

Research Article

AN ANTIBIOTIC PHENOTYPE AND GENOTYPE ASSESSMENT OF *Aeromonas* spp. ISOLATES FROM STRIPED CATFISH WITH MOTILE *Aeromonas septicemia* IN VIETNAM

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ABSTRACT

Motile *Aeromonas* Septicemia (MAS), caused by *Aeromonas hydrophila* and *Aeromonas dhakensis*, is a major disease affecting striped catfish (*Pangasianodon hypophthalmus*) aquaculture in the Mekong Delta, Vietnam. The overuse of antibiotics to treat MAS disease in catfish farming has contributed to the emergence of antimicrobial resistance (AMR) in these bacterial species. This study aims to provide an insight into antibiotic susceptibility in *Aeromonas* spp. associated with MAS in striped catfish. Forty-four isolates were analyzed through disk diffusion susceptibility testing and genomic analysis for antibiotic gene, plasmid and integron detection. A phylogenetic tree was also constructed to examine resistance patterns and their associations with epidemiological metadata. Results showed that *A. hydrophila*, *A. dhakensis*, *A. veronii*, and *A. enteropelogenes* exhibited multidrug resistance, especially to sulfonamide-trimethoprim, tetracycline, and norfloxacin. Genomic analyses revealed antibiotic resistance in genes across *Aeromonas* spp. except for *A. salmonicida* and *A. rivipollensis*, with statistical tests confirming significant associations between *sul12*, *dfrA1* and resistance to STX and *tetA/R* to tetracycline resistance, respectively. Class 1 integrons containing resistance cassettes were also found exclusively in *A. hydrophila*, whereas *A. dhakensis* carried diverse plasmid determinants. Phylogenetic reconstruction grouped isolates into five species-specific clades of (1) *A. hydrophila*, (2) *A. dhakensis*, (3) *A. veronii*, (4) *A. enteropelogenes*, and (5) *A. salmonicida* and *A. rivipollensis*; each displaying distinct resistome signatures and regional clustering. These findings indicate that the intensifying use of antibiotics in *pangasius* aquaculture accelerates AMR development in both species through different genetic pathways. This study provides important insights into antibiotic resistance capability and potential dissemination

mechanisms of different *Aeromonas* spp., thus urging the need for improved antibiotic stewardship and surveillance under a One Health approach.

Keywords: *Aeromonas*, antibiotic resistance gene, integron, phylogenetic tree, plasmid, susceptibility.

INTRODUCTION

Pangasius (*Pangasianodon hypophthalmus*) farming represents a cornerstone of Vietnam's aquaculture industry and is one of the country's most important export sectors, reaching approximately 1.8 billion USD annually in gross and accounting for over one-third of Vietnam's seafood export value (Tran *et al.*, 2025). The Mekong Delta serves as the "heart" of this industry, with its extensive river systems, nutrient-rich waters, and warm tropical climate providing ideal conditions for fish cultivation. Major farming provinces include Dong Thap, An Giang, Can Tho, Ben Tre, Tien Giang, and Vinh Long (Erickson *et al.*, 2023). However, despite this success, the pangasius industry faces persistent challenges in disease management, particularly under intensive farming conditions characterized by high stocking densities and deteriorating water quality, which favor the proliferation of bacterial pathogens. Motile *Aeromonas* Septicemia (MAS), commonly referred to as "red spot disease," remains one of the most serious and widespread problems affecting pangasius aquaculture. The disease is primarily caused by opportunistic *Aeromonas* species, historically attributed to *A. hydrophila* but more recently associated with *A. dhakensis*, which has emerged as the predominant pathogen in many outbreaks. MAS can lead to acute mortality rates reaching 80% in untreated cases, particularly during the juvenile and fry stages, causing severe economic losses for farmers (Le *et al.*, 2018).

Due to the lack of effective vaccines or alternative control measures, antibiotics were still the primary method to treat MAS, as surveys have shown that nearly 80% of pangasius farming households in the Mekong Delta use antibiotics during production, commonly including tetracyclines, β -lactams, sulfonamides, phenicols, and fluoroquinolones (Dao *et al.*, 2022). However, this extensive antibiotic use has contributed to the emergence of multidrug-resistant (MDR) *Aeromonas* strains, notably *A. hydrophila* and *A. dhakensis*, undermining treatment efficacy and posing broader risks to aquatic and public health (Luu *et al.*, 2021). Antibiotic resistance (AMR) genes in these bacteria are often disseminated through mobile genetic elements such as plasmids and integrons—key drivers of horizontal gene transfer (HGT). For instance, the fluoroquinolone resistance gene *qnrS* has been detected on plasmids of *A. dhakensis*, while class 1 integrons frequently harbor diverse ARG cassettes that enhance bacterial adaptability under antibiotic pressure. Despite growing evidence, previous studies have often lacked genomic resolution to characterize these mobile elements or failed to integrate epidemiological and geographic data to trace clonal dissemination. This highlights an urgent need for a more comprehensive, integrated genomic and epidemiological approach to understand and mitigate AMR in *Aeromonas* populations associated with pangasius aquaculture in the Mekong Delta.

MATERIALS AND METHODS

Biological samples, DNA extraction and whole genome sequencing

A total of 44 *Aeromonas* strains were included in this study, which were previously recovered from MAS-diseased *pangasius* in Tien Giang (Cai Lay) and Vinh Long (Mang Thit, Vung Liem, Tam Binh) provinces, Vietnam (Table S1). Genomic DNA was extracted from the overnight culture of the *Aeromonas* isolates using the Wizard Genomic DNA Extraction Kit (Promega, USA) following the manufacturer's recommendations. DNA library preparation was done using a Nextera XT kit (Illumina, USA), followed by genome sequencing on an Illumina Miseq of 75 bp paired-end reads (for catfish- and water-related isolates). Illumina reads quality assessment, *de novo* assembly, species delineation by Average Nucleotide Identity (ANI) method, and molecular typing performed by SRST2 v0.2.0 were performed as described previously (Truong *et al.*, 2024). The isolation and taxa details of all isolates were listed in Table S1. The raw Illumina sequencing reads of each isolate have been deposited in the European Nucleotide Archive (ENA) with the project accession PRJEB65955.

