

# Detection of NDM-1-Positive *Aeromonas caviae* from Bacteremia by Using Whole-Genome Sequencing

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**Purpose:** Nosocomial infections caused by New Delhi metallo- $\beta$ -lactamase (NDM)-producing bacteria are prevalent worldwide. However, such diseases caused by NDM-producing *Aeromonas caviae* had never been reported. Our study aimed to elucidate the genomic characteristics of NDM-1-producing *A. caviae* isolated from hospital patients.

**Methods:** Bacterial genomic features and possible origins were assessed by whole-genome sequencing (WGS) and phylogenetic analysis. Subsequent investigations include antimicrobial susceptibility testing and multilocus sequence typing (MLST).

**Results:** We identified here two NDM-1-producing *A. caviae* isolates from bacteremia. Susceptibility testing showed that two isolates were multi-drug resistant and shared a similar resistance profile and were only sensitive to amikacin and trimethoprim/sulfamethoxazole. Both *A. caviae* isolates carry the carbapenem resistance gene *bla*<sub>NDM-1</sub> and also have antibiotic resistance genes such as  $\beta$ -lactams, AmpC enzymes, macrolides, aminoglycosides, and quinolones. S1-PFGE and Southern blot analysis were negative. Whole-genome sequencing and comparative analysis revealed that these two isolates shared a close relationship.

**Conclusion:** To the best of our knowledge, this work describes the first detection of non-plasmid encoded *bla*<sub>NDM-1</sub> in *A. caviae*. The *A. caviae* isolated in this study has a broad drug resistance spectrum. Phenotypic and molecular analysis indicated the two isolates belong to the same clone. Routine genomic surveillance of this species is now necessary to effectively curb the further dissemination of carbapenem-resistant bacteria in the region.

**Keywords:** *Aeromonas caviae*, New Delhi metallo- $\beta$ -lactamase, whole-genome sequencing, SNP, phylogenetic analysis

## Introduction

The Genus *Aeromonas* belongs to the *Aeromonadaceae*, and it is a group of Gram-negative bacteria widely distributed in the aquatic environment.<sup>1,2</sup> However, *Aeromonas* can cause a wide range of diseases in humans and animals, and the main pathogenic species of clinical relevance are *Aeromonas hydrophila* and *Aeromonas caviae*.<sup>3</sup> They are emerging opportunistic human pathogens that can cause host wound infections, gastrointestinal infections, and even bacteremia.<sup>4,5</sup>

The prevalence of carbapenemase-resistant *Enterobacteriaceae* (CRE) has risen since the 2000s.<sup>6</sup> New Delhi metallo- $\beta$ -lactamase (NDM) is a type of metallo- $\beta$ -lactamase (MBL) able to hydrolyze most  $\beta$ -lactams (including carbapenems).<sup>7,8</sup> Since the isolation of NDM-1-producing *Klebsiella pneumoniae* strains in India in 2008, NDM-1 has been found in various species of *Enterobacteriaceae*, *Acinetobacter*, and *Pseudomonas*.<sup>9,10</sup> Since then, NDM-1 has become widely popular around the world. Successful dissemination of carbapenemase-producing bacteria poses an enormous global public health challenge.<sup>11,12</sup>

However, there is no detailed genomic information on *A. caviae* carrying *bla*<sub>NDM-1</sub> has been reported. In this study, two *A. caviae* strains carrying *bla*<sub>NDM-1</sub> were isolated from patients with sepsis. The two clinically derived strains were further characterized by whole-genome sequencing and phylogenetic analysis. To the best of our knowledge, this is the first report that describes the detection of NDM-1-producing *A. caviae*.

