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The mobile gene cassette carrying tetracycline resistance genes in *Aeromonas veronii* strain Ah5S-24 isolated from catfish pond sediments shows similarity with a cassette found in other environmental and foodborne bacteria

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Aeromonas veronii is a Gram-negative bacterium ubiquitously found in aquatic environments. It is a foodborne pathogen that causes diarrhea in humans and hemorrhagic septicemia in fish. In the present study, we used whole-genome sequencing (WGS) to evaluate the presence of antimicrobial resistance (AMR) and virulence genes found in *A. veronii* Ah5S-24 isolated from catfish pond sediments in South-East, United States. We found *cphA4*, *dfrA3*, *mcr-7.1*, *valF*, *bla_{FOX-7}*, and *bla_{OXA-12}* resistance genes encoded in the chromosome of *A. veronii* Ah5S-24. We also found the tetracycline *tet(E)* and *tetR* genes placed next to the IS5/IS1182 transposase, integrase, and hypothetical proteins that formed as a genetic structure or transposon designated as IS5/IS1182/*hp/tet(E)/tetR/hp*. BLAST analysis showed that a similar mobile gene cassette (MGC) existed in chromosomes of other bacteria species such as *Vibrio parahaemolyticus* isolated from retail fish at markets, *Aeromonas caviae* from human stool and *Aeromonas media* from a sewage bioreactor. In addition, the IS5/IS1182/*hp/tet(E)/tetR/hp* cassette was also found in the plasmid of *Vibrio alginolyticus* isolated from shrimp. As for virulence genes, we found the tap type IV pili (*tapA* and *tapY*), polar flagellae (*flgA* and *flgN*), lateral flagellae (*ifgA* and *lfgL*), and fimbriae (*pefC* and *pefD*) genes responsible for motility and adherence. We also found the hemolysin genes (*hylIII*, *hylA*, and *TSH*), *aerA* toxin, biofilm formation, and quorum sensing (*LuxS*, *mshA*, and *mshQ*) genes. However, there were no MGCs encoding virulence genes found in *A. veronii* Ah5S-24. Thus, our findings show that MGCs could play a vital role in the spread of AMR genes between chromosomes and plasmids among bacteria in aquatic environments. Overall, our findings are suggesting that MGCs encoding AMR genes could play a vital role in the spread of resistance acquired from high usage of antimicrobials in aquaculture to animals and humans.

KEYWORDS

Aeromonas veronii, antimicrobial resistance, mobile gene cassette, virulence, tetracycline, environment, foodborne

1. Introduction

Aeromonas veronii is a Gram-negative bacterium ubiquitously found in different aquatic environments. It was first reported by Hickman-Brenner et al. (1987) as a new species in 1983. It is pathogenic to several fish species that include the top farmed species such as common carp (*Cyprinus carpio*), channel catfish (*Ictalurus punctatus*), tilapia (*Oreochromis niloticus*), and pangasius (*Pangasius hypophthalmus*) (González-Serrano et al., 2002; Smyrli et al., 2017, 2019; Wang et al., 2022). It causes hemorrhagic septicemia and skin ulcers in fish (Hoai et al., 2019; Tekedar et al., 2020) and diarrhea in humans (Roberts et al., 2006). Strain variations have been linked to virulence leading to studies aimed at identifying the virulence factors associated with mortalities (González-Serrano et al., 2002; Smyrli et al., 2017, 2019; Wang et al., 2022). The high mortalities experienced in aquaculture have led to use of antibiotics, thereby contributing to increase of antimicrobial resistance (AMR) (Roberts et al., 2006). As mentioned in our previous studies (Dubey et al., 2022a,b), the major limitation with most studies aimed at identifying AMR genes in bacteria is that they are mostly done by PCR that only detects AMR genes based on the primers used in the assay. This poses the risk of omitting important AMR genes whose primers are not included in PCR assays. Besides, PCR-based assays do not determine whether the AMR genes are intrinsically encoded in the chromosomes or extrinsically in plasmids. So, the use of whole-genome sequencing (WGS) able to detect all genes and their location in bacteria genomes is a better approach for elucidating the role of different bacteria species in the spread of AMR and virulence genes than PCR-based assays.

The spreading of AMR genes by horizontal transfer is contributing to involvement of bacteria species outside the 12 bacteria families enlisted to pose the greatest AMR threat to human health by the World Health Organization (WHO) (Willyard, 2017). As pointed out by White et al. (2001), the spread of AMR genes is enhanced when they form part of mobile gene cassettes (MGCs) or transposons. The MGCs were first identified as integrated AMR genes found in integrons in the early 1980s (Ward and Grinsted, 1982; Meyer et al., 1983; White et al., 2001). Although studies done this far have focused on cassettes carrying AMR genes, it is likely that the packaging in cassettes includes other genes such as virulence factors. As stated by White et al. (2001), MGCs facilitate horizontal gene transfer using various mechanisms that include mobilization of individual cassettes by integrons (Collis and Hall, 1992), movement of integrons having cassettes by transposases (Brown et al., 1996; Craig, 1996; Minakhina et al., 1999), dissemination of larger transposons carrying integrases (Liebert et al., 1999), and translocation of conjugative plasmids having integrases among bacteria (White et al., 2001). It is likely that most of the AMR genes associated with infections in aquaculture, livestock and humans are part of MGCs (Recchia and Hall, 1995). Yet, gene cassettes conferring resistance to antibiotics used in aquaculture have not been widely investigated as done in mammalian studies. Hence, it is unknown whether the AMR genes selected against drugs like

tetracycline, sulphonamide, and trimethoprim widely used in aquaculture are packaged in MGCs. Thus, although previous studies have focused on identifying individual genes associated with resistance, the cassettes responsible for the spread of AMR genes has not been widely investigated for bacteria found in aquaculture.

In the present study we used WGS to profile all AMR and virulence genes found in *A. veronii* Ah5S-24 isolated from pond sediment obtained from the South East, USA by DePaola et al. (1988). Although in the previous study, they detected presence of Oxytetracycline-resistance (OTc^r) and tetracycline-resistance (Tc^r) by selecting for isolates that replicated on MacConkey agar containing oxytetracycline or tetracycline antibiotics, they did not determine whether the resistance gene was located in the chromosome or plasmids. Even though they showed the transfer of OTc^r and Tc^r resistance from the *Aeromonas* isolate to *Escherichia coli*, they did not determine whether the transfer was plasmid mediated or MGC. Thus, we wanted to determine whether the OTc^r and Tc^r resistance in the isolate was encoded in the chromosome or plasmid. We also wanted to determine whether the resistance detected was associated with a tetracycline genetic structure similar to that found in other bacteria species. We anticipate that data presented herein will underscore the importance of screening for MGCs carrying AMR genes from aquatic organisms with potential transmission to animals and humans.

2. Methodology

2.1. Bacteria culture, characterization, and antibiotic diffusion test

A suspected *Aeromonas hydrophila* isolated from pond sediments in the South-Eastern USA by DePaola et al. (1988) in 1988 was retrieved from the -80°C freezer at the Norwegian University of Life Sciences (NMBU), Ås, Norway. The isolate was kindly provided by Dr. Angelo DePaola, Gulf Coast Seafood Laboratory, United States. After thawing, the bacteria isolate was streaked on blood agar and incubated at 10°C for 5–7 days. Single colonies were streaked on tryptone soy agar (TSA) for purification followed by characterization using the Matrix-assisted laser Desorption/Ionization-Time of Flight (MALDI-TOF) mass spectrometry while DNA was extracted based on manufacturer's protocol (Qiagen, Germany). Identification of the bacteria species was done by PCR using universal 16S rRNA primers 27F and 1492R. Phenotypic characterization of antibiotic resistance was done using the Kirby-Bauer disk diffusion test (Joseph et al., 2011). The commercial antibiotic discs (Neo-Sensitabs™, Rosco) used consisted of Penicillin (PEN-10 µg), Amoxicillin (AMOXY-30 µg), Ampicillin (AMP-10 µg), Ciprofloxacin (CIPR-5 µg), Cefoxitin (CFO-30 µg), Cephalothin (CEP-30 µg), Tetracycline (TET-30 µg), Gentamycin (GEN-10 µg), Rifampicin (RIF-5 µg), Sulfonamide (SULFA-240 µg), Trimethoprim (TRIM-5 µg), Erythromycin (Ery-15 µg), Nitrofurantoin (NI-300 µg), and (Colistin-CO-150 µg)

Table 1 Overview of antibiotic resistance genes detected in the draft genome of *Aeromonas veronii* AhS5-24 together with phenotypic antibiotic susceptibility testing results using disk diffusion assay.

