Infection and Drug Resistance

Dovepress
open access to scientific and medical research

Open Access Full Text Article

ORIGINAL RESEARCH

Molecular Epidemiological Characteristics of Carbapenem Resistant *Aeromonas* from Hospital Wastewater

Qianwen Zhang[1,*], Shengcen Zhang[1,*], Binbin Xu[1], Luyan Dong[1], Zhichang Zhao[2], Bin Li[1]

[1]Department of Clinical Laboratory, Fujian Medical University Union Hospital, Fujian, 350001, People's Republic of China; [2]Department of Pharmacy, Fujian Medical University Union Hospital, Fuzhou, Fujian, People's Republic of China

*These authors contributed equally to this work

Correspondence: Bin Li, Department of Clinical Laboratory, Fujian Medical University Union Hospital, Fujian, 350001, People's Republic of China, Email leonlee307@hotmail.com; Zhichang Zhao, Department of Pharmacy, Fujian Medical University Union Hospital, Fuzhou, Fujian, People's Republic of China, Email slip2046@126.com

Background: Hospital wastewater (HWW) promotes the spread of carbapenem resistance genes (CRGs). *Aeromonas* carry a large number of CRGs in HWW, they may play a role as a suitable reservoir for CRGs, while resistomes in HWW are still poorly characterized regarding carbapenem resistant *Aeromonas*. Thus, the aim of the study was to evaluate the molecular epidemiological characteristics of carbapenem resistant *Aeromonas* in HWW.

Methods: A total of 33 carbapenem resistant *Aeromonas* were isolated from HWW. Antimicrobial susceptibility testing and polymerase chain reaction (PCR) were used to assess the antimicrobial resistance profiles. Molecular typing was performed using enterobacterial repetitive intergenic consensus PCR (ERIC-PCR) and multilocus sequence typing (MLST). The horizontal transmission mode of bla_{KPC} was explored through conjugation and transformation experiments. The stability of bla_{KPC}-IncP-6 plasmids was assessed through plasmid stability and in vitro competition test. The PCR mapping method was used to investigate the structural diversity of bla_{KPC}.

Results: The detection rates of bla_{KPC} and *cphA* in *Aeromonas* were 97.0% and 39.4% respectively. *Aeromonas caviae* were grouped into 13 clusters by ERIC-PCR and 12 STs by MLST. *Aeromonas veronii* were grouped into 11 clusters by ERIC-PCR and 4 STs by MLST. 56.3% bla_{KPC} were located on mobilizable IncP-6 plasmids. bla_{KPC}-IncP-6 plasmid showed high stability and low cost fitness.

Conclusion: Carbapenem resistant *Aeromonas* from HWW mainly carried bla_{KPC}, which exhibited great structural diversity. *Aeromonas* might serve as reservoirs for bla_{KPC} and bla_{KPC} might spread mainly through transformation in HWW.

Keywords: hospital wastewater, carbapenem resistance, *Aeromonas*, IncP-6, bla_{KPC}

Introduction

Carbapenems were generally considered as last resort for treating infections from multi-resistant Gram-negative pathogenic bacteria.[1] But the widespread prevalence of carbapenem resistant bacteria (CRBs) has seriously limited the effectiveness of carbapenems. Carrying carbapenem resistance genes (CRGs) is an important mechanism of carbapenem resistance. CRGs are generally found on mobile plasmids, exhibiting strong dissemination capabilities.[2] Accordingly, effective control of CRG dissemination is critical to slow the spread of resistance.

Hospital wastewater (HWW) is considered significant reservoirs of CRGs. HWW connects human activities with the ecological environment, promoting the spread of clinically important CRGs such as bla_{NDM}, bla_{KPC}, and bla_{VIM} from clinical bacteria to environmental bacteria.[3] The slow flow rate of HWW and high-density bacterial communities promote the horizontal transfer of resistance genes within and between bacterial species. Meanwhile, a large amount of antimicrobial residues promotes the proliferation of antimicrobial resistant bacteria.[3] In addition to providing selective advantages, antimicrobial residues may induce the SOS-response and hence upregulate expression of conjugation

Received: 22 January 2024
Accepted: 22 May 2024
Published: 18 June 2024

associated genes and T4SS genes to enhance conjugative transfer.[4] Furthermore, antimicrobial residues could also promote gene transformation and transduction by increasing the fraction of competent cells and inducing prophage activity.[4] Hospital wastewater treatment plants could not eliminate antimicrobial resistant genes (ARGs) in wastewater and could even promote conjugations and transformations of ARGs.[5–7] Therefore, compared with other wastewater conditions, HWW may play a more integral role in the dissemination of CRGs.

Aeromonas is a widespread organism in aquatic environment and carry large numbers of CRGs.[8] Previous studies showed that *cphA* is prevalent in carbapenem resistant *Aeromonas*. Notably, more and more CRGs have been detected in *Aeromonas*, such as bla_{KPC} or bla_{NDM}.[9,10] Especially, plasmid-associated bla_{KPC-2} is becoming the most prevalent CRG in *Aeromonas* from wastewater. Given that *cphA* is located on the chromosome, the spread of plasmid-associated bla_{KPC-2} is even more concerning.[11,12] Bacteria of the *Aeromonas* genus only has a 4–5 Mbp genome, which is smaller than that of other Gram-negative bacteria. The core genome of *Aeromonas* comprises only 16% of the entire genome, with the remaining 84% consisting of variable represented genes, including plasmids, transposons, genomic islands or integron gene cassettes in order to compensate for its relatively small genome size. Thus, *Aeromonas* has a significant capacity to acquire various mobile genetic elements.[13,14] Considering the strong ability of *Aeromonas* to take-up exogenous DNA, it might play a significant role in the transmission of CRGs.