## Materials and Methods

### Sample Collection

Two carbapenem-resistant strains, HZ574 and HZ578, were isolated from two female patients with sepsis hospitalized in a tertiary hospital in Huzhou, China. Bacterial species were identified using matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF/MS) (Bruker, Bremen, Germany). In addition, High throughput ANI analysis is used to compare the whole genome sequencing results to reveal distinct species of *Aeromonadaceae* (Figure S1). The carbapenemase genes (*bla*<sub>KPC</sub>, *bla*<sub>NDM</sub>, *bla*<sub>OXA-48</sub>, *bla*<sub>VIM</sub> and *bla*<sub>IMP</sub>) were identified by polymerase chain reaction (PCR)<sup>13</sup> (Table S1).

### Antibiotic Susceptibility Testing

Minimum inhibitory concentrations (MICs) were determined by VITEK 2 system with AST-GN16 panel.<sup>14</sup> The results were interpreted using European Committee on Antimicrobial Susceptibility Testing breakpoints, version 6.0, and Clinical and Laboratory Standards Institute document M100-S25.

### Plasmid Characterization and Conjugation Assay

The number and size of the plasmid of the two isolates were characterized by the S1 Nuclease-Pulsed Field Gel Electrophoresis (S1-PFGE).<sup>15</sup> Briefly, DNA plugs were digested using S1 Nase restriction enzyme (Takara Bio Inc., Kyoto, Japan) for 30 minutes. S1-PFGE was undertaken on a CHEF-DR III (Bio-Rad, Hercules, CA, USA) using the following parameters: running time 16 hours, temperature 14 °C, field strength 6 V/cm<sup>2</sup>, angles 120°, initial pulse time 2.2 s, final pulse time 63.8 s. The location of *bla*<sub>NDM-1</sub> was confirmed by Southern blotting and hybridization with a digoxigenin-labelled *bla*<sub>NDM-1</sub> probe using DIG-High Prime DNA Labeling and Detection Starter Kit II (Roche Diagnostics). Plasmid conjugation experiments were performed by mating *E. coli* J53/EC600 as the recipient strain. Transconjugants were selected on agar (OXOID, Hampshire, UK) medium at a concentration of 200 mg/L sodium azide and two mg/L meropenem. Finally, MALDI-TOF-MS was used to identify transconjugants, and the target gene was verified by PCR. The location and size of plasmids were characterized by S1-PFGE as described previously.

### Whole-Genome Sequencing

Whole-genome sequencing (WGS) was performed by Novo Gene Co., Ltd. Beijing, China, using the Illumina HiSeq (Illumina, San Diego, California) platform for all isolates. Alignment of antimicrobial resistance genes was performed through the ResFinder platform (<https://cge.cbs.dtu.dk/services/ResFinder/>). Multilocus sequence typing (MLST) was performed on bacteria using the website (<https://cge.cbs.dtu.dk/services/MLST/>). After annotating the strains using Prokka (rapid prokaryotic genome annotation), the genetic environment of carbapenemase encoding genes was characterized using Easyfig 2.2.3. Virulence genes were identified by blasting the VFDB database (<http://www.mgc.ac.cn/VFs/main.htm>).

### Phylogenetic Reconstruction and Analysis

Thirty complete *A. caviae* genomes were downloaded from the National Center for Biotechnology Information (NCBI) for phylogenetic analysis (Table S2). Snippy (rapid haploid variant calling and core genome alignment) was used to compare genomic differences between strains.<sup>16</sup> The alignment file was filtered from variants with elevated densities of base substitutions as putative recombination events by Gubbins version 2.4.1.<sup>17</sup> The filtered core-genome alignment file was used to construct a maximum likelihood tree using FastTree with the GTR+CAT model.

