



# Emergence and Characterization of a Novel IncP-6 Plasmid Harboring *bla*<sub>KPC-2</sub> and *qnrS2* Genes in *Aeromonas taiwanensis* Isolates

Xinjun Hu<sup>1,2†</sup>, Xiao Yu<sup>2†</sup>, Yibing Shang<sup>1</sup>, Hao Xu<sup>2</sup>, Lihua Guo<sup>2</sup>, Yile Liang<sup>1</sup>, Yixin Kang<sup>1</sup>, Li Song<sup>1</sup>, Jifeng Sun<sup>1</sup>, Feng Yue<sup>1</sup>, Yimin Mao<sup>3\*</sup> and Beiwen Zheng<sup>2\*</sup>

<sup>1</sup> Department of Infectious Diseases, The First Affiliated Hospital, College of Clinical Medicine, Henan University of Science and Technology, Luoyang, China, <sup>2</sup> Collaborative Innovation Center for Diagnosis and Treatment of Infectious Diseases, State Key Laboratory for Diagnosis and Treatment of Infectious Diseases, The First Affiliated Hospital, College of Medicine, Zhejiang University, Hangzhou, China, <sup>3</sup> Department of Respiratory Medicine, The First Affiliated Hospital, College of Clinical Medicine, Henan University of Science and Technology, Luoyang, China

## OPEN ACCESS

### Edited by:

Ziad Daoud,  
University of Balamand, Lebanon

### Reviewed by:

Roberto Gustavo Melano,  
Public Health Ontario, Canada  
Christopher Morton Thomas,  
University of Birmingham,  
United Kingdom

### \*Correspondence:

Yimin Mao  
yimin6107@sina.com  
Beiwen Zheng  
zhengbw@zju.edu.cn

†These authors have contributed  
equally to this work

### Specialty section:

This article was submitted to  
Antimicrobials, Resistance  
and Chemotherapy,  
a section of the journal  
Frontiers in Microbiology

Received: 05 May 2019

Accepted: 30 August 2019

Published: 12 September 2019

### Citation:

Hu X, Yu X, Shang Y, Xu H, Guo L,  
Liang Y, Kang Y, Song L, Sun J, Yue F,  
Mao Y and Zheng B (2019)  
Emergence and Characterization of a  
Novel IncP-6 Plasmid Harboring  
*bla*<sub>KPC-2</sub> and *qnrS2* Genes  
in *Aeromonas taiwanensis* Isolates.  
*Front. Microbiol.* 10:2132.  
doi: 10.3389/fmicb.2019.02132

The dissemination of *Klebsiella pneumoniae* carbapenemases (KPCs) among Gram-negative bacteria is an important threat to global health. However, KPC-producing bacteria from environmental samples are rarely reported. This study aimed to elucidate the underlying resistance mechanisms of three carbapenem-resistant *Aeromonas taiwanensis* isolates recovered from river sediment samples. Pulsed-field gel electrophoresis (PFGE) and whole genome sequencing (WGS) analysis indicated a close evolutionary relationship among *A. taiwanensis* isolates. S1-PFGE, Southern blot and conjugation assays confirmed the presence of *bla*<sub>KPC-2</sub> and *qnrS2* genes on a non-conjugative plasmid in these isolates. Plasmid analysis further showed that pKPC-1713 is an IncP-6 plasmid with a length of 53,205 bp, which can be transformed into DH5 $\alpha$  strain and mediated carbapenems and quinolones resistance. The plasmid backbone of p1713-KPC demonstrated 99% sequence identity to that of IncP-6-type plasmid pKPC-cd17 from *Aeromonas* spp. and IncP-6-type plasmid: 1 from *Citrobacter freundii* at 74% coverage. A 14,808 bp insertion sequence was observed between *merT* gene and hypothetical protein in p1713-KPC, which include the quinolone resistance *qnrS2* gene. Emergence of plasmid-borne *bla*<sub>KPC</sub> and *qnrS2* genes from *A. taiwanensis* isolates highlights their possible dissemination into the environment. Therefore, potential detection of such plasmids from clinical isolates should be closely monitored.

**Keywords:** *Aeromonas taiwanensis*, *bla*<sub>KPC-2</sub>, *qnrS2*, whole-genome sequencing, plasmid analysis

## INTRODUCTION

The global spread of *Klebsiella pneumoniae* carbapenemases (KPCs) among Gram-negative bacteria, has become a major public health concern in recent decades (Grundmann et al., 2017). Infections caused by KPC-producing bacteria have been associated with a high mortality rate of 53%, which presents tremendous challenges for clinicians and healthcare providers (Munoz-Price et al., 2013). Although most KPCs are commonly found in *Enterobacteriaceae*, the production

of KPCs appeared to be less dominance in *Aeromonadaceae* spp. (Picao et al., 2013; Montezzi et al., 2015; Lamba and Ahammad, 2017; Paschoal et al., 2017; Tuo et al., 2018; Xu et al., 2018; Zheng et al., 2018b). The emergence of *bla*<sub>KPC</sub> gene on broad-host-range plasmids has facilitated its rapid dissemination to *Enterobacteriaceae* and other Gram-negative families (van Duin and Doi, 2017). More importantly, KPC-type carbapenemases are often associated with quinolone resistance determinants, which can be modulated by *qnrS* gene (Montezzi et al., 2015). The quinolone resistance gene, *qnrS2*, shares 92% identity with the *qnrS* gene, was first detected on a highly mobile plasmid that is isolated from wastewater treatment plant bacterial population (Bonemann et al., 2006). Thus far, the co-existence of *bla*<sub>KPC</sub> and *qnrS2* in the same plasmid has not been reported.