To date, the wastewater resistomes have been poorly characterized regarding carbapenem resistant *Aeromonas*. A previous study mainly focused on prevalence and antimicrobial resistance characteristics of carbapenem resistant *Aeromonas* in HWW.[15] The transmission characteristics of CRGs from carbapenem resistant *Aeromonas* remain relatively unexplored. Therefore, we deciphered the molecular epidemiological characteristics of carbapenem resistant *Aeromonas* and the potential transmission mechanism of bla_{KPC} in *Aeromonas*, helping to gain a more in-depth insight into the contribution of *Aeromonas* to CRGs dissemination and enhance the public consciousness about resistance-related risks.

Materials and Method

Aeromonas Isolates

The HWW samples were collected from the raw sewage influent of on-site HWW treatment plant in Fujian medical university union hospital (Fujian, China). A total of three HWW samples were taken from November 3 to November 25, 2020. Each HWW sample was independent and was cultured individually. The HWW samples were diluted and then plated on LB agar (Hopebio, China) containing Imipenem (IPM) at 2 μg/mL to select imipenem-non-susceptible Gram-negative bacteria at 35 °C. All imipenem-non-susceptible Gram-negative bacteria were cultured separately and identified through MALDI-TOF MS (Autobio, China). All isolates, identified as *Aeromonas*, were then reconfirmed by housekeeping gene sequencing (*gyrB*).

Antimicrobial Susceptibility Testing

Antimicrobial susceptibility testing was performed using the VITEK 2 Compact system (Vitek-AST-GN16) (bioMerieux, France) and the disk diffusion method (K-B method) (Oxoid, UK) according to the Clinical and Laboratory Standards Institute File.[16] The antimicrobials included Piperacillin/Tazobactam (TZP), Cefoxitin (FOX), Ceftriaxone (CRO), Cefepime (FEP), Aztreonam (ATM), Ertapenem (ETP), IPM, Amikacin (AN), Gentamicin (CN), Ciprofloxacin (CIP), Levofloxacin (LVX) and Trimethoprim/Sulfamethoxazole (SXT). *E.coli* ATCC 25922 was used as a quality control.

Detection of Carbapenemase Genes

All isolates were subjected to a polymerase chain reaction (PCR) for confirmation of carbapenemase genes, including bla_{KPC}, bla_{NDM}, bla_{VIM}, bla_{IMP}, bla_{OXA-48}, *cphA,* bla_{SME}, bla_{GIM}, bla_{IMI} and bla_{GES}. The sequences and product lengths of primers are summarized in Table S1. The positive products were confirmed by sequencing (Sangon Biotech, China).

Enterobacterial Repetitive Intergenic Consensus PCR (ERIC-PCR)

The sequences of primers and PCR procedures were described previously.[17] The results of ERIC-PCR were analyzed using Quantity One software (version 4.6.2). The phylogenetic tree was determined using the unweighted pair-group method with arithmetic means (UPGMA) clustering method and Dice similarity.

Multilocus Sequence Typing (MLST)

Six housekeeping genes (*gyrB, groL, gltA, metG, ppsA, recA*) were amplified using PCR. The sequences of primers and the PCR procedures were described previously.[18] The products were sent to Sangon Biotech for DNA sequencing, and the results were compared with known alleles in the MLST database. The new STs were deposited in the *Aeromonas* PubMLST database (http://pubmlst.org/*aeromonas*). The phylogenetic trees were inferred using the UPGMA clustering method with MEGA11 software.

Conjugation

Conjugation experiments were performed to test the transferability of bla_{KPC} using filter mating. Rifampin-resistant *E. coli* EC600 was used as the recipient. Donors and recipients were cultured to log-phase in LB broth (Hopebio, China), respectively. Donors and recipients were mixed in an initial ratio of 1:4 and then incubated on a 0.22 μM filter, which was placed on a Columbia blood agar plate, and left overnight at 35 °C. The next day, the mating mixture was washed from the filter and spread onto LB agar containing Rifampin at 500 μg/mL and IPM at 1 μg/mL. The transconjugants harboring bla_{KPC} were identified by PCR.

Transformation and Plasmid Replicon Types Analysis

Plasmid DNA was isolated using the alkaline lysis extraction procedure and introduced to competent *E.coli* DH5*α* (Weidibio, China) through chemical transformation. The transformants were selected on LB agar supplemented with IPM at 1 μg/mL. The transformants harboring bla_{KPC} were identified by PCR. Plasmids were classified according to their incompatibility group using the PCR-based replicon typing scheme (PBRT)[19] and the sequences of IncP-6 primers are listed in Table S2.

Plasmid Stability Assay and Fitness Assessment

The stability of plasmids was assessed as previously described with minor modifications.[20] Briefly, bla_{KPC}-carrying *E. coli* DH5*α* was inoculated into LB broth. Subcultures were diluted daily, and each dilution was plated on LB agar every 3 days. The plates were then incubated overnight at 35 °C. A total of 50 colonies were randomly collected from the LB agar and the presence of bla_{KPC} was identified using PCR.

The fitness costs of plasmids were evaluated through in vitro growth competition experiment. Plasmid-carrying *E. coli* DH5*α* (IPM-R) and plasmid-free *E. coli* DH5*α* (IPM-S) were cultured in an initial ratio of 1:1. The subcultures were then transferred to fresh LB broth. After 1 day, cultures were inoculated overnight on LB agar. Then colonies were collected from the LB agar and the presence of bla_{KPC} was identified by PCR. The fitness costs were calculated using the following equation:[21]

$$\mathrm{Fit} = 1 + \ln\left[\left(\frac{\frac{R}{S}}{\frac{R0}{S0}}\right)^{1/17}\right]$$

where Fit represents the fitness cost of the bla_{KPC}-carrying plasmid in the host *E. coli* DH5*α*; R denotes number of the bla_{KPC}-carrying *E. coli* DH5*α*; S represents the number of bla_{KPC}-free *E. coli* DH5*α*; R_0 indicates the initial number of bla_{KPC}-carrying *E. coli* DH5*α* in the competition experiments, and S_0 represents the initial number of bla_{KPC}-free *E.coli* DH5*α* in the competition experiments.

