Environmental Research 239 (2023) 117273

Contents lists available at ScienceDirect

Environmental Research

journal homepage: www.elsevier.com/locate/envres

Comparative resistome analysis of *Aeromonas* species in aquaculture reveals antibiotic resistance patterns and phylogeographic distribution

HyeongJin Roh *, Dhamotharan Kannimuthu

Pathogen Transmission and Disease Research Group, Institute of Marine Research, PO Box 1870, Nordnes, 5870, Bergen, Norway

ARTICLE INFO

Keywords:
Multi-drug resistant
Antibiotic-resistant gene
Resistome
Horizontal and vertical gene transfer
Aeromonas

ABSTRACT

The overuse of antibiotics in aquaculture drives the emergence of multi-drug-resistant bacteria, and antibiotic-resistant genes (ARGs) can be disseminated to other bacteria through vertical- and horizontal gene transfer (VGT and HGT) under selective pressure. Profiling the antibiotic resistome and understanding the global distribution of ARGs constitutes the first step in developing a control strategy. Hence, this study utilized extensive genomic data from hundreds of *Aeromonas* strains in aquaculture to profile resistome patterns and explores their association with isolation year, country, and species characteristics. Overall, ~400 *Aeromonas* genomes were used to predict the ARGs from *A. salmonicida*, *A. hydrophila*, *A. veronii*, *A. media*, and *A. sobria*. ARGs such as *sul1*, *tet(A)*, and *tet(D)*, which display a similar proportion of positive strains among species, were subjected to phylodynamic and phylogeographic analyses. More than a hundred ARGs were identified, some of which exhibited either species-specific or non-species-specific patterns. *A. salmonicida* and *A. media* were found to have a higher proportion of species-specific ARGs than other strains, which might lead to more distinct patterns of ARG acquisition. Overall, ~25% of strains have either *sul1*, *tet(A)*, or *tet(D)* gene(s), but no significant difference was observed in the proportion of positive strains by species. Phylogeographic analysis revealed that the abundant numbers of *sul1*, *tet(A)*, and/or *tet(D)* introduced in a few East Asian and North American countries could spread to both adjacent and faraway countries. In recent years, the proportions of these ARGs have dramatically increased, particularly in strains sourced from aquatic environments, suggesting control is required of the overuse of antibiotics in aquaculture. The findings of this research offer significant insights into the global dissemination of ARGs.

1. Introduction

Aquaculture is one of the fastest-growing sectors for the resource of high-quality proteins; hence, a large number of developed and developing countries are promoting the aquaculture industry (e.g., Gui et al., 2018; Béné et al., 2016; Hersoug, 2015). The trend toward developing aquaculture with industrialization has contributed significantly to massive fish production with high efficiency (Sikveland et al., 2022). However, without sufficient regulation, the rapid growth of aquaculture can lead to substantial water pollution and increase the outbreak of diseases (Carter, 2018; Greaker et al., 2020). Among multiple relevant problems that arise, bacterial disease is one of the most prominent and problematic causes of high mortality rates and economic losses (Mzula et al., 2021). Whilst numerous environmentally sustainable treatment modalities have been devised for managing bacterial diseases in aquaculture, it is undeniable that, in the short term, the utilization of antibiotics remains the most instantaneous and cost-effective remedy. (Roh et al., 2018; Pereira et al., 2022; Vignesh et al., 2011; Burridge et al., 2010). However, the misuse and overuse of antibiotics in the aquaculture environment has raised multiple concerns, not only in terms of public health issues but also the reduction of effective and valid antibiotics for bacterial infection in aquatic animals (Shao et al., 2018; Kim et al., 2018). Regarding the former, the indiscriminate use of chemotherapy with antibiotics induces a selective pressure on bacterial pathogens, which generate multi-drug resistant bacteria (Rasul and Majumdar, 2017). Moreover, the residual antibiotics following chemotherapy are released without any elimination or inactivation procedures through effluent water, which increases propagation of antimicrobial resistances in natural microbiome environments (Silva et al., 2021). A more severe problem is that many ARGs are located within mobile genetic elements such as integrase, transposase, and plasmid. Therefore, ARGs can be replicated and delivered continuously if the bacteria are in

* Corresponding author.
E-mail address: HyeongJin.Roh@hi.no (H. Roh).

https://doi.org/10.1016/j.envres.2023.117273
Received 2 August 2023; Received in revised form 27 September 2023; Accepted 28 September 2023
Available online 5 October 2023

environments that require antibiotic resistance for survival (Ellabaan et al., 2021; Hu et al., 2022). In addition, ARGs can be horizontally transferred to other bacteria. Eventually, the spread of ARGs in nature will raise public health concerns about emerging multi-drug-resistant bacteria that are infectious in humans. Thus, profiling the current status of ARG distribution and its propagation pattern is vital for gaining a comprehensive understanding of this issue, and implementing effective countermeasures.

Among the bacterial pathogens, the genus *Aeromonas* is a major waterborne opportunistic pathogen that can cause bacterial infection in both aquatic and terrestrial animals (Conte et al., 2021). In the *Aeromonas* species, some of the important bacterial pathogens in fish are *Aeromonas salmonicida*, *Aeromonas hydrophila*, *Aeromonas veronii*, *Aeromonas sobria*, and *Aeromonas media*. Of these, *A. salmonicida* is the causative agent of fish furunculosis in salmonid and non-salmonid fish by typical (subsp. *salmonicida*) and atypical subspecies (subsp. *masoucida*, *achromogenes*, *smithia*, and *pectinolytica*) (Cipriano and Bullock, 2001; Torres-Corral et al., 2022). Furthermore, *A. hydrophila* and *A. veronii* are ubiquitous bacterial pathogens with diverse host ranges, including fish, reptiles, and amphibians, and can lead to motile *Aeromonas* hemorrhagic septicemia (Wang et al., 2003; Li et al., 2019b). They occasionally cause tremendous mortality and economic losses in the aquaculture and ornamental fish industries through primary infections or co-infection with other pathogens (e.g., Jun et al., 2013; Esteve et al., 1993; Roh et al., 2018; Suresh et al., 2023). Likewise, *A. sobria* and *A. media* are etiological agents causing skin hemorrhages, ulceration, lesions, and abnormal swimming behavior in a large number of aquatic animals (Majtán et al., 2012; Lü et al., 2016).

There are several advantages in using *Aeromonas* as the genus for profiling waterborne ARGs worldwide. Firstly, genomic data on *Aeromonas* from various countries are highly diverse, providing robust information about isolation year, country, and host available in public databases (e.g., Genbank). Secondly, many cases, reports, and studies about *Aeromonas* that can acquire or spread ARGs from or to the surrounding environment or microbiome highlight the usefulness of the genus in profiling global resistome patterns (e.g., Conte et al., 2021; Roh et al., 2018; Baron et al., 2017; Sahoo et al., 2016). For these reasons, this study collected genomic data on five major *Aeromonas* pathogens in aquaculture *A. salmonicida*, *A. hydrophila*, *A. veronii*, *A. sobria*, and *A. media* from more than 30 countries, and identified ARGs and antibiotic class resistances by species and strain. In addition, among more than a hundred genes, ARGs with no species-specific differences in mutation patterns and a relatively even distribution in several species, namely *Tet(A)*, *tet(D)*, and *sul1*, were selected and subjected to phylodynamic and phylogeographic analyses to profile the dissemination of ARGs. The use of such metadata and big data is expected to make an enormous contribution to the establishing of strategies for controlling the propagation and movement of ARGs.

2. Material and methods

2.1. Collection of Aeromonas genomes

The genome data of major *Aeromonas* pathogens (*A. salmonicida*, *A. hydrophila*, *A. veronii*, *A. media*, and *A. sobria*) were obtained from NCBI GenBank. To ensure data reliability and completeness, we only used genomes with publicly available information on the isolation country or site, isolation year, and hosts. A total of 399 *Aeromonas* genomes meeting these criteria were included in the analysis. Based on information on the isolation source, the type of site - either terrestrial or aquatic environment was assigned. Detailed information regarding the bacterial genomes, such as species, strain, genome size, BioSample, environmental type, BioProject, and assembly accession number is available in Table S1. To generate maps for visualization of the data, we retrieved geographic information, including GPS coordinates, from Google Maps.