Resistance mechanism	Resistance gene	Antibiotic class	Antibiotic	Results
Antibiotic inactivation	<i>bla</i> _{FOX-7}	Cephamycin	Cefoxitin (CFO30)	R
	<i>bla</i> _{OXA-12}	Cephalosporin	Cephalothin (CEP 30)	R
	<i>cpmA4</i>	β -lactams	Amoxicillin (AMOXY)	R
Antibiotic efflux	<i>tet(E)</i>	Tetracycline	Tetracycline (TET30)	R
	<i>MexB</i>	Sulfonamide, β -lactams	Sulfonamide (SULFA)	I
	<i>CRP</i>	Macrolide	Erythromycin (ERY15)	S
Antibiotic target alteration	<i>mcr-7.1</i>	Peptide	Colistin (CO150)	S
	<i>vatF</i>	–	–	–
Antibiotic target replacement	<i>dfrA3</i>	Diaminopyrimidine	Trimethoprim (TRIM5)	I
Other resistance mechanism		Fluoroquinolone	Ciprofloxacin (CIPR5)	S
		Aminoglycoside	Gentamicin (GEN10)	S
		Nitrofurantoin	Nitrofurantoin (NI300)	S
		Rifamycin	Rifampicin (RIF5)	S

(Table 1). A volume of 100 μ l containing freshly cultured bacteria diluted at McFarland concentration of 10^8 CFU/ml was spread on Müller Hinton agar followed by putting the antibiotic discs on the bacteria lawn. Next, the plates were incubated at 30°C overnight followed by measuring the susceptibility or resistance based on the Clinical and Laboratory Standards Institute (CLSI) guidelines (Kahlmeter et al., 2006; Cockerill et al., 2012).

2.2. DNA extraction

Genomic DNA (gDNA) was extracted as previously described (Becker et al., 2016) using the MagAttract[®] HMW DNA kit based on the manufacturer protocols (Qiagen GmbH, Hilden, Germany). Briefly, a 1 ml volume of approximately 2×10^9 CFU/ml of freshly overnight cultured bacteria was spanned in 2 ml Eppendorf tubes followed by suspending the pellets in 180 μ l buffer ATL (tissue lysis buffer, Qiagen GmbH, Hilden, Germany). Next, Proteinase K was added to each vial at a concentration of 20 mg/ml followed by incubation at 56°C in an Eppendorf thermomixer for 30 min. Afterward, 4 μ l RNase was added and the vials were pulse vortexed

followed by adding 15 μ l of MagAttract Suspension G and 280 μ l Buffer MB to each vial (Tarumoto et al., 2017). The suspension from each tube was transferred onto the MagAttract holder followed by mixing for 1 min on an Eppendorf thermomixer. The magnetic beads having the gDNA were separated on the MagAttract magnetic rack for approximately 1 min. Supernatants were removed without disturbing the beads followed by washing the magnetic beads twice using MW1 and PE buffer (Becker et al., 2016; Tarumoto et al., 2017). The remaining suspension was removed by washing the beads twice using 1 ml RNAase free water (Qiagen GmbH, Hilden, Germany) (Becker et al., 2016). The gDNA was harvested by eluting in 100 μ l buffer EB while purity was evaluated using the NanoDrop (Thermo Fisher, United States) and gel electrophoresis using 1% agarose. Quantification of gDNA was carried out using the Qubit double-stranded DNA high-CHS kit following the manufacturer's guidelines (Life Technologies Inc., Carlsbad, CA, United States) (Guan et al., 2020).

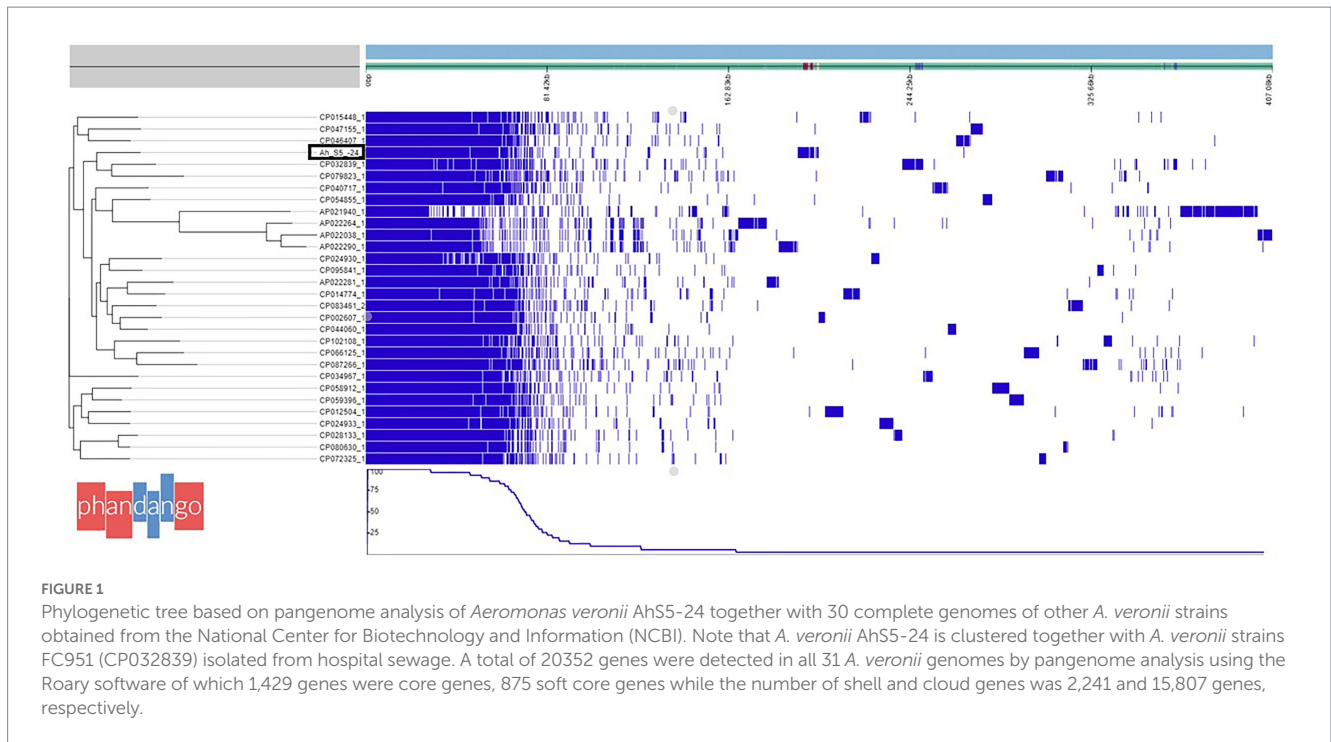
2.3. Library preparation, sequencing, and bioinformatics analysis

Library preparation was carried out using Nextera DNA Flex (Tagmentation Illumina Inc. San Diego, CA, United States) while Illumina MiSeq were used with a paired-end read length of 2×300 bp. The bacterial raw DNA reads were analyzed using the online Galaxy platform¹ version 21.05. Quality of both forward and reverse raw reads were analyzed using the FastQC Version 0.11.9 software (Bioinformatics B, 2011), while the Trimmomatic version 0.38.1 was used to remove the adapters and low-quality reads from paired-end sequences (Bolger et al., 2014). The resulting paired-end sequence reads were *de novo* assembled using SPAdes v. 3.12.0 (Coil et al., 2015) with 33 to 91 k-mers (Bankevich et al., 2012) while genome annotation was done using the prokaryotic genome annotation pipeline (PGAP) (Tatusova et al., 2016) from the National Center for Biotechnology and Information (NCBI) and Prokka (Seemann, 2014). Online Galaxy platform (see Footnote 1) version 21.05 was used for bioinformatic analysis.

2.4. Pangenome analysis

Pangenome analysis of *A. veronii* AhS5-24 together with 30 complete genomes of other *A. veronii* isolates retrieved from the NCBI was carried out using Roary v. 3.13.0 using general feature files 3 (.gff) file generated from Prokka v. 1.14.5. The phylogenetic tree was made using the Phandango software using Gene_presence_absence and Newick files obtained from Roary v. 3.13.0. The average nucleotide identity (ANI) of all 31 *A. veronii* genomes was computed using FastANI v1.3 using *A. veronii* FC951 (CP032839) as a reference strain. Antimicrobial resistance (AMR) genes were identified using staramr version 0.7.2 (Tran et al., 2021) and ABRicate v1.0.1 (Seemann, 2016) in the Comprehensive antimicrobial resistance database (CARD) (Alcock et al., 2020) and staramr v. 0.7.2 with the identification threshold set at 80%. Plasmidfinder v 2.0 (Ullah et al., 2020) was used

¹ <https://usegalaxy.no/>



to identify plasmids in the bacterial genomes while virulence genes were identified using virulence factors database (VFDB). Genome circular maps were created using Proksee.²

2.5. Phylogenetic analysis of antimicrobial resistance genes

Phylogenetic comparison of the *tet(E)* and *tetR* genes from strain AhS5-24 with other *A. veronii* isolates was done using the Molecular Evolutionary Genetic Analysis version 7 (MEGA-7) software (Kumar et al., 2016). The *tet(E)* and *tetR* sequences from strain AhS5-24 were retrieved after screening using ABRicate version 1.0.1 followed by comparison with *tet(E)* and *tetR* sequences from other *A. veronii* isolates retrieved from NCBI. Phylogenetic trees were produced using the Neighbor-joining and BioNJ algorithm to a pairwise matrix estimated using JTT model and expressed as number of base substitution per site (Jones et al., 1992).