bla_{KPC} Genetic Region Analysis

The sequences and lengths of the primer products are summarized in Table S3. These primers were designed based on the sequence of the template plasmid p10265-KPC.[22] Plasmid DNA was extracted using the GeneJET Kit (Thermo

Scientific, US). PCR products were purified using the FastPure Gel DNA Extraction Mini Kit (Vazyme, China). Then, the purified DNA samples were digested using different enzymes. Fragment I, II and III were digested by HindIII (NEB, England). Fragment IV and V were digested by BamHI (NEB, England). Fragment VI was digested by HinfI (Takara, Japan). The grouping was based on the electrophoresis results of the six fragments' enzymatic digestion. The PCR products were sent to Sangon Biotech for Sanger sequencing, and the fragments were assembled using the CAP3 program in SnapGene software (version 5.2). One isolate from each type was sequenced and annotated using the RAST (Rapid Annotation using Subsystems Technology) annotation website to determine the bla_{KPC} region.

Statistical Analysis

GraphPad Prism software (version 8) was used for statistical analysis. Fisher's exact test was performed to analyze statistical significance. Only $p < 0.05$ was considered statistically significant.

Results

Prevalence of Carbapenem-Resistant *Aeromonas*

16 *Aeromonas caviae* and 17 *Aeromonas veronii* isolates from HWW were collected in this study. The percentage of carbapenem resistant *Aeromonas* was 26.83%. The percentage of other carbapenem resistant organisms detected have been listed in Table S4.

Antimicrobial Susceptibility Testing

16 *Aeromonas caviae* and 17 *Aeromonas veronii* isolates from HWW were collected in this study. *Aeromonas caviae* demonstrated high resistance to FOX (87.5%), CRO (100.0%), FEP (100.0%), ATM (100.0%), ETP (100.0%) and IPM (100.0%). The resistance to TZP (37.5%), AN (25.0%) and LVX (12.5%) was less than 40.0% in *Aeromonas caviae*. Meanwhile, *Aeromonas veronii* exhibited high resistance to CRO (100.0%), FEP (94.1%), ATM (88.2%), ETP (100.0%), IPM (94.1%), while showing low resistance to TZP (35.3%), AN (35.3%), CIP (29.4%) and SXT (35.3%). The Results are listed in Table 1.

Carbapenemase Genes Profiles

All 33 *Aeromonas* isolates in our study contained carbapenemase genes which included bla_{KPC} and *cphA* (Table 2). 16 *Aeromonas caviae* isolates carried bla_{KPC-2}. Among the 17 *Aeromonas veronii* isolates, 1 isolate carried *cphA*, 4 isolates carried bla_{KPC-2}, 4 isolates carried both *cphA* and bla_{KPC-24}, 8 isolates carried *cphA* and bla_{KPC-2} simultaneously.

Table 1 Antimicrobial Susceptibility Analysis of *Aeromonas caviae* and *Aeromonas veronii*

Drugs	*Aeromonas caviae* (n=16)			*Aeromonas veronii* (n=17)			*p*-value
	Susceptible	Intermediate	Resistant	Susceptible	Intermediate	Resistant	
TZP	-	62.5%	37.5%	5.9%	58.8%	35.3%	>0.05
FOX	12.5%	-	87.5%	5.9%	29.4%	64.7%	>0.05
CRO	-	-	100.0%	-	-	100.0%	-
FEP	-	-	100.0%	5.9%	-	94.1%	>0.05
ATM	-	-	100.0%	11.8%	-	88.2%	>0.05
ETP	-	-	100.0%	-	-	100.0%	-
IPM	-	-	100.0%	5.9%	-	94.1%	>0.05
AN	68.7%	6.3%	25.0%	41.2%	23.5%	35.3%	>0.05
CN	56.2%	-	43.8%	23.5%	17.6%	58.8%	>0.05
CIP	12.5%	31.3%	56.3%	47.1%	23.5%	29.4%	>0.05
LVX	56.2%	31.3%	12.5%	88.2%	11.8%	-	>0.05
SXT	43.7%	-	56.3%	64.7%	-	35.3%	>0.05

Table 2 The Frequency of Carbapenemase Genes Among *Aeromonas caviae* and *Aeromonas veronii*

Genes	Total population; n(%)	*Aeromonas caviae*; n(%)	*Aeromonas veronii* n(%)	*p*
bla_{KPC}	32 (97.0)	16 (100.0)	16 (94.1)	>0.05
cphA	13 (39.4)	–	13 (76.5)	<0.05

ERIC-PCR

The fingerprints of *Aeromonas* isolates consisted of various amplification bands, ranging in size from 100 to 5000 bp. The similarity coefficient of *Aeromonas caviae* ranged from 0.34 to 0.82 and that of *Aeromonas veronii* ranged from 0.19 to 0.94 (Figure 1). When the similarity coefficient was 0.70, *Aeromonas caviae* were divided into 13 clusters and *Aeromonas veronii* were divided into 11 clusters. The results of ERIC-PCR in this study divided *Aeromonas* into various clusters, confirming the high genetic diversity in *Aeromonas*.