The objective of the present study was to identify plasmid-borne *bla*<sub>KPC-2</sub> genes in *Aeromonas taiwanensis* from river sediments in China and to analyze the plasmids carrying them. Three strains were isolated and found to harbor IncP-6 plasmids carrying both *bla*<sub>KPC-2</sub> and *qnrS2*. We characterized the phenotypic and molecular features of these strains in order to assess their genomic epidemiology profiles. Additionally, the complete nucleotide sequence of p1713-KPC was determined by plasmid sequencing. To the best of our knowledge, this is the first study to indicate the co-occurrence of *bla*<sub>KPC-2</sub> and *qnrS2* genes in the same plasmid and this plasmid harboring these two gene were founded in *A. taiwanensis* isolates for the first time.

## MATERIALS AND METHODS

### Bacterial Isolation and Pulsed-Field Gel Electrophoresis (PFGE)

The KPC-2-producing *A. taiwanensis* isolates were previously identified from river sediment samples from China (Xu et al., 2018). A total of three *A. taiwanensis* isolates were further characterized to uncover the underlying resistance mechanisms. The genomic diversity of *A. taiwanensis* isolates was assessed by *Xba*I-pulsed-field gel electrophoresis (PFGE) as described previously (Zheng et al., 2016). A dendrogram of PFGE profiles was constructed with BioNumerics v7.6 by using UPGMA (unweighted pair group method with averages) clustering. Isolates with a similarity cut-off of  $\geq 80\%$  were considered as pulsotypes.

### Whole-Genome Sequencing (WGS) and Assembly

To characterize the genomic features and resistome of *A. taiwanensis* isolates, WGS was conducted on all the three isolates. Genomic DNA was extracted and sequenced with HiSeq X Ten sequencing platform (Illumina, San Diego, CA, United States). Subsequently, *de novo* genome assembly and bioinformatics analysis were carried out as previously described (Xu et al., 2018; Zheng et al., 2018a). After obtaining the raw reads, the genome sequence of pKPC-1713 was assembled using plasmidSPAdes (Antipov et al., 2016).

## Phylogenetic Reconstruction and Analysis

The pan-genome analysis was performed with Roary: the pangenome pipeline (version 3.6.0) using the Prokka annotation (Seemann, 2014; Page et al., 2015). The complete genome sequences of all 20 *Aeromonas* spp. strains were downloaded from NCBI genome database (current as of March 30, 2019). Roary software was used to cluster the above genomes, The software clustered the genomes based on the genes carried by each strain. According to the distribution of each gene among the strains, the genes were divided into core genes and accessory genes. Core genes were defined as genes carried by 95% or more of the strains. Accessory genes were those carried by less than 95% of the strains. Then the core genome of these strains is obtained. A whole-genome phylogenetic tree was built from the core-genome SNPs of *Aeromonas* spp. and the three studied isolates. Phylogenetic reconstruction and analysis was performed using the R package phangorn (Schliep, 2011).

## Plasmid Characterization

The size of plasmid was determined by S1-nuclease PFGE analysis, as previously described (Zheng et al., 2015). Southern blot hybridization of plasmid DNA was performed on *Aeromonas hydrophila* isolates by using DIG-labeled *bla*<sub>KPC</sub>- and *qnrS*-specific probes, according to manufacturer's instructions (Roche Diagnostics, Germany) (Zheng et al., 2016). Plasmid conjugation experiments were carried out by filter mating using *Escherichia coli* J53 and EC600 as recipient strains, at a ratio of 1:1 in broth culture (Zheng et al., 2015). The pKPC-1713 plasmid was transferred into chemically competent *E. coli* DH5 $\alpha$  cells via transformation process. The transformants were selected on Luria-Bertani agar plates supplemented with 2 mg/L meropenem. The presence of *bla*<sub>KPC</sub> and *qnrS2* genes was then screened by PCR and sequencing. Plasmid DNA was extracted from conjugants with Qiagen Plasmid Midi Kit (Qiagen, Valencia, CA). To obtain the complete sequence of the plasmid co-expressing *bla*<sub>KPC</sub> and *qnrS2*, the combined application of PCR walking method with targeted primers (Supplementary Table S1) and Illumina sequencing technique were performed to specifically investigate the representative plasmid pKPC-1713. The RAST annotation pipeline was chosen to perform rapid annotation of the plasmids (Overbeek et al., 2014). Plasmid replicons and antibiotic resistance genes were identified using CGE server<sup>1</sup>. The sequence of pKPC-1713 was BLAST for homology against the NCBI plasmids database.

## Susceptibility Testing

The isolated *A. taiwanensis* 1713, *E. coli* DH5 $\alpha$  and DH5 $\alpha$ : pKPC-1713 were cultured overnight, while *E. coli* ATCC 25922 was used as a quality control. Minimum inhibitory concentrations (MICs) of amikacin, ampicillin, ampicillin-sulbactam, aztreonam, cefazolin, ceftazidime, cefotetan, ceftriaxone, ciprofloxacin, ertapenem, gentamicin, imipenem, levofloxacin, nitrofurantoin, piperacillin-tazobactam, tobramycin, and trimethoprim

<sup>1</sup><https://cge.cbs.dtu.dk/services/>

antibiotics were determined by VITEK 2 system with AST-GN16 panel. The results of antimicrobial susceptibility testing were interpreted according to 2017's CLSI guidelines.

## Plasmid Mobilization

Plasmid conjugation was performed as previously described with some modification (Yi et al., 2012). *A. taiwanensis* isolates were electrotransformed with a plasmid pEC1002-MCR(CP021205) which contains a *tra* module. Isolates containing the resident and pEC1002-MCR plasmids were used as donor strains. Plasmid conjugative transfer was performed by using donor and recipient sodium azide resistant *E. coli* J53 cells mixed in a 1:1 ratio as described previously. Transconjugants were selected on MacConkey agar containing 2 mg/L imipenem and 100 mg/L sodium azide. Transconjugants were confirmed by susceptibility testing and PCR.