Antibiotic susceptibility testing

Antimicrobial susceptibility of *A. hydrophila* and *A. dhakensis* isolates was determined using the Kirby-Bauer disk diffusion method following CLSI VET04 guidelines. Test cultures were revived from cryopreserved stocks (−70°C) by thawing gradually in 4°C racks before spreading on TSA (Merck, Germany) and sub-cultured on MHA (Merck, Germany) plates at 28°C for

18–20 h to ensure purity and active growth. Bacterial suspensions were adjusted to a 0.5 McFarland standard ($\sim 1.5 \times 10^8$ CFU/mL) in sterile 0.9% saline and evenly spread on MHA plates to obtain confluent lawn growth. Five antibiotic discs representing different classes relevant to Vietnamese aquaculture pathogens (Table 1) (Luu *et al.*, 2021) were aseptically placed on the agar surface and incubated at 28°C for 18–20 h. The diameters of inhibition zones were measured from the underside of the plates using reflected light, with readings rounded to the nearest millimeter; if growth reached the disc edge, the inhibition zone was recorded as 0 mm. Tests were performed in duplicate, and *Escherichia coli* ATCC 25922 was used as the quality control strain to validate the diffusion procedure. Results were interpreted according to CLSI VET04 breakpoints and classified as “susceptible” or “non-susceptible”.

Gene content analysis

Antimicrobial resistance (AMR) genes were identified using ARIBA v2.14.7 (Hunt *et al.*, 2017) against the CARD database (Alcock *et al.*, 2023) (accessed 18 December 2024). Assembled contigs were screened for plasmid-associated sequences with MOB-suite v3.1.0 using the PLSDB database (Molano *et al.*, 2025) and for integrons using IntegronFinder v2.0.6 with the NCBI RefSeq database (Néron *et al.*, 2022) (accessed 6 January 2025). A Mash neighbor distance threshold of 0.06 was applied to define plasmid clusters (Robertson and Nash, 2018), while only integrons with >90% identity and >80% coverage were retained. All analyses were conducted using default parameters unless otherwise specified.

Table 1. Antibiotics used in this study to assess antibiotic susceptibility testing

Antibiotics	Abbreviated name	Concentration on disc (µg)
Sulfamethoxazole-trimethoprim	STX	23.75-1.25
Ampicillin	AMP	10
Amoxicillin	AMC	10
Cefotaxime	CTX	30
Tetracycline	TE	30
Doxycycline	DOX	30
Gentamicin	GM	10
Norfloxacin	NOR	10
Florfenicol	FF	30

Epidemiologic analysis of *Aeromonas* spp. isolates

Genome assemblies from *Aeromonas* spp. clean were annotated using Prokka v1.14.6 (Seemann, 2014), and the pangenome was reconstructed by Roary v3.13.0 (Page *et al.*, 2015). Core gene alignments generated by Roary were then processed with SNP-sites v2.5.1 (Page *et al.*, 2016) to extract single nucleotide polymorphisms (SNPs). A maximum likelihood phylogenetic tree was constructed using IQ-TREE (Nguyen *et al.*, 2015) v3.0.0 in 1,000 ultrafast bootstrap replicates. The resulting tree was visualized using the Interactive Tree of Life (iTOL) v6 (Letunic and Bork, 2021) platform.

Statistical analysis

All experiments were performed in at least two independent replicates to ensure reproducibility. To evaluate associations between antibiotic resistance phenotypes and corresponding resistance genes, Fisher's exact test was performed on GraphPad

Prism. In addition, odds ratios (ORs) with 95% confidence intervals (CIs) were calculated to assess the strength and direction of observed associations. A *p*-value < 0.05 was considered statistically significant.

RESULTS

Antibiotic susceptibility profiles of *Aeromonas* isolates

The antibiotic susceptibility test was performed on diffusion disks with the diameter of the inhibition zones and was measured in millimeters with precision to the nearest whole millimeter (Figure S1). Phenotypic testing showed a universal resistance to β -lactam antibiotics (amoxicillin-clavulanate and AMP) across the genus (Figure 1, Table S2). In contrast, non-susceptibility to CTX and FF was exclusive to *A. dhakensis* (39%, 9/23 and 30%, 7/23) and *A. hydrophila* (25%, 3/12 and 17%, 2/12), respectively, whereas DOX resistance was detected only in *A. veronii*

(60%, 3/5). STX resistance was observed in *A. hydrophila* (92%, 11/12), *A. veronii* (60%, 3/5), *A. enteropelogenes* (50%, 1/2), *A. dhakensis* (35%, 8/23). Similarly, TE resistance showed a similar pattern in *A. hydrophila* (83%, 10/12), *A. veronii* (60%, 3/5), *A. enteropelogenes* (50%, 1/2), *A. dhakensis* (43%, 10/23). NOR resistance

was found in all *A. enteropelogenes* isolates, while only appeared in 40% of *A. veronii* (2/5), 35% of *A. dhakensis* (8/23), 8% of *A. hydrophila* (1/12). Meanwhile, GM resistance rate, albeit lower, was also detected in multiple species namely *A. enteropelogenes* (50%, 1/2), *A. veronii* (20%, 1/5) and *A. hydrophila* (8%, 1/12).