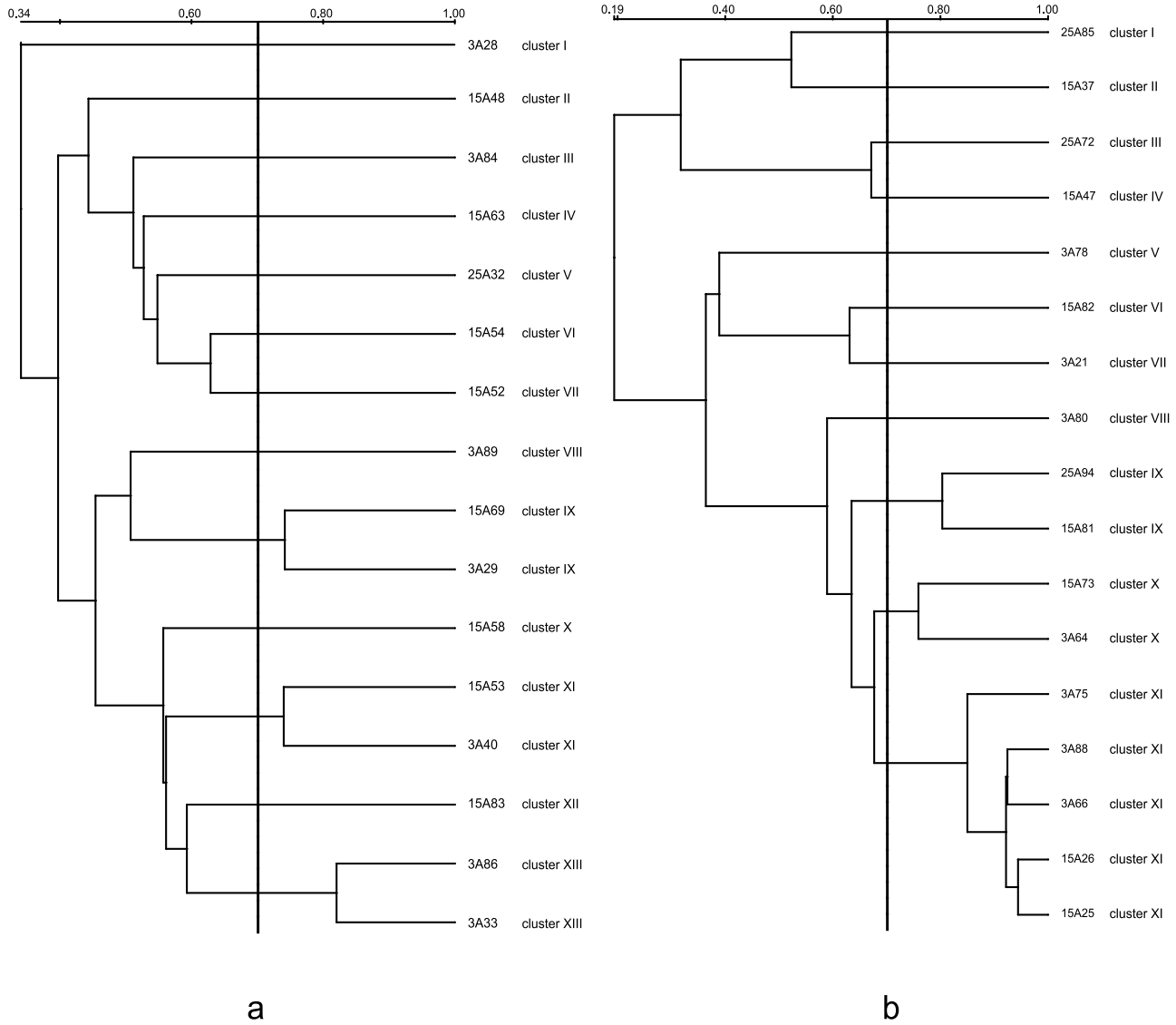

Figure 1 ERIC-PCR analysis of *Aeromonas caviae* (**a**) and *Aeromonas veronii* (**b**).

MLST

16 *Aeromonas caviae* isolates from HWW showed a high level of genetic diversity. 12 STs were identified in our study, including ST2170, ST2171, ST2379, ST2172, ST2173, ST2380, ST2174, ST2178, ST2179, ST2180, ST2181 and ST747. Additionally, 11 STs were novel founded (except ST747). ST2174 (3/16, 18.8%) was the most prevalent in *Aeromonas caviae*. Among 17 *Aeromonas veronii* isolates, 4 novel STs were identified, including ST2381, ST2175, ST2176 and ST2177. ST2176 (10/17, 58.8%) was the most prevalent ST in *Aeromonas veronii*. The evolutionary trees (Figure 2) could more directly illustrate the diversity among *Aeromonas caviae* and *Aeromonas veronii*. Notably, with the identification of 15 novel STs, this study also importantly contributed to the diversity in the MLST database of *Aeromonas*.

Conjugation, Transformation and Plasmid Replicon Types Analysis

None of 32 bla_{KPC}-positive *Aeromonas* isolates were able to transfer bla_{KPC} into *E.coli* EC600 successfully. A total of 18 bla_{KPC}-positive *Aeromonas* (56.3%) isolates could transfer bla_{KPC} into *E.coli* DH5*α* through chemical transformation successfully, which included 5 *Aeromonas caviae* and 13 *Aeromonas veronii* isolates. All of the replicon types of mobilizable bla_{KPC}-carrying plasmids were IncP-6.

Plasmid Stability Assay and Fitness Assessment

The plasmid stability assay showed bla_{KPC}-IncP-6 plasmids could be stably maintained in *E.coli* DH5*α* for 30 days without any loss. But the bla_{KPC}-IncFII plasmids were not as perfect as bla_{KPC}-IncP-6 plasmids, bla_{KPC}-IncFII-31 began to lose stability from day 12 and plasmid bla_{KPC}-IncFII-1008 began to lose stability from day 18. However, plasmid bla_{KPC}-IncFII still showed a high level of stability, the rate of bla_{KPC} remaining above 96.0%. The results of plasmid stability were shown in Figure 3.

The fitness assessment was shown by in vitro growth competition experiment. The relative fitness costs of bla_{KPC}-IncP-6 plasmids were 1.024, 1.018 and 0.981. The relative fitness costs of bla_{KPC}-IncFII plasmids were 0.962, 1.016 and 1.039. There was 1 isolate (33.3%) with IncP-6 plasmid and 1 isolate (33.3%) with IncFII plasmid that increased the fitness cost of the host in our study. The results of fitness assessment were listed in Table 3.