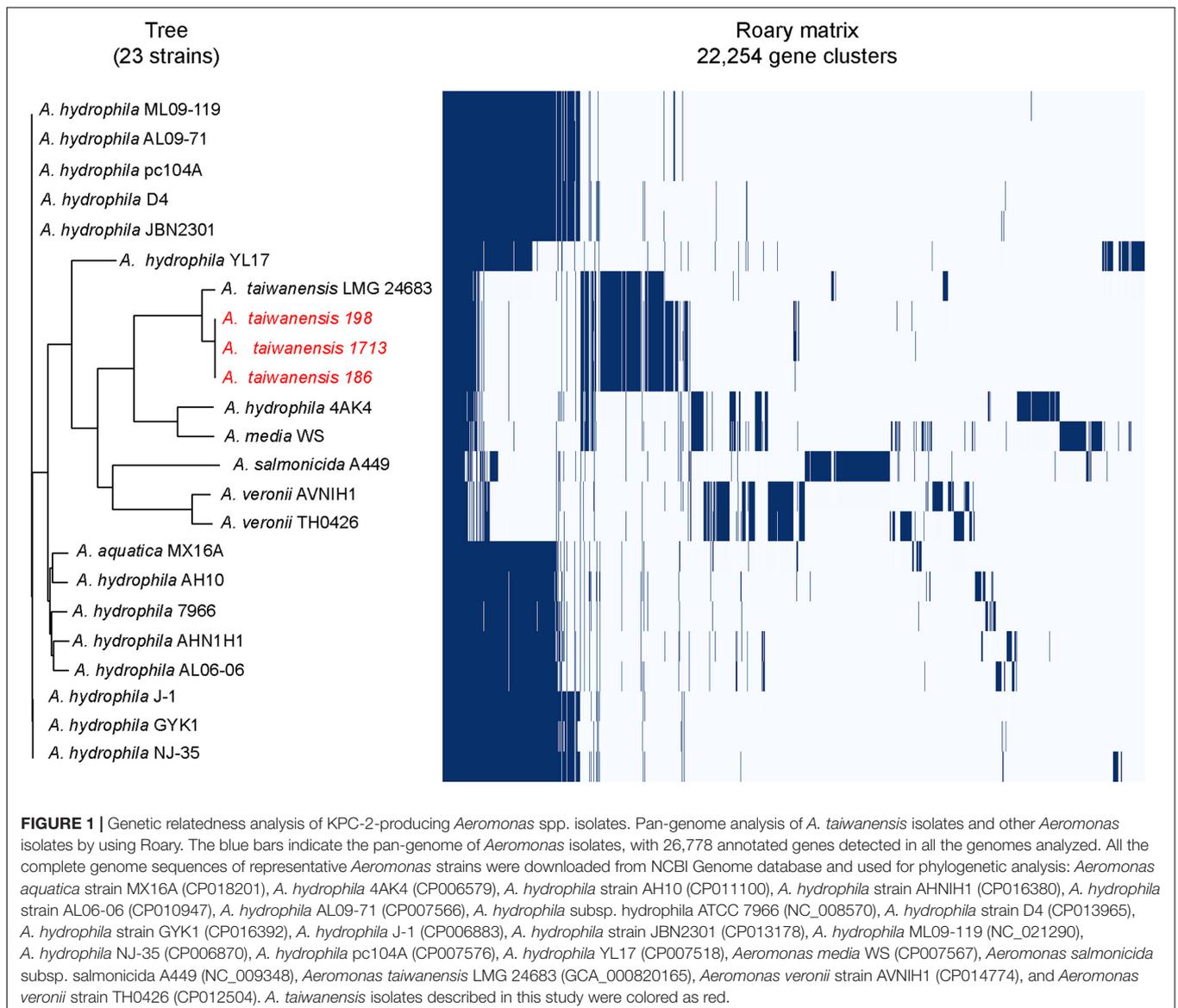
## Accession Numbers

The whole genome shotgun project of the *A. taiwanensis* isolates has been deposited into DDBJ/EMBL/GenBank under the Bioproject accession number PRJNA478520. The nucleotide sequence of plasmid p1713-KPC has been deposited into the GenBank database under GenBank accession number MH624132.

## RESULTS AND DISCUSSION

### Comparative Genomic Analysis of Carbapenem-Resistant *A. taiwanensis* Isolates

Pulsed-field gel electrophoresis analysis revealed that two pulsotypes from *A. taiwanensis*, namely 198 and 186, exhibited



**FIGURE 1 |** Genetic relatedness analysis of KPC-2-producing *Aeromonas* spp. isolates. Pan-genome analysis of *A. taiwanensis* isolates and other *Aeromonas* isolates by using Roary. The blue bars indicate the pan-genome of *Aeromonas* isolates, with 26,778 annotated genes detected in all the genomes analyzed. All the complete genome sequences of representative *Aeromonas* strains were downloaded from NCBI Genome database and used for phylogenetic analysis: *Aeromonas aquatica* strain MX16A (CP018201), *A. hydrophila* 4AK4 (CP006579), *A. hydrophila* strain AH10 (CP011100), *A. hydrophila* strain AHN1H1 (CP016380), *A. hydrophila* strain AL06-06 (CP010947), *A. hydrophila* AL09-71 (CP007566), *A. hydrophila* subsp. *hydrophila* ATCC 7966 (NC\_008570), *A. hydrophila* strain D4 (CP013965), *A. hydrophila* strain GYK1 (CP016392), *A. hydrophila* J-1 (CP006883), *A. hydrophila* strain JBN2301 (CP013178), *A. hydrophila* ML09-119 (NC\_021290), *A. hydrophila* NJ-35 (CP006870), *A. hydrophila* pc104A (CP007576), *A. hydrophila* YL17 (CP007518), *Aeromonas media* WS (CP007567), *Aeromonas salmonicida* subsp. *salmonicida* A449 (NC\_009348), *Aeromonas taiwanensis* LMG 24683 (GCA\_000820165), *Aeromonas veronii* strain AVNIH1 (CP014774), and *Aeromonas veronii* strain TH0426 (CP012504). *A. taiwanensis* isolates described in this study were colored as red.