2.2. Prediction of antibiotic-resistant genes (ARG)

All 399 *Aeromonas* genome data were utilized for the prediction of ARG sequences. Specifically, we utilized the resistance gene identifier (RGI) in the comparative antibiotic resistance database (CARD) to obtain the resistome, which included protein and nucleotide sequences. To ensure accuracy, we used the options 'Perfect and strict hits only' (Pass_Bitscore $\geq$50, and Best_Hit_Bitscore $\geq$80), 'Exclude nudge', and 'High quality/coverage' (Percentage length of reference sequence $\geq$50%) when blasting the genomes. We profiled the distribution of each ARG by species and strain. Species-specific ARGs were identified as those found exclusively in one species and accounting for more than 5% of those present. This prevented the inclusion of species-specific ARGs that might be coincidentally identified due to rare events. The ARGs observed from all *Aeromonas* species used in this study were regarded as non-species-specific ARGs. The copy numbers of specific ARGs were counted for each strain (number of specific ARG copy number/1 genome). Following this, the patterns of ARG compositions were hierarchically clustered using the Euclidean distance method. Each strain was visualized differentiated by the presence of ARG sulfonamide resistance; *sul1*, tetracycline resistance; *tet(D)* and/or *tet(A)* gene in the genomes, and circular dot colors differentiated by species. The identified ARGs were used for estimating the resistance of antibiotic class, and the following formula was applied to calculate the relative proportion of each antibiotic class. The antibiotic classes were then compared by species.

A. **sal** = Number of resistant strains in each class from *A. salmonicida* / total number of *A. salmonicida* (1)

A. **hyd** = Number of resistant strains in each class from *A. hydrophila* / total number of *A. hydrophila* (2)

A. **ver** = Number of resistant strains in each class from *A. veronii* / total number of *A. veronii* (3)

A. **sob** = Number of resistant strains in each class from *A. sobria* / total number of *A. sobria* (4)

A. **med** = Number of resistant strains in each class from *A. media* / total number of *A. media* (5)

Relative proportion of *A. salmonicida* in each antibiotic class = **A**. **sal** / (**A**. **sal** + **A**. **hyd** + **A**. **ver** + **A**. **sob** + **A**. **med**) (6)

Relative proportion of *A. hydrophila* in each antibiotic class = **A**. **hyd** / (**A**. **sal** + **A**. **hyd** + **A**. **ver** + **A**. **sob** + **A**. **med**) (7)

Relative proportion of *A. veronii* in each antibiotic class = **A**. **ver** / (**A**. **sal** + **A**. **hyd** + **A**. **ver** + **A**. **sob** + **A**. **med**) (8)

Relative proportion of *A. sobria* in each antibiotic class = **A. sob** / (**A. sal** + **A. hyd** + **A. ver** + **A. sob** + **A. med**) (9)

Relative proportion of *A. media* in each antibiotic class = **A. med**/ (**A. sal** + **A. hyd** + **A. ver** + **A. sob** + **A. med**) (10)

Among all 399 *Aeromonas* strains, the identified 138 ARGs exhibited gene copy numbers ranging from 0 to 7 copies per strain. To characterize the distribution patterns of ARG copies and investigate their relationship with antibiotics indicative of resistance across strain and species levels, principal component analysis (PCA) was performed based on the number of ARGs within each antibiotic class. All data were analyzed and visualized using 'dendextend', 'circlize', 'dplyr', 'plyr', and 'factoextra' packages in R (Ver. 4.2.2) (Galili, 2015; Gu et al., 2014; Wickham et al., 2018; Wickham, 2011; Kassambara and Mundt, 2017).

2.3. Phylodynamic and phylogeographic analyses of non-species-specific ARGs

For the phylodynamic and phylogeographic analyses of non-species-specific antimicrobial resistance genes (ARGs) in *Aeromonas*, we selected *tet(A)*, *tet(D)*, and *sul1*, which were less dominant but shared a similar proportion of positive strains among the species, and *AdeF*, which had high dominance among all species. In total, we utilized 47 *tet(D)*, 41 *tet(A)*, and 94 *sul1* sequences. All epidemiological analyses were conducted using Nextstrain (Hadfield et al., 2018). The nucleotide sequences of each ARG (*tet(D)*, *tet(A)*, and *sul1*) were grouped by country, isolated year, and species, and 'sequences-per-group' was set to 20. To construct the phylogenetic tree, the sequences were aligned with '–fill-gap' option without a custom reference. The Time-Resolved Tree was obtained using the 'refine' command, with options of 'coalescent = opt', 'data-confidence', and 'data-inference = marginal' for assigning times to the internal node. The trait, inferred ancestral sequences, and amino-acid mutation sites were annotated and profiled by 'traits', 'ancestral', and 'translate' commands, and the results were visualized by Auspice using a 'nextstrain view' command line. Likewise, *adeF* sequences were analyzed using a similar approach to *tet(A)*, *tet(D)*, and *sul1*, with a slight modification of 'sequences-per-group' to 100. Due to the higher abundance of *adeF* sequences, a 'sequence-per-group' value of 100 was assigned. Time-resolved and unrooted phylogenetic trees for *sul1*, *tet(A)*, *tet(D)*, and *adeF* were visualized according to country, isolation year, and species. Similarly, the number and proportion of each ARG by country and predicted transmission routes were illustrated. In addition, Pearson's correlation analysis was performed using the 'PerformanceAnalytics' package in R (ver 4.2.2) to evaluate the relationship between the proportion of *sul1*, *tet(D)*, and *tet(A)* positive strains and isolated years (Peterson et al., 2018).

2.4. Statistical analysis

The proportion of ARGs-positive strains derived from terrestrial or aquatic environments was calculated within whole years or isolation year intervals (pre-1999, 2000–2009, 2010–2019, and 2020–2022). Chi-square analysis was applied to evaluate the frequency of specific ARG-positive strains between the sites using 'gmodels' package in R (Warnes et al., 2015). A *p*-value less than 0.05 was regarded as a significant difference.

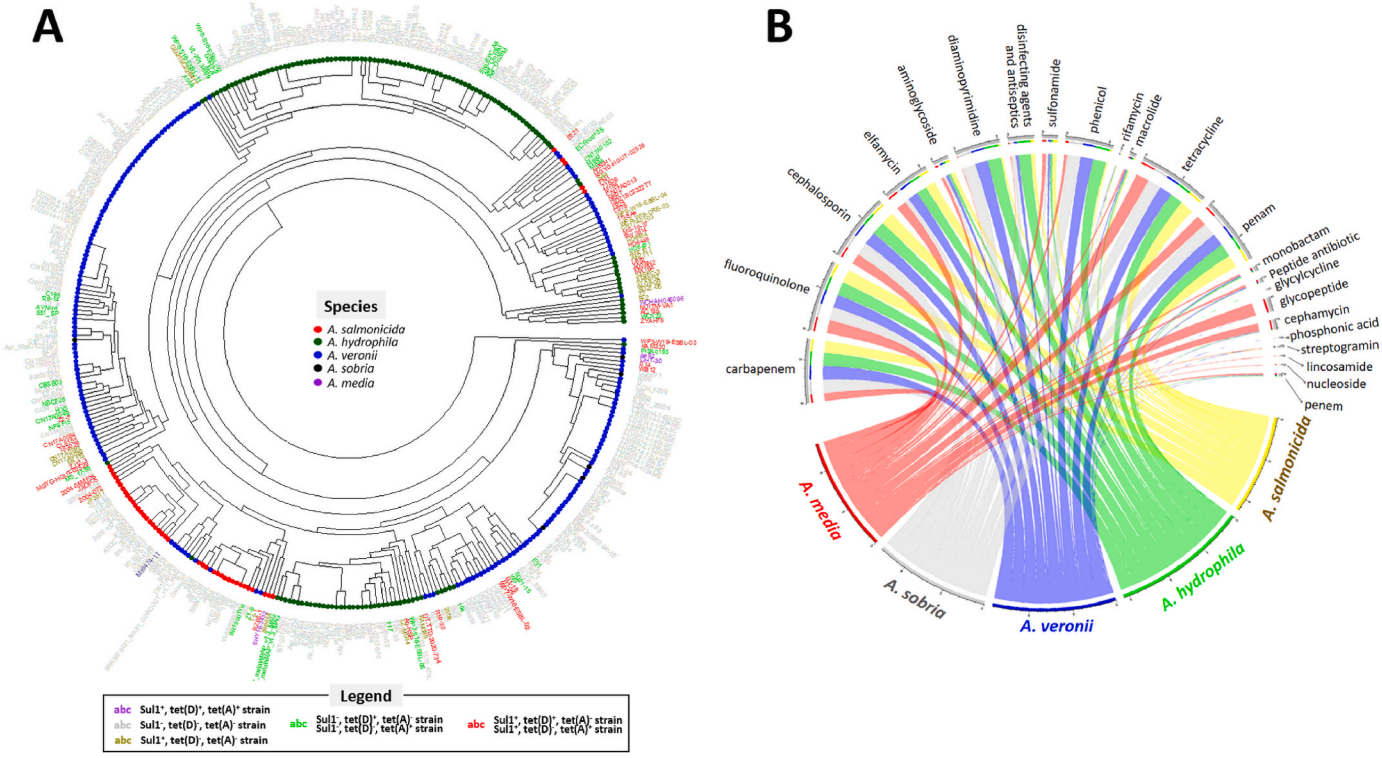

Fig. 1. Dendrogram based on the overall number of antibiotic-resistant genes identified in this study from 399 *Aeromonas* genomes. Strain name with the letter colors differentiated by the presence of ARGs of sulfonamide resistance; *Sul1*, tetracycline resistance; *tet(D)*, and/or *tet(A)* gene in the genomes and circular dot colors by species (A). More detailed information is available in Table S2. The relative proportion of strains that harbored ARG(s) of the antibiotic class) from each species (B).