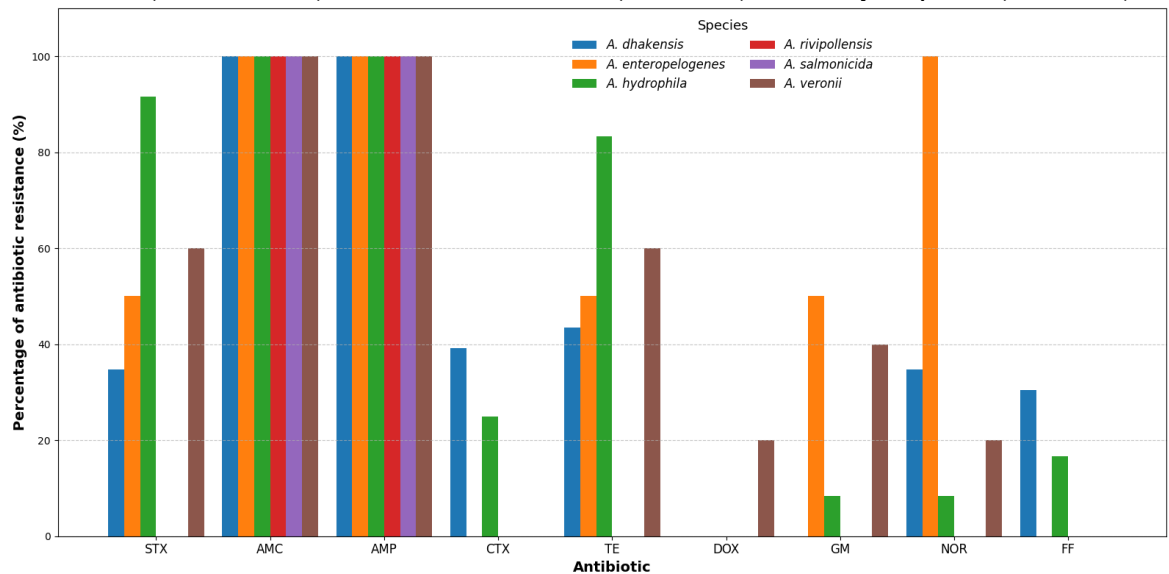


Figure 1. Antibiotic non-susceptibility distribution of different *Aeromonas* spp. The bars represent the percentage of isolates from the corresponding species with non-susceptibility to the antibiotic in the x-axis. STX: sulfamethoxazole-trimethoprim, AMC: amoxicillin, AMP: ampicillin, CTX: cefotaxime; TE: tetracycline, DOX: doxycycline, GM: gentamicin, NOR: norfloxacin, FF: florfenicol.

Antibiotic resistance gene profiles of *Aeromonas* isolates

Multiple AMR determinants were detected across 44 *Aeromonas* genomes with species-specific patterns (Figure 2). However, *A. rivipollensis* lacked detectable AMR genes, whereas *A. salmonicida* harbored only the metallo- β -lactamase *imiH*. Nevertheless, β -lactamase genes were diverse and species-specific. Particularly, *A. dhakensis* predominantly carried *blaAQU* (96%, 22/23) together with *imiH* (100%, 23/23), while all *A. hydrophila* and *A. veronii* isolates possessed AmpC *cepH*. Another β -

lactamase detected included *blaTRU-1* in *A. enteropelogenes* (2/2, 100%), *blaOXA-12* in *A. veronii* (20%, 1/5), and *blaMOX-8* in *A. hydrophila* (1/12, 8%). The *sul12-dfrA1* module associated with STX resistance was frequent in *A. hydrophila* (75–83%), *A. dhakensis* (39%, 8/23) and *A. veronii* (40–60%), respectively. TE-resistance *tetA/tetR* occurred mainly in *A. hydrophila* (83%, 11/12) and *A. veronii* (60%), was less common in *A. dhakensis* (35%, 8/23), and in one *A. enteropelogenes* isolate (50%). Quinolone-class resistance genes showed contrasting distributions: *aac(6)-Ib-cr* was characteristic of *A. hydrophila* (83%, 11/12)

but sporadic elsewhere, whereas *qnrS2* was enriched in *A. dhakensis* (48%) and absent from other species. The only GM-resistant determinant found was *aadA1*, which was only harbored in one isolate from each of *A.*

hydrophila, *A. veronii*, and *A. enteropelogenes*. Meanwhile, FF-resistant determinants *floR* mainly occurred in *A. dhakensis* (35%, 8/23).

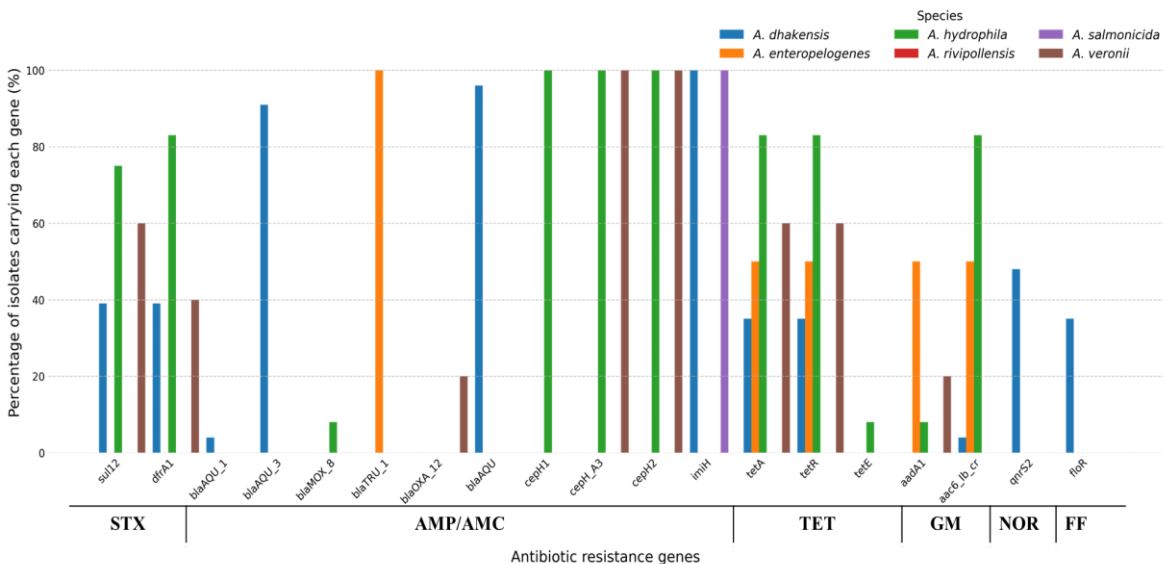


Figure 2. Antibiotic resistant genes distribution of different *Aeromonas* spp. STX: sulfamethoxazole-trimethoprim, AMC: amoxicillin, AMP: ampicillin, CTX: cefotaxime; TE: tetracycline, DOX: doxycycline, GM: gentamicin, NOR: norfloxacin, FF: florfenicol.

Using Fisher's exact tests, we assessed whether phenotypic non-susceptibility aligns with the presence of the corresponding resistance genes (Figure S2). While the presence of different β -lactamases in all isolates bar *A. rivipollensis* were justifiable with the natural resistance of AMP and AMC natural resistance, there were no significant correlation between AMR genes presence and susceptibility in the case of CTX, DOX, GM, NOR, and FF. Noticeably, STX non-susceptible isolates showed a strong genotype–phenotype match with both *dfrA1* and *sul12* (Figure 3). Isolates carrying *dfrA1* had markedly higher

odds of being STX non-susceptible (odds ratio, OR = 133.3; $p < 0.0001$). Likewise, the presence of *sul12* was strongly associated with STX non-susceptibility (OR = 90.25; $p < 0.0001$). Together, these data indicate a clear and biologically coherent genotype–phenotype linkage for STX in our *Aeromonas* collection. Second, TE non-susceptibility was significantly associated with *tetA* and *tetR*. Carriage of *tetA* increased the odds of TE non-susceptibility (OR = 7.29; $p = 0.0058$). A nearly identical pattern was observed for *tetR* (OR = 7.29; $p = 0.0058$), consistent with the co-occurrence of these determinants in many isolates.

