Infection and Drug Resistance

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ORIGINAL RESEARCH

# NDM-1 and OXA-48-Like Carbapenemases (OXA-48, OXA-181 and OXA-252) Co-Producing *Shewanella xiamenensis* from Hospital Wastewater, China

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**Background:** *Shewanella* genus, as an important carrier of resistance genes, has the potential to transmit resistance to many antimicrobials in many circumstances, especially in aquatic environment. The aim of the study was to describe the risk of *Shewanella xiamenensis* in hospital environment through analysis of genomic comparison and resistance status.

**Methods:** Seven *S. xiamenensis* strains were isolated from hospital wastewater. PCR and Sanger sequencing were carried out for detection of common carbapenemase genes. Antimicrobial susceptibility testing was performed to determine the antimicrobial profile. Whole genome sequencing was applied, and sequences were further used for genomic analysis.

**Results:** Seven *Shewanella xiamenensis* were all positive for $bla_{NDM}$ and $bla_{OXA-48}$. Antimicrobial susceptibility testing showed all *Shewanella xiamenensis* were resistant to cefotaxime, ceftazidime, imipenem, meropenem, gentamycin and trimethoprim-sulfamethoxazole. Whole genome sequencing and phylogenetic analysis demonstrated the diversity of *Shewanella xiamenensis* despite isolating from one wastewater pool.

**Conclusion:** To the best of our knowledge, this is the first report of detection of three types $bla_{OXA-48-like}$ genes in one hospital in China. And we have detected multi-drug resistant *S. xiamenensis* from hospital wastewater. This emphasizes that the presence of naturally existing carbapenemases in the environment may be significantly overlooked and that the $bla_{OXA-48-like}$ genes in China may originate through the horizontal gene transfer from *S. xiamenensis to Enterobacterales* rather than import from other countries.

**Keywords:** OXA-48, NDM, *Shewanella xiamenensis*, hospital wastewater, reservoir, horizontal transmission, surveillance, New Delhi metallo-β-Lactamase

## Introduction

Antimicrobial resistance has become a threat for decades, and one representative example is carbapenem-resistant *Enterobacterales* (CRE), which have spread all over the world. CRE has received great attention in recent years due to its high morbidity, mortality, and lack of effective treatment solutions.[1] And the main mechanism of carbapenem resistance is the production of different carbapenemases, and *Klebsiella pneumoniae* carbapenemase (KPC), New Delhi metallo-β-Lactamase (NDM), Verona integron-mediated metallo-β-lactamase (VIM), imipenemase (IMP), and oxacillinase-48 (OXA-48) are the most common carbapenemases over the world.[2] Specifically, KPC and NDM are widely spread in America and China, while OXA-48-like is prevalent in Europe, North Africa, Middle East, and Indian subcontinent. OXA-48-like enzymes remain uncommon in American and most Asian countries, including China.[3] In China, OXA-48-like producers

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have been reported in Shanghai, Jiangsu, Sichuan, Zhejiang, Guangdong. But few cases were related to foreign stay, and whether these $bla_{\text{OXA-48-like}}$ genes were originated from abroad was unclear.[4–6]

Surveillance has been widely regarded as the key point of administration of antibiotic resistance, which is informative for guidance on empirical treatment before antimicrobial susceptibility profile is determined.[7,8] To make surveillance data more informative, samples from both clinical patients and healthy individuals should be included. However, this is demanding and difficult to achieve, thus hospital wastewater has been increasingly recognized as an alternative of classical surveillance due to the abundance of antibiotic residual and pathogenic bacteria released from large quantity of hospitalized population.[9] Additionally, antimicrobial resistant genes (ARG) in wastewater have also been suggested as a suitable marker to predict clinical resistance prevalence in the hospitals.[10] This provides the possibility to evaluate the antimicrobial resistant bacteria (ARB) communities and ARG prevalence in less developing regions with low input of expenditure and labor. On the other hand, detection of uncommon ARG from wastewater in a non-epidemic region indicates a risk of further transmission, with probably much more severe consequences than those have already been circulating in pathogens.[8]

The genus *Shewanella*, facultative anaerobic, motile gram-negative bacteria, is widely distributed in various environments, such as spoilt food and aquatic environments.[11,12] In recent years, *Shewanella* infection cases have been rapidly increased and have been reported over the world. Most of cases are reported in tropical regions, and temperate countries and coastal cities like Australia, Spain, Martinique, Canary Islands, also have sporadic cases.[13,14] *Shewanella*-related infections can involve multiple parts of human body, and could be divided into several categories, including ear, nose, and throat (ENT) disorders, central nervous system (CNS) disorders, chest infections, cardiovascular diseases, bloodstream infections, intra-abdominal infections, bone arthropathy, skin and soft-tissue infections (SSTIs).[13] Marine exposure, compromised immune system, invasive procedures and poor hygiene in hospital are risk factors of *Shewanella* infections.[11,15–17] Although no antimicrobial treatment guideline for *Shewanella* infections has been published yet, the empirical treatment could efficiently control infections.[13] With the increase of antimicrobial resistance, however, clinicians and laboratory researchers should raise awareness to occurrence and dissemination of multi-drug resistant strains.