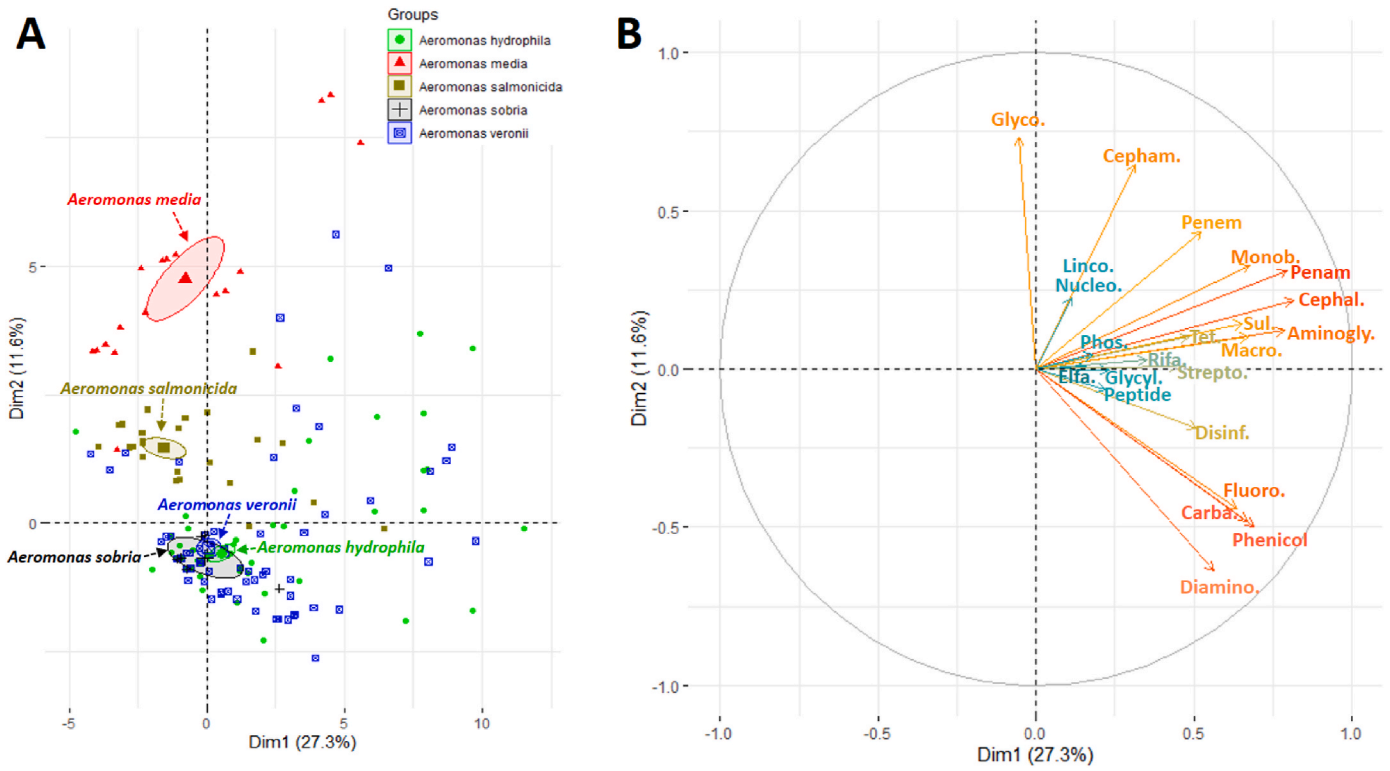

Fig. 2. The principal component analysis (PCA) plot displays the individual patterns of ARG counts within each antibiotic class at the level of strains from five *Aeromonas* species (*A. salmonicida*, *A. hydrophila*, *A. veronii*, *A. sobria*, and *A. media*) (A). The PCA plot illustrates the patterns of antibiotic variables (Carbapenem (Carba.), Fluoroquinolone antibiotic (Fluoro.), Cephalosporin (Cephal.), Elfamycin antibiotics (Elfa.), Aminoglycoside antibiotic (Aminogly.), Diaminopyrimidine antibiotic (Diamino.), Disinfecting agents and antiseptics (Disinf.), Sulfonamide antibiotic (Sul.), Phenicol antibiotic (Phenicol), Rifamycin antibiotic (Rifa.), Macrolide antibiotic (Macro.), Tetracycline antibiotic (Tet.), Penam, Monobactam (Monob.), Peptide antibiotic (Peptide), Glycylcycline (Glycyl.), Glycopeptide antibiotic (Glyco.), Cephamycin (Cepham.), Phosphonic acid antibiotic (Phos.), Streptogramin antibiotic (Strepto.), Lincosamide antibiotic (Linco.), Nucleoside antibiotic (Nucleo.), and Penem) (B).

3. Results

3.1. Characteristics of genomic data

The *Aeromonas* genomes used in this study were isolated between 1968 and 2022. Of the 399 strains of *Aeromonas*, 252 strains, including ~170 strains from fish and aquatic animals, originated from aquatic environments. By comparison, ~37% of *Aeromonas* (147 strains) came from territorial animals or environments. All five *Aeromonas* species have been reported in fish (see Table S1).

3.2. Resistome data analysis reveals antibiotic-resistant patterns in *Aeromonas* species

In total, 138 ARGs belonging to 23 types of antibiotic classes were identified (Figs. 1 and 2; Table S2). Species-specific ARGs, which are present in more than 5% of each species, were identified. One or two species-specific ARG(s) was/were identified for each species. Beta-lactamase genes related to the *cphA* family have been identified in several *Aeromonas*, but *cphA2*, *cphA5*, *cphA6*, and *cphA8* were only observed in *A. hydrophila*, *A. salmonicida*, *A. veronii*, and *A. sobria*, respectively. Likewise, the carbapenem-resistant gene, *OXA-917* and beta-lactamase gene, *CepS* were only found in *A. media* (13/23) and *A. hydrophila* strains (36/45) (Table S3). The proportions of non-species specific ARGs present (to some extent) in all *Aeromonas* species are presented in Table S4. Almost all *Aeromonas* strains harbored *adeF* and *EF-Tu*, but *tet(D)*, *sul1*, and *tet(A)* were observed in some strains regardless of species (Table S4). Hence, this study mainly investigated the non-species specific ARGs (*tet(D)*, *sul1*, and *tet(A)*). The proportion of ARG positive strains derived from territorial and aquatic environments for non-species-specific ARGs and species-specific ARGs are presented in Table 1. The proportion of species-specific ARG positive strains did not display any significant difference depending on the type

Table 1
The proportion of species-specific ARGs (*cphA2*, *cphA5*, *cphA6*, *cphA8*, *CepS*, *OXA.917*, and *vanT*) and non-species-specific ARGs (*tet(D)*, *sul1*, and *tet(A)*) derived from the aquatic and terrestrial environments.

Applicable bacterial species (Number of strains)	ARGs	Environment		*P-value*
		Aquatic	Terrestrial	
A. salmonicida (45)	*cphA5*	76% (29/38)	100% (7/7)	0.1500
A. hydrophila (149)	*CepS*	36% (39/108)	44% (18/41)	0.3736
	cphA2	41% (44/108)	29% (12/41)	0.1966
A. veronii (176)	*cphA6*	5% (4/81)	5% (5/95)	0.9223
A. sobria (6)	*cphA8*	33% (2/6)	–	0.2789
A. media (23)	*OXA.917*	58% (11/19)	50% (2/4)	0.7722
	vanT	95% (18/19)	75% (3/4)	0.2029
All species (399)	***tet(D)***	**14% (36/252)**	**7% (11/147)**	**0.0420**[a]
A. salmonicida				
A. hydrophila	*sul1*	19% (49/252)	13% (19/147)	0.0948
A. veronii				
A. sobria	*tet(A)*	12% (30/252)	7% (10/147)	0.1017
A. media				

[a] The *p*-value less than 0.05 based on the Chi-square analysis indicates a statistically different proportion of the ARG positive strains between aquatic and terrestrial environments.