3. Results

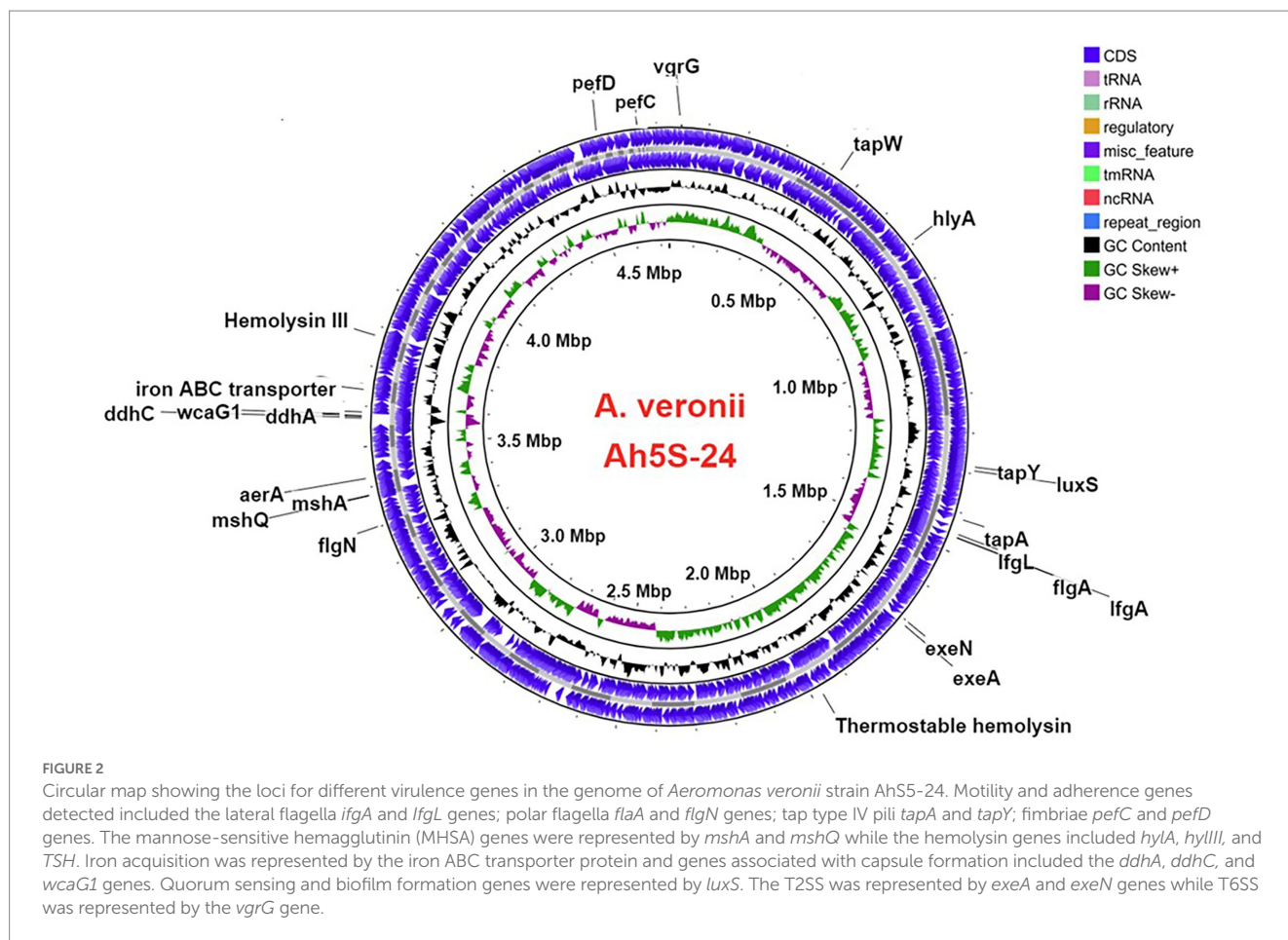
3.1. Genome organization and pangenome analysis

The draft genome of *A. veronii* AhS5-24 showed a high similarity with other *A. veronii* genomes, as shown in Figure 1. The draft genome of strain AhS5-24 had a size of 4,748,224 bp with G+C content of 58.48%. It contained 157 contigs with an N50 value of 115,408. A total

of 4,493 genes were predicted with 4,334 genes coding for proteins. The genome contained a total of 108 genes of RNA consisting of 99 tRNA and 5 rRNAs. The total number of genes detected from the 31 *A. veronii* genomes based on pangenome analysis was 20,352 of which 1,429 genes were core-, 875 softcore-, 2,241 shell-, and 15,807 cloud genes. The phylogenetic tree divided the genomes into three groups of which strain AhS5-24 was closely related to the human CP032839 (FC951) and hospital sewage CP079823 (HD6454) isolates (Figure 1). The average nucleotide identity (ANI) analysis using FastANI showed high similarity (>93%) of all 31 *A. veronii* isolates despite coming from different host species and geographic locations. The ANI of strain AhS5-24 was 96.31% similar with the *A. veronii* CF951 (CP032839) human clinical isolate and 96.20% similar with *A. veronii* HD6454 (CP079823) from hospital sewage.

The virulence genes found in *A. veronii* AhS5-24 comprised the motility and adherence genes that included the (i) lateral flagella proteins consisting of *lfgA* and *lfgL*, (ii) polar flagella that were represented by *flgA* and *flgN*, (iii) members of the tap type IV pili that included *tapA*, *tapW* and *tapY*, and (iv) fimbrial adherence determinants that included *pefC* and *pefD* genes (Figure 2). The mannose-sensitive hemagglutinin (MHSA) is encoded by the genes *mshA* and *mshQ* (Figure 2). Genes associated with capsule formation and immune evasion included *ddhA*, *ddhC*, and *wcaG1*. The hemolysin genes detected were *hlyA*, *hlyIII*, and thermostable hemolysin (*TSH*) while toxin genes consisted of aerolysin *aerA*. Genes associated with iron acquisition consisted of the Iron ABC transporter while biofilm formation and quorum sensing genes were represented by *luxS* and *MshA-Q* pilus. We detected genes belonging to the type II secretion systems (T2SS) represented by *exeA* to *exeN* (Supplementary Table S1) and *vgrG*, which is part of T6SS (Figure 2). Overall, the virulence genes detected belonged to motility, adherence, secretion systems, iron acquisition, biofilm formation, quorum sensing, and immune evasion groups.

² <https://proksee.ca/>



3.2. Phenotype characterization of antimicrobial resistance genes

Results of the disk diffusion test showed that strain Ah5S-24 was resistant to CFO30, CEP30, AMOXY30, and TET30, whereas it showed intermediate resistance against SULFA240 and TRIM5 (Table 1). However, it was susceptible to ERY15, CO150, CIPR5, GEN10, NI300, and RIF5. We found an overall correlation kappa score of 82% (Cohen's $k=0.8235$) with a specificity of 91.66% and sensitivity of 93% between the phenotypic profile based on the disk diffusion test and genotypic profile based on the genes identified using the CARD (Alcock et al., 2020).

3.3. Genotype characterization of antimicrobial resistance genes

Identification of AMR genes using the CARD (Alcock et al., 2020) showed that strain Ah5S-24 encoded multiple AMR genes that included the β -lactamase like *bla*_{FOX-7}, *bla*_{OXA-12}, and *cphA4*. Other genes detected included the colistin *crp* and *mcr-7.1* genes as well as the streptogramin A acetyl transferase *vatF* gene (Figure 3). There were no integrase and transposases located near the *bla*_{FOX-7}, *bla*_{OXA-12}, *cphA4*, *crp*, *mcr-7.1*, and *vatF* genes. The trimethoprim *dfrA3* gene was placed together with the sulfurtransferase, DUF2541 family protein, mog, DUF3135 domain-containing protein, threonine exporter

protein, and phosphoadenyl-sulfate reductase (Figure 3). The efflux pumps detected included the resistance-nodulation-cell division (RND) *mexB* and *smeD* that were placed next to each other together with the IS5 transposase (Figure 3).

Our findings show that the repressor of the tetracycline resistance element gene *tetR* was placed next to *tet(E)* together with the IS5/IS1182 transposase, helicase, integrase, tyrosine type recombinase/integrase, and the site-specific integrase all in one cassette (Figure 3). The cassette found in *A. veronii* Ah5S-24 showed a high similarity with cassettes found in *Vibrio parahaemolyticus* (MN199028.1) isolated from a fish market, *Vibrio alginolyticus* plasmid (MN865127.1) from shrimp, *Aeromonas caviae* (CP110176) from human stool, and *Aeromonas media* (CP03844.1) from a sewage bioreactor (Figure 4). They all had a similar genetic structure or transposon consisting of the IS5/IS1182 transposase followed by a gene encoding a hypothetical protein (*hp*), *Tet(E)*, *tetR*, and another hypothetical protein (*hp*), thereby forming a MGC designated as IS5/IS1182/*hp/tet(E)/tetR/hp* (Figure 4). Suffice to point out that the cassette from *Vibrio alginolyticus* (MN865127.1) was from a plasmid, while the cassettes from *A. veronii* Ah5S-24, *Vibrio parahaemolyticus* (MN199028.1), *A. media* (CP03844.1), and *Aeromonas caviae* (CP110176) were from chromosomes. This findings demonstrate that the IS5/IS1182/*hp/tet(E)/tetR/hp* cassette can be found both in chromosomes and plasmids of different bacteria species. It is noteworthy that the cassette for *Vibrio alginolyticus* plasmid (MN865127.1) had the *IShfr9* transposase, and not the IS5/IS1182 transposase, despite having a