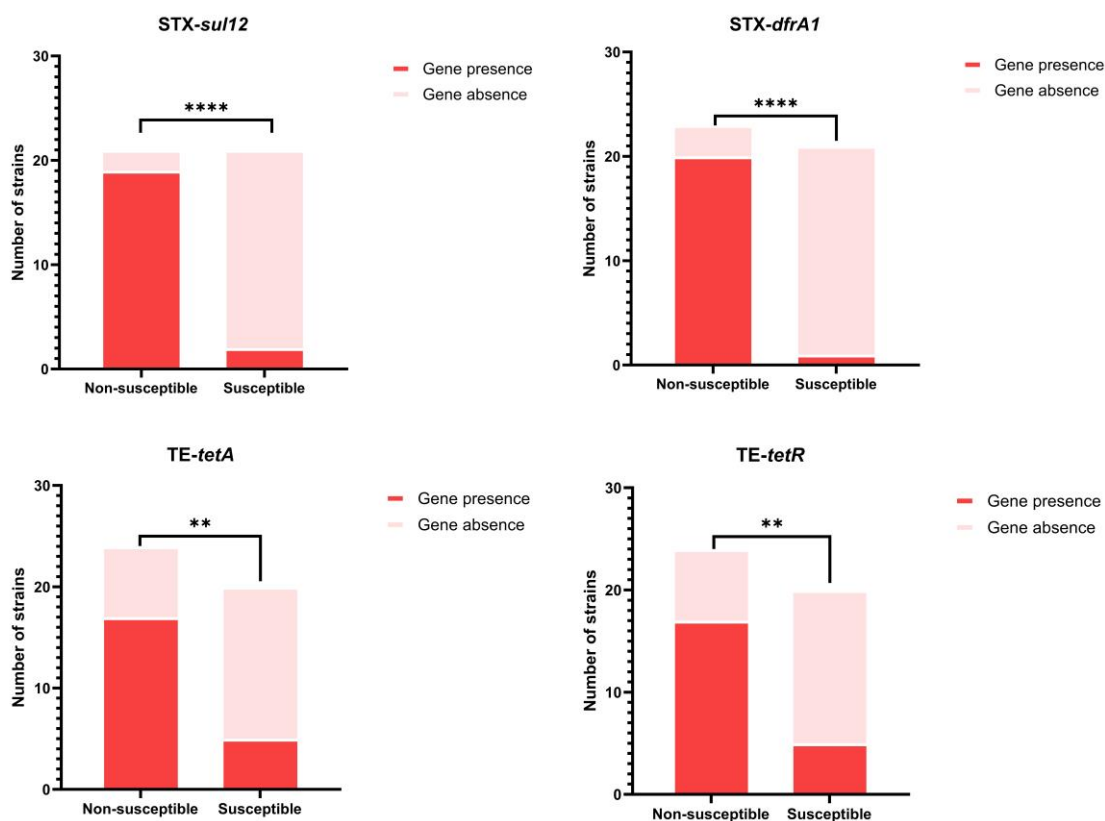


Figure 3. Correlation between genotype and phenotype of STX resistance and TE resistance. The susceptibility tests were performed in duplicates, with Fisher's exact test was performed to find the relationship between genotype and phenotype and odds ratios were calculated the confidence level of the test (* $p \leq 0.05$, ** $p \leq 0.01$, *** $p \leq 0.001$, **** $p \leq 0.0001$).

Plasmid and integron profiles

Screening for mobile genetic elements (MGEs) revealed two contrasting, species-biased patterns. Class 1 integrons were restricted to *A. hydrophila*: 75% of isolates carried a class 1 integron, detected in two cassette organizations: *arr-2-aac(6')-Ib-cr-dfrA1-qacEΔ1* (4/12, 33%) and *qacEΔ1-dfrA1-aac(6')-Ib-cr-arr-2* (5/12, 42%). No class 1 integron was detected in *A. dhakensis* or the other species. The gene content of these cassettes (trimethoprim/sulfonamide, aminoglycoside and rifamycin resistance plus the integron marker *qacEΔ1*) is consistent with the elevated STX and

aminoglycoside phenotypes reported for *A. hydrophila*. In contrast, plasmid signatures were concentrated in *A. dhakensis*: 78% (18/23) of isolates harbored at least one plasmid-like contig. The most frequent matches were pS44-5 (12/23, 52%) and IncQ1 (9/23, 39%), followed by hits to *Citrobacter* sp. S39 plasmid pS39-4 (3/12, 13%), pAsa7 (2/23, 9%), pAH06-06-3 (2/23, 9%), pS121-4 (1/23, 4%), and *Klebsiella pneumoniae* plasmid pC51_005 (1/23, 4%). No plasmid hit was recorded for *A. hydrophila* on this screen, except for one *A. hydrophila* ST251 strain harboring a pAH06 plasmid.

Table 2. Distribution of integrons and plasmid in different *Aeromonas* spp.

ARGs located on MGEs	Class 1 integron		Plasmid			
	<i>arr-2 – aac(6')-lb-cr – dfrA1 – qacEΔ1</i>	<i>qacEΔ1 – dfrA1 – aac(6')-lb-cr – arr-2</i>	IncQ1	pS44-5	pAsa7	pAH06-06-3
<i>A. hydrophila</i> (n = 12)	33%	42%				0.1%
<i>A. dhakensis</i> (n = 23)	0%		39%	52%	13%	9%

Epidemiologic analysis of antibiotic resistance profiles in dominant *Aeromonas* isolates

The phylogenetic tree of all *Aeromonas* spp. showed each *Aeromonas* species to form a separate cluster, highlighting the genetic diversity within the genus (Figure 3). The first cluster comprised the *A. dhakensis* isolated during 2017-2020, which were mainly ST656. Most notably, *A. dhakensis* reflected a potential resistance to 6-8 antibiotics, while also being a reservoir for different plasmids, and containing all five to seven major AMR genes. The *A. hydrophila* (n = 12) cluster, which was dominated by *A. hydrophila* ST251 (11/12), also possessed the capacity to resist 6-9 antibiotics, but only one to two isolates could resist NOR or FF, and most *A. hydrophila* showed the resistant genotype of *sul*⁺-*dfrA*⁺-*tet*⁺-*aac*⁺, with the exception of a novel *A. hydrophila* STNF. *A.*

hydrophila also demonstrated the risk of antibiotic transmission, with 8/12 isolates possessing the integron Class 1 *AttC/AttI*.