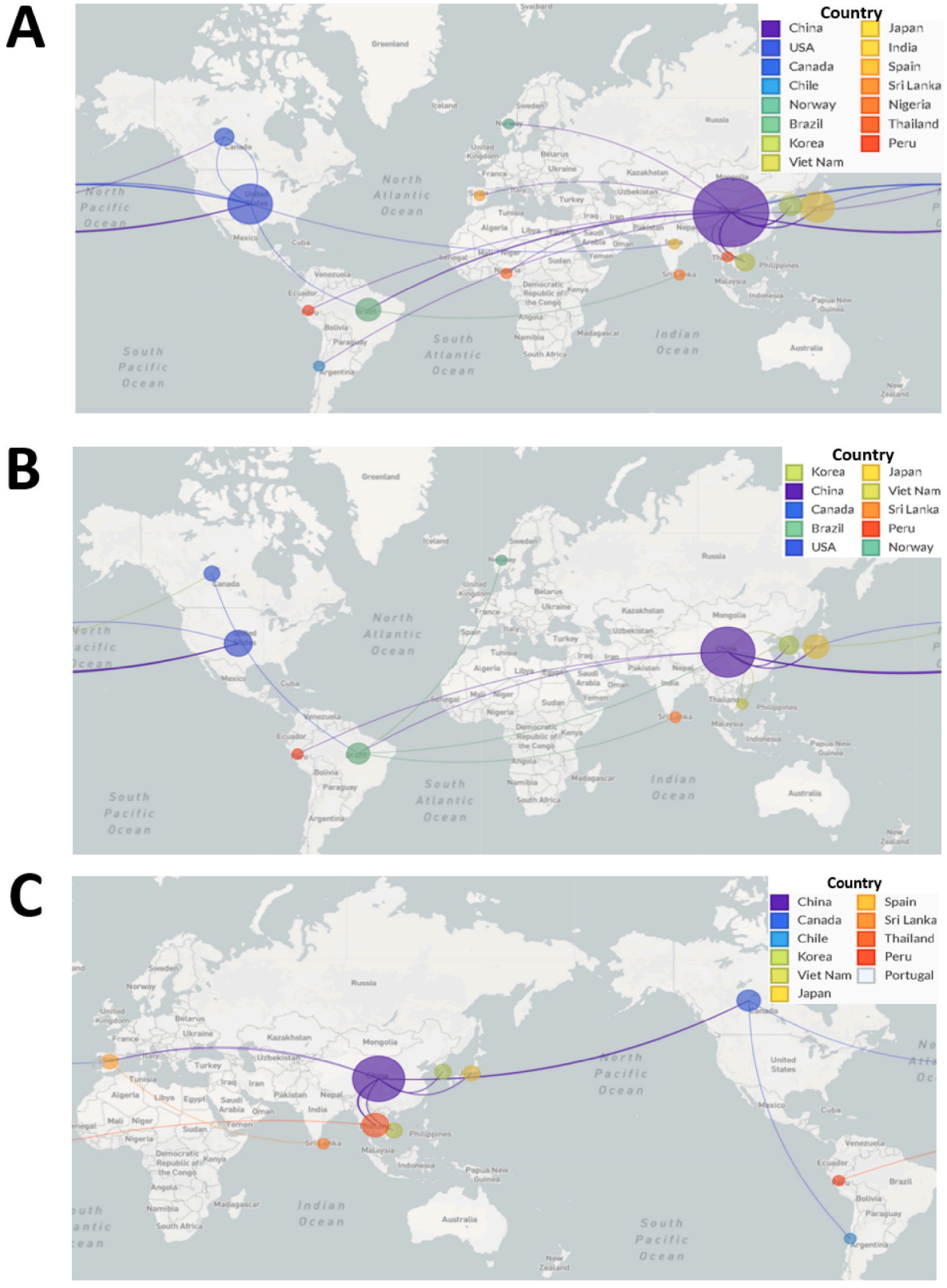

Fig. 3. Genetic diversity and geographic transmission of *sul1*, *tet(D)*, and *tet(A)* across the country (A, B, and C). The pie chart sizes indicate the number of genomes used from each country.

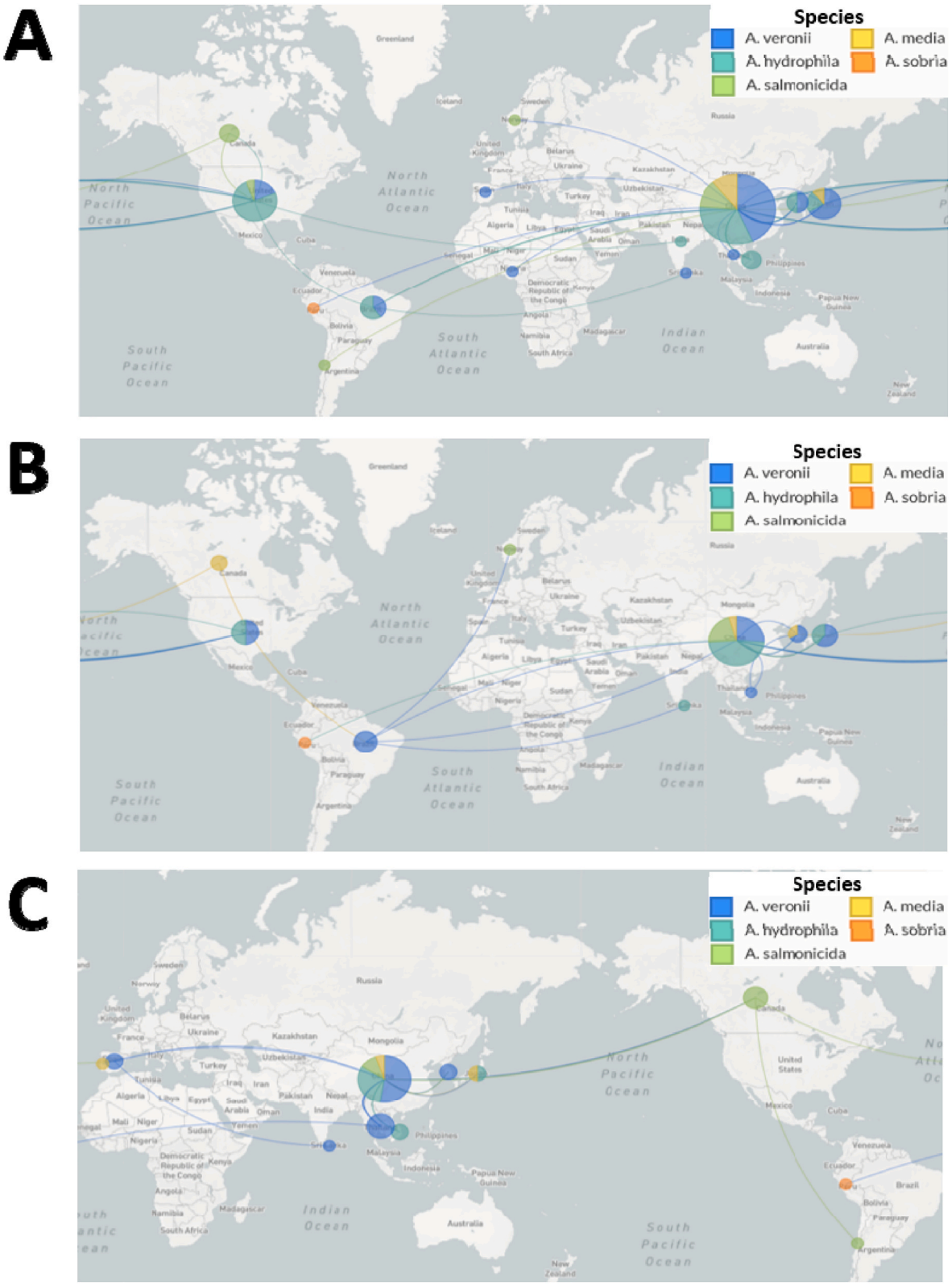

Fig. 4. Genetic diversity and geographical transmission of *sul1*, *tet(D)*, and *tet(A)* among *Aeromonas* species (A, B, and C). The pie charts represent the proportion of different species of *Aeromonas* genomes used from each country. The pie chart sizes indicate the number of genomes used from each country.

of environments. However, *tet(D)* positive strains from aquatic environments, as one of the non-species-specific ARGs, were higher in number than territorial strains. Likewise, although not statistically significant, the proportions of *sul1* and *tet(A)* positive strains from aquatic environments were higher than those of terrestrial strains (Table 1).