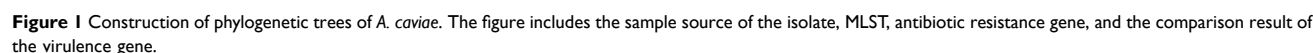
Isolate	MIC (mg/L) <sup>a</sup>										
	AMC	TZP	CAZ	CRO	CPM	ETP	IPM	AMK	LVX	TGC	SXT
HZ574	32	128	64	64	16	8	16	2	8	8	20
HZ578	32	128	32	64	16	8	8	2	8	4	20

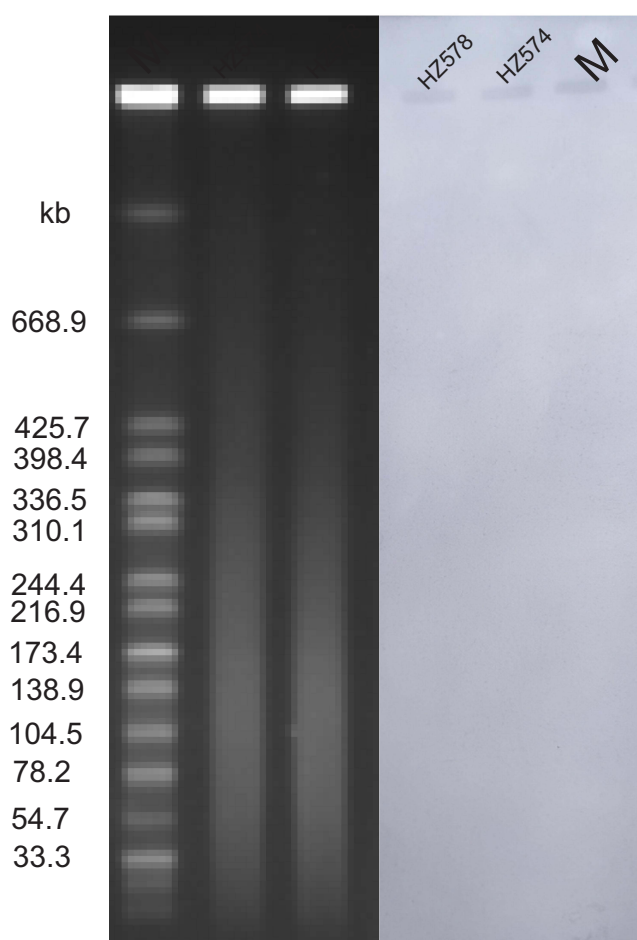
**Abbreviations:** AMC, amoxicillin/clavulanic acid; TZP, piperacillin/tazobactam; CAZ, ceftazidime; CRO, ceftriaxone; CPM, cefepime; ETP, ertapenem; IPM, imipenem; AMK, amikacin; LVX, levofloxacin; TE, tetracycline; TGC, tigecycline; SXT, trimethoprim/sulfamethoxazole.

The whole-genome sequences of the *A. caviae* were submitted to GenBank under the following BioProject numbers: PRINA799930

The results of the MIC of antibiotics are shown in Table 1. The two *A. caviae* strains in this study have a broad drug resistance spectrum and belong to multidrug-resistant bacteria. Antibiotics to which bacteria are resistant include amoxicillin/clavulanic acid, piperacillin/tazobactam, ceftazidime, ceftriaxone, cefepime, ertapenem, imipenem, levofloxacin, tetracycline, tigecycline. HZ574 and HZ578 are only sensitive to amikacin and trimethoprim/sulfamethoxazole. Two isolates shared similar antimicrobial profiles.

We provide data on antimicrobial resistance genes in [Figure 1](#). Both HZ574 and HZ578 carry the carbapenem resistance gene *bla*<sub>NDM-1</sub>, which is the first reported *A. caviae* carrying *bla*<sub>NDM-1</sub> so far. In addition, bacteria also carry antibiotic resistance genes such as  $\beta$ -lactams, AmpC enzymes, macrolides, aminoglycosides, quinolones, etc. ([Figure 1](#)). We also compared the resistance genes carried by 30 *A. caviae* strains downloaded from NCBI, and the results showed that 30 *A. caviae* strains mainly had AmpC enzyme (86.21%), followed by  $\beta$ -lactamase (24.14%) ([Figure 1](#)).