The third cluster comprised five *A. veronii* isolates isolated in 2019-2022, in which most were untyped (STNF). These isolates were positioned in distant branches with higher average SNP distances, indicating greater genetic divergence compared with the first cluster. All *A. veronii* showed non-susceptibility to all antibiotics apart from CTX and FF, while carrying the average of four AMR genes. Meanwhile, *A. enteropelogenes* cluster and *A. rivipollensis*-*A. salmonicida* cluster showed susceptibility to 7/9 antibiotics, except for one novel *A. enteropelogenes*, while also having fewer AMR genes and transposable elements compared to the three major MAS-causing *Aeromonas* spp.

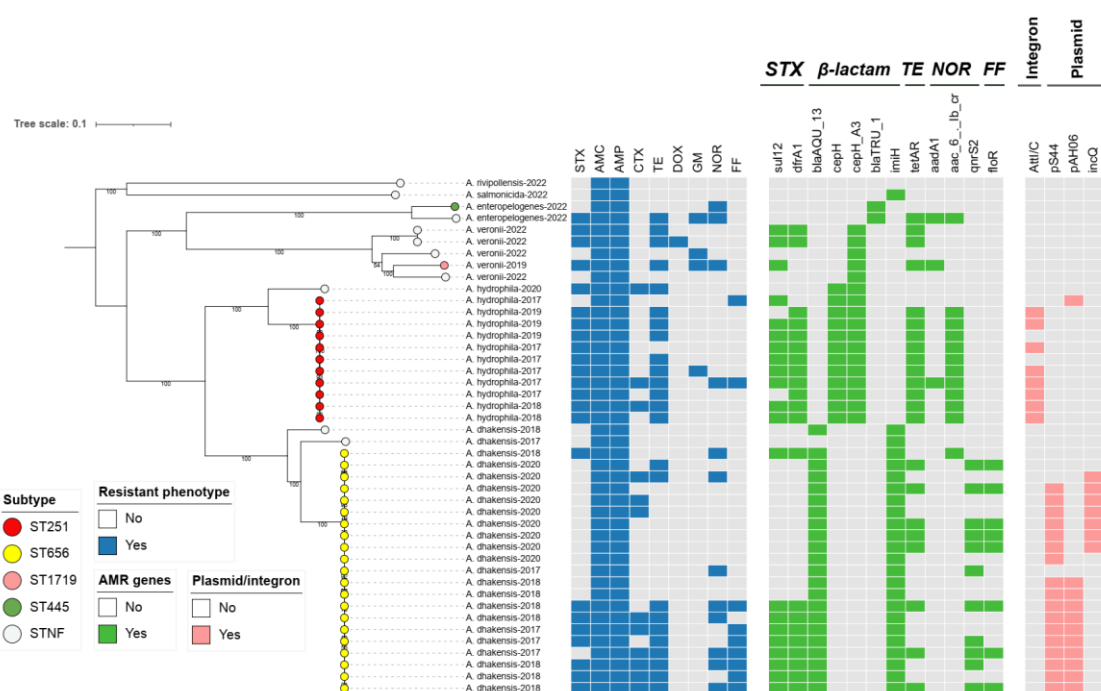


Figure 4. Maximum likelihood phylogenetic tree of *Aeromonas* spp. (n = 44) based on the total SNPs of core genes, extracted using SNP-sites. The node is colored based on the sequence types (STs) of isolates. The first heatmap illustrates antibiotic susceptibility, while the second heatmap shows presence of acquired AMR genes. The third heatmap depicts the presence of *Aeromonas*-related plasmids and integrons. The tree scale represents the number of substitutions per site. STX: sulfamethoxazole-trimethoprim, AMC: amoxicillin, AMP: ampicillin, CTX: cefotaxime; TE: tetracycline, DOX: doxycycline, GM: gentamicin, NOR: norfloxacin, FF: florfenicol.

DISCUSSION

In this study, the antibiotic susceptibility situations of different *Aeromonas* spp. were characterized. *A. dhakensis* and *A. hydrophila* carried the most diverse and frequent AMR repertoires and carried multiple resistance phenotypes, which were consistent with their roles as major aquatic pathogens (Bartie et al., 2023; Truong et al., 2024). However, the emergence of other multi-resistant strains in *Aeromonas* spp., particularly *A. veronii* and *A. enteropelogenes*, has not been examined. Particularly, 80% of *A. veronii* showed resistance phenotypes to STX and TET,

which were commonly used in Vietnam fish production (Luu et al., 2021), while one *A. enteropelogenes* isolate exhibited resistance to six out of nine antibiotics, most notably GM, which the majority of *Aeromonas* spp. isolates were susceptible to. Antibiotic gene detection also reflected the abundance in AMR genes in *A. dhakensis*, *A. hydrophila*, *A. veronii*, and *A. enteropelogenes*. In contrast, *A. salmonicida* and *A. rivipollensis* harbored few or no AMR genes, potentially reflecting limited exposure to antimicrobial selection or reduced capacity for horizontal gene transfer. While AMR gene prediction has been investigated in catfish *Aeromonas* spp., there has been no confirmation about

the correlation between the genotype and phenotype (Erickson *et al.*, 2023). Here, we demonstrated that only *tetAR* and *sul12-dfrA1* had significant contributions to the resistance of TET and STX, respectively. While AMP and AMC were naturally resisted in *Aeromonas* spp., the resistance was attributed to different species-specific β -lactamases. However, there were not sufficient data to prove the effect of *aad/aac*, *qnrS2*, and *floR* on the resistance of GM, NOR, and FF, respectively. This discrepancy emphasizes the need to reassess the culprits behind these antibiotics, while *tetAR* and *sul12-dfrA1* could be candidates for more rapid detection of TET and STX resistance by PCR assays.