3.3. Distribution of antibiotic resistance genes among diverse Aeromonas species

All strains were clustered based on the presence and quantity of ARGs, and ~74% of strains did not harbor either *sul1*, *tet(D)*, or *tet(A)*

(Fig. 1). The clustering of ARGs did not necessarily correspond with each species, and no specific pattern of *sul1*, *tet(D)*, and *tet(A)* between the species was identified. Based on the functional characteristics of identified ARGs, the resistance of the antibiotic classes was predicted for each strain. The relative proportion of each antibiotic class among species is depicted in Fig. 2A. Resistance against carbapenem, fluoroquinolone, cephalosporin, elfamycin, aminoglycoside, tetracycline, and the penam class was more or less evenly distributed across all species, while resistance against the other classes varied between species. For example, *A. hydrophila*, *A. veronii*, and *A. sobria* had some strains resistant to diaminopyrimidine and phenicol class, but a much smaller number of strains were found in *A. salmonicida* and *A. media* (Fig. 2A). The overall patterns of the number of ARGs per each antibiotic class in *A. hydrophila*, *A. veronii*, and *A. media* were similar, but *A. salmonicida* and *A. media* strains exhibited slightly different clustering in the PCA plot. Nonetheless, several strains exhibited distinct patterns of ARG composition compared to the majority (Fig. 2B).

3.4. Phylodynamic and phylogeographic patterns by sul1, tet(D), tet(A), and adeF

The *sul1* sequences from the genome tended to be clustered by isolation year, but no significant pattern in terms of *Aeromonas* species was observed. Although no clear differences in *sul1* by geographical factors were found, USA strains tended to cluster in one large distinct group, whereas most of strains of Chinese and other countries were scattered and distributed in two other groups (Fig. S1). With respect to geographical region, the numbers of *Aeromonas* genomes released from China and the USA were the first and second highest, respectively. A relatively high number of *sul1* were isolated from *A. veronii* in East Asian countries, including China, Korea, and Japan. However, the major source of the *sul1* gene from *A. hydrophila* was found to be the USA and Brazil (Fig. 3A; 4A) (see Fig. 3 and Fig. 4).

The similarity of *tet(D)* sequences was associated more closely with geographical distribution than the year of isolation or species. All *tet(D)* genes isolated from China were clustered in the same branch and were not identified in other phylogenetic branches (Fig. S2). Similar to *sul1*, the first and second highest number of segregated regions in genomes containing *tet(D)* sequences were in China and the USA, respectively. Although *A. veronii* had the lowest ratio of *tet(D)* genes compared to other *Aeromonas* species, almost half of the *tet(D)* genes identified in the *Aeromonas* genomes from the USA and China were from *A. veronii.* However, in other countries where multiple *Aeromonas* genomes are reported to harbor the *tet(D)* gene, a higher number of *tet(D)* genes were isolated from *A. hydrophila* than from *A. veronii* (Fig. 3B; 4B).

Phylogenetic analysis of *tet(A)* sequences revealed that the distributions differed from other ARGs in terms of their association with the country, isolation year, and species. Specifically, *tet(A)* sequences were not well clustered based on these factors (Fig. S3). Among the countries with high numbers of *Aeromonas* genomes containing *tet(A)* sequences, China and Thailand ranked first and second, respectively. Moreover, *A. veronii* was found to be the primary source of *tet(A)* genes in numerous Asian countries, contributing to more than half of the total amount. By contrast, *tet(A)* was not identified in any of the *Aeromonas* genomes from the USA, unlike many *tet(D)* and *sul1* genes (Fig. 3C; 4C). In addition, the proportion of *sul1*, *tet(D)*, and *tet(A)* positive strains increased significantly and correlated with the year of isolation (Fig. S4). Moreover, there was a significant increase over time in the proportion of *sul1*, *tet(D)*, and *tet(A)* positive strains from aquatic environments (Table 2).

In the case of *adeF*, the sequence similarities were closely related to the origin species rather than country and isolation date. *A. hydrophila* and *A. veronii* strains exhibited clear delineation, with no instances of them coalescing into a single group. Instead, each species primarily dominated its own cluster in two of the groups (Fig. S5). The phylogeographic estimation revealed that China, USA, and Brazil were geographically important countries with regard to *adeF* transmission (Fig. S6).

4. Discussion

The continuous and frequent use of antibiotics has been identified as a trigger for the dissemination of antibiotic resistance genes (ARGs) not only in specific bacterial pathogens but also in various aquatic

Table 2
Proportions of *sul1*, *tet(D)*, and *tet(A)* positive strains from aquatic and terrestrial sources across year intervals: ~pre-1999, 2000–2009, 2010–2019, and 2020–2022.

ARGs	Isolation year	Proportion of positive strain		Chi-square	
		Aquatic	Terrestrial	χ^2	p-value
Sul1	~1999	6.3% (1/16)	0.0% (0/4)	0.26	0.608
	2000 - 2009	9.4% (5/53)	12.5% (1/8)	0.07	0.786
	2010 - 2019	20.9% (32/153)	13.7% (14/102)	2.14	0.144
	2020 - 2022	**36.7% (11/30)**	**12.1% (4/33)**	**5.22**	**0.022***
Tet(D)	~1999	0.0% (0/16)	0.0% (0/4)	-	-
	2000 - 2009	9.4% (5/53)	12.5% (1/8)	0.07	0.786
	2010 - 2019	15.7% (24/153)	9.8% (10/102)	1.83	0.176
	2020 - 2022	**23.3% (7/30)**	**0.0% (0/33)**	**8.66**	**0.003****
Tet(A)	~1999	0.0% (0/16)	0.0% (0/4)	-	-
	2000 - 2009	5.7% (3/53)	12.5% (1/8)	0.53	0.466
	2010 - 2019	11.1% (17/153)	6.9% (7/102)	1.3	0.255
	2020 - 2022	**33.3% (10/30)**	**6.1% (2/33)**	**7.58**	**0.006****

[+] Chi-square analyses of aquatic- and terrestrial strains at the same year intervals, displaying both the Chi-square (χ^2) and p-value (B – D). Asterisks indicate statistical significance (*; $P < 0.05$ and **; $P < 0.01$).

environments, including groundwater, seawater, surface water, sewage, and drinking water (Zhang et al., 2009; Blanco-Picazo et al., 2020). The characteristics of each region for phylogeographic analysis are ultimately determined by the interaction between terrestrial and aquatic environments. Therefore, for the phylogeographic analysis, we included both terrestrial and aquatic strains. Nonetheless, the *Aeromonas* species used in this study have been reported to cause significant damage to aquatic animals, and the usage of antibiotics in aquaculture and aquatic environments could be more problematic than in terrestrial environments due to the method of antibiotic administration (Sørum, 2005; Quiñones et al., 2019). This difference can have substantial implications which underscores the importance of aquatic environments. The resistance of aquaculture bacterial pathogens can be derived from the acquisition of ARGs through horizontal gene transfer (HGT) and/or incidental gene mutation, both of which can directly and indirectly impact the phenotypic characteristics of bacteria (Von Wintersdorff et al., 2016). HGT between bacteria has been raised as the major concern regarding the spread of ARGs and a potential threat to public health (Cooper et al., 2017). HGT can occur among inter-species bacteria, as demonstrated by numerous studies, such as between *Acinetobacter baumannii* and *Escherichia coli*, and among environmental microbiota like the gut, aquatic ecosystem, soil, and biofilms (e.g., Cooper et al., 2017; Giaouris et al., 2015; Aminov, 2011). Thus, several types of HGT with different mechanisms, including conjugation, transformation, and transduction, have been reported. Conjugation occurs via direct interaction between bacteria through cell surface components (e.g., adhesin and pili); while transformation involves the uptake of free DNA in the environment; and transduction is mediated by the bacteriophage (reviewed by Von Wintersdorff et al., 2016). The multiple routes of HGT increase the likelihood of sharing ARGs, which can occur more frequently than anticipated. Whilst HGT is commonly recognized as a major pathway, recent evidence has highlighted the notable contribution of vertical gene transfer (VGT) in transmitting ARGs through reproductive processes to progeny. VGT mediates and forms the transconjugants that can drive further dissemination of ARGs from donor to recipients (Li et al., 2019a). In nature, HGT and VGT coexist, leading to the dissemination of antibiotic resistance genes (ARGs) under selective pressure (Qiu et al., 2018). In the aquaculture environment, Preena et al. (2020) cautioned against the high prevalence of bacterial infection and increased use of antibiotics, both of which have contributed in the proliferation of antibiotic-resistant bacteria. In addition, anthropogenic activities, such as the international trade in live fish and their products, might spread the ARGs from regions where many multi-drug resistant bacteria are prevalent to others, thereby introducing these genes into natural bacterial populations through HGT and VGT. For example, Roh et al. (2018) isolated a bacterial pathogen (*Aeromonas veronii*) from imported ornamental fish (Discus; *Symphysodon discus*). They found ARGs such as *sul1*, *tet(A)*, and *qcuC* in the ARG integron and transposon cassettes closely relevant to HGT between bacteria (Domingues et al., 2012). These findings underscore the importance of international cooperation in sharing information to prevent the transmission of ARGs in aquaculture systems. Moreover, it is crucial to understand the phylodynamic and phylogeographic patterns of ARGs distribution to effectively combat the emergence and spread of antibiotic resistance.