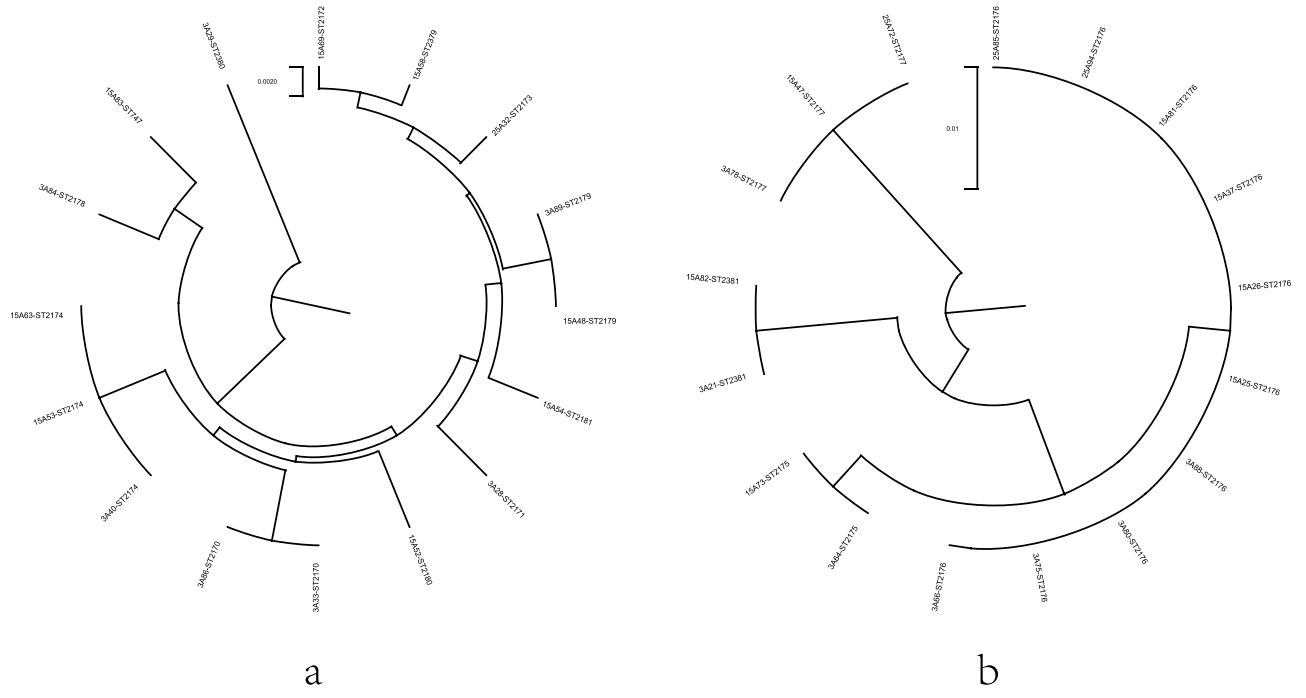

Figure 2 Phylogenetic tree analysis of *Aeromonas caviae* (**a**) and *Aeromonas veronii* (**b**).

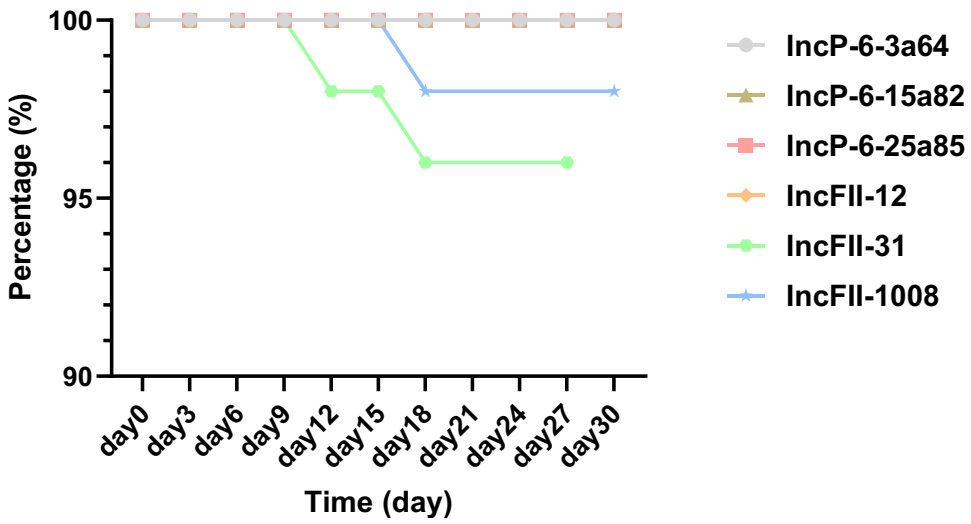

Figure 3 Results of plasmid stability for bla_{KPC}-IncP-6 and bla_{KPC}-IncFII in *E.coli* DH5*α*.

bla_{KPC} Genetic Region Analysis

In template plasmid p10265-KPC, from the genetic content we found that it had undergone two main evolutionary events from ΔIS*Kpn6*-bla_{KPC-2}-IS*Kpn27*: (i) the insertion of Δbla_{TEM-1} between IS*Kpn27* and Tn3, and (ii) the insertion of IS*Apu1*-ORF-IS*Apu2* into the Tn*3 tnpA* gene, disrupting the fragment into two separate parts.[22] The genetic region of bla_{KPC} might undergoing the events of gene acquisition and lost in our study. Figure 4 showed these types of bla_{KPC} genetic regions.

Type A1 has the same genetic region with the reference sequence p10265-KPC, as ΔIS*Kpn6*-bla_{KPC-2}-Δbla_{TEM-1}-IS*Kpn27*-tnpR-ΔtnpA-IS*Apu2*-ORF-IS*Apu1*-ΔtnpA. Among type A2, there was a transposase element IS*Kmi1*, which was inserted between the ORF and IS*Apu1*. There was a transposase element IS*As3* inserted between IS*Apu1* and ΔtnpA in type A3. The loss of Δbla_{TEM-1} was found in type A4 and A5. Deletion of downstream fragments of tnpR occurred in type B. Type C received bla_{KPC-24} instead of bla_{KPC-2} and experienced loss of the downstream fragments of ΔtnpA.