Here, we identified seven *Shewanella xiamenensis* strains that coproduce NDM-1 and OXA-48-like (OXA-48, OXA-181 and OXA-252) carbapenemases from hospital wastewater in Suzhou, China, where OXA-48-like-producing bacteria have not been detected before. We aimed to describe the risk of *Shewanella xiamenensis* in hospital environment through analysis of genomic comparison and resistance analysis.

# Materials and Methods

## Sample Collection, Culture Condition and Species Determination

To screen carbapenem-resistant bacteria in hospital wastewater, we performed the assay as described previously with minor modification.[18] Briefly, water samples were collected from main sewer outlet at different depth of 0.5 to 1 m below the surface using 50mL sterile bottles. (Samples were chilled on ice during the sampling procedure and after being collected.) The samples were concentrated by 0.45 μm membrane filters, and ten-fold serial dilutions (10–1000 times) of each water sample were made in sterile saline solution. A total of 100 μL of each dilution was then plated onto LB agar plates supplemented with 2 mg/L meropenem. After incubated for 18 to 24 hours at 37°C, bacterial colonies with distinct coloration and morphologies were randomly picked and subcultured onto meropenem plates for further purification. Then each isolate was stored at −80 °C in 30% (vol/vol) glycerol for further investigation. And the species preliminary identification was determined using matrix-assisted laser desorption/ionization time of flight mass spectrometry (MALDI-TOF-MS, Bruker).

## Carbapenemase Gene Assay and Antimicrobial Susceptibility Testing

To determine resistance mechanism to carbapenem of these strains, common carbapenemase genes were detected ($bla_{\text{KPC}}$, $bla_{\text{NDM}}$, $bla_{\text{IMP}}$, $bla_{\text{VIM}}$, $bla_{\text{OXA-48}}$) using specific primer pairs followed by Sanger sequencing for their subtypes (Sangon, Shanghai). Primers were synthesized by Sangon and were listed in Table 1. Reaction buffer 2× Taq Mater Mix was provided by Vazyme (Nanjing).

**Table 1** Primers Used in This Study

| Primer | Sequence (5'→ 3') |
|---|---|
| KPC-F | ATGTCACTGTATCGCCGTCT |
| KPC-R | TTTTCAGAGCCTTACTGCCC |
| NDM-F | ATGGAATTGCCCAATATTATGC |
| NDM-R | TCAGCGCAGCTTGTCGG |
| IMP-F | GGCAGTCGCCCTAAAACAAA |
| IMP-R | TAGTTACTTGGCTGTGATGG |
| VIM-F | TTATGGAGCAGCAACGATGT |
| VIM-R | CAAAAGTCCCGCTCCAACGA |
| OXA-48-F | GCGTGGTTAAGGATGAACAC |
| OXA-48-R | CATCAAGTTCAACCCAACCG |

To characterize the antimicrobial susceptibility profile, we performed antimicrobial susceptibility testing. Isolates were suspended in 0.9% saline to 0.5 McFarland. Panel Phoenix NMIC/ID (BD) was used to determine MIC values on Phoenix M50 Automated System (BD, USA) and a total of 21 antibiotics were tested, including ampicillin, piperacillin, amoxicillin-clavulanate, ampicillin-sulbactam, piperacillin-tazobactam, imipenem, meropenem, cefazolin, cefepime, cefotaxime, ceftazidime, aztreonam, chloramphenicol, colistin, tetracycline, trimethoprim-sulfamethoxazole, amikacin, gentamycin, ciprofloxacin, levofloxacin and moxifloxacin. Results were interpreted according to "Other Non-Enterobacterales" in Clinical and Laboratory Standards Institute criteria (CLSI, 2020).

## Whole Genome Sequencing

To further characterize the strains, we have performed genome sequencing. Bacterial genomic DNA were extracted by The Omega Bio-Tek Bacterial DNA Kit (Doraville, GA, USA) following instructions provided. Whole genome sequencing used the Illumina NovaSeq 6000 platform (Illumina, CA, USA) and then the corrected reads were assembled de novo utilizing SPAdes v3.11. In addition, sequencing of the complete genome sequence of isolate HD6416 and HD6452 were respectively performed with a sheared DNA library with average size of 10 kb on a Nanopore PromethION sequencer (Oxford Nanopore Technologies, OX, UK). The paired-end short Illumina reads were used to correct the long Nanopore reads, and then the corrected Nanopore reads were assembled de novo utilizing *Unicycler* v0.4.9 (https://github.com/rrwick/Unicycler).

## Phylogenetic