Our findings demonstrate variations in the composition of ARG across different *Aeromonas* species. Notably, the ARG composition differs according to the *Aeromonas* species. *A. salmonicida* and *A. media* exhibited more distinct patterns in the ARG count belonging to 23 antibiotic classes than other *Aeromonas* species. This difference was correlated with the percentage of species-specific ARGs, although all *Aeromonas* species harbored one or two own species-specific ARGs. The *cphA5* and *vanT* as species-specific ARGs in *A. salmonicida* and *A. media* was 80% and 91%, respectively, which was two - three times higher the existence of species-specific ARGs in other *Aeromonas* species (*CepS*, *cphA2*, *cphA6*, and *cphA8)*. Notably, four out of the seven species-specific ARGs found in this study belong to *cphA* genes. *CphA* is the enzyme that can hydrolyze the penems and carbapenems classes of antibiotics (Segatore et al., 1993). In this study, whole genome based resistome analysis showed that ~58% of *A. hydrophila* harbored at least one *cphA* gene, including *cphA2* (38%; 56/149), *cphA3* (20%; 30/149), or *cphA7* (less than 1%; 1/149). However, this could be highly variable depending on the site and region. For example, several studies investigated the presence of *cphA* from *A. hydrophila* isolated from Southeastern Brazil or southern Taiwan and found that they showed more than 90% of *Aeromonas* harbored the *cphA* gene (Balsalobre et al., 2009; Wu et al., 2012). In addition, ~80% of *A. salmonicida* genomes profiled in this study harbored *cphA5*. All these results could raise concerns about the spreading of *cphA* worldwide. Although each *Aeromonas* species seems to have a specific subtype of *cphA* regarding species-specific ARGs (according to the results in Table S3), multiple subtypes of *cphA* have been detected from the same *Aeromonas* species. For instance, Tekedar et al. (2020) detected *cphA1*, *cphA2*, *cphA6*, *cphA7*, and *cphA8* genes from *A. veronii*. This difference might arise from the varying compositions of strain pools and methods for investigating ARGs; however, releasing more genomes and ARGs databases will decrease such errors.

With respect to shared ARGs among all *Aeromonas* species, *adeF* and *Ef-Tu* are most abundant in all strains. In particular, *adeF* provides resistance against tetracycline and fluoroquinolone classes, which have been widely used in fish farms. The acquisition of *adeF* in almost all *Aeromonas* strains can drive the emergence of multidrug-resistant bacteria in aquaculture (Quesada et al., 2013; Guidi et al., 2018; Li et al., 2022). A former study (Li et al., 2022) revealed the possibility that *adeF* frequently found in activated sludge and wastewater treatment plants could be enriched through HGT from Betaproteobacteria and Alphaproteobacteria to Gammaproteobacteria, supporting the hypothesis that *adeF* can transfer to other genera of bacterial pathogens. The relatively high acquisition of *adeF* genes in most *Aeromonas* species will increase the risk of multidrug-resistant bacteria in aquaculture environments. *Tet (D)* and *tet(A)* are the ordinary resistant genes against the tetracycline class found in gram-negative bacteria, and *sul1* is a resistant gene against the sulfonamide class. These three genes have been reported in numerous bacteria and/or environments in aquaculture and are known to contribute to multi-drug resistance in the surrounding microbiota via multiple types of HGT (Chopra and Roberts, 2001; Grape et al., 2005; Minogue et al., 2012; Kim et al., 2018; Roh et al., 2019). Although they were not dominant, a relatively even distribution among *Aeromonas* species was observed in this study, and their sequence similarity was not highly dependent on *Aeromonas* species. This suggests that *tet(D)*, *sul1*, and *tet(A)* are the appropriate ARGs for phylogeographic analysis.

Although there can be multiple pathways through which bacteria acquire ARGs, they tend to acquire them readily from the surrounding environment or microbial community through various gene transfer mechanisms when the conditions requiring ARGs arise for their survival. The acquisition and modification of various genes can happen in the environmental microbiome, which could drive the proliferation and dissemination of ARGs by overusing antibiotics. Even for the same level of antibiotic resistance, a 100% match in nucleotide sequence is not guaranteed; variations in sequence can occur, and the sequence patterns can be specific to the geographic origin. These patterns of variation over time and location can be determined through phylogeographic analysis which may provide a way to trace ARG transmission and dynamics (Hadfield et al., 2018). For instance, supposing that an ARG with a unique or specific pattern had been identified from region A, and an ARG with the same or very similar mutation pattern was then detected in another region B. We could speculate that the ARG introduced from region A would have spread to region B (Laamarti et al., 2023; Chakraborty et al., 2022; Hadfield et al., 2018; Baele et al., 2017). Based on calculations in dozens or hundreds of ARG sequences, the best estimation of ARG spreading between and among the countries could be obtained by placing lines onto a geographical map. For instance, East Asian and North American counties have reported multiple *Aeromonas* genomes that harbored *sul1*, *tet(D)*, and/or *tet(A)*. These genes exhibit the

possibility for transferring from China to the USA, as well as in the opposite direction. Likewise, there is a substantial likelihood of transferring *sul1*, *tet(D)*, and *tet(A)* genes among East and East South Asian countries. Notably, this study found that the emergence of *tet(D)* positive strains from waterborne environments is approximately twice as frequent as that of, terrestrial strains. Given that the most frequently used antibiotics in aquaculture is the tetracycline class, this pattern underscores the need for caution and control of the overuse of antibiotics, especially in aquatic environments (Schar et al., 2020; Thiang et al., 2021). Moreover, when estimating the proportions of *sul1*, *tet(D)*, and *tet(A)* positive strains in aquatic and terrestrial strains across each isolation year interval (pre-1999, 2000–2009, 2010–2019, and 2020–2022), our study revealed a substantial increase in positive organisms over time exclusively among aquatic strains. By contrast, terrestrial strains did not exhibit a comparable surge in positivity relative to the isolation years. This could be attributable to the different form of antibiotics used in aquaculture. One prevalent method for administering antibiotics to farmed fish is immersion, which exposes the aquatic environment and surrounding microorganisms to a considerably higher amount of antibiotics compared to individual administration to terrestrial animals (Sørum, 2005; Quiñones et al., 2019). This highlights the need for significantly greater caution in the use of antibiotics in aquaculture environments compared to terrestrial settings. In addition, previous studies have verified multiple types of ARGs in surface sediments and waterborne environments in the country where many ARGs positive strains were identified in the current study, which raises further concern about the pervasive influx of ARGs into natural aquatic environments (Qiao et al., 2018; Ohore et al., 2019). However, given that the analysis of this study was conducted only on genomes and data that have been freely available to date, which creates a heavily biased sampling distribution, it cannot be the basis for judging whether the situation in a particular country is better or worse. Also, the possibility that similar or identical mutations in ARGs may occur in different countries by chance rather than transmission may be a limitation of this study. Nevertheless, we observed a significant increase in the proportion of *sul1*, *tet(D)*, and *tet(A)* positive strains over the years. These results indicate that the prevalence of these ARGs in *Aeromonas* genomes has been rising over time, potentially reflecting the increased use of antibiotics and the resulting selective pressure on bacterial populations. This emphasizes the need for a global strategy and a unitary health approach to controlling the spread of ARGs.

In conclusion, this is the first study to profile the resistome in major *Aeromonas* species, which was achieved by exploring the phylodynamic and phylogenetic distribution of ARGs among approximately 400 bacterial genomes. Among more than a hundred ARGs identified in this study, *sul1*, *tet(D)*, and *tet(A)*, were appropriate for phylodynamic and phylogeographic analyses of *Aeromonas* species. We found that *Aeromonas* strains derived from aquatic environments displayed a significantly higher proportion of ARG compositions compared to those originating from terrestrial sources. This underscores the urgent need and importance for effective control and management of antibiotics usage, including addressing potential overuse, in aquaculture practices. The spread of these ARGs was not limited to adjacent countries and thus revealed the possibility of spreading to distant regions. Therefore, the control of ARGs spread in the water system is not only a concern for specific countries but a global issue. To effectively combat the spread of ARGs, it is imperative to monitor their distribution and implement mitigation measures with full transparency through international cooperation, one health approach and information sharing.