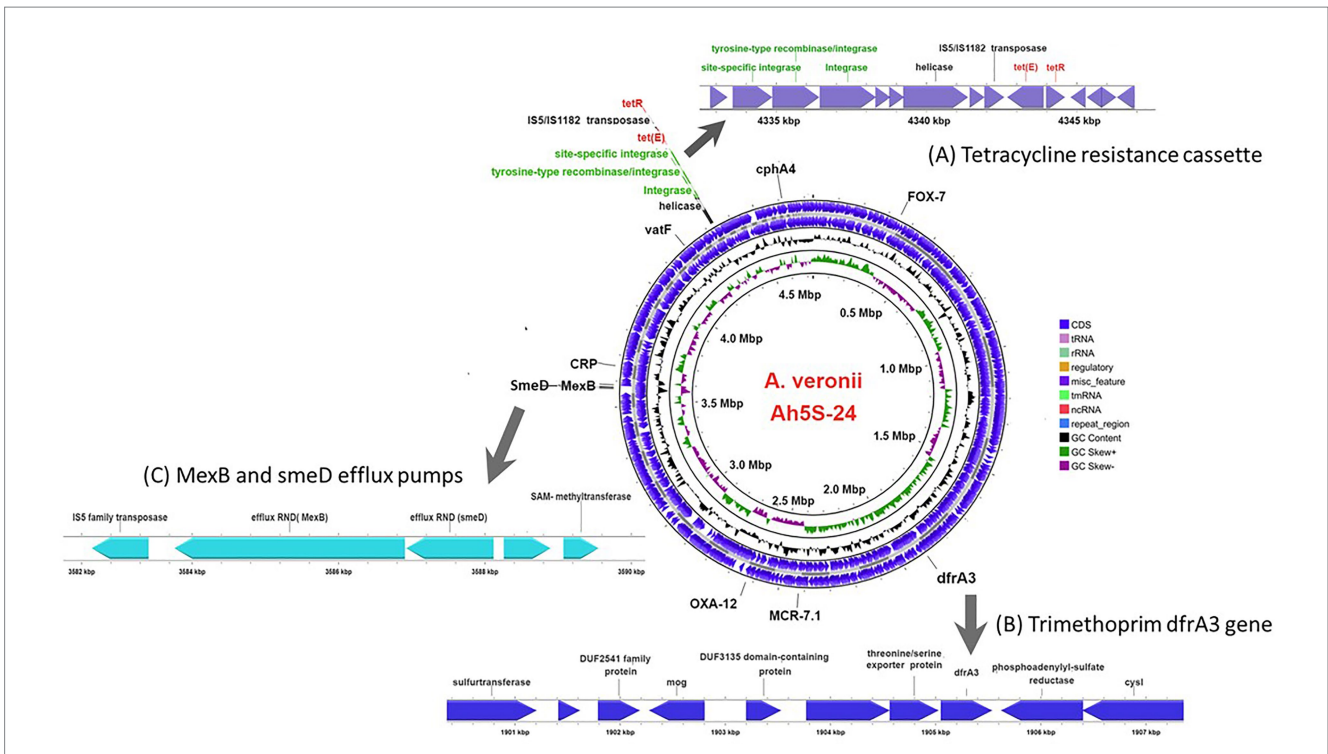


FIGURE 3 Circular genomic map of *Aeromonas veronii* AhS5-24 showing the loci for antimicrobial resistance (AMR) genes. The AMR genes detected included the β -lactam *bla*_{OXA-12} and *bla*_{FOX-7} genes together with *cphA4*, *dfrA3*, *mcr-7.1*, and *vatF* genes while the efflux pump proteins detected were *CRP*, *smeD*, and *mexB*. The extended linear map (A) shows the cassette encoding the site-specific integrase, tyrosine-type recombinase/integrase, integrase, helicase, *IS5/IS1182* transposase, *tet(E)* efflux pump protein gene and *tetR* gene designated as *IS5/IS1182/hp/tet(E)/tetR/hp*. The extended line map (B) shows the linear relationship between the trimethoprim *dfrA3* gene and other genes that includes sulfurtransferase, DUF2541 family protein, mog, DUF3135 domain-containing protein, threonine/serine exporter protein, *dfrA3* and phosphoadenyl-sulfate reductase in the genome of *A. veronii* AhS5-24. The extended map (C) shows the linear relationship between the *smeD* and *mexB* efflux pumps in the genome of *A. veronii* AhS5-24.

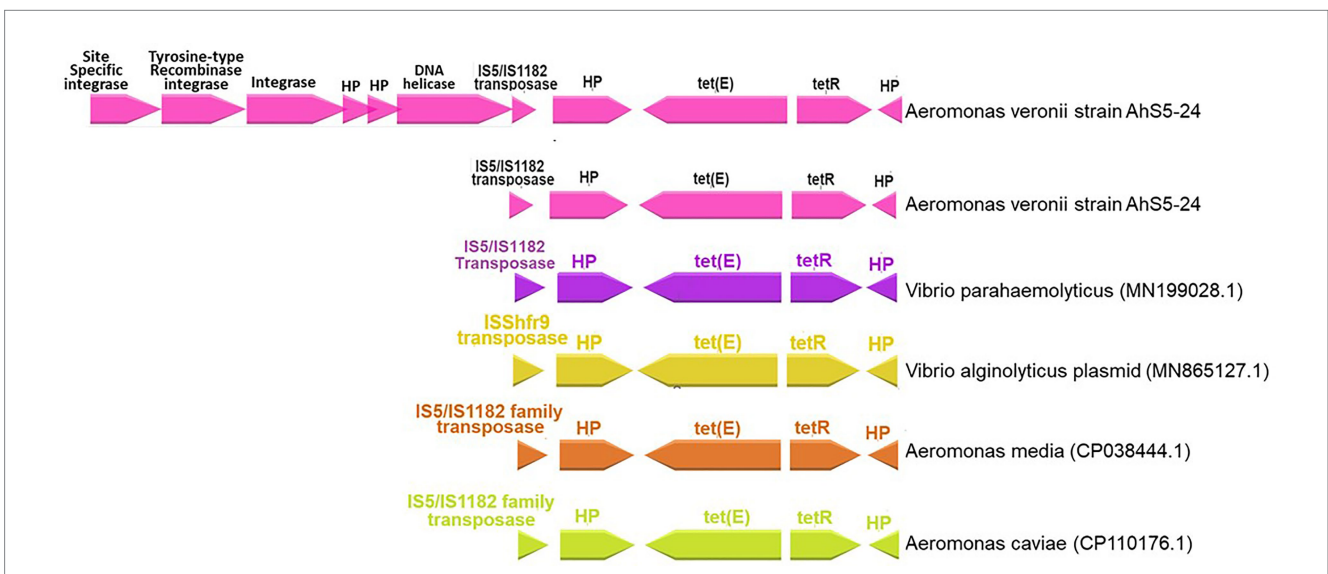


FIGURE 4 Comparison of the *IS5/IS1182/hp/tet(E)/tetR/hp* gene cassettes for *Aeromonas veronii* strain AhS5-24 from pond sediments, *Vibrio parahaemolyticus* (MN199028) isolated from retail fish from a market, *Vibrio alginolyticus* plasmid (MN865127.1) from shrimp, *Aeromonas media* (CP038444.1) from sewage bioreactor and *Aeromonas caviae* (CP110176.1) from human stool. Note that all isolates had the hypothetical proteins (*hp*), *tetR*, *tet(E)*, and *IS5/IS1182* transposase forming a gene cassette designated as *IS5/IS1182/hp/tet(E)/tetR/hp*. The uppermost linear map shows *Aeromonas veronii* strain AhS5-24 having the *IS5/IS1182/hp/tet(E)/tetR/hp* cassette linked to the DNA helicase, two hypothetical proteins, integrase, tyrosine-type recombinase/integrase and site-specific integrase.

similar *hp/tet(E)/tetR/hp* component with other bacteria species used in the comparison (Figure 4).

Phylogenetic analysis showed that the *tet(E)* gene from *A. veronii* AhS5-24 had a 100% similarity with *tet(E)* genes from different bacteria species that included *Escherichia coli* (AIL23572.1, CAC20135.1, and WP_20194468.1), *Aeromonas caviae* (BBR12376.1, WP_244056220.1, and WP_201964468.1), *Yersinia ruckeri* (APO36645.1, APO36648.1, and APO36646.1), *Klebsiella pneumoniae* (EIW8806435.1), *Aeromonas* spp. (QEV84027.1 and WP_017780889.1), and *Enterobacter cloacae* (ASF90526.1) (Figure 5). Phylogenetic analysis also showed that the *tetR* gene from *A. veronii* AhS5-24 had a 100% similarity with *tetR* genes from different bacteria species that included *E. coli* (AAA98409.1), Gammaproteobacteria (W_P011899269.1 and WP_017411289.1), *Aeromonas salmonicida* (QJR83010.1), *Aliivibrio salmonicida* (CAC81917.1), and *A. caviae* (WP_223946105.1 and WP_223945860.1) (Figure 6). Altogether, our findings show that *tet(E)* and *tetR* genes were highly similar with those found in different bacteria species.