Compared with previous data from Erickson *et al.* (2023) collected from 2019 to 2022, the result here was generally consistent with prior reports on aquaculture *Aeromonas* (Erickson *et al.*, 2023). Particularly, FF resistance was found to only occur in 17% of *A. hydrophila* and *A. dhakensis*, which corresponded to 100% susceptibility from *A. hydrophila* and 50% susceptibility from *A. dhakensis* in the previous research. Similarly, GM showed to be highly susceptible to *Aeromonas* spp. in both studies. However, contrasting trends were found in *Aeromonas* resistance to STX, where the previous results showed a low resistance in *A. hydrophila* (1/5, 20%) and high resistance from *A. dhakensis* (20/25, 80%). TE resistance had also been attributed to *A. dhakensis* (19/25, 76%), while exhibiting moderate resistance in both *Aeromonas* spp. here. The discrepancy was likely caused by the unbalanced sampling of *A. hydrophila*, as the previous research only used 5 isolates, compared to 12 isolates being used here.

Phylogenetic analysis proved the genomic distinction of each species despite sharing the same pathogenic symptoms. The *A. dhakensis* cluster was shown to be a plasmid reservoir, in which the IncQ plasmid has been detected in *Aeromonas* from aquaculture environments in China, India, and Korea, often carried quinolone-resistant *qnrS2*, and was found in nearly half of all *A. dhakensis* isolates (Majumdar *et al.*, 2011). Meanwhile, two-thirds of all *A. hydrophila* cluster possessed Class 1 integron, which had the capacity to transfer GM-resistant determinant *aac(6')-Ib-cr*, trimethoprim AMR *dfrA1*, and rifamycin-resistant gene *arr2*, which has been reported in Vietnam catfish-infected vAH *A. hydrophila* (Bartie *et al.*, 2023), while reported in Thailand aquaculture *Aeromonas* spp., which could be closely related to the Vietnam strain (Lukkana *et al.*, 2012). However, the presence of one pAH06 plasmid-carrying *A. hydrophila* could hint at transmission between *A. dhakensis* and *A. hydrophila*. The presence of such mobile elements may be expected to facilitate the transmission of these resistant phenotypes in the aquatic environment. Nonetheless, further integration experiments are necessary to confirm the dissemination of MGE from both *A. hydrophila* and *A. dhakensis*.

CONCLUSION

In conclusion, the research attempted to provide a comprehensive characterization of both AMR genotypes and phenotypes of aquatic *Aeromonas* spp., some of which were first reported. While *A. dhakensis* and *A. hydrophila* still remained the primary culprit for the MAS outbreak, the prevalence of multi-resistant *A. veronii* and *A. enteropelogenes* should also be considered

when formulating future vaccine research and detection assay.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

SUPPLEMENTARY MATERIALS

Figure S1. Typical antibiotic susceptibility testing results by diffusion disk methods.

Figure S2. Fisher's test of correlation between an antibiotic susceptibility and the corresponding AMR genes.

Table S1. Isolate details including collection date and geographic origin, host species and statuses, species identification methods, ANI values, and sequence typing results.

Table S2. The average inhibition zone diameters obtained from the antibiotic susceptibility testing (mm).

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Table S1. Isolate details including collection date and geographic origin, host species and statuses, species identification methods, ANI values, and sequence typing results.

Full ID	Species	ST	Farm location	Fish status
10-AH37	<i>A. dhakensis</i>	656	Tien Giang	Juvenile
12-AH41	<i>A. dhakensis</i>	656	Tien Giang	Juvenile
19-AD19	<i>A. dhakensis</i>	NF	Dong Thap	Fingerling
20-AH38	<i>A. dhakensis</i>	656	Tien Giang	Juvenile
22-AH43	<i>A. dhakensis</i>	656	Tien Giang	Juvenile
24-AH100	<i>A. dhakensis</i>	656	An Giang	Juvenile
32-AD24	<i>A. dhakensis</i>	656	Tien Giang	Fingerling
4-AD25	<i>A. dhakensis</i>	656	Tien Giang	Fingerling
8-AD10	<i>A. dhakensis</i>	NF	Dong Thap	Juvenile
11-AH39	<i>A. dhakensis</i>	656	Tien Giang	Juvenile
1-AD01	<i>A. dhakensis</i>	656	An Giang	Juvenile
21-AH42	<i>A. dhakensis</i>	656	Tien Giang	Juvenile
9-AH36	<i>A. dhakensis</i>	656	Tien Giang	Juvenile
18-AD18	<i>A. dhakensis</i>	656	Can Tho	Juvenile
23-AH44	<i>A. dhakensis</i>	656	Tien Giang	Juvenile
AD02	<i>A. dhakensis</i>	656	Vinh Long	Fingerling
11-AD27	<i>A. dhakensis</i>	656	Tien Giang	Fingerling
26-AD08	<i>A. dhakensis</i>	656	Vinh Long	Juvenile
9-AD29	<i>A. dhakensis</i>	656	Tien Giang	Fingerling
AD21	<i>A. dhakensis</i>	656	Dong Thap	Fingerling
6-AD9	<i>A. dhakensis</i>	656	Vinh Long	Juvenile
7-AD16	<i>A. dhakensis</i>	656	An Giang	Fingerling
8-AD23	<i>A. dhakensis</i>	656	Tien Giang	Fingerling
30-AH129	<i>A. enteropelogenes</i>	445	An Giang	Juvenile
28-AH127	<i>A. enteropelogenes</i>	NF	An Giang	Juvenile
AH13	<i>A. hydrophila</i>	251	An Giang	Fingerling
31-AH40	<i>A. hydrophila</i>	NF	Tien Giang	Juvenile
10-AH34	<i>A. hydrophila</i>	251	Vinh Long	Juvenile
4-AH04	<i>A. hydrophila</i>	251	Vinh Long	Juvenile
5-AD32	<i>A. hydrophila</i>	251	Vinh Long	Fingerling
5-AH05	<i>A. hydrophila</i>	251	Vinh Long	Juvenile

8-AH22	<i>A. hydrophila</i>	251	Ben Tre	Juvenile
10-AD26	<i>A. hydrophila</i>	251	Tien Giang	Fingerling
37-AH33	<i>A. hydrophila</i>	251	Vinh Long	Juvenile
AH07	<i>A. hydrophila</i>	251	Vinh Long	Juvenile
3-AH03	<i>A. hydrophila</i>	251	Vinh Long	Juvenile
24-AH06	<i>A. hydrophila</i>	251	Vinh Long	Juvenile
29-AH125	<i>A. rivipollensis</i>	NF	An Giang	Juvenile
25-AH121	<i>A. salmonicida</i>	NF	An Giang	Juvenile
27-AH126	<i>A. veronii</i>	NF	An Giang	Juvenile
28-AH124	<i>A. veronii</i>	NF	An Giang	Juvenile
27-AH123	<i>A. veronii</i>	NF	An Giang	Juvenile
6-AH35	<i>A. veronii</i>	1719	Vinh Long	Juvenile
26-AH122	<i>A. veronii</i>	NF	An Giang	Juvenile