Credit author statement

HR: Conceptualization, Data curation, Methodology, Visualization, Formal analysis, Writing, Revising, Approving. DK: Writing, Revising, Writing – review & editing

Financial disclosure

No financial disclosures were reported by the authors of this paper.

Declaration of competing interest

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

Data availability

Data will be made available on request.

Acknowledgement

The authors thank Institute of Marine Research (Havforskningsinstituttet) for funding the open access of this article.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.envres.2023.117273.

References

Aminov, R.I., 2011. Horizontal gene exchange in environmental microbiota. Front. Microbiol. 2, 158.
Baele, G., Suchard, M.A., Rambaut, A., Lemey, P., 2017. Emerging concepts of data integration in pathogen phylodynamics. Syst. Biol. 66, e47–e65.
Balsalobre, L.C., Dropa, M., Lincopan, N., Mamizuka, E.M., Matté, G.R., Matté, M.H., 2009. Detection of metallo-β-lactamases-encoding genes in environmental isolates of *Aeromonas hydrophila* and *Aeromonas jandaei*. Lett. Appl. Microbiol. 49, 142–145.
Baron, S., Granier, S.A., Larvor, E., Jouy, E., Cineux, M., Wilhelm, A., Gassilloud, B., Le Bouquin, S., Kempf, I., Chauvin, C., 2017. *Aeromonas* diversity and antimicrobial susceptibility in freshwater—an attempt to set generic epidemiological cut-off values. Front. Microbiol. 8, 503.
Béné, C., Arthur, R., Norbury, H., Allison, E.H., Beveridge, M., Bush, S., Campling, L., Leschen, W., Little, D., Squires, D., 2016. Contribution of fisheries and aquaculture to food security and poverty reduction: assessing the current evidence. World Dev. 79, 177–196.
Blanco-Picazo, P., Roscales, G., Toribio-Avedillo, D., Gómez-Gómez, C., Avila, C., Ballesté, E., Muniesa, M., Rodríguez-Rubio, L., 2020. Antibiotic resistance genes in phage particles from antarctic and mediterranean seawater ecosystems. Microorganisms 8, 1293.
Burridge, L., Weis, J.S., Cabello, F., Pizarro, J., Bostick, K., 2010. Chemical use in salmon aquaculture: a review of current practices and possible environmental effects. Aquaculture 306, 7–23.
Carter, C., 2018. The Politics of Aquaculture: Sustainability Interdependence, Territory and Regulation in Fish Farming. Routledge.
Chakraborty, C., Bhattacharya, M., Sharma, A.R., Dhama, K., 2022. Evolution, epidemiology, geographical distribution, and mutational landscape of newly emerging monkeypox virus. Geroscience 44, 2895–2911.
Chopra, I., Roberts, M., 2001. Tetracycline antibiotics: mode of action, applications, molecular biology, and epidemiology of bacterial resistance. Microbiol. Mol. Biol. Rev. 65, 232–260.
Cipriano, R.C., Bullock, G.L., 2001. Furunculosis and Other Diseases Caused by Aeromonas Salmonicida. National Fish Health Research Laboratory.
Conte, D., Palmeiro, J., Bavaroski, A., Rodrigues, L., Cardozo, D., Tomaz, A., Camargo, J., Dalla-Costa, L., 2021. Antimicrobial resistance in *Aeromonas* species isolated from aquatic environments in Brazil. J. Appl. Microbiol. 131, 169–181.
Cooper, R.M., Tsimring, L., Hasty, J., 2017. Inter-species population dynamics enhance microbial horizontal gene transfer and spread of antibiotic resistance. Elife 6, e25950.
Domingues, S., da Silva, G.J., Nielsen, K.M., 2012. Integrons: vehicles and pathways for horizontal dissemination in bacteria. Mobile Genet. Elem. 2, 211–223.
Ellabaan, M.M., Munck, C., Porse, A., Imamovic, L., Sommer, M.O., 2021. Forecasting the dissemination of antibiotic resistance genes across bacterial genomes. Nat. Commun. 12, 2435.
Esteve, C., Biosca, E.G., Amaro, C., 1993. Virulence of *Aeromonas hydrophila* and some other bacteria isolated from European eels *Anguilla anguilla* reared in fresh water. Dis. Aquat. Org. 16, 15–20.
Galili, T., 2015. Dendextend: an R package for visualizing, adjusting and comparing trees of hierarchical clustering. Bioinformatics 31, 3718–3720.
Giaouris, E., Heir, E., Desvaux, M., Hébraud, M., Møretrø, T., Langsrud, S., Doulgeraki, A., Nychas, G., Kačániová, M., Czaczyk, K., 2015. Intra-and inter-species interactions within biofilms of important foodborne bacterial pathogens. Front. Microbiol. 6, 841.