relatively identical PFGE patterns (**Supplementary Figure S1**). Meanwhile, Roary matrix-based gene sequence analysis generated a large pan-genome matrix of 26,778 gene clusters across 23 genomes (**Figure 1**). Moreover, the 186, 198, and 1713 isolates were found to be genetically closely related by Roary matrix-based gene sequence analysis (**Figure 1**). These findings are consistent with the results of PFGE profiles, suggesting a possible clonal spread of KPC-producing *A. taiwanensis*. So far, only few reports described the detection of plasmid-mediated *bla*<sub>KPC</sub> determinants in *Aeromonadaceae* (Picao et al., 2013; Montezzi et al., 2015). Notably, the presence of *bla*<sub>KPC</sub> in *Aeromonadaceae* considered predominantly environmental is remarkable, and the spread of KPC-producing *A. taiwanensis* in aquatic environments deserves attention.

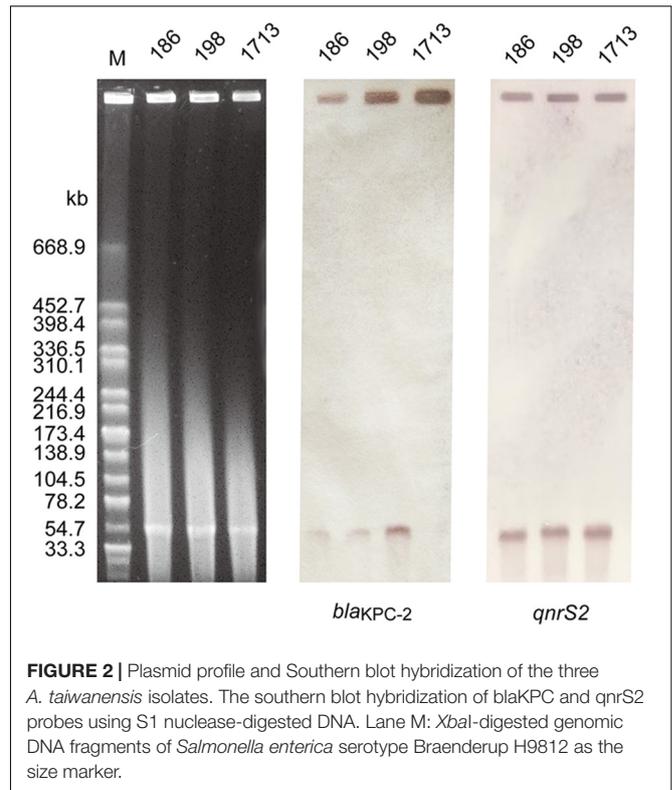
## Identification of the Plasmid Harboring Both *bla*<sub>KPC-2</sub> and *qnrS2* Genes

S1-PFGE and Southern blot analysis demonstrated that all *A. taiwanensis* isolates contained a ~54 kb plasmid, harboring both *bla*<sub>KPC-2</sub> and *qnrS2* genes (**Figure 2**). Moreover, nearly identical plasmid sequences of the three isolates were assembled from WGS data by SPAdes (data not shown), suggesting that these isolates shared the same plasmid profile. Notably, none of the plasmids could be transferred to recipient strains via conjugation, despite repeated attempts, which suggests that *bla*<sub>KPC-2</sub> and *qnrS2* are located on a non-conjugative plasmid. Occasionally, *qnrS2* has been identified in the plasmids of *Aeromonas* spp. (Arias et al., 2010; Marti and Balcazar, 2012; Marti et al., 2014; Yang et al., 2017); however, the co-occurrence of *bla*<sub>KPC</sub> and *qnrS2* genes in the same plasmid has never been described before. To our knowledge, this is the first study to indicate the co-occurrence of *bla*<sub>KPC</sub> and *qnrS2* genes in the same plasmid. As a consequence, we selected a representative sample of 1713 isolate for further plasmid characterization.

Plasmid transformation and MICs determination revealed that carbapenem and quinolone resistant genes were successfully transferred from a donor strain to *E. coli* DH5 $\alpha$ . Antibiotic susceptibility testing showed that the MIC values of ertapenem, imipenem, ciprofloxacin, and levofloxacin were increased from 0.125 to 8 mg/L, 0.125 to 16 mg/L, 0.25 to 8 mg/L, and 1 to 4 mg/L, respectively (**Table 1**). These results confirmed that carbapenem and quinolone resistance were successfully transferred to recipient cells.

## Complete Sequence of pKPC-1713 Plasmid

Sequence analysis of representative pKPC-1713 plasmid revealed the total size of 53,205 bp with an average G + C content of 58% and 66 open reading frames. The plasmid pKPC-1713 harbored five functional regions of genes, including *bla*<sub>KPC-2</sub> encoding region, IS5045 elements, an insertion region, genes involved in plasmid maintenance, and plasmid replication (**Figure 3**). The search against nr/nt database revealed a 99% identity with *Aeromonas* sp. ASNIH3 IncP-6-type plasmid pKPC-cd17 at 74% coverage, and a 99% identity with *Citrobacter freundii* IncP-6-type plasmid: 1 at 74% coverage (**Figure 3**).