ANI: Average Nucleotide Identity, ST: Sequence Type, NF: Not Found

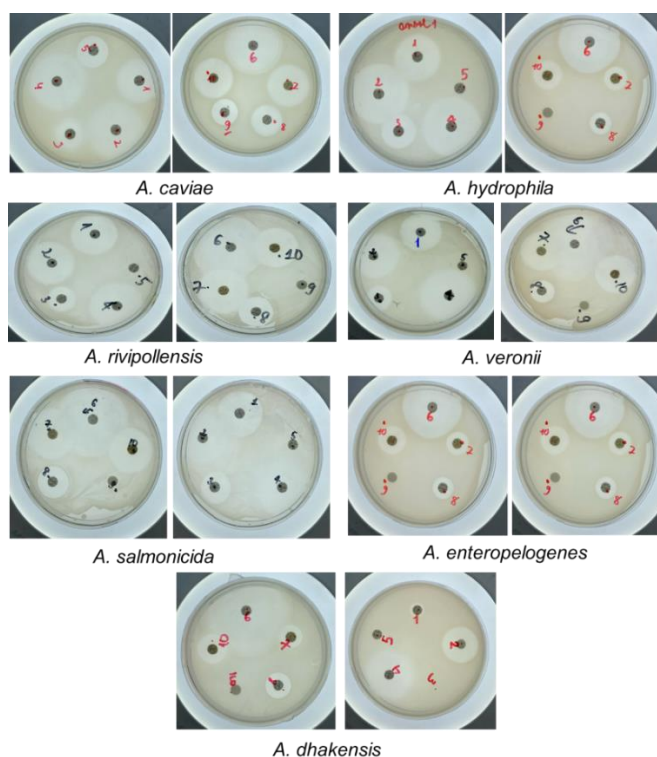


Figure S1. Typical antibiotic susceptibility testing results by diffusion disk methods. A total of 10 antibiotics were tested and numbered, including: 1. Sulfonamide-trimethoprim, 2. Norfloxacin, 3. Erythromycin, 4. Florfenicol, 5. Ampicillin, 6. Cefotaxime, 7. Tetracycline, 8. Gentamicin, 9. Amoxicillin, and 10. Doxycycline. Each antibiotic test was performed in duplicates.

Table S2. The average inhibition zone diameters obtained from the antibiotic susceptibility testing (mm).

Full ID	Species	AMC	AMP	CTX	TE	DOX	GM	STX	NOR	FF
1-AD01	<i>A. dhakensis</i>	0	0	26.4	22.5	22.9	18.7	26.3	20.1	27.7
AD02	<i>A. dhakensis</i>	0	0	25.3	12.2	18.3	20	20	20	10.8
3-AH03	<i>A. hydrophila</i>	0	0	25.5	14.5	20.7	18.3	0	23.3	26.9
4-AH04	<i>A. hydrophila</i>	0	0	25.2	13.1	20.2	18.2	0	22.7	28.4
5-AH05	<i>A. hydrophila</i>	0	0	31.9	15.2	21.9	18.4	0	22.1	26.9
24-AH06	<i>A. hydrophila</i>	0	0	32.9	14.8	31.2	9.5	0	21	26.8
AH07	<i>A. hydrophila</i>	0	0	27.2	11.7	17.5	18.7	0	22.7	27.4
26-AD08	<i>A. dhakensis</i>	0	0	26.2	10.8	19.6	20.3	0	22.2	11.2
6-AD9	<i>A. dhakensis</i>	0	0	25.2	10.8	18.8	17.9	0	21.6	11
8-AD10	<i>A. dhakensis</i>	0	0	26.7	26.2	23.9	19.1	25.8	24.6	24
AH13	<i>A. hydrophila</i>	0	0	26.2	25.7	25	20.5	23.2	19.3	27.4
7-AD16	<i>A. dhakensis</i>	0	0	21.3	11.7	18.6	18.5	0	11.7	26.8
18-AD18	<i>A. dhakensis</i>	0	0	29.3	25.8	25.3	17.3	0	18.1	28.3
19-AD19	<i>A. dhakensis</i>	0	0	28.1	25.5	24	24	22.8	27.3	26.6
AD21	<i>A. dhakensis</i>	0	0	33.1	11.9	18.3	21.9	0	19.6	9.7
8-AH22	<i>A. hydrophila</i>	0	0	31.1	14.5	24.2	18.2	0	21.9	27.9
8-AD23	<i>A. dhakensis</i>	0	0	24.3	12.6	19.2	18.3	0	20.2	12.4
32-AD24	<i>A. dhakensis</i>	0	0	32.1	31.3	27.2	26.2	23.5	29.3	29.3
4-AD25	<i>A. dhakensis</i>	0	0	34.2	26.8	25.8	23.9	25.6	23.3	31.3
10-AD26	<i>A. hydrophila</i>	0	0	24.9	11.7	17.1	18.5	0	16	10.1
11-AD27	<i>A. dhakensis</i>	0	0	23.3	13	17	17.7	0	20.3	13
9-AD29	<i>A. dhakensis</i>	0	0	24.6	12	18.5	19.1	0	21.8	11.2
5-AD32	<i>A. hydrophila</i>	0	0	31.3	13.2	22.5	15	0	19.6	28.8
37-AH33	<i>A. hydrophila</i>	0	0	32	13.9	22	19.3	0	22.4	32
10-AH34	<i>A. hydrophila</i>	0	0	32	13.8	21.9	19.1	0	21.2	27.1
6-AH35	<i>A. veronii</i>	0	0	33.3	13.2	17.3	11.5	0	18.3	25.5
9-AH36	<i>A. dhakensis</i>	0	0	29.7	10.8	27.6	23.4	30.2	34.9	31.8
10-AH37	<i>A. dhakensis</i>	0	0	26	24.2	24.2	21.5	24.6	29.8	27.4
20-AH38	<i>A. dhakensis</i>	0	0	26.6	23.9	23.8	21.2	26.9	32.7	26.5
11-AH39	<i>A. dhakensis</i>	0	0	24.6	25.9	24.4	19.8	28.3	31	29.2
31-AH40	<i>A. hydrophila</i>	0	0	26.5	24.9	23.3	19.6	24.7	28	29.2
12-AH41	<i>A. dhakensis</i>	0	0	28	26.3	26.1	20.1	29.7	25.3	26.8