4. Discussion

In this study, we have shown that the bacteria isolated from pond sediments in the South East USA previously classified as *A. hydrophila* using the API 20E system in DePaola et al. (1988) was characterized as *A. veronii* AhS5-24 using WGS and pangenome analysis. We have also shown that the T⁺ and TO⁺ detected by DePaola et al. (1988) could be linked to the *tetR* and *tet(E)* genes found in the same isolate

designated as strain AhS5-24 in this study. In addition, strain AhS5-24 encoded several virulence and AMR genes of which tetracycline resistance genes were placed in the same genetic structure with an integrase, transposase and recombinase and can be defined as a transposon. These findings demonstrate that *Aeromonas* spp. isolated from aquatic environments have the potential to transmit AMR genes to other bacteria using transposons carrying different AMR genes.

Pangenome analysis showed a high similarity of strain AhS5-24 with other *A. veronii* strains linked to different diseases in aquatic organism and humans. For example, strains CP032839.1 and CP046407.1 shown to be closely related with *A. veronii* AhS5-24 were from human clinical cases (Ragupathi et al., 2020) and diseased rohu (*Labeo rohita*) (Tyagi et al., 2022), respectively. Besides, *A. veronii* AhS5-24 had several virulence genes linked to adherence, biofilm formation, quorum sensing, immune evasion, toxins and intracellular secretion systems (TSS) found in other pathogenic *A. veronii* strains (Arechaga and Cascales, 2022). Detection of the *Msh* pili, tap type IV pili, lateral and polar flagellar genes associated with intestinal adherence, colonization, and biofilm formation (Ro, 2006; Hadi et al., 2012) is suggestive that these genes play a vital role in the pathogenicity of strain AhS5-24. The presence of the *LuxS* and *mshQ* genes is suggestive that strain AhS5-24 has the capacity for biofilm formation and quorum sensing as seen in other bacteria species (Enos-Berlage et al., 2005; Trappetti et al., 2011) while presence of the iron ABC transporter is suggestive that strain AhS5-24 uses this protein in acquiring iron from infected hosts (Delepelaire, 2019). Detection of *ddhA*, *ddhC*, and *wcaG1* associated with capsule formation (Mobine, 2008) is suggestive strain AhS5-24 has the ability to form a capsule as a defense mechanism against host immune responses while presence of *hlyA*, *hlyIII*, and *TSH* together with aerolysin *aerA* is suggestive that these genes might be linked to pore formation and intracellular release of enterotoxins by strain AhS5-24 as seen in other bacteria species (Honda et al., 1992; Baida and Kuzmin, 1996; Abrami et al., 2000; Maté et al., 2014). Besides, several scientists (Wong et al., 1998; Heuzenroeder et al., 1999; Wu et al., 2007; Castilho et al., 2009) have shown that a combination of *hlyA*(+) and *aerA*(+) is a major virulence determinant in *Aeromonas* spp. Castilho et al. (2009) found a high prevalence of hemolytic and cytotoxic *Aeromonas* spp. that had both *hlyA*(+) and *aerA*(+) from human clinical, food, and environmental samples in Brazil while Wu et al. (2007) showed that the absence of *hlyA*(-) and *aerA*(-) in *Aeromonas* spp. from fish and human samples in Taiwan was associated with low virulence. Heuzenroeder et al. (1999) and Wong et al. (1998) showed that deletion or attenuation of the *hlyA*(+) and *aerA*(+) double mutant significantly reduced the pathogenicity of *A. hydrophila* in mice. They also showed that cytotoxicity to buffalo green monkey kidney cells and hemolysis on horse blood agar was only eliminated in the double and not in the single mutants of *A. veronii*, *A. hydrophila*, and *A. caviae*. Our findings show that *A. veronii* AhS5-24 had both *hlyA*(+) and *aerA*(+), indicating that it shares the two key virulence determinants with other pathogenic *Aeromonas* spp.

Several studies have shown that *Aeromonas* spp. intrinsically carry various *bla*_{OXA} genes in their genomes that include the *bla*_{OXA-12} gene (Dubey et al., 2022a,b) previously detected in *A. media*, *A. jandaei*, *A. sobria*, *A. dhakensis*, and *A. hydrophila* (Rasmussen et al., 1994; Alksne and Rasmussen, 1997; Hilt et al., 2020; Huang et al., 2020; Dubey et al., 2022a) being in line with its presence in strain AhS5-24 while *bla*_{FOX-7} previously reported in *A. media* and

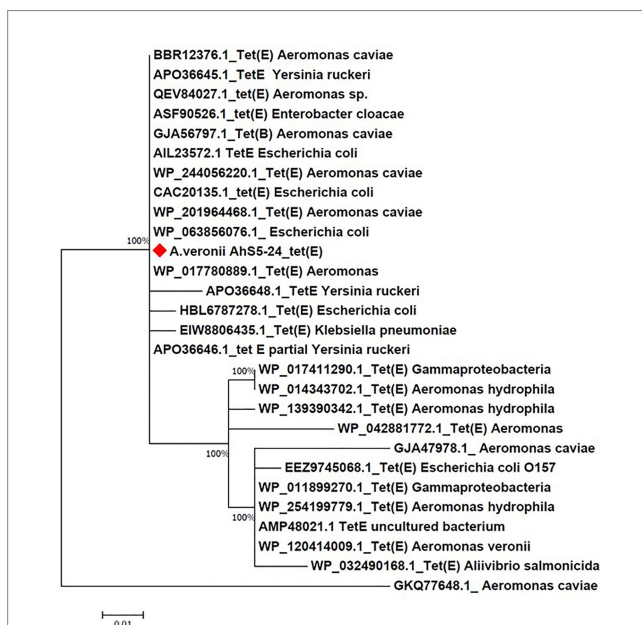
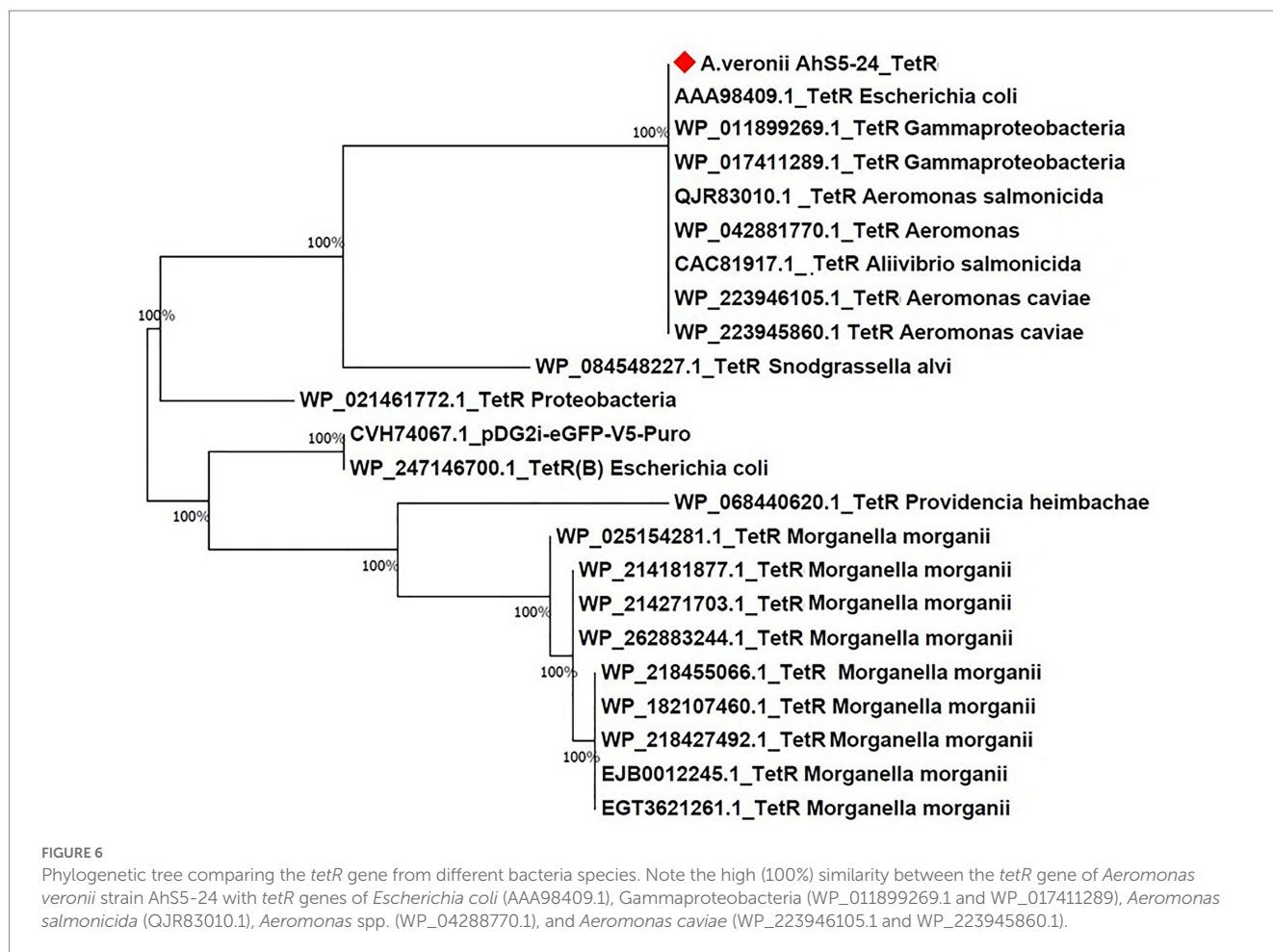


FIGURE 5

Phylogenetic tree comparing the *tet(E)* gene from different bacteria species. Note that the *tet(E)* *Aeromonas veronii* strain AhS5-24 had 100% similarity with *tet(E)* genes from other bacteria species that include *Aeromonas caviae* (BBR12376.1, GJA56797.1, and WP/244056220.1 and WP_20964468.1), *Yersinia ruckeri* (Apo36645.1, APO36646.1, and APO36648.1), *Enterobacter cloacae* (ASF90526.1), *Escherichia coli* (AIL23572.1, CAC20135.1, WP_063856076.1 and HBL6787278.1), and *Klebsiella pneumoniae* (EIW8806435.1).