Discussion

Aeromonas is a widespread organism in aquatic environment and carry large numbers of CRGs, it might play a significant role in the transmission of CRGs. Previous studies showed that *cphA* and bla_{KPC} are prevalent in carbapenem resistant *Aeromonas*.[15,23] Notably, the most prevalent carbapenemase gene in *Aeromonas* from wastewater was the plasmid-associated bla_{KPC}.[15] In this study, 97.0% *Aeromonas* isolates carried bla_{KPC} indicating the potential transmission risk of bla_{KPC}. The high prevalence of *cphA* and bla_{KPC} suggests that carbapenem resistant *Aeromonas* may play a role in the transmission of carbapenem resistance. Although *Aeromonas* were not the predominant species, the high prevalence of CRGs and strong capacity to acquire various mobile genetic elements demonstrate the contribution of carbapenem resistant *Aeromonas* to CRGs' dissemination. Given that *cphA* is located on bacterial chromosomes, plasmid-associated bla_{KPC-2} might exhibit stronger dissemination capabilities.[24] The high prevalence and transmission risk of bla_{KPC} indicate that *Aeromonas* might serve as a potential reservoir for bla_{KPC} transmission.

Table 3 Different Types of bla_{KPC}-Carrying Plasmids and Their Fitness Cost Results

Plasmid	Inc group	Host	Relative Adaptive Fitness
p3a64	IncP-6	*E.coli* DH5*α*	1.024
p15a82	IncP-6	*E.coli* DH5*α*	1.018
p25a85	IncP-6	*E.coli* DH5*α*	0.981
p12	IncFII	*E.coli* DH5*α*	0.962
p31	IncFII	*E.coli* DH5*α*	1.016
p1008	IncFII	*E.coli* DH5*α*	1.039

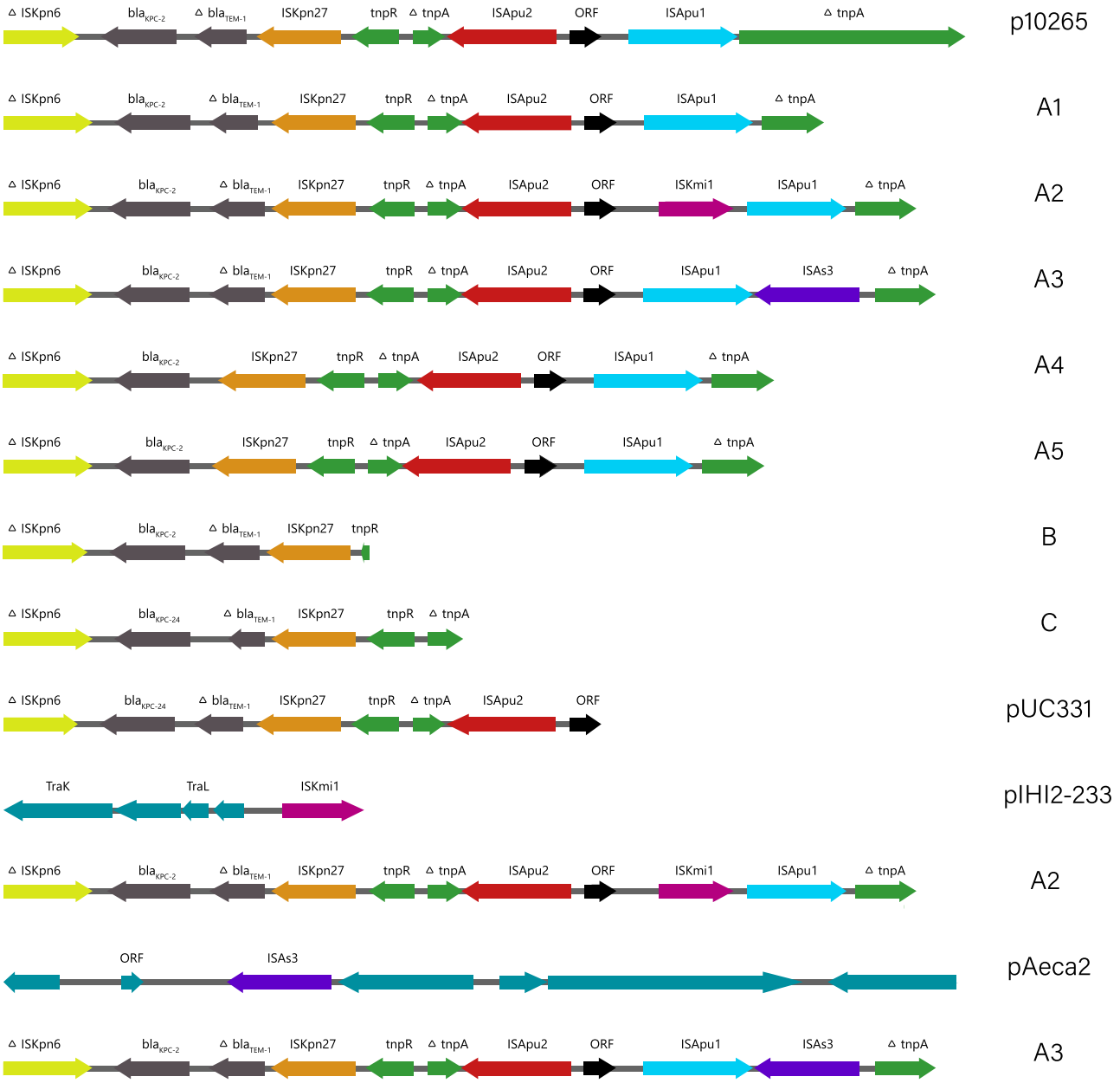

Figure 4 *bla*$_{KPC}$ regions from IncP-6 plasmids and comparison with the related plasmids.