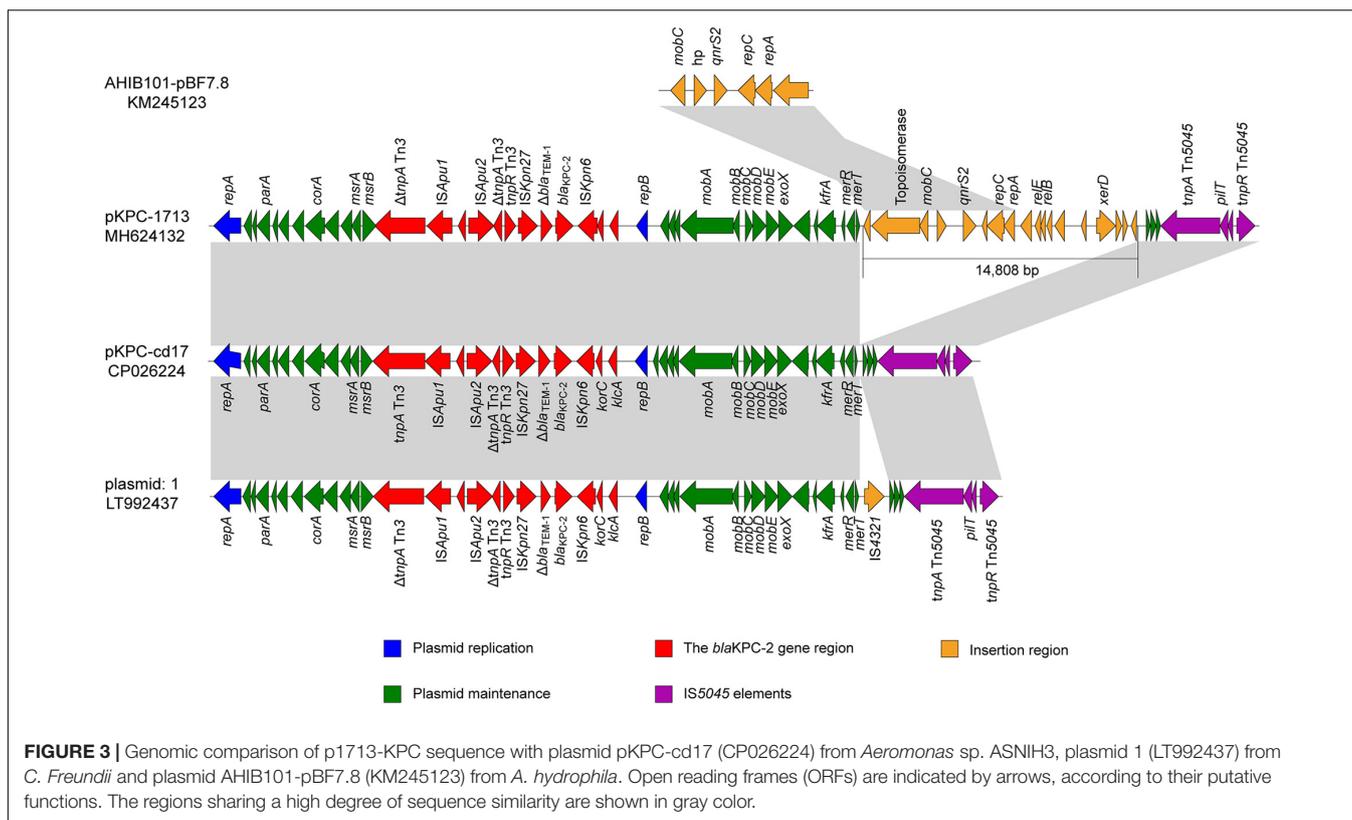
**FIGURE 2 |** Plasmid profile and Southern blot hybridization of the three *A. taiwanensis* isolates. The southern blot hybridization of *bla*<sub>KPC</sub> and *qnrS2* probes using S1 nuclease-digested DNA. Lane M: *Xba*I-digested genomic DNA fragments of *Salmonella enterica* serotype Braenderup H9812 as the size marker.

**TABLE 1 |** Characteristics of *A. taiwanensis* 1713 and its transformant strain<sup>a</sup>.

Antibiotics	<i>A. taiwanensis</i> 1713	DH5 $\alpha$ : pKPC-1713	<i>E. coli</i> E DH5 $\alpha$
Ampicillin	≥128/R	≥128/R	1/S
Amoxicillin/clavulanate	≥128/R	64/R	1/S
Piperacillin/tazobactam	≥128/R	≥128/R	1/S
Cefazolin	≥128/R	≥128/R	1/S
Ceftriaxone	≥128/R	≥128/R	0.125/S
Ceftazidime	16/R	16/R	0.25/S
Ceftaxitin	≥128/R	≥128/R	0.25/S
Aztreonam	≥128/R	≥128/R	0.25/S
Ertapenem	8/R	8/R	0.125/S
Imipenem	16/R	16/R	0.125/S
Ciprofloxacin	8/R	8/R	0.25/S
Levofloxacin	4/I	4/I	1/S
Amikacin	1/S	0.25/S	0.25/S
Gentamicin	0.5/S	0.25/S	0.25/S
Tobramycin	8/I	0.25/S	0.25/S
Tigecycline	1/S	0.25/S	0.125/S
Nitrofurantoin	16/S	16/S	16/S
Trimethoprim/sulfamethoxazole	≥320/R	20/S	20/S

<sup>a</sup>MIC for antimicrobial drugs tested, mg/L. The MICs were interpreted according to the CLSI guidelines.