A. allosaccharophila was also found in strain AhS5-24 (Ebmeyer et al., 2019). Other AMR genes detected included the *cphA4* gene known to be intrinsically encoded in various *Aeromonas* spp. (Dubey et al., 2022a,b) as well as the colistin-resistance *mcr-7.1* gene also reported from different *Aeromonas* spp. (Dubey et al., 2022a,b). Despite so, the *bla*_{OXA-12}, *bla*_{FOX-7}, and *mcr-7.1* genes detected in strain AhS5-24 were not associated with integrases, recombinases or transposases suggesting that these genes could not be easily transferred or acquired from other bacteria species. Similarly, although trimethoprim and sulfonamide are among the most widely used antibiotics linked to AMR in aquaculture (Gao et al., 2012; Muziasari et al., 2014; Phu et al., 2015), the trimethoprim resistance gene *dfrA3* detected in the present study was not linked to integrases and transposases. Thus, the sulfonamide and trimethoprim resistance observed in the disc diffusion test could have been mediated by the *MexB* and *smeD* pumps that have been associated with resistance of several drugs that include sulfonamide, fluoroquinolone, cephalosporins, carbapenem, and trimethoprim. The trimethoprim and sulfonamide resistance observed on the disc diffusion test was intermediate (I) unlike the tetracycline resistance (R), which was highly expressed suggesting that the impact of trimethoprim and sulfonamide in conferring resistance was not as high as tetracycline in strain AhS5-24. Despite so, we found a high correlation of kappa score of 82% (Cohen's $k=0.8235$) with a specificity of 91.66% and sensitivity of 93% between the phenotype characterization based on the disc diffusion test and genotypic

characterization based on the CARD (Alcock et al., 2020), indicating that the two diagnostic tests were highly in agreement.

Tetracycline is one of the most widely used antibiotics in aquaculture, which has been linked to resistance in farmed aquatic organisms (Seyfried et al., 2010; Tamminen et al., 2011). Thus, it is likely that selection of the Tet E operon in strain AhS5-24 occurred in pond sediments used for aquaculture where tetracycline was used for the treatment of fish diseases. Although the absence of plasmids is suggestive that strain AhS5-24 had lesser chances of transferring AMR genes to other bacteria, detection of the Tet E operon together with the integrase and IS5/IS1182 transposase suggests that *tetR* and *tet(E)* genes could be transferred or acquired from other bacteria using the IS5/IS1182/*hp/tet(E)/tetR/hp* cassette encoded in strain AhS5-24. Besides, DePaola et al. (1988) used the same isolate to transfer the OT^r and T^r resistance to *E. coli* suggesting that the IS5/IS1182/*hp/tet(E)/tetR/hp* cassette found in strain AhS5-24 could have been responsible for transferring the tetracycline resistance to *E. coli*. Also, detection of the same cassette in *V. parahaemolyticus*, *V. alginolyticus* (MN199028.1), *A. media* (CP038444.1), *A. caviae* (CP110176.1), and *A. caviae* (CP038445.1) emanating from fish market, shrimp, sewage bioreactor and human stool is suggesting that the IS5/IS1182/*hp/tet(E)/tetR/hp* transposon could be involved in interspecies transmission of the *tet(E)* and *tetR* genes in different bacteria species. These findings also suggest that the IS5/IS1182/*hp/tet(E)/tetR/hp* transposon might be in existence in different bacteria species found in different aquatic environments hosted by species that include

shrimps, fish, animals and humans. Its presence in *V. parahaemolyticus* (MN199028.1) isolated from retail fish at markets and *V. alginolyticus* (MN865127.1) from shrimps is indicative that it could play a vital role in transmission of *tet(E)* and *tetR* genes to humans through food.

The similarity of the IS5/IS1182/hp/tet(E)/tetR/hp cassette found in the chromosomes of strain *A. veronii* AhS5-24, *V. parahaemolyticus* (MN199028.1) and *A. media* (CP038445.1), with the transposon found in the plasmid of *V. alginolyticus* (MN865127.1) is suggesting that the IS5/IS1182/hp/tet(E)/tetR/hp transposon can be transferable between chromosomes and plasmids of different bacteria species. Also, the high similarity of the *tet(E)* and *tetR* genes detected in strain AhS5-24 with those found in *E. coli*, *K. pneumoniae*, and *Aeromonas* spp. shown in the phylogenetic analysis consolidates our view that *tet(E)* and *tetR* genes could be transmissible between different bacteria species using MGCs. Thus, it is likely that the transfer of the OT^r and T^r resistance to *E. coli* observed by DePaola et al. (1988) was not plasmid mediated but it was done by the IS5/IS1182/hp/tet(E)/tetR/hp transposon found in strain AhS5-24. Therefore, our findings indicate that the resistance acquired by different *Aeromonas* spp. in aquatic environments could play a vital role in the transfer of AMR genes to foodborne, environmental, nosocomial and other bacteria species using MGCs. However, future studies should seek to demonstrate the transfer of *tet(E)* and *tetR* genes using the IS5/IS1182/hp/tet(E)/tetR/hp cassette to other bacteria spp. including nosocomial, foodborne and environmental bacteria.

5. Conclusion

In this study, we have shown that *A. veronii* AhS5-24 is a multidrug-resistant bacterium encoding several AMR and virulence genes. It encoded a tetracycline resistance operon Tet E placed in a transposon designated as IS5/IS1182/hp/tet(E)/tetR/hp found in different bacteria species inhabiting different aquatic environments and infecting different host species suggesting that the Tet E operon could be transferred to other bacteria. Overall, this study shows that MGCs encoding AMR genes found in bacteria inhabiting aquatic environments could play a vital role in the spread of AMR genes to other bacteria infecting animals and humans.

Data availability statement

The *Aeromonas veronii* whole genome shotgun (WGS) project has the project accession JAJVCX000000000. This version of the project (01) has the accession number JAJVCX010000000 and consists of sequences JAJVCX010000001-JAJVCX010000157.

References

- Abrami, L., Fivaz, M., and Van Der Goot, F. G. (2000). Adventures of a pore-forming toxin at the target cell surface. *Trends Microbiol.* 8, 168–172. doi: 10.1016/S0966-842X(00)01722-4
- Alcock, B. P., Raphenya, A. R., Lau, T. T., Tsang, K. K., Bouchar, M., Edalatmand, A., et al. (2020). Antibiotic resistance surveillance with the comprehensive antibiotic resistance database. *Nucleic Acids Res.* 48, D517–D525. doi: 10.1093/nar/gkz935
- Alksne, L. E., and Rasmussen, B. A. (1997). Expression of the AsbA1, OXA-12, and AsbM1 beta-lactamases in *Aeromonas jandaei* AER 14 is coordinated by a two-component regulon. *J. Bacteriol.* 179, 2006–2013. doi: 10.1128/jb.179.6.2006-2013.1997
- Arechaga, I., and Cascales, E. (2022). Editorial: bacterial secretion systems, volume II. *Front. Microbiol.* 13:13. doi: 10.3389/fmicb.2022.917591
- Baida, G. E., and Kuzmin, N. P. (1996). Mechanism of action of hemolysin III from *Bacillus cereus*. *Biochim Biophys Acta* 1284, 122–124. doi: 10.1016/S0005-2736(96)00168-X
- Bankevich, A., Nurk, S., Antipov, D., Gurevich, A. A., Dvorkin, M., Kulikov, A. S., et al. (2012). SPAdes: a new genome assembly algorithm and its applications to single-cell sequencing. *J. Comput. Biol.* 19, 455–477. doi: 10.1089/cmb.2012.0021
- Becker, L., Steglich, M., Fuchs, S., Werner, G., and Nübel, U. (2016). Comparison of six commercial kits to extract bacterial chromosome and plasmid DNA for MiSeq sequencing. *Sci. Rep.* 6:28063, 1–5. doi: 10.1038/srep28063
- Bioinformatics B. (2011). *FastQC: A Quality Control Tool for High Throughput Sequence Data*. Cambridge: Babraham Institute.