In this study, we found that *bla*$_{KPC}$-carrying plasmids cannot be transferred through conjugation in *Aeromonas*, but can be transferred through transformation. All of the replicon types of mobilizable *bla*$_{KPC}$-carrying plasmids were IncP-6. The failed conjugation might be attributed to the lack of necessary genetic elements for conjugation in IncP-6 plasmids (such as the *traA* and *traB* operons).[25] Meanwhile, *Aeromonas* is equipped with the type VI secretion system which might be implicated in transformation.[13] Previous studies found that *bla*$_{KPC}$ cannot be transferred through conjugation in *Aeromonas* with or without the presence of self-transmissible plasmid.[25,26] Therefore, transformation might be an important mechanism for the transmission of *bla*$_{KPC}$ in *Aeromonas*.

To further investigate the transmission risk of *bla*$_{KPC}$, we analyzed the stability of *bla*$_{KPC}$-IncP-6 plasmids by plasmid stability and in vitro competition test. The *bla*$_{KPC}$-IncP-6 plasmids had a strong stability and low fitness cost in this study. The reason for the high stability of *bla*$_{KPC}$-IncP-6 plasmids might be due to them carrying some genetic elements (such as the ParA/B/C operon), which can maintain their existence in the host.[20] A previous study found that *bla*$_{KPC}$-IncP-6 plasmids were broad host range plasmids and present in diverse environmental bacterial species of opportunistic, indicating that it might be a suitable vector of

bla$_{KPC}$ in HWW.[9] The plasmid persistence depends on plasmid stability and their effect on the host fitness.[27] The high stability of IncP-6 plasmids is beneficial to the persistence of plasmids in host and the low fitness cost of IncP-6 plasmids could be an important advantage of carbapenem-resistant bacteria propagation. Therefore, the high stability of IncP-6 plasmids might promote the transmission of CRGs.

The wide spread of CRGs has posed a major public health threat. Previous research has suggested some solutions to control the spread of carbapenem resistance in clinic, such as antimicrobial restriction systems.[28] However, HWW, an important source of CRGs, had received increasing attention. HWW connects human activities with the ecological environment, promoting the spread of clinically important CRGs from clinical bacteria to environmental bacteria.[3] Compared to other wastewater environments, HWW may have a stronger promoting effect on ARGs' dispersion. Therefore, more emphasis should be placed on the contribution of HWW on the CRGs' dissemination and an efficient method of controlling the CRGs' dissemination is also urgently needed to relieve the current crisis of antimicrobial resistance.

There is a limitation to our study: whole genome sequencing was not applied in our study. Therefore, we have no idea about the genetic environment and molecular evolutionary relationships of carbapenem resistant *Aeromonas* in HWW, which might limit the exploration of contribution of *Aeromonas* in transmission of *bla*$_{KPC}$. In our further study, the phylogenomic analysis of carbapenem resistant Aeromonas will be further explored. Despite these limitations, this study deciphered the molecular epidemiological characteristics of carbapenem resistant *Aeromonas* and the potential transmission mechanism of *bla*$_{KPC}$ in *Aeromonas*, helping to gain a more in-depth insight into the contribution of *Aeromonas* to CRGs' dissemination and enhance public awareness about resistance-related risks.

Conclusions

Carbapenem resistant *Aeromonas* in HWW showed high genetic diversity. *Aeromonas* mainly carried *bla*$_{KPC}$, which exhibited structural diversity. Transformation might be an important mechanism for the transmission of *bla*$_{KPC}$ in *Aeromonas*. *Aeromonas* might serve as reservoirs for *bla*$_{KPC}$ and promote its spread in HWW.

Data Sharing Statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Author Contributions

All authors contributed to data analysis, drafting or revising the article, have agreed on the journal to which the article will be submitted, gave final approval of the version to be published, and agree to be accountable for all aspects of the work.

Funding

This work was supported by Fujian Research and Training Grants for Young and Middle-aged Leaders in Healthcare.

Disclosure

The 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.

References

1. Patel G, Bonomo RA. "Stormy waters ahead": global emergence of carbapenemases. *Front Microbiol*. 2013;4:48. doi:10.3389/fmicb.2013.00048
2. Nordmann P, Dortet L, Poirel L. Carbapenem resistance in *Enterobacteriaceae*: here is the storm! *Trends Mol Med*. 2012;18(5):263–272. doi:10.1016/j.molmed.2012.03.003
3. Zhu L, Shuai XY, Lin ZJ, et al. Landscape of genes in hospital wastewater breaking through the defense line of last-resort antibiotics. *Water Res*. 2022;209:117907. doi:10.1016/j.watres.2021.117907
4. Lopatkin AJ, Sysoeva TA, You L. Dissecting the effects of antibiotics on horizontal gene transfer: analysis suggests a critical role of selection dynamics. *BioEssays*. 2016;38(12):1283–1292. doi:10.1002/bies.201600133
5. Zhang S, Xu B, Chen M, et al. Profile and actual transmissibility of Carbapenem resistance genes: intracellular and extracellular DNA in hospital wastewater. *J Environ Manage*. 2023;329:117085. doi:10.1016/j.jenvman.2022.117085
6. Wang Z, Fu L, Gu JD, Deng S, Huang C, Luo L. The factors controlling antibiotic resistance genes in different treatment processes of mainstream full-scale wastewater treatment plants. *Sci Total Environ*. 2023;900:165815. doi:10.1016/j.scitotenv.2023.165815