In contrast to pKPC-cd17, an insertion of 14,808 bp DNA fragment and IS4321 gene were observed between *merT* gene and hypothetical protein in p1713-KPC and plasmid: 1, respectively. The insertion sequence of p1713-KPC harbored 18 different



genes, which include the quinolone resistance *qnrS2* gene. Interestingly, BLAST search of this insertion sequence revealed a 99% identity with *A. hydrophila* IB101 plasmid AHIB101-pBF7.8 at 100% coverage (Figure 3). We speculate that p1713-KPC has been formed by an IncP-6 plasmid fusing with the *qnrS* plasmid after one of them has acquired extra DNA with *xerD* etc. Furthermore, *bla*<sub>KPC-2</sub> gene was found to be located on a 14.5 kb Tn3 transposon element in p1713-KPC with the linear structure:  $\Delta trpA$  Tn3-ISApu1-ISApu2- $\Delta trpA$ -Tn3-*trpR* Tn3-ISKpn27- $\Delta bla_{TEM-1}$ -*bla*<sub>KPC-2</sub>- $\Delta ISKpn6$  (Figure 3). Previous studies of *bla*<sub>KPC-2</sub> have indicated its association with Tn3-based transposon and responsible for its widespread among *Enterobacteriaceae* in different geographical locations of China (Shen et al., 2009; Yang et al., 2013).

## Plasmid Mobilization

IncP-6 plasmids are found in various species from both clinical and environmental sources, suggesting that they have a broad host range and potential for long-term persistence in the environment (Naas et al., 2013; Dai et al., 2016; Wang et al., 2017; Yao et al., 2017). Interestingly, most of these *bla*<sub>KPC-2</sub> bearing IncP-6 plasmids were detected in China. Our findings further revealed that *bla*<sub>KPC-2</sub> carrying IncP-6 plasmid has moved into *Aeromonas* spp. Of note, p1713-KPC is lacking of a *tra* module encoding primary pilus, which explains the failure of plasmid conjugation. Studies had shown that plasmids could be able to transfer by conjugation if the right self-transmissible plasmids are present (Smillie et al., 2010). However, due to unknown reasons,

mobilization transfer from donor *A. taiwanensis*, which harbored resident plasmid and electrotransformed plasmid containing a *tra* module, to the recipient cells was not successful. Nevertheless, acquisition of free DNA is a general feature of *Aeromonas* isolates (Huddlestone et al., 2013). And the IncP plasmid, which is a broad-host-range incompatibility plasmid, has also been proved with the potential to mediate the dissemination of antibiotic resistant genes from the *Enterobacteriaceae* to other Gram-negative bacteria, such as *Pseudomonas aeruginosa* (Zhao et al., 2017). Since the complex mechanism of plasmid transfer is still not fully understood and these 3 IncP plasmids do encode mobilization functions, potential detection of such incP plasmids from clinical isolates should be closely monitored. Therefore, it is suggested that IncP-6 plasmids may act as an important vector responsible for the genetic transmission of *bla*<sub>KPC</sub> and *qnrS2* among the aquatic *Aeromonas* spp.

## CONCLUSION

The analysis of plasmids carrying different resistance genes is pivotal for characterization of bacterial isolates, since they play a significant role in transmission of antibiotic resistance (Galata et al., 2018). In this study, we reported the complete sequence of p1713-KPC, a novel IncP-6 plasmid identified from environmental *A. taiwanensis* isolates. To our knowledge, this is the first report describing the co-existence of *bla*<sub>KPC-2</sub> and *qnrS2* on the same plasmid in *A. taiwanensis* isolates. The emergence and dissemination of such plasmids in environmental

isolates deserve special attention. Given that *Aeromonas* spp. are ubiquitous organisms isolated from a wide range of environmental niches, they might act as important vectors for the dissemination of plasmid-mediated carbapenem- and quinolone-resistance genes. Therefore, potential detection of such plasmids from clinical isolates should be closely monitored.

## DATA AVAILABILITY

The whole genome shotgun project of the *A. taiwanensis* isolates has been deposited into DDBJ/EMBL/GenBank under the Bioproject accession number PRJNA478520. The complete nucleotide sequence of plasmid p1713-KPC has been deposited into the GenBank database under GenBank accession number MH624132.

## AUTHOR CONTRIBUTIONS

XH, YM, and BZ conceived and designed the experiments. XH, XY, YS, HX, YL, YK and LS performed the experiments. LG, JS, and FY analyzed the data. BZ and XH wrote the manuscript.