Author contributions

SD, HS, and HM: conceptualization, methodology, mobilizing resources, supervision, data curation, and bioinformatics analysis. SD, EA-W, BP, AD, HS, and HM: manuscript preparation, editing, and submission. All authors contributed to the article and approved the submitted version.

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Conflict of interest

AD was employed by Angelo DePaola Consulting LLC.

The remaining authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Supplementary material

The Supplementary material for this article can be found online at: <https://www.frontiersin.org/articles/10.3389/fmicb.2023.1112941/full#supplementary-material>

- Bolger, A. M., Lohse, M., and Usadel, B. (2014). Trimmomatic: a flexible trimmer for Illumina sequence data. *Bioinformatics* 30, 2114–2120. doi: 10.1093/bioinformatics/btu170
- Brown, H. J., Stokes, H., and Hall, R. M. (1996). The integrons In0, In2, and In5 are defective transposon derivatives. *J. Bacteriol.* 178, 4429–4437. doi: 10.1128/jb.178.15.4429-4437.1996
- Castilho, M. C., Castro, T. L., Araújo, V. S., Trajano, R. S., Santos, P. A., Pimenta, P., et al. (2009). High frequency of hemolytic and cytotoxic activity in *Aeromonas* spp. isolated from clinical, food and environmental in Rio de Janeiro, Brazil. *Antonie Van Leeuwenhoek* 96, 53–61. doi: 10.1007/s10482-009-9335-6
- Cockerill, F. R., Wikler, M., Bush, K., Dudley, M., Eliopoulos, G., and Hardy, D. (2012). Clinical and laboratory standards institute. Performance standards for antimicrobial susceptibility testing: twenty-second informational supplement. CLSI, Pennsylvania USA.
- Coil, D., Jospin, G., and Darling, A. E. (2015). A5-miseq: an updated pipeline to assemble microbial genomes from illumina MiSeq data. *Bioinformatics* 31, 587–589. doi: 10.1093/bioinformatics/btu661
- Collis, C. M., and Hall, R. M. (1992). Gene cassettes from the insert region of integrons are excised as covalently closed circles. *Mol. Microbiol.* 6, 2875–2885. doi: 10.1111/j.1365-2958.1992.tb01467.x
- Craig, N. (1996). Transposon Tn7. *Curr Top Microbiol Immunol* 204, 27–48. doi: 10.1007/978-3-642-79795-8_2
- Deleplaire, P. (2019). Bacterial ABC transporters of iron containing compounds. *Res. Microbiol.* 170, 345–357. doi: 10.1016/j.resmic.2019.10.008
- DePaola, A., Flynn, P., McPhearson, R. M., and Levy, S. (1988). Phenotypic and genotypic characterization of tetracycline- and oxytetracycline-resistant *Aeromonas hydrophila* from cultured channel catfish (*Ictalurus punctatus*) and their environments. *Appl. Environ. Microbiol.* 54, 1861–1863. doi: 10.1128/aem.54.7.1861-1863.1988
- Dubey, S., Ager-Wiick, E., Peng, B., Evensen, O., Sorum, R. H., and Munang'andu, H. M. (2022a). Characterization and comparative analysis of virulence and antimicrobial resistance genes of *Aeromonas media* strain SD/21-15 from marine sediments with other *Aeromonas* spp. *Front. Microbiol.* 4217. doi: 10.3389/fmicb.2022.1022639
- Dubey, S., Anger-Wick, E., Kumar, J., Karunasagar, I., Karunasagar, I., Peng, B., et al. (2022b). *Aeromonas* species isolated from aquatic organisms, insects, chicken, and humans in India show similar antimicrobial resistance profiles. *Front. Microbiol.* 4364. doi: 10.3389/fmicb.2022.1008870
- Ebmeyer, S., Kristiansson, E., and Larsson, D. J. (2019). The mobile FOX AmpC beta-lactamases originated in *Aeromonas allosaccharophila*. *Int. J. Antimicrob. Agents* 54, 798–802. doi: 10.1016/j.ijantimicag.2019.09.017
- Enos-Berlage, J. L., Guvener, Z. T., Keenan, C. E., and McCarter, L. L. (2005). Genetic determinants of biofilm development of opaque and translucent *Vibrio parahaemolyticus*. *Mol. Microbiol.* 55, 1160–1182. doi: 10.1111/j.1365-2958.2004.04453.x
- Gao, P., Mao, D., Luo, Y., Wang, L., Xu, B., and Xu, L. (2012). Occurrence of sulfonamide and tetracycline-resistant bacteria and resistance genes in aquaculture environment. *Water Res.* 46, 2355–2364. doi: 10.1016/j.watres.2012.02.004
- González-Serrano, C., Santos, J., García-López, M., and Otero, A. (2002). Virulence markers in *Aeromonas hydrophila* and *Aeromonas veronii* biovar *sobria* isolates from freshwater fish and from a diarrhoea case. *J. Appl. Microbiol.* 93, 414–419. doi: 10.1046/j.1365-2672.2002.01705.x
- Guan, G., He, X., Chen, J., Bin, L., and Tang, X. (2020). Identifying the mechanisms underlying the protective effect of tetramethylpyrazine against cisplatin-induced in vitro ototoxicity in HEI-OC1 auditory cells using gene expression profiling. *Mol. Med. Rep.* 22, 5053–5068. doi: 10.3892/mmr.2020.11631
- Hadi, N., Yang, Q., Barnett, T. C., Tabei, S. M. B., Kirov, S. M., and Shaw, J. G. (2012). Bundle-forming pilus locus of *Aeromonas veronii* bv *sobria*. *Infect. Immun.* 80, 1351–1360. doi: 10.1128/IAI.06304-11
- Heuzenroeder, M. W., Wong, C. Y., and Flower, R. L. (1999). Distribution of two hemolytic toxin genes in clinical and environmental isolates of *Aeromonas* spp.: correlation with virulence in a suckling mouse model. *FEMS Microbiol. Lett.* 174, 131–136. doi: 10.1111/j.1574-6968.1999.tb13559.x
- Hickman-Brenner, F., Mac Donald, K., Steigerwalt, A., Fanning, G., Brenner, D. J., and Farmer, J. 3rd. (1987). *Aeromonas veronii*, a new ornithine decarboxylase-positive species that may cause diarrhea. *J. Clin. Microbiol.* 25, 900–906. doi: 10.1128/jcm.25.5.900-906.1987
- Hilt, E. E., Fitzwater, S. P., Ward, K., de St Maurice, A., Chandrasekaran, S., Garner, O. B., et al. (2020). Carbapenem resistant *Aeromonas hydrophila* carrying Bla(cphA7) isolated from two solid organ transplant patients. *Front. Cell. Infect. Microbiol.* 10:563482. doi: 10.3389/fcimb.2020.563482
- Hoai, T. D., Trang, T. T., Van Tuyen, N., Giang, N. T. H., and Van Van, K. (2019). *Aeromonas veronii* caused disease and mortality in channel catfish in Vietnam. *Aquaculture* 513:734425. doi: 10.1016/j.aquaculture.2019.734425
- Honda, T., Ni, Y., Miwatani, T., Adachi, T., and Kim, J. (1992). The thermostable direct hemolysin of *Vibrio parahaemolyticus* is a pore-forming toxin. *Can. J. Microbiol.* 38, 1175–1180. doi: 10.1139/m92-192
- Huang, M., Chen, H., Li, C., Liu, Y., Gan, C., MAE-G, E.-S. A., et al. (2020). Rapid fulminant progression and mortality secondary to *Aeromonas dhakensis* septicemia with hepatitis B virus infection following the ingestion of snakehead fish in mainland China: a case report. *Foodborne Pathog. Dis.* 17, 743–749. doi: 10.1089/fpd.2019.2780
- Jones, D. T., Taylor, W. R., and Thornton, J. M. (1992). The rapid generation of mutation data matrices from protein sequences. *Bioinformatics* 8, 275–282. doi: 10.1093/bioinformatics/8.3.275
- Joseph, N. M., Sistla, S., Dutta, T. K., Badhe, A. S., Rasitha, D., and Parija, S. C. (2011). Reliability of Kirby-Bauer disk diffusion method for detecting meropenem resistance among non-fermenting gram-negative bacilli. *Indian J. Pathol. Microbiol.* 54, 556–560. doi: 10.4103/0377-4929.85092
- Kahlmeter, G., Brown, D. F., Goldstein, F. W., MacGowan, A. P., Mouton, J. W., Odenholt, I., et al. (2006). European Committee on Antimicrobial Susceptibility Testing (EUCAST) Technical Notes on antimicrobial susceptibility testing. *Clin Microbiol Infect.* 12, 501–503. doi: 10.1111/j.1469-0691.2006.01454.x
- Kumar, S., Stecher, G., and Tamura, K. (2016). MEGA7: molecular evolutionary genetics analysis version 7.0 for bigger datasets. *Mol. Biol. Evol.* 33, 1870–1874. doi: 10.1093/molbev/msw054
- Liebert, C. A., Hall, R. M., and Summers, A. O. (1999). Transposon Tn 21, flagship of the floating genome. *Microbiol. Mol. Biol. Rev.* 63, 507–522. doi: 10.1128/MMBR.63.3.507-522.1999
- Maté, S. M., Vázquez, R. F., Herlax, V. S., Millone, M. A. D., Fanani, M. L., Maggio, B., et al. (2014). Boundary region between coexisting lipid phases as initial binding sites for *Escherichia coli* alpha-hemolysin: a real-time study. *Biochim Biophys Acta* 1838, 1832–1841. doi: 10.1016/j.bbame.2014.02.022
- Meyer, J. F., Nies, B. A., and Wiedemann, B. (1983). Amikacin resistance mediated by multiresistance transposon Tn2424. *J. Bacteriol.* 155, 755–760. doi: 10.1128/jb.155.2.755-760.1983
- Minakhina, S., Kholodii, G., Mindlin, S., Yuriyeva, O., and Nikiforov, V. (1999). Tn5053 family transposons are res site hunters sensing plasmid res sites occupied by cognate resolvases. *Mol. Microbiol.* 33, 1059–1068. doi: 10.1046/j.1365-2958.1999.01548.x
- Mobine, H. R. (2008). *Pheochromocytoma-Induced Cardiomyopathy: A Model of Synergistic Effects of Multifactorial Tumor Secretions* Massachusetts Institute of Technology. Doctoral dissertation, MIT, USA.
- Muziasari, W. I., Managaki, S., Pärnänen, K., Karkman, A., Lyra, C., Tamminen, M., et al. (2014). Sulphonamide and trimethoprim resistance genes persist in sediments at Baltic Sea aquaculture farms but are not detected in the surrounding environment. *PLoS One* 9:e92702. doi: 10.1371/journal.pone.0092702
- Phu, T. M., Scippo, M.-L., Phuong, N. T., Tien, C. T. K., Son, C. H., and Dalsgaard, A. (2015). Withdrawal time for sulfamethoxazole and trimethoprim following treatment of striped catfish (*Pangasianodon hypophthalmus*) and hybrid red tilapia (*Oreochromis mossambicus* × *Oreochromis niloticus*). *Aquaculture* 437, 256–262. doi: 10.1016/j.aquaculture.2014.12.009
- Ragupathi, N. K. D., Sethuvel, D. P. M., Anandan, S., Murugan, D., Asokan, K., Mohan, R. G. N., et al. (2020). First hybrid complete genome of *Aeromonas veronii* reveals chromosome-mediated novel structural variant mcr-3.30 from a human clinical sample. *Access Microbiol.* 2:acmi000103. doi: 10.1099/acmi.0.000103
- Rasmussen, B. A., Keeney, D., Yang, Y., and Bush, K. (1994). Cloning and expression of a cloxacillin-hydrolyzing enzyme and a cephalosporinase from *Aeromonas sobria* AER 14M in *Escherichia coli*: requirement for an *E. coli* chromosomal mutation for efficient expression of the class D enzyme. *Antimicrob. Agents Chemother.* 38, 2078–2085. doi: 10.1128/AAC.38.9.2078
- Recchia, G. D., and Hall, R. M. (1995). Gene cassettes: a new class of mobile element. *Microbiology* 141, 3015–3027. doi: 10.1099/13500872-141-12-3015
- Ro, C. (2006). Altarriba M, Vilches S, Horsburgh G, Shaw JG, Tomás JM, merino S: analysis of the lateral flagellar gene system of *Aeromonas hydrophila* AH-3. *J. Bacteriol.* 188, 852–862. doi: 10.1128/JB.188.3.852-862.2006
- Roberts, M., Enoch, D., Harris, K., and Karas, J. (2006). *Aeromonas veronii* biovar *sobria* bacteraemia with septic arthritis confirmed by 16S rDNA PCR in an immunocompetent adult. *J. Med. Microbiol.* 55, 241–243. doi: 10.1099/jmm.0.46295-0
- Seemann, T. (2014). Prokka: rapid prokaryotic genome annotation. *Bioinformatics* 30, 2068–2069. doi: 10.1093/bioinformatics/btu153
- Seemann, T. (2016). ABRicate: Mass screening of contigs for antibiotic resistance genes. GitHub. Available at: <https://github.com/tseemann/abricate>
- Seyfried, E. E., Newton, R. J., Rubert, K. F., Pedersen, J. A., and McMahon, K. D. (2010). Occurrence of tetracycline resistance genes in aquaculture facilities with varying use of oxytetracycline. *Microb. Ecol.* 59, 799–807. doi: 10.1007/s00248-009-9624-7
- Smyrli, M., Prapas, A., Rigos, G., Kokkari, C., Pavlidis, M., and Katharios, P. (2017). *Aeromonas veronii* infection associated with high morbidity and mortality in farmed European seabass *Dicentrarchus labrax* in the Aegean Sea, Greece. *Fish Pathol.* 52, 68–81. doi: 10.3147/jsfp.52.68
- Smyrli, M., Triga, A., Dourala, N., Varvarigos, P., Pavlidis, M., Quoc, V. H., et al. (2019). Comparative study on a novel pathogen of European seabass. Diversity of *Aeromonas veronii* in the Aegean Sea. *Microorganisms* 7:504. doi: 10.3390/microorganisms7110504
- Tamminen, M., Karkman, A., Lohmus, A., Muziasari, W. I., Takasu, H., Wada, S., et al. (2011). Tetracycline resistance genes persist at aquaculture farms in the absence of selection pressure. *Environ. Sci. Technol.* 45, 386–391. doi: 10.1021/es102725n