**Figure 2** S1 Nuclease-pulsed field gel electrophoresis (SI-PFGE) and southern blot. The number and size of the plasmid of the two isolates were characterized by the S1 Nuclease-pulsed field gel electrophoresis (SI-PFGE).

## Virulence Genes

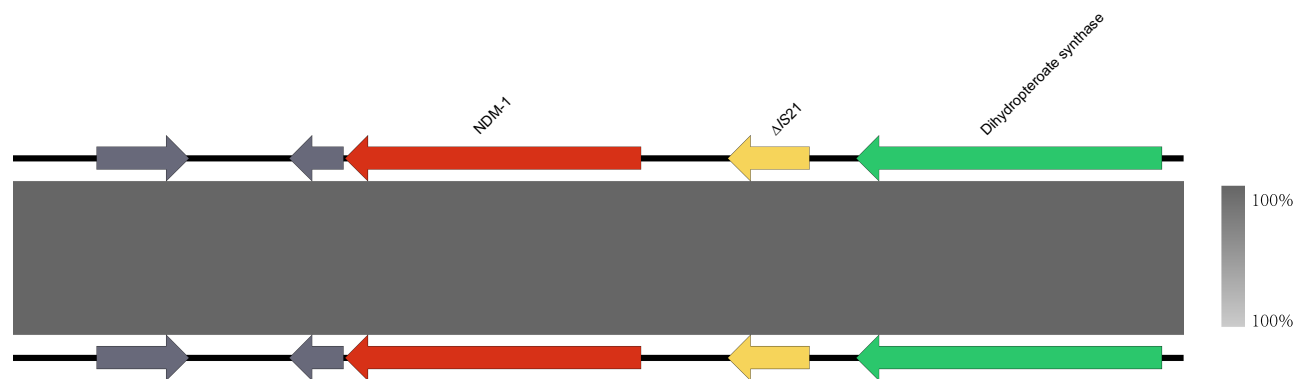
The results of the alignment of virulence genes are shown in [Figure 1](#). The virulence genes carried by HZ574 and HZ578 are related genes of the Type VI secretion system, Tap type IV pili, Polar flagella, twitching ATPase, and Hemolysin A. The strains downloaded from NCBI mainly carried secretion pathway protein (83.33%) and related genes of Polar flagella (100%).

## Molecular Characteristics of *A. caviae*

MLST typing analysis of 32 *A. caviae* strains showed that only six strains had a known ST type, including ST141, ST313, and ST645 ([Figure 1](#)). S1-PFGE and Southern blot demonstrated that the *bla*<sub>NDM-1</sub> gene is not carried by plasmid in isolates HZ574 and HZ578 ([Figure 2](#)). Moreover, two isolates were both negative for genes of plasmid typing replicon. These results indicated that *bla*<sub>NDM-1</sub> gene is chromosome encoded in these two isolates. Phylogenetic analysis of all the strains showed that HZ574 and HZ578 were located in the same clade and had a close relationship with GCA\_000959705 from Brazil ([Figure 1](#)). Moreover, the *bla*<sub>NDM-1</sub> genes of HZ574 and HZ578 share the same genetic environment ([Figure 3](#)).

## Discussion

With the widespread of carbapenemase-producing bacteria, *bla*<sub>NDM-1</sub> has been detected in various bacteria worldwide, but there is no detailed genomic information on *A. caviae* carrying *bla*<sub>NDM-1</sub> has been reported.<sup>10,18</sup> In this study, two



**Figure 3** The genetic environment of the *bla*<sub>NDM-1</sub> gene in *A. caviae* was isolated from clinical sources. The arrows represent the direction of transcription. The red open reading frame (ORF) indicates the *bla*<sub>NDM-1</sub> gene, the yellow ORF indicates the mobile element, the green ORF indicates enzymes, and the grey ORF indicates other genes or genes of unknown function.

strains of *A. caviae* carrying NDM-1 were isolated from patients. The two clinically derived strains were characterized by genome and phylogenetic analysis using whole-genome sequencing technology.