## REFERENCES

- Antipov, D., Hartwick, N., Shen, M., Raiko, M., Lapidus, A., and Pevzner, P. A. (2016). plasmidSPAdes: assembling plasmids from whole genome sequencing data. *Bioinformatics* 32, 3380–3387.
- Arias, A., Seral, C., Navarro, F., Miro, E., Coll, P., and Castillo, F. J. (2010). Plasmid-mediated QnrS2 determinant in an *Aeromonas caviae* isolate recovered from a patient with diarrhoea. *Clin. Microbiol. Infect.* 16, 1005–1007. doi: 10.1111/j.1469-0691.2009.02958.x
- Bonemann, G., Stiens, M., Puhler, A., and Schluter, A. (2006). Mobilizable IncQ-related plasmid carrying a new quinolone resistance gene, *qnrS2*, isolated from the bacterial community of a wastewater treatment plant. *Antimicrob. Agents Chemother.* 50, 3075–3080. doi: 10.1128/aac.00378-06
- Dai, X., Zhou, D., Xiong, W., Feng, J., Luo, W., Luo, G., et al. (2016). The IncP-6 Plasmid p10265-KPC from *Pseudomonas aeruginosa* carries a novel DeltaISEc33-associated bla KPC-2 gene cluster. *Front. Microbiol.* 7:310. doi: 10.3389/fmicb.2016.00310
- Galata, V., Fehlmann, T., Backes, C., and Keller, A. (2018). PLSDB: a resource of complete bacterial plasmids. *Nucleic Acids Res.* 47, D195–D202. doi: 10.1093/nar/gky1050
- Grundmann, H., Glasner, C., Albigier, B., Aanensen, D. M., Tomlinson, C. T., Andrasevic, A. T., et al. (2017). Occurrence of carbapenemase-producing *Klebsiella pneumoniae* and *Escherichia coli* in the European survey of carbapenemase-producing Enterobacteriaceae (EuSCAPE): a prospective, multinational study. *Lancet Infect. Dis.* 17, 153–163. doi: 10.1016/S1473-3099(16)30257-2
- Huddleston, J. R., Brokaw, J. M., Zak, J. C., and Jeter, R. M. (2013). Natural transformation as a mechanism of horizontal gene transfer among environmental *Aeromonas* species. *Syst. Appl. Microbiol.* 36, 224–234. doi: 10.1016/j.syapm.2013.01.004
- Lamba, M., and Ahammad, S. Z. (2017). Sewage treatment effluents in Delhi: a key contributor of beta-lactam resistant bacteria and genes to the environment. *Chemosphere* 188, 249–256. doi: 10.1016/j.chemosphere.2017.08.133
- Marti, E., and Balcazar, J. L. (2012). Multidrug resistance-encoding plasmid from *Aeromonas* sp. strain P2G1. *Clin. Microbiol. Infect.* 18, E366–E368. doi: 10.1111/j.1469-0691.2012.03935.x
- Marti, E., Huerta, B., Rodriguez-Mozaz, S., Barcelo, D., Jofre, J., and Balcazar, J. L. (2014). Characterization of ciprofloxacin-resistant isolates from a wastewater

## FUNDING

This study was supported by funding from the National Natural Science Foundation of China (Nos. 81600512 and 81741098); the grant from Henan Science and Technology Department (No. 162102310509); the National Key Research and Development Program of China (No. 2016YFD0501105); and the Zhejiang Provincial Natural Science Foundation of China (No. LY17H190003).

## SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: <https://www.frontiersin.org/articles/10.3389/fmicb.2019.02132/full#supplementary-material>

**FIGURE S1** | PFGE profiles of *A. taiwanensis* strains. A dendrogram of PFGE profiles was constructed with BioNumerics v7.6 by using UPGMA (unweighted pair group method with averages) clustering. Isolates with a similarity cut-off of  $\geq 80\%$  were considered as pulsotypes. The scale bar indicates the percentage of genetic relatedness.

**TABLE S1** | Primers used in the plasmid sequencing of novel IncP-6 plasmid pKPC-1713, harbouring *bla*<sub>KPC-2</sub> and *qnrS2* genes.

- treatment plant and its receiving river. *Water Res.* 61, 67–76. doi: 10.1016/j.watres.2014.05.006
- Montezzi, L. F., Campana, E. H., Correa, L. L., Justo, L. H., Paschoal, R. P., da Silva, I. L., et al. (2015). Occurrence of carbapenemase-producing bacteria in coastal recreational waters. *Int. J. Antimicrob. Agents* 45, 174–177. doi: 10.1016/j.ijantimicag.2014.10.016
- Munoz-Price, L. S., Poirel, L., Bonomo, R. A., Schwaber, M. J., Daikos, G. L., Cormican, M., et al. (2013). Clinical epidemiology of the global expansion of *Klebsiella pneumoniae* carbapenemases. *Lancet Infect. Dis.* 13, 785–796. doi: 10.1016/S1473-3099(13)70190-7
- Naas, T., Bonnin, R. A., Cuzon, G., Villegas, M. V., and Nordmann, P. (2013). Complete sequence of two KPC-harboring plasmids from *Pseudomonas aeruginosa*. *J. Antimicrob. Chemother.* 68, 1757–1762. doi: 10.1093/jac/dkt094
- Overbeek, R., Olson, R., Pusch, G. D., Olsen, G. J., Davis, J. J., Disz, T., et al. (2014). The seed and the rapid annotation of microbial genomes using subsystems technology (RAST). *Nucleic Acids Res.* 42, D206–D214.
- Page, A. J., Cummins, C. A., Hunt, M., Wong, V. K., Reuter, S., Holden, M. T., et al. (2015). Roary: rapid large-scale prokaryote pan genome analysis. *Bioinformatics* 31, 3691–3693. doi: 10.1093/bioinformatics/btv421
- Paschoal, R. P., Campana, E. H., Correa, L. L., Montezzi, L. F., Barrueto, L. R. L., da Silva, I. R., et al. (2017). Concentration and variety of carbapenemase producers in recreational coastal waters showing distinct levels of pollution. *Antimicrob. Agents Chemother.* 61:e1963-17. doi: 10.1128/AAC.01963-17
- Picao, R. C., Cardoso, J. P., Campana, E. H., Nicoletti, A. G., Petrolini, F. V., Assis, D. M., et al. (2013). The route of antimicrobial resistance from the hospital effluent to the environment: focus on the occurrence of KPC-producing *Aeromonas* spp. and *Enterobacteriaceae* in sewage. *Diagn. Microbiol. Infect. Dis.* 76, 80–85. doi: 10.1016/j.diagmicrobio.2013.02.001
- Schliep, K. P. (2011). Phangorn: phylogenetic analysis in R. *Bioinformatics* 27, 592–593. doi: 10.1093/bioinformatics/btq706
- Seemann, T. (2014). Prokka: rapid prokaryotic genome annotation. *Bioinformatics* 30, 2068–2069. doi: 10.1093/bioinformatics/btu153
- Shen, P., Wei, Z., Jiang, Y., Du, X., Ji, S., Yu, Y., et al. (2009). Novel genetic environment of the carbapenem-hydrolyzing beta-lactamase KPC-2 among *Enterobacteriaceae* in China. *Antimicrob. Agents Chemother.* 53, 4333–4338. doi: 10.1128/AAC.00260-09