- Tarumoto, N., Sakai, J., Sujino, K., Yamaguchi, T., Ohta, M., Yamagishi, J., et al. (2017). Use of the Oxford Nanopore MinION sequencer for MLST genotyping of vancomycin-resistant enterococci. *J. Hosp. Infect.* 96, 296–298. doi: 10.1016/j.jhin.2017.02.020
- Tatusova, T., DiCuccio, M., Badretin, A., Chetvernin, V., Nawrocki, E. P., Zaslavsky, L., et al. (2016). NCBI prokaryotic genome annotation pipeline. *Nucleic Acids Res.* 44, 6614–6624. doi: 10.1093/nar/gkw569
- Tekedar, H. C., Arick, M. A., Hsu, C.-Y., Thrash, A., Blom, J., Lawrence, M. L., et al. (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. doi: 10.3389/fcimb.2020.00348
- Tran, T. T., Scott, A., Tien, Y.-C., Murray, R., Boerlin, P., Pearl, D. L., et al. (2021). On-farm anaerobic digestion of dairy manure reduces the abundance of antibiotic resistance-associated gene targets, and the potential for plasmid transfer. *Appl. Environ. Microbiol.* 87, 02980–02920. doi: 10.1128/AEM.02980-20
- Trappetti, C., Potter, A. J., Paton, A. W., Oggioni, M. R., and Paton, J. C. (2011). LuxS mediates iron-dependent biofilm formation, competence, and fratricide in *Streptococcus pneumoniae*. *Infect. Immun.* 79, 4550–4558. doi: 10.1128/IAI.05644-11
- Tyagi, A., Sharma, C., Srivastava, A., Kumar, B. N., Pathak, D., and Rai, S. (2022). Isolation, characterization and complete genome sequencing of fish pathogenic *Aeromonas veronii* from diseased *Labeo rohita*. *Aquaculture* 553:738085:738085. doi: 10.1016/j.aquaculture.2022.738085
- Ullah, S. R., Majid, M., and Andleeb, S. (2020). Draft genome sequence of an extensively drug-resistant neonatal *Klebsiella pneumoniae* isolate harbouring multiple plasmids contributing to antibiotic resistance. *J. Glob. Antimicrob. Resist.* 23, 100–101. doi: 10.1016/j.jgar.2020.08.008
- Wang, H., Lin-Zhao, Z., Jie-An, D., Lin-Wang, J., Tong-Yang, B., Huan-Kang, Y., et al. (2022). The lip gene contributes to the virulence of *Aeromonas veronii* strain TH0426. *Microb. Pathog.* 167:105566. doi: 10.1016/j.micpath.2022.105566
- Ward, J., and Grinsted, J. (1982). Physical and genetic analysis of the Inc-W group plasmids R388, Sa, and R7K. *Plasmid* 7, 239–250. doi: 10.1016/0147-619X(82)90005-1
- White, P. A., McIver, C. J., and Rawlinson, W. D. (2001). Integrons and gene cassettes in the Enterobacteriaceae. *Antimicrob. Agents Chemother.* 45, 2658–2661. doi: 10.1128/AAC.45.9.2658-2661.2001
- Willyard, C. (2017). Drug-resistant bacteria ranked. *Nature* 543:15. doi: 10.1038/nature.2017.21550
- Wong, C. Y., Heuzenroeder, M. W., and Flower, R. L. (1998). Inactivation of two haemolytic toxin genes in *Aeromonas hydrophila* attenuates virulence in a suckling mouse model. *Microbiology* 144, 291–298. doi: 10.1099/00221287-144-2-291
- Wu, C.-J., Wu, J.-J., Yan, J.-J., Lee, H.-C., Lee, N.-Y., Chang, C.-M., et al. (2007). Clinical significance and distribution of putative virulence markers of 116 consecutive clinical *Aeromonas* isolates in southern Taiwan. *J. Infect.* 54, 151–158. doi: 10.1016/j.jinf.2006.04.002