7. Jin M, Liu L, Wang DN, et al. Chlorine disinfection promotes the exchange of antibiotic resistance genes across bacterial genera by natural transformation. *ISME J*. 2020;14(7):1847–1856. doi:10.1038/s41396-020-0656-9
8. Conte D, Palmeiro JK, Bavaroski AA, et al. Antimicrobial resistance in *Aeromonas* species isolated from aquatic environments in Brazil. *J Appl Microbiol*. 2021;131(1):169–181. doi:10.1111/jam.14965
9. Yao Y, Lazaro-Perona F, Falgenhauer L, et al. Insights into a Novel $bla_{(KPC-2)}$-encoding IncP-6 plasmid reveal carbapenem-resistance circulation in several *Enterobacteriaceae* species from wastewater and a hospital source in Spain. *Front Microbiol*. 2017;8:1143. doi:10.3389/fmicb.2017.01143
10. Luo X, Mu K, Zhao Y, et al. Emergence of $bla_{(NDM-1)}$-Carrying *Aeromonas caviae* K433 isolated from patient with community-acquired pneumonia. *Front Microbiol*. 2022;13:825389. doi:10.3389/fmicb.2022.825389
11. Wu CJ, Chen PL, Wu JJ, et al. Distribution and phenotypic and genotypic detection of a metallo-β-lactamase, CphA, among bacteraemic *Aeromonas* isolates. *J Med Microbiol*. 2012;61(Pt 5):712–719. doi:10.1099/jmm.0.038323-0
12. Sinclair HA, Heney C, Sidjabat HE, et al. Genotypic and phenotypic identification of *Aeromonas* species and CphA-mediated carbapenem resistance in Queensland, Australia. *Diagn Microbiol Infect Dis*. 2016;85(1):98–101. doi:10.1016/j.diagmicrobio.2016.02.005
13. Lamy B, Baron S, Barraud O. *Aeromonas*: the multifaceted middleman in the One Health world. *Curr Opin Microbiol*. 2022;65:24–32. doi:10.1016/j.mib.2021.09.012
14. Huddleston JR, Brokaw JM, Zak JC, Jeter RM. Natural transformation as a mechanism of horizontal gene transfer among environmental *Aeromonas* species. *Syst Appl Microbiol*. 2013;36(4):224–234. doi:10.1016/j.syapm.2013.01.004
15. Drk S, Puljko A, Dželalija M, Udiković-Kolić N. Characterization of third generation cephalosporin- and carbapenem-resistant *Aeromonas* isolates from municipal and hospital wastewater. *Antibiotics*. 2023;12(3):513. doi:10.3390/antibiotics12030513
16. CLSI. Methods for antimicrobial dilution and disc susceptibility testing of infrequently isolated or fastidious bacteria. In: *CLSI Guideline M45*. Wayne, PA: Clinical and Laboratory Standards Institute; 2016.
17. Szczuka E, Kaznowski A. Typing of clinical and environmental *Aeromonas* sp. strains by random amplified polymorphic DNA PCR, repetitive extragenic palindromic PCR, and enterobacterial repetitive intergenic consensus sequence PCR. *J Clin Microbiol*. 2004;42(1):220–228. doi:10.1128/jcm.42.1.220-228.2004
18. Martino ME, Fasolato L, Montemurro F, et al. Determination of microbial diversity of *Aeromonas* strains on the basis of multilocus sequence typing, phenotype, and presence of putative virulence genes. *Appl Environ Microbiol*. 2011;77(14):4986–5000. doi:10.1128/AEM.00708-11
19. Carattoli A, Bertini A, Villa L, Falbo V, Hopkins KL, Threlfall EJ. Identification of plasmids by PCR-based replicon typing. *J Microbiol Methods*. 2005;63(3):219–228. doi:10.1016/j.mimet.2005.03.018
20. Rahube TO, Viana LS, Koraimann G, Yost CK. Characterization and comparative analysis of antibiotic resistance plasmids isolated from a wastewater treatment plant. *Front Microbiol*. 2014;5:5. doi:10.3389/fmicb.2014.00558
21. Li S, Yin Y, Chen H, Wang Q, Wang X, Wang H. Fitness cost of daptomycin-resistant *Staphylococcus aureus* obtained from in vitro daptomycin selection pressure. *Front Microbiol*. 2017;8:2199. doi:10.3389/fmicb.2017.02199
22. Dai X, Zhou D, Xiong W, et al. The IncP-6 Plasmid p10265-KPC from *Pseudomonas aeruginosa* Carries a Novel ΔIS*Ec33*-Associated bla_{KPC-2} Gene Cluster. *Front Microbiol*. 2016;7:310. doi:10.3389/fmicb.2016.00310
23. Wang Y, Liu H, Zhang L, Sun B. Application of modified carbapenem inactivation method and its derivative tests for the detection of carbapenemase-producing *Aeromonas*. *Infect Drug Resist*. 2021;14:3949–3960. doi:10.2147/idr.S330115
24. Adams RJ, Mathys DA, Mollenkopf DF, Whittle A, Daniels JB, Wittum TE. Carbapenemase-producing *aeromonas veronii* disseminated in the environment of an equine specialty hospital. *Vector Borne Zoonot Dis*. 2017;17(6):439–442. doi:10.1089/vbz.2016.2083
25. Zhu Z, Wu S, Zhu J, et al. Emergence of *Aeromonas veronii* strain co-harboring $bla_{(KPC-2)}$, *mcr-3.17*, and *tmexC3.2-tmexD3.3-toprJ1b* cluster from hospital sewage in China. *Front Microbiol*. 2023;14:1115740. doi:10.3389/fmicb.2023.1115740
26. Hu X, Yu X, Shang Y, et al. Emergence and Characterization of a Novel IncP-6 Plasmid Harboring $bla_{(KPC-2)}$ and *qnrS2* genes in *Aeromonas taiwanensis* Isolates. *Front Microbiol*. 2019;10:2132. doi:10.3389/fmicb.2019.02132
27. Wein T, Hülter NF, Mizrahi I, Dagan T. Emergence of plasmid stability under non-selective conditions maintains antibiotic resistance. *Nat Commun*. 2019;10(1):2595. doi:10.1038/s41467-019-10600-7
28. Wanla W, Katip W, Supakul S, Apiwatnakorn P, Khamsarn S. Effects of an antimicrobial restriction system on appropriate carbapenem use in a hospital without infectious diseases consultation. *Int J Gene Med*. 2017;10:443–449. doi:10.2147/ijgm.S145133