The study results on the resistance of *A. caviae* showed that *A. caviae* is resistant to penicillin and first-generation cephalosporins.<sup>19,20</sup> However, it is susceptible to monobactams, carbapenems, third- and fourth-generation cephalosporins, aminoglycosides, and fluoroquinolones.<sup>21</sup> As *A. caviae* has been isolated, its resistance spectrum has changed, most notably due to the presence of genes encoding the production of  $\beta$ -lactamases, resulting in increased resistance to  $\beta$ -lactam antimicrobials.<sup>22</sup> The HZ574 and HZ578 isolates in this study were both resistant to ertapenem and imipenem. The results of whole-genome sequencing showed that HZ574 and HZ578 carried *bla*<sub>NDM-1</sub>, indicating that the resistant phenotype was consistent with the genotype. In addition, HZ574 and HZ578 also carry antibiotic resistance genes such as  $\beta$ -lactams, AmpC enzymes, macrolides, aminoglycosides, quinolones and have multidrug-resistant phenotypes.<sup>23</sup> Notably, *A. caviae* in this study had a broader spectrum of resistance compared with *A. caviae* available at NCBI (Figure 1).

The virulence potential and pathogenic mechanism of *A. caviae* remain unclear.<sup>24</sup> Through the analysis of virulence genes, we found that HZ574 and HZ578 in this study contained many kinds of virulence genes, such as Type VI secretion system, Tap type IV pili, Polar flagella, twitching ATPase, Hemolysin A. These virulence factors can help bacteria attach to the host surface, cause host cell and tissue damage, evade the host immune response, and allow bacteria adhere to the cell surface to form biofilms.<sup>25,26</sup> The strains downloaded from NCBI mainly carried secretion pathway protein (83.33%) and related genes of Polar flagella (100%), suggesting that *A. caviae* in this study may have a more substantial virulence potential (Figure 1).<sup>27</sup>

The phylogenetic analysis of HZ574 and HZ578 and the complete *A. caviae* genome downloaded from NCBI showed three evolutionary clones of *A. caviae*. HZ574 and HZ578 were related in the second clone (Figure 1). This is probably due to the isolation of these two strains being in the same period of time, and both patients were hospitalized in the same hospital. Phylogenetic analysis showed that HZ574 and HZ578 were located in the same branch as GCA\_000959705 from Brazil. We speculate that HZ574 and HZ578 belong to the same clone, although two isolates were recovered from different patients. Analysis of the gene-environment of *bla*<sub>NDM-1</sub> found that there are mobile transfer elements around *bla*<sub>NDM-1</sub>, which increases the risk of spreading the drug resistance gene, and also suggests that we should carry out routine testing and appropriately treat patients (Figure 3).<sup>28</sup> This work may have important implications on the transmission of *bla*<sub>NDM-1</sub> gene in the hospital. We emphasize the importance of improved multisectoral surveillance for carbapenemase-producing isolates, which may contribute to the spread of antimicrobial resistance genes in clinical settings and communities.

## Conclusions

We report the *A. caviae* carrying *bla*<sub>NDM-1</sub> from bacteremia. The study demonstrated the genomic characteristics of *A. caviae* carrying *bla*<sub>NDM-1</sub> by whole-genome sequencing. The *A. caviae* isolated in this study has a broad drug

resistance spectrum, and the virulence genes carried by the strains pose challenges to clinical treatment. Routine genomic surveillance of this clone is now necessary to effectively curb the dissemination of drug-resistant bacteria in this region.

## Ethical Statement

The study protocol was reviewed, approved by and carried out following the recommendations of the Ethics Committee of the Ningbo Medical Center Lihuili Hospital. Written informed consents were obtained from patients. The study complies with the Declaration of Helsinki.

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## Disclosure

The authors report no conflicts of interest in this work.

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