- Smillie, C., Garcillan-Barcia, M. P., Francia, M. V., Rocha, E. P., and de la Cruz, F. (2010). Mobility of plasmids. *Microbiol. Mol. Biol. Rev.* 74, 434–452. doi: 10.1128/MMBR.00020-10
- Tuo, H., Yang, Y., Tao, X., Liu, D., Li, Y., Xie, X., et al. (2018). The prevalence of colistin resistant strains and antibiotic resistance gene profiles in Funan River, China. *Front. Microbiol.* 9:3094. doi: 10.3389/fmicb.2018.03094
- van Duin, D., and Doi, Y. (2017). The global epidemiology of carbapenemase-producing *Enterobacteriaceae*. *Virulence* 8, 460–469. doi: 10.1080/21505594.2016.1222343
- Wang, J., Yuan, M., Chen, H., Chen, X., Jia, Y., Zhu, X., et al. (2017). First report of klebsiella oxytoca strain simultaneously producing NDM-1, IMP-4, and KPC-2 carbapenemases. *Antimicrob. Agents Chemother.* 61:e877-17. doi: 10.1128/AAC.00877-17
- Xu, H., Wang, X., Yu, X., Zhang, J., Guo, L., Huang, C., et al. (2018). First detection and genomics analysis of KPC-2-producing citrobacter isolates from river sediments. *Environ. Pollut.* 235, 931–937. doi: 10.1016/j.envpol.2017.12.084
- Yang, J., Ye, L., Guo, L., Zhao, Q., Chen, R., Luo, Y., et al. (2013). A nosocomial outbreak of KPC-2-producing *Klebsiella pneumoniae* in a Chinese hospital: dissemination of ST11 and emergence of ST37, ST392 and ST395. *Clin. Microbiol. Infect.* 19, E509–E515. doi: 10.1111/1469-0691.12275
- Yang, Q., Zhao, M., Wang, K. Y., Wang, J., He, Y., Wang, E. L., et al. (2017). Multidrug-resistant *Aeromonas veronii* recovered from channel catfish (*Ictalurus punctatus*) in China: prevalence and mechanisms of fluoroquinolone resistance. *Microb. Drug Resist.* 23, 473–479. doi: 10.1089/mdr.2015.0296
- Yao, Y., Lazaro-Perona, F., Falgenhauer, L., Valverde, A., Imirzalioglu, C., Dominguez, L., et al. (2017). Insights into a novel blaKPC-2-encoding IncP-6 plasmid reveal carbapenem-resistance circulation in several *Enterobacteriaceae* species from wastewater and a hospital source in Spain. *Front. Microbiol.* 8:1143. doi: 10.3389/fmicb.2017.01143
- Yi, H., Cho, Y. J., Yong, D., and Chun, J. (2012). Genome sequence of *Escherichia coli* J53, a reference strain for genetic studies. *J. Bacteriol.* 194, 3742–3743. doi: 10.1128/JB.00641-12
- Zhao, F., Feng, Y., Lu, X., McNally, A., and Zong, Z. (2017). IncP plasmid carrying colistin resistance gene mcr-1 in *Klebsiella pneumoniae* from hospital sewage. *Antimicrob. Agents Chemother.* 61:e2229-16. doi: 10.1128/AAC.02229-16
- Zheng, B., Dong, H., Xu, H., Lv, J., Zhang, J., Jiang, X., et al. (2016). Coexistence of MCR-1 and NDM-1 in clinical *Escherichia coli* Isolates. *Clin. Infect. Dis.* 63, 1393–1395. doi: 10.1093/cid/ciw553
- Zheng, B., Feng, C., Xu, H., Yu, X., Guo, L., Jiang, X., et al. (2018a). Detection and characterization of ESBL-producing *Escherichia coli* expressing mcr-1 from dairy cows in China. *J. Antimicrob. Chemother.* 74, 321–325. doi: 10.1093/jac/dky446
- Zheng, B., Huang, C., Xu, H., Yu, X., Zhang, J., Wang, X., et al. (2018b). Complete nucleotide sequences of two KPC-2-encoding plasmids from the same *Citrobacter freundii* isolate. *J. Antimicrob. Chemother.* 73, 531–533. doi: 10.1093/jac/dkx381
- Zheng, B., Zhang, J., Ji, J., Fang, Y., Shen, P., Ying, C., et al. (2015). Emergence of *Raoultella ornithinolytica* coproducing IMP-4 and KPC-2 carbapenemases in China. *Antimicrob. Agents Chemother.* 59, 7086–7089. doi: 10.1128/AAC.01363-15

**Conflict of Interest Statement:** 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.

Copyright © 2019 Hu, Yu, Shang, Xu, Guo, Liang, Kang, Song, Sun, Yue, Mao and Zheng. This is an open-access article distributed under the terms of the Creative Commons Attribution License (CC BY). The use, distribution or reproduction in other forums is permitted, provided the original author(s) and the copyright owner(s) are credited and that the original publication in this journal is cited, in accordance with accepted academic practice. No use, distribution or reproduction is permitted which does not comply with these terms.