Grape, M., Farra, A., Kronvall, G., Sundström, L., 2005. Integrons and gene cassettes in clinical isolates of co-trimoxazole-resistant gram-negative bacteria. Clin. Microbiol. Infection 11, 185–192.
Greaker, M., Vormedal, I., Rosendal, K., 2020. Environmental policy and innovation in Norwegian fish farming: resolving the sea lice problem? Mar. Pol. 117, 103942.
Gu, Z., Gu, L., Eils, R., Schlesner, M., Brors, B., 2014. Circlize implements and enhances circular visualization in R. Bioinformatics 30, 2811–2812.
Gui, J., Tang, Q., Li, Z., Liu, J., De Silva, S.S., 2018. Aquaculture in China: Success Stories and Modern Trends. John Wiley & Sons.
Guidi, L.R., Santos, F.A., Ribeiro, A.C.S., Fernandes, C., Silva, L.H., Gloria, M.B.A., 2018. Quinolones and tetracyclines in aquaculture fish by a simple and rapid LC-MS/MS method. Food Chem. 245, 1232–1238.
Hadfield, J., Megill, C., Bell, S.M., Huddleston, J., Potter, B., Callender, C., Sagulenko, P., Bedford, T., Neher, R.A., 2018. Nextstrain: real-time tracking of pathogen evolution. Bioinformatics 34, 4121–4123.
Hersoug, B., 2015. The greening of Norwegian salmon production. Maritime Studies 14, 1–19.
Hu, F., Zhang, T., Liang, J., Xiao, J., Liu, Z., Dahlgren, R.A., 2022. Impact of biochar on persistence and diffusion of antibiotic resistance genes in sediment from an aquaculture pond. Environ. Sci. Pollut. Control Ser. 29, 57918–57930.
Jun, J.W., Kim, J.H., Shin, S.P., Han, J.E., Chai, J.Y., Park, S.C., 2013. Protective effects of the *Aeromonas* phages pAh1-C and pAh6-C against mass mortality of the cyprinid loach (*Misgurnus anguillicaudatus*) caused by *Aeromonas hydrophila*. Aquaculture 416, 289–295.
Kassambara, A., Mundt, F., 2017. Factoextra: extract and visualize the results of multivariate data analyses. R Package Version 1, 337–354.
Kim, A., Lim, Y., Kim, N., Luan Nguyen, T., Roh, H.J., Park, C., Han, H., Jung, S., Cho, M., Kim, D., 2018. A comparison of genotypic and phenotypic methods for analyzing the susceptibility to sulfamethoxazole and trimethoprim in *Edwardsiella piscicida*. Microb. Drug Resist. 24, 1226–1235.
Laamarti, M., El Fathi Lalaoui, Y., Elfermi, R., Daoud, R., El Allali, A., 2023. Afro-TB dataset as a large scale genomic data of Mycobacterium tuberuclosis in Africa. Sci. Data 10, 212.
Li, B., Qiu, Y., Song, Y., Lin, H., Yin, H., 2019a. Dissecting horizontal and vertical gene transfer of antibiotic resistance plasmid in bacterial community using microfluidics. Environ. Int. 131, 105007.
Li, D., Gao, J., Dai, H., Wang, Z., Zhao, Y., Cui, Y., 2022. Higher spreading risk of antibacterial biocide and heavy metal resistance genes than antibiotic resistance genes in aerobic granular sludge. Environ. Res. 212, 113356.
Li, F., Wu, D., Gu, H., Yin, M., Ge, H., Liu, X., Huang, J., Zhang, Y., Wang, Z., 2019b. *Aeromonas hydrophila* and *Aeromonas veronii* cause motile *Aeromonas* septicaemia in the cultured Chinese sucker, *Myxocyprinus asiaticus*. Aquacult. Res. 50, 1515–1526.
Lü, A., Hu, X., Li, L., Sun, J., Song, Y., Pei, C., Zhang, C., Kong, X., 2016. Isolation, Identification and Antimicrobial Susceptibility of Pathogenic *Aeromonas media* Isolated from Diseased Koi Carp (*Cyprinus carpio* Koi).
Majtán, J., Černy, J., Ofúkaná, A., Takáč, P., Kozánek, M., 2012. Mortality of therapeutic fish *Garra rufa* caused by *Aeromonas sobria*. Asian Pac. J. Trop. Biomed. 2, 85–87.
Minogue, E., Barry, T., Carroll, C., Smith, P., 2012. Setting epidemiological cut-off values for *Aeromonas salmonicida* disc diffusion data capable of discriminating between strains on the basis of their possession of sul1 genes. Aquaculture 364, 329–332.
Mzula, A., Wambura, P.N., Mdegela, R.H., Shirima, G.M., 2021. Present status of aquaculture and the challenge of bacterial diseases in freshwater farmed fish in Tanzania; A call for sustainable strategies. Aquaculture and Fisheries 6, 247–253.
Ohore, O.E., Addo, F.G., Zhang, S., Han, N., Anim-Larbi, K., 2019. Distribution and relationship between antimicrobial resistance genes and heavy metals in surface sediments of Taihu lake, China. Journal of Environmental Sciences 77, 323–335.
Pereira, W.A., Mendonça, C.M.N., Urquiza, A.V., Marteinsson, V.þ., LeBlanc, J.G., Cotter, P.D., Villalobos, E.F., Romero, J., Oliveira, R.P., 2022. Use of probiotic bacteria and bacteriocins as an alternative to antibiotics in aquaculture. Microorganisms 10, 1705.
Peterson, B.G., Carl, P., Boudt, K., Bennett, R., Ulrich, J., Zivot, E., Cornilly, D., Hung, E., Lestel, M., Balkissoon, K., 2018. Package 'performanceanalytics'. R Team Cooperation 3, 13–14.
Preena, P.G., Swaminathan, T.R., Kumar, V.J.R., Singh, I.S.B., 2020. Antimicrobial resistance in aquaculture: a crisis for concern. Biologia 75, 1497–1517.
Qiao, M., Ying, G., Singer, A.C., Zhu, Y., 2018. Review of antibiotic resistance in China and its environment. Environ. Int. 110, 160–172.
Qiu, Y., Zhang, J., Li, B., Wen, X., Liang, P., Huang, X., 2018. A novel microfluidic system enables visualization and analysis of antibiotic resistance gene transfer to activated sludge bacteria in biofilm. Sci. Total Environ. 642, 582–590.
Quesada, S.P., Paschoal, J.A.R., Reyes, F.G.R., 2013. Considerations on the aquaculture development and on the use of veterinary drugs: special issue for fluoroquinolones—a review. J. Food Sci. 78, R1321–R1333.
Quiñones, R.A., Fuentes, M., Montes, R.M., Soto, D., León-Muñoz, J., 2019. Environmental issues in Chilean salmon farming: a review. Rev. Aquacult. 11, 375–402.
Rasul, M., Majumdar, B., 2017. Abuse of antibiotics in aquaculture and it's effects on human, aquatic animal and environment. The Saudi Journal of Life Sciences 2, 81–88.
Roh, H.J., Kang, G.S., Kim, A., Kim, N.E., Nguyen, T.L., Kim, D., 2018. Blue light-emitting diode photoinactivation inhibits Edwardsiellosis in fancy carp (*Cyprinus carpio*). Aquaculture 483, 1–7.
Roh, H.J., Kim, B., Kim, A., Kim, N.E., Lee, Y., Chun, W., Ho, T.D., Kim, D., 2019. Whole-genome analysis of multi-drug-resistant *Aeromonas veronii* isolated from diseased discus (*Symphysodon discus*) imported to korea. J. Fish. Dis. 42, 147–153.
Sahoo, P., Swaminathan, T.R., Abraham, T.J., Kumar, R., Pattanayak, S., Mohapatra, A., Rath, S., Patra, A., Adikesavalu, H., Sood, N., 2016. Detection of goldfish haematopoietic necrosis herpes virus (Cyprinid herpesvirus-2) with multi-drug resistant *Aeromonas hydrophila* infection in goldfish: first evidence of any viral disease outbreak in ornamental freshwater aquaculture farms in India. Acta Trop. 161, 8–17.
Schar, D., Klein, E.Y., Laxminarayan, R., Gilbert, M., Van Boeckel, T.P., 2020. Global trends in antimicrobial use in aquaculture. Sci. Rep. 10, 21878.
Segatore, B., Massidda, O., Satta, G., Setacci, D., Amicosante, G., 1993. High specificity of cphA-encoded metallo-beta-lactamase from *Aeromonas hydrophila* AE036 for carbapenems and its contribution to beta-lactam resistance. Antimicrob. Agents Chemother. 37, 1324–1328.
Shao, S., Hu, Y., Cheng, J., Chen, Y., 2018. Research progress on distribution, migration, transformation of antibiotics and antibiotic resistance genes (ARGs) in aquatic environment. Crit. Rev. Biotechnol. 38, 1195–1208.
Sikveland, M., Tveterås, R., Zhang, D., 2022. Profitability differences between public and private firms: the case of Norwegian salmon aquaculture. Aquacult. Econ. Manag. 26, 414–438.
Silva, C.P., Louros, V., Silva, V., Otero, M., Lima, D.L., 2021. Antibiotics in aquaculture wastewater: is it feasible to use a photodegradation-based treatment for their removal? Toxics 9, 194.
Sørum, H., 2005. Antimicrobial drug resistance in fish pathogens. Antimicrobial resistance in bacteria of animal origin 213–238.
Suresh, T., Nithin, M., Kushala, K., Girisha, S., Shivakumar, V., Dheeraj, S., Puneeth, T., Kishan, K., Vinay, T., 2023. Largescale mortality of *Oreochromis mossambicus* in lakes and reservoirs of Karnataka due to coinfection of tilapia lake virus (TiLV) and multidrug-resistant *Aeromonas veronii*: an emerging fish disease in India. Aquaculture 565, 739077.
Tekedar, H.C., Arick, M.A., Hsu, C., Thrash, A., Blom, J., Lawrence, M.L., Abdelhamed, H., 2020. Identification of antimicrobial resistance determinants in *Aeromonas veronii* strain MS-17-88 recovered from channel catfish (*Ictalurus punctatus*). Front. Cell. Infect. Microbiol. 10, 348.
Thiang, E.L., Lee, C.W., Takada, H., Seki, K., Takei, A., Suzuki, S., Wang, A., Bong, C.W., 2021. Antibiotic residues from aquaculture farms and their ecological risks in southeast asia: a case study from Malaysia. Ecosys. Health Sustain. 7, 1926337.
Torres-Corral, Y., González-Barreiro, O., Riaza, A., Santos, Y., 2022. Establishment of different challenge models for *Aeromonas salmonicida* subsp. *achromogenes* in turbot and sole. Aquaculture 555, 738261.
Vignesh, R., Karthikeyan, B., Periyasamy, N., Devanathan, K., 2011. Antibiotics in aquaculture: an overview. South Asian J Exp Biol 1, 114–120.
Von Wintersdorff, C.J., Penders, J., Van Niekerk, J.M., Mills, N.D., Majumder, S., Van Alphen, L.B., Savelkoul, P.H., Wolffs, P.F., 2016. Dissemination of antimicrobial resistance in microbial ecosystems through horizontal gene transfer. Front. Microbiol. 7, 173.
Wang, G., Clark, C.G., Liu, C., Pucknell, C., Munro, C.K., Kruk, T.M., Caldeira, R., Woodward, D.L., Rodgers, F.G., 2003. Detection and characterization of the hemolysin genes in *Aeromonas hydrophila* and *Aeromonas sobria* by multiplex PCR. J. Clin. Microbiol. 41, 1048–1054.
Warnes, G.R., Bolker, B., Lumley, T., Johnson, R.C., 2015. Gmodels: Various R Programming Tools for Model Fitting, vol. 2. R Package Version.
Wickham, H., 2011. The split-apply-combine strategy for data analysis. J. Stat. Software 40, 1–29.
Wickham, H., François, R., Henry, L., Müller, K., 2018. Dplyr: A Grammar of Data Manipulation. R package version 0.7. 6. Computer Software]. CRAN.R-Project.org/package= Dplyr.
Wu, C., Chen, P., Wu, J., Yan, J., Lee, C., Lee, H., Lee, N., Chang, C., Lin, Y., Chiu, Y., 2012. Distribution and phenotypic and genotypic detection of a metallo-β-lactamase, CphA, among bacteraemic *Aeromonas* isolates. J. Med. Microbiol. 61, 712–719.
Zhang, X., Zhang, T., Fang, H.H., 2009. Antibiotic resistance genes in water environment. Appl. Microbiol. Biotechnol. 82, 397–414.