 2011 and 2024 and identified *E. faecium* and *E. faecalis* in twenty-four rivers across thirteen different countries, with *E. faecalis* as the dominant species across the studied rivers. *E. faecium* and *E. faecalis* were prevalent in Canada's South Nation River and the USA's Passaic River. Conversely, only *E. faecium* was found in the Ebro basin River in France, and only *E. faecalis* was found in the river in Veracruz, Mexico. A small fraction of the isolates from these rivers were found to be MDR.

We found that *van*A and *van*B continue to be widespread, while *erm*B remains a major resistance mechanism in these waterbodies. The prevalence of *tet*M may be linked to agricultural practices near some of the sampled rivers. The detection of *poxt*A in one river in 2022 suggests that such resistance genes can emerge and potentially become more widespread in environmental isolates [28].

4.2. Staphylococcus aureus

Hospital-associated methicillin-resistant *S. aureus* was first isolated in the hospital environment in the 1960s, and community-associated MRSA infections appeared in the

1990s, with no associated risk factors [108,109]. S. aureus is not a common bacterium in surface water; hence, its recovery from surface water may be an indication of recent anthropogenic contamination. Since humans are the primary reservoir of *S. aureus*, recreational activities such as swimming may contribute to the spread of Staphylococci in the aquatic environments [110].

A strain of MRSA, known as USA300, is known for causing various infections, such as those affecting the skin and soft tissues (SSTIs), and is associated with severe complications in infections acquired both in the community and healthcare settings [111,112]. Gerken et al. (2021) [47] documented the presence of methicillin-susceptible *S. aureus* (MSSA), MRSA isolates of USA300 ST8 SCCmec IVa CA-MRSA with the Panton-Valentine leukocidin (PVL) toxin, and other MRSA isolates in Lehia, Richardson's, Honoliʻi, Pohoiki, Kealoha, Onekahakaha beach parks, Wailuku River Estuary, anchialine pools, and sand in locations with limited human activity on the Hawaiʻi Island. Similarly, Lepuschitz and his group reported the first draft genome sequence of the MRSA USA300 isolate obtained from a surface water sample from the River Mur in the province of Carinthia, southern Austria. They observed the presence of CA-MRSA USA300, characterized by relevant features such as sequence type 8 (ST8), spa type t008, staphylococcal cassette chromosome mec element type IV (SCCmec IV), PVL, and the arginine catabolic mobile element (ACME) cluster. Their findings also suggested a potential health risk associated with the detection of the CA-MRSA USA300 isolate in aquatic environments, which is closely associated with clinical isolates [113].

The *mec*A gene plays a key role in the resistance of *S. aureus* to methicillin by producing a penicillin-binding protein, PBP2a, which exhibits low binding affinity for β-lactam antibiotics, thereby rendering it ineffective [114,115]. Similarly, *bla*Z encodes beta-lactamase, an enzyme that hydrolyzes beta-lactam antibiotics, making them ineffective for the treatment regimen [115,116]. *mec*A and *bla*Z genes work synergistically; for example, regulatory factors linked to *bla*Z help *mec*A acquisition stay stable and make antibiotic resistance more visible in the phenotype [117]. An investigation revealed the simultaneous presence of these genes and other genes in a river that receives water from the Bon Accord dam located at the Tshwane University of Technology (TUT) research farm in Honingnestkranz, Pretoria North, South Africa. Ramaite et al. [49] identified *S. aureus* ARGs (*mec*A, *erm*A, and *bla*Z), enterotoxins (*sec* and *seq* genes), and STs (ST80, ST728, ST1931, ST2030, ST3247, and ST5440) associated with clonal complex 80 (CC80) in environmental samples. They cautioned about the potential for the widespread dissemination of ARGs and virulent bacterial strains due to the combination of these ARGs and enterotoxins found in livestock-associated MRSA. Similarly, Ramessar & Olaniran [50] identified ARGs (*bla*Z, *erm*C, *aac(6′)/aph(2′′)*, *msr*A, and *tet*K) and VGs such as *hla*, *hld*, *luk*S/FPV, and *sea* in the upstream and downstream points of receiving rivers of two wastewater treatment plants (WWTPs) in the Durban area, South Africa. Their findings suggested that the treated effluent might pose a threat to the environment and human health. The association between ARGs and virulence factors could complicate the treatment of infections caused by *S. aureus*, necessitating broader strategies for their management. In addition, Levin-Edens et al. [56] identified 22 MRSA isolates carrying the *mec*A gene, with a predominant prevalence of SCCmec type IV, of which 17 showed MDR harboring *tet*(K), *erm*(C), *msr*(A), and *aad*D ARGs. These isolates were found on popular recreational beaches in marine waters, freshwater, and lakes on the west side of Lake Washington. They suggested that the MRSA strains might be linked to humans and animals based on MLST typing, which could have significant implications for individuals visiting recreational beaches in colder climates. Akanbi et al. [52] identified *bla*Z, *mec*A, *erm*B, *rpo*B, and *tet*M ARGs in some *S. aureus* isolates collected from 10 beaches in the Eastern Cape Province of South Africa. They observed a higher frequency of these