21-AH42	<i>A. dhakensis</i>	0	0	25.3	25.9	24.7	22.1	27.4	30.8	30
22-AH43	<i>A. dhakensis</i>	0	0	25.8	23.7	24.7	19.7	26.9	29.8	28
23-AH44	<i>A. dhakensis</i>	0	0	24.9	10.4	18.1	17.4	21.7	17.1	24.1
24-AH100	<i>A. dhakensis</i>	0	0	30.8	25.3	27.4	17.2	24	25	28.9
25-AH121	<i>A. salmonicida</i>	0	0	42.8	24.5	26.6	16.6	27	31.8	31
26-AH122	<i>A. veronii</i>	0	0	41.2	14	8.9	14.7	7.3	22.3	31.4
27-AH123	<i>A. veronii</i>	0	0	46.3	12.8	16.1	15.1	6.8	22.5	33.3
28-AH124	<i>A. veronii</i>	0	0	42.8	29.6	27.3	9.5	28	28.3	36.9
29-AH125	<i>A. rivipollensis</i>	0	0	35.6	26.7	20	19.7	22	29.3	31.1
27-AH126	<i>A. veronii</i>	0	0	46.4	28.3	25.7	15.9	25.1	27.8	33.3
28-AH127	<i>A. enteropelogenes</i>	0	7.4	33.9	11.3	16.5	12.7	0	17.1	28.2
30-AH129	<i>A. enteropelogenes</i>	0	17.5	30.4	18.9	18.7	15.9	19.8	17.6	30.3

Red: Resistant, Blue: Intermediate, Orange: Sensitive; STX: sulfamethoxazole-trimethoprim, AMC: amoxicillin, AMP: ampicillin, CTX: cefotaxime; TE: tetracycline, DOX: doxycycline, GM: gentamicin, NOR: norfloxacin, FF: florfenicol.

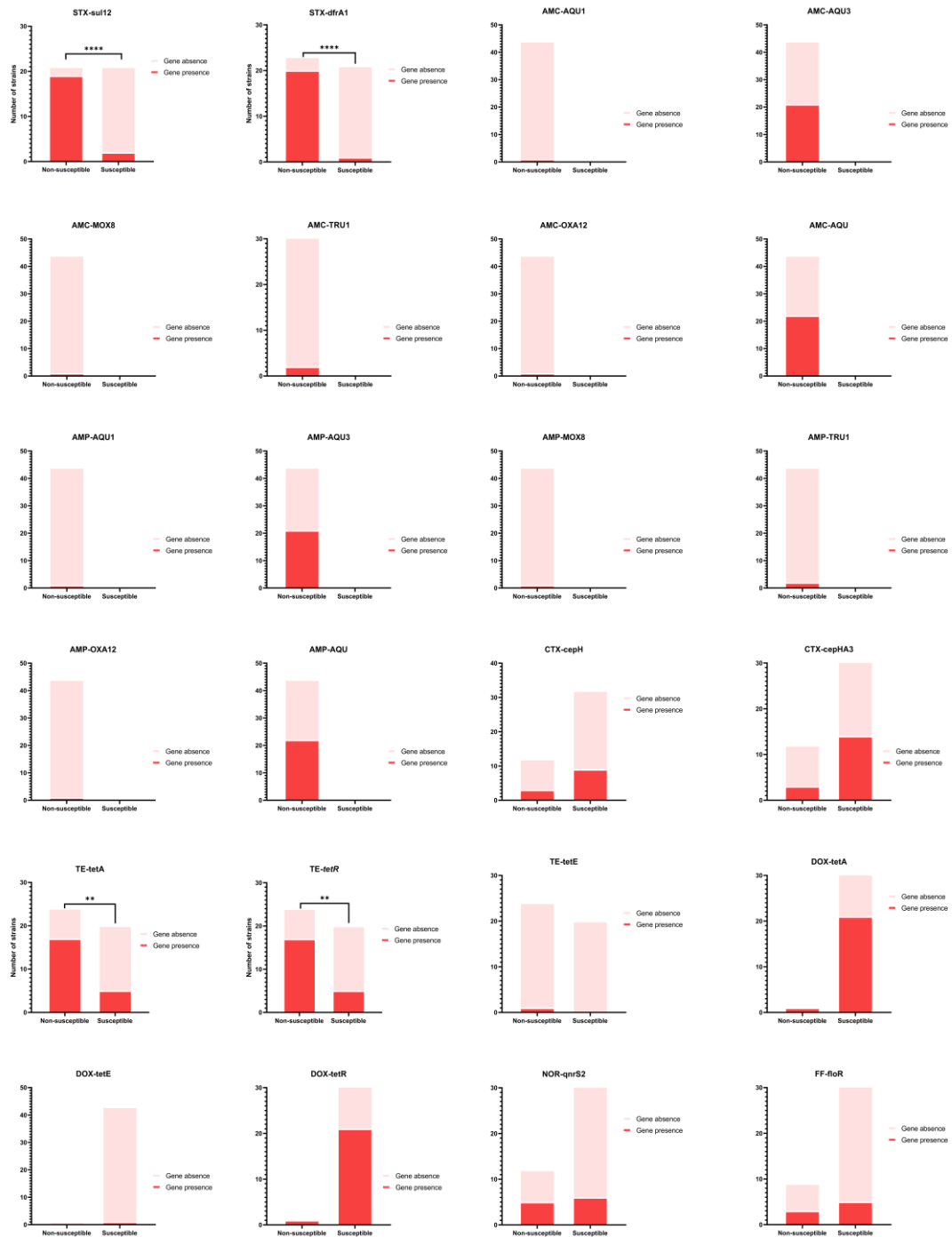


Figure S2. Fisher's test of correlation between an antibiotic susceptibility and the corresponding AMR genes. The susceptibility tests were performed in duplicates, with Fisher's exact test was performed to find the relationship between genotype and phenotype and odds ratios were calculated the confidence level of the test (* $p \leq 0.05$, ** $p \leq 0.01$, *** $p \leq 0.001$, **** $p \leq 0.0001$)