genes in beach water than in sand isolates; moreover, they confirmed the presence of the *fem*A gene in all *mec*A-positive isolates, indicating functional methicillin resistance.

The *mec*C gene is a variant of the *mec*A gene, which is responsible for methicillin resistance in Staphylococci. It encodes a distinct penicillin-binding protein (PBP2c) with a lo w affinity for beta-lactam antibiotics, contributing to the methicillin resistance characteristic [118]. Some studies have reported the co-occurrence of these genes in bacterial isolates from rivers. For example, *mec*C-MRSA isolates were detected in river water that runs through a game estate in Spain; these isolates were closely related to isolates of ST425 *mec*C-MRSA found in wild animals. These isolates were resistant to benzylpenicillin and cefoxitin and carried the resistance genes mecC and *bla*ZLGA251, highlighting the potential role of water in the dissemination of *mec*C-MRSA [54]. In a similar study, Silva et al. [119] also identified three MRSA, resistant to penicillin and cefoxitin, and harboring the *bla*Z-SCCmecIX characteristic of *mec*C-MRSA strains, in the Douro River Basin in Portugal.

The presence of MDR *S. aureus* in river environments poses significant public health risks, particularly in areas impacted by anthropogenic activities. For instance, 65% of *S. aureus* isolates in a study from Brazil were resistant to at least three classes of antibiotics, with the highest resistance to trimethoprim–sulfamethoxazole and clindamycin and gentamicin; these isolates were obtained from the Extrema River Spring in central Brazil, a river known to be the target dumping ground of pharmaceutical products from surrounding industries [46]. The implication of the study's observation is that the release of pharmaceutical waste into surrounding waterbodies can trigger the development of AMR in resident bacteria. In a similar study, Soge et al. identified five MDR MRSA-SCCmec type I, which carried the *erm*A gene and were PVL-negative. These characteristics are commonly associated with hospital MRSA rather than US CA-MRSA isolates from public beaches in Washington State, USA, which were not near hospitals [57]. Analysis of water from the Ganga River in India (a river extensively used as a drinking water source and for religious bathing and cleaning purposes) found a high prevalence of MRSA isolates primarily resistant to erythromycin, augmentin, tetracycline and streptomycin in the lower region of the riverbanks of due to extensive anthropologic activities in the state of Uttarakhand, India [55]. Furthermore, Skariyachan et al. (2015) [53] reported that the preliminary antimicrobial testing of all *S. aureus* isolates recovered from River Cauvery, a significant drinking water source in Karnataka, India, showed MDR to the present generation of antibiotics.

This review documented the presence of a few *S. aureus* isolates in natural aquatic environments from 2009 to 2023, as shown in Table 3. We focused on beaches and rivers as habitats for *S. aureus* due to the similarity in anthropogenic activities, such as human and recreational interactions, that contribute to its dissemination in these environments. The prevalence of MRSA and MSSA was notable in developing countries like India and South Africa [50,55]. It is worth noting that only a small proportion of the MRSA and MSSA strains reviewed in this study were MDR. In addition, at least one of the three *erm* genes associated with macrolide resistance (*erm*A, *erm*B, or *erm*C) was recovered from most parts of the river, suggesting that macrolide resistance is common in river water, likely due to human activity, agriculture, and environmental factors. Some sequence types (STs) and clonal complexes (CCs), such as ST8 (CC8), ST5, ST398, and ST80 (CC80), which are considered dangerous due to their association with MRSA, virulence factors, and global dissemination, were identified [47,56,119].

4.3. Klebsiella pneumoniae

K. pneumoniae has been increasingly recovered in aquatic environments, particularly in rivers, lakes, and estuaries [120–122]. *K. pneumoniae* in the aquatic ecosystem could disrupt the ecosystem via interactions with other bacteria and competition for nutrients. In a bid to

compete, *K. pneumoniae* could secrete toxic substances that can eliminate other microbial populations from the environment [123].

Research has indicated that MDR *K. pneumoniae*, which carries the *bla*KPC-2 gene alongside other crucial resistance genes, is not only present in clinical environments but can also be found in natural water sources. For example, Nascimento et al. documented resistance to carbapenems (*bla*KPC-2), beta-lactams (*bla*SHV-11), fluoroquinolones (*oqx*A and *oqx*B), trimethoprim (*dfr*A30), tetracycline (*tet*A), and fosfomycin (*fos*A) by a strain of *K. pneumoniae* isolated from Ibirapuera Lake in São Paulo, Brazil. WGS analysis confirmed that this strain belonged to the high-risk hospital-associated clone ST11/CC258, showing that the *bla*KPC-2 gene was contained on a conjugative IncN plasmid found within a Tn4401b, which means it can potentially spread this resistance to other bacteria [68]. In a related study from Croatia, a KPC-producing *K. pneumoniae* was isolated from the Krapina River, situated 300 m downstream of the sewage outlet from the Zabok General Hospital. Four isolates from the river shared characteristics (carbapenem and MDR, plus the *bla*KPC-2 gene associated with an IncFII plasmid) with a clinical isolate marked "ST258" from a nearby hospital. This discovery indicated that the *K. pneumoniae* isolates from the river likely have a clinical origin, with the KPC-producing *K. pneumoniae* ST258 being able to survive in river water for extended periods, highlighting their potential to spread ARGs within the natural bacterial population [66]. Recent findings in China by Zou et al. also included the detection of four carbapenem-resistant *K. pneumoniae* strains carrying *bla*KPC-2 and *bla*NDM in the Dongluo, Quanfu, and Shunhe rivers of Jinan City, China. Among these, two carbapenem-resistant hypervirulent *K. pneumoniae* (CR-hvKP) clones of ST11-KL64 were retrieved from the Dongluo River, suggesting transmission between hospital and urban aquatic environments, given the relative abundance of *bla*KPC and *bla*NDM genes [61]. Additionally, Piedra-Carrasco et al. identified carbapenemase-producing *K. pneumoniae* (KPC-2) carrying carbapenemase-encoding plasmid genes (*vag*CD and *par*AB) and expressing low levels of virulence-associated factors in the Llobregat River, Catalunya, Spain, proposing these isolates as low-risk pathogens [70]. The emergence of KPC-producing strains raises concern due to their resistance to carbapenems, which are often the antibiotics of last resort for treating severe bacterial infections [124].

Several studies have reported the presence of significant carbapenemases and other antimicrobial resistance genes in rivers, raising concerns for public health [67,71]. In Austria, Lepuschitz et al. identified three ESBL-producing strains and two carbapenem-resistant strains in samples from various rivers (Inn, Drau, Glan, Traun, and Danube). They discovered the first case of *K. pneumoniae* strains positive for *bla*VIM-1 in an Austrian river, emphasizing their alarming presence due to the limited treatment options available for this antimicrobial-resistant strain [67]. Finally, Tafoukt et al. described the first identification of three *K. pneumoniae* isolates carrying OXA-48 carbapenemase with different STs from Soummam River, Algeria, emphasizing the importance of monitoring and controlling such water environments to prevent gene dissemination [71].

MDR *K. pneumoniae* and hypervirulent *K. pneumoniae* are normally classified in distinct groups, with MDR strains being prevalent in healthcare settings and hypervirulent strains being associated with severe community-acquired infections [125,126]. In 2020, Furlan et al. identified three MDR and hypervirulent *K. pneumoniae* isolates—EW158, EW160, and EW185—in Sertãozinho Stream, Moquem Stream (Euclides Morelli Lake), and Monjolinho River, respectively, in several cities of São Paulo State, Brazil [65].

Donato et al. reported an MDR frequency of 10% for *K. pneumoniae* among 59 environmental isolates collected from dams and various points spanning from the upper to the lower parts of the Lerma River basin in Mexico. WGS of two isolates revealed the presence of significant ARGs, including common genes (*aac(6′)-Ib-cr*, *fos*A, *sul*2, *bla*TEM-1B,

*bla*CTX-M-15, *oqx*A, *oqx*B), intrinsic genes (*fos*A, *bla*SHV-11), and unusual genes (*aac(3)-IIa*, *str*A, *str*B, *dfr*A14, *Qnr*S1, *Qnr*B66, *tet*(A), *bla*OXA-1, *bla*OXA-232, *cat*B4), as well as virulence genes such as standard genes (*fim*H) and dangerous genes (*ppd*D, *dam*). The study emphasized that the detection of MDR strains in areas where such strains were previously absent can cause significant environmental and public health concern [62]. Similarly, Lobato et al. reported the first evidence of *bla*BKC-1-producing *K. pneumoniae* belonging to the high-risk ST11/CC258 clone with an extensive drug-resistant phenotype in Guajará Bay (intersection of the Guamá and Acará rivers), Brazil. They also identified the presence of common genes (*bla*CTX-M-15, *bla*SHV-11, *aph (3′)*-VIa, *aac(6′)-Ib-cr*, *qnr*E1, *qnr*B19, *cat*B3, and *sul*1), intrinsic genes (*fos*A6), rare genes (*rsm*A, *emr*R, *crp*, *arr*-3, and *aac(3)-IIa*), and various virulence-related genes in the same river [63]. Sahoo et al. (2023) [60] reported the first case of a *K. pneumoniae* isolate expressing a rare gene, *bla*NDM-5, common resistance determinants (*bla*CTX-M and *bla*TEM), an intrinsic gene (*bla*SHV), as well as the virulence factors *fim*H, *mrk*D, and *ent*B (standard virulence genes) and *irp*-1 and *ybt*S (dangerous virulence genes) in samples collected from the Kathajodi River in Odisha, India. The study suggested that this strain originated from infected people or healthcare facilities [60].

Studies from Northern Portugal and China reported the isolation of ESBL *K. pneumoniae* harboring *bla*SHV from samples collected from the Douro River Basin and Qinhuai River, respectively [58,59]. Another study on the Tigris River in Iraq found that 87.5% of the 40 *K. pneumoniae* isolates carried the *bla*CTX-M gene, indicating a significant prevalence of ESBL-producing strains [72]. In Africa, Hassen et al. (2020) identified two *K. pneumoniae* isolates with a predominance of *blaCTX*-M-15, *bla*SHV, and high genetic diversity in river water from the Rouriche River, Tunis City, Tunisia [64].

Tigecycline is a last-resort antibiotic used for treating MDR infections, particularly when microorganisms have already developed resistance to carbapenems and/or colistin [127]. However, the rising cases of tigecycline resistance in Enterobacterales strains are raising concerns regarding the efficacy of this antimicrobial agent [127,128]. Hladicz et al. identified two tigecycline-resistant *K. pneumoniae* isolates in the River Mur, Austria; the two belonged to different, unrelated MLST types—ST2392 and ST2394—and both exhibited resistance to tetracycline and tigecycline. They postulated that river water may contain substances such as mutagenic agents that can cause mutations, leading to overexpression of the efflux pump [69].

This review identified a small number of *K. pneumoniae* isolates in waterbodies between 2015 and 2024, as summarized in Table 4. However, it is concerning that most of these isolates are MDR, carrying ARGs, including ESBL genes (*bla*SHV, *bla*CTX-M), as well as genes associated with aminoglycoside resistance (*aad*A) and sulfonamide resistance (*sul*1). Additionally, hypervirulent *K. pneumoniae* isolates resistant to multiple antibiotics were also identified in environmental settings, which is quite alarming [65]. Notably, *K. pneumoniae* isolated from the River Mur in Austria was found to be resistant to tigecycline, a last-resort antibiotic for treating MDR infections [69].

4.4. Acinetobacter baumannii

A. baumannii is a notable species within the genus *Acinetobacter* that has garnered significant research interest due to its role in hospital-acquired infections [129,130]. *A. baumannii* is often found in water, soil, and on surfaces in healthcare settings [74,131]. Its ability to survive in diverse environments, combined with its MDR nature, has made it a growing concern in hospital-associated infections [131,132].

Its introduction into river systems through various anthropogenic activities, including hospital effluents and agricultural runoff discharge, may contribute to the dissemination of the antibiotic-resistant strain [131,133]. Tsai et al. observed higher rates of *A. baumannii*

in livestock wastewater channels (21.4%) and tributaries near livestock farms (15.4%) compared with areas adjacent to the Puzi River in Taiwan. Genotyping using ERIC-PCR identified two primary clusters of *A. baumannii*, suggesting that livestock wastewater contributes to the dissemination of the bacterium in aquatic ecosystems [80].

Recent research has raised concerns about the prevalence of extensively drug-resistant (XDR) *A. baumannii* in river environments, showing the potential transmission of resistance genes from clinical settings to natural waterbodies [75]. For instance, Sahoo et al. found high levels of XDR carbapenem-resistant *A. baumannii* in a river catchment near Cuttack City, Eastern India. This indicates the direct disposal of biomedical wastes into the river system [75]. Similarly, Havenga et al. (2022) [76] identified XDR *A. baumannii* isolates (AB14) in the Plankenbrug River, located near a local health clinic in South Africa, demonstrating antibiotic resistance to amikacin, ciprofloxacin, gentamicin, imipenem, levofloxacin, sulfamethoxazole/trimethoprim, and tobramycin. The isolate also exhibited biofilm formation and shared genetic relatedness with clinical isolates [76].

In a European study, Kettinger et al. identified the presence of classic intrinsic OXA carbapenemase (OXA-23 and OXA-51) in *A. baumannii* strains resistant to carbapenems (meropenem and imipenem), as well as strains carrying the genes encoding carbapenemase VIM-2 and broad-spectrum β-lactamases TEM-1 throughout the Danube River in Europe [82]. Studies in the United States and China documented the emergence of *A. baumannii* harboring *msr*E, and *mph*E genes both midstream (proximal to effluent pipe) and downstream (Mount Vernon WWTP) of the Kokosing River, a rural river in Ohio, and in the Qinhuai River. The studies observed a substantial increase in various ARGs because of the WWTP discharge [59,79]. Another study identified the MDR *A. baumannii* L13 strain harboring 314 resistance genes, including the *bla*OXA-69 gene, cephalosporin-hydrolyzing class C-β-lactamase gene (*bla*ADC-2), glycopeptide resistance genes, and Na^+-driven multidrug efflux pump genes (*abe*S, *abe*M, *ade*K, *ade*I, *ade*J, *ade*H, *ade*R, *ade*G, *ade*F, *ade*N, *ade*A, and *ade*S), in the Tarim River in Xinjiang Uygur Autonomous Region of China, using draft genome sequencing to identify correlated MDR genes for further study of antimicrobial resistance mechanisms [81]. Adewoyin et al. discovered *A. baumannii* isolates in Great Fish, Keiskamma, and Tyhume Rivers in Eastern Cape Province, South Africa; these isolates carried a variety of resistance genes for β-lactamases, aminoglycosides, fluoroquinolones, and tetracyclines. They attributed the presence of MDR *A. baumannii* in these freshwater sources to likely wastewater discharge from nearby animal farms [78].

Wang et al. (2021) [77] discovered *A. baumannii* isolates carrying the *bla*OXA-2 ARG and mercury resistance operon downstream of the Xiangjiang River. These bacteria, identified as ampicillin-resistant opportunistic pathogens, were associated with areas impacted by metal mining activities. The researchers suggested that heavy metals might facilitate plasmid conjugative transfer, leading to the proliferation of resistant opportunistic pathogenic bacteria in the river [77]. Additionally, Turano et al. (2016) identified *bla*OXA-23-producing ST79 *A. baumannii* strains in Tietê and Pinheiros Rivers in São Paulo, Brazil, indicating the presence of clinically significant bacteria outside of hospital settings and showing the escalating public health risk [83]. In a similar study, Sotomayor et al. recently demonstrated that carbapenem-resistant *A. baumannii* were predominant in healthcare facilities and polluted river environments in Quito, Ecuador, with river-derived isolates only carrying the *bla*OXA-51 and possibly the *bla*OXA-259 genes [73].

In a study conducted in France, Hamidian et al. (2022) [74] identified ARGs in *A. baumannii* strain isolated from the Seine River, including genes encoding the tetracycline efflux protein (*tet*A), an ISAba1-activated chromosomal cephalosporinase (*amp*C), an oxacillinase (OXA-23), and an aminoglycoside 3′-phosphotransferase (*str*AB). The researchers

suggested that this strain, which may have originated in nosocomial environments, have been released into the environment [74].

In this review, we found a limited number of reports specific to *A. baumannii* isolates from rivers between 2016 and 2024, as seen in Table 5, despite numerous studies on other *Acinetobacter* species in the same environments. Both the Kathajodi River in India and the Plankenbrug River in South Africa had a co-occurrence of MDR and XDR *A. baumannii*. Of the 42 isolates from the Kathajodi River, 41 were identified as MDR and XDR, while 4 of the seven isolates from the Plankenbrug River exhibited the same co-occurrence [75,76].

The presence of XDR *A. baumannii* in these rivers raises concerns about its potential impact on the local aquatic ecosystem. Additionally, other rivers, such as the Danube in Europe, also reported the presence of MDR *A. baumannii* carrying ESBL and/or carbapenemase, although at a lower prevalence [82]. The current trend shows increasing resistance in XDR and MDR *A. baumannii* strains, largely due to the dissemination of acquired resistance genes such as *bla*OXA-23, as demonstrated in this review. Only one study actively investigated the presence of the *mcr* variant (colistin resistance), a commonly sought acquired gene in environmental samples, but did not detect it in the *A. baumannii* strains isolated from the Plankenbrug River [76].

4.5. Pseudomonas aeruginosa

P. aeruginosa is a ubiquitous environmental Gram-negative bacterium inhabiting terrestrial and aquatic environments. Its wide-ranging metabolic adaptability helps it spread, proliferate, and survive despite unfavorable physical and chemical conditions [134,135]. *P. aeruginosa*'s resistance to multiple antibiotics is due to its low outer membrane permeability and a robust efflux pump system, which together enable it to survive in aquatic environments [136,137].

Multiple studies have shown that *P. aeruginosa* strains isolated from river environments often display high levels of resistance to antibiotics. The SPM-1 metallo- beta-lactamase is particularly concerning, as it confers resistance to a broad spectrum of β-lactam antibiotics that are crucial for treating severe infections [83]. For example, a study in southern Brazil identified the presence of *bla*SPM-1-producing *P. aeruginosa* strains in two locations in the metropolitan area of São Paulo (Tietê and Pinheiros Rivers). MLST analysis revealed that the isolated strains were closely related and belonged to sequence type ST277, which is environmentally persistent [83]. In a similar study, Fontes et al. identified MDR *P. aeruginosa* carrying *bla*SPM-1, *bla*OXA-56, *rmt*D1, *aac*A4, *aad*A7, *sul*1, and *dhfr* genes in the Tietê River in São Paulo state, Brazil. They suggested that strains carrying *bla*SPM-1 and *rmt*D1 genes are not limited to hospitals and can spread in communities through river environments [91].

Furthermore, the presence of MDR *P. aeruginosa* in rivers raises concerns about its potential to acquire and spread ARGs. Hosu et al. (2021) detected MDR *P. aeruginosa* harboring rare and important resistance genes such as ESBL (*bla*TEM, *bla*SHV, and *bla*CTX-M) and MBL (*bla*VIM) genes from the wastewater of the Umzikantu Red Meat Abattoir, Zimbane Mthatha, Mthatha River, and Mthatha Dam, Province of South Africa, with an MAR index > 0.2, indicating that the isolates originated from high-risk contamination sources [135]. Another study analyzed MDR strains of *P. aeruginosa* from seven distinct sites along the Buriganga River in Bangladesh, revealing resistance to common antibiotics such as cefotaxime, tetracycline, and ciprofloxacin, as well as to important antibiotics such as cefepime, imipenem, meropenem, and amikacin. They concluded that sources such as chemical waste, medical waste, and sewage disposal points may play an important role in the spread of MDR *P. aeruginosa* in the river [85]. Additionally, a previous study in Bangladesh observed similar MDR resistance patterns in strains from ponds, lakes, and

rivers in Dhaka city, with resistance to common antibiotics such as ampicillin, tetracycline, and gentamicin [88].

Suzuki et al. (2013) [90] reported the distribution of *P. aeruginosa*, with counts ranging from 2 to 46 cfu/100 mL, and AMR to various antibiotics from both upstream and downstream locations of two rivers, the Kiyotake River and the Yae River, which flow through Miyazaki City, Japan. Between 0.2% and 2% of all *P. aeruginosa* isolates (516 isolates from the two rivers) exhibited resistance to piperacillin, cefotaxime, and imipenem [90]. Although the observed patterns of resistance to piperacillin, cefotaxime, and imipenem are relatively low, the presence of resistance to these antibiotics, especially imipenem, remains a significant threat [90]. Similarly, Xiao et al. (2024) [59] detected an abundance of *P. aeruginosa* in the Qinhuai River, Nanjing, China (the river receives effluent from many municipal WWTPs). The *P. aeruginosa* carried various ARGs, including *bla*OXA-101, *mph*F, *bla*TEM-1, LCR-1, and *bla*OXA-827, further emphasizing the microbial risks in urban rivers influenced by municipal wastewater. In Brazil, Magalhães et al. (2016) [89] detected the presence of MDR *P. aeruginosa* in the Mindu stream in Manaus, which drains into the Rio Negro.

Additionally, a recent study by Rojo-Bezares et al. in Spain recovered *P. aeruginosa* isolates from the Iregua River [84]. These isolates exhibited a low range of AMR patterns, with only one strain resistant to carbapenems (imipenem, meropenem, and doripenem) due to a mutation in the OprD porin gene. Notably, the isolates exhibited a high level of virulence factors, motility, as well as pigment production and elastase activity. A related study characterized a hemolytic and antibiotic-resistant strain of *P. aeruginosa*, designated S3, isolated from the Mahananda River in India. This strain exhibited significant hemolytic activity and multiple virulence factors, rendering it pathogenic to fish [86].

Relatively few studies have focused on analyzing the presence and AMR of *P. aeruginosa* in river water due to the primary focus on clinical settings. This review highlights the growing prevalence of antibiotic-resistant *P. aeruginosa* in river environments from 2011 to 2024, as shown in Table 6, indicating significant challenges to public health due to threats to animal, human, and environmental safety. Recent studies from diverse geographical locations, including Brazil, South Africa, and Bangladesh, have consistently highlighted the alarming presence of MDR strains and the dissemination of critical resistance genes [83,85,135]. The detection of *bla*SPM-1-producing strains and other resistance determinants such as *bla*OXA-56 and *rmt*D1 shows the potential for these bacteria to escape hospital confines and establish in community settings, particularly through aquatic ecosystems [91].

4.6. Enterobacter spp.

Enterobacter spp. are mobile Gram-negative rods and form part of the gut microbiota of animals and humans [138]. *Enterobacter* spp. are commonly found in the natural environment, including in soil and surface water [139,140]. Studies have shown that various *Enterobacter* spp., including *E. cloacae*, can carry multiple carbapenemase genes. In a 2018 study conducted near Netanya, Israel, *Enterobacter* spp. were identified in the Alexander River estuary, with two carbapenemase-producing *Enterobacter* isolates (*E. asburiae* and *E. bugandensis*) detected, indicating significant contamination in these aquatic environments. Genome sequencing revealed a high degree of genetic similarity between these isolates, suggesting a common source of contamination [95]. Furthermore, a study in the Philippines isolated carbapenemase-producing *E. cloacae*, *E. kobei*, *E. tabaci*, *E. xiangfangensis*, and *E. hormaechei* from different points along the Metro Manila River, identifying different carbapenemases, with the most prevalent gene being *bla*NDM [94]. Additionally, Adachi et al. reported the presence of imipenem and meropenem-resistant *E. asburiae* 17Nkhm-UP2 and *Enterobacter* spp. 18A13 in a river contaminated with discharge from a sewage treatment

plant in Osaka, Japan. They found that *E. asburiae* 17Nkhm-UP2 harbored an FRI-4-like gene, while *Enterobacter* sp. 18A13 harbored FRI-8. The researchers concluded that FRI carbapenemase genes may have spread within the genus *Enterobacter* spp. in the river [93].

Environmental studies have drawn attention to the presence of ESBL and carbapenemase-producing *Enterobacter* spp. in aquatic environments, particularly in rivers [98]. In a study conducted in Germany, samples taken from the Danube River revealed the presence of antibiotic-resistant Enterobacteriaceae, including *E. cloacae*, *E. cancerogenus*, and *E. asburiae* [98]. The study highlighted the presence of multiple resistant strains, emphasizing the role of the river in the dissemination of these pathogens [98]. This corresponds with findings from a study in Switzerland, where researchers detected ESBL *E. cloacae* and *E. amnigenus* strains in the Landquart and Lorze rivers in the German-speaking part of Switzerland. The study concluded that the prevalence of ESBL producing Enterobacteriaceae in surface waters is highly concerning [99]. Additionally, in Spain, Piedra-Carrasco et al. (2017) identified one *E. cloacae* that was positive for *bla*KPC-2 and one that was positive for *bla*IMI-2 (the first IMI-2 determinant identified in environmental bacteria in Europe) and harbored new STs; they were collected from sediment and water samples at the Sant Joan Despí station of the Llobregat river, Catalunya, Spain [70]. Zarfel et al. also identified two *bla*VIM-1 and *bla*TEM-1-harboring *E. cloacae* in the Mur River in Austria [141]. Finally, a *bla*CTX-M-harboring *E. cloacae* isolate was also identified in water samples from the Choqueyapu River in La Paz, Bolivia. This river frequently receives untreated household, industrial, and hospital waste. WGS of the isolate confirmed the presence of conjugative plasmids, including *Inc*H groups, which are commonly associated with *ESBL* and carbapenemase genes [97].

Bartholin et al. (2023) [92] reported the presence of MDR *E. cloacae* in the Cachapoal River and Villarrica Lake in Chile. They also identified the *bla*TEM gene, which encodes β-lactamase in *E. cloacae* [92]. MDR *Enterobacter* spp. were also detected in the Tsomo and Tyhume rivers in the Eastern Cape Province, South Africa. These bacteria harbored ESBLs (*bla*CTX-M, *bla*SHV) and the pAmpC gene (*bla*FOX), as well as non-β-lactam resistance genes (*tet*A, *tet*B, *tet*D, *sul*1, and *cat*II) [96]. Xu et al. (2011) [100] reported the identification of *fos*A2 in *E. cloacae*. This *fos*A2 gene confers fosfomycin resistance in an MDR *E. cloacae* from the Salmon River in south-central British Columbia, Canada [100].

In this review, we found a limited number of studies on *Enterobacter* spp. isolates from rivers between 2011 and 2024, as shown in Table 7. The presence of MDR *Enterobacter* spp. harboring carbapenemase and ESBL genes, among other dangerous genes, in riverine environments is a growing concern worldwide. The detection of novel *bla*FRI-4 and *bla*FRI-8 carbapenemase genes in river water means that these ARGs are now present and mobile in the environment, increasing the risk of dissemination to human and animal pathogens through water, food, and ecological contact [93].

Finally, from the data in this review, we identified studies on individual ESKAPE pathogens in 109 major rivers, including springs, dams, beaches, and lakes across 32 different countries worldwide, with each pathogen exhibiting distinct resistance patterns. Some exhibited patterns similar to those observed in clinical settings. The resistance pattern in clinical settings is frequently characterized by XDR or MDR [142]. ESKAPE bacteria in these environments may increase the risk of human infections, especially in vulnerable populations. This literature review demonstrates the current trends in the prevalence and risks associated with ESKAPE bacteria in waterbodies, particularly rivers. Available data indicate that MDR Gram-positive ESKAPE pathogens, including *E. faecium/faecalis* and *S. aureus*, harbor critical resistance genes against vancomycin, aminoglycosides, and tetracyclines; similarly, data show that MDR Gram-negative ESKAPE pathogens carry resistance determinants targeting β-lactams and carbapenems. These resistance patterns

closely mirror those observed in clinical settings, posing a serious threat given the essential role of these antibiotics in managing infections caused by these pathogens.

Limitations of this review.

i. Data collected for the analysis of the prevalence of individual ESKAPE pathogens in water systems in various regions are unevenly distributed across the globe.
ii. Important data, such as VGs, ARGs, and MDR, were not included in some of the studies analyzed, limiting the available information on the patterns and mechanisms of resistance of the ESKAPE pathogens in the studied literature.

5. Conclusions

This review supports the fact that contaminated surface water such as rivers can be a source of pathogens associated with severe infections in the community. We reported the current trends in the identification of ESKAPE pathogens in aquatic environments. It is worth noting that the rate of recovery of this group of bacteria from aquatic environments is low. This could be due to the continuous flow of water, which often dislodges bacteria from surfaces; however, despite the lower rate of recovery, antimicrobial resistance among ESKAPE pathogens is on the increase, highlighting the possible transfer of resistant strains or genes into the aquatic environment, which could be due to anthropogenic activities or the influence of agricultural activities. This observation highlights the importance of adequate management of water before it is used in agriculture or as portable water to prevent the spread of resistant pathogens to the public.

The studies analyzed in this review used different methodologies, including cultural media, PCR, automated systems, and WGS, to identify the bacteria and their resistant genes, as well as the antimicrobial patterns in these pathogens. Although only a few members of the ESKAPE family were recovered from these rivers, and only a few of these were MDR, the presence of MDR ESKAPE pathogens could still pose a risk to the health of an immunocompromised individual who comes into contact with this waterbody. Carbapenemase and *tet* genes appear to be more prevalent in Gram-negative bacteria, while the *van* and *tet* genes are shared among two ESKAPE Gram-positive bacteria. A new carbapenemase gene, the *bla*FRI gene, a member of a group of carbapenemase genes found primarily in *Enterobacter cloacae* and other related bacteria, was isolated in one of the rivers reviewed in this study. Genomic technologies were well-represented in some of the studies; however, most did not conduct genomic analysis, resulting in less-documented genomic results. It is important that continuous surveillance and monitoring of waterbodies are conducted regularly to prevent the spread or acquisition of AMR ESKAPE pathogens. Studies determining the abundance, diversity, and survival of ESKAPE pathogens in different waterbodies will be helpful in this regard. Given these findings, it is crucial to implement robust monitoring and management strategies to mitigate the spread of antibiotic resistance in aquatic environments. Collaborative efforts by public health officials, environmental scientists, and healthcare providers are essential to address this pressing issue. Continued research is needed to better understand the resistance and virulence mechanisms of ESKAPE pathogens and to develop innovative approaches for controlling their spread in both environmental and clinical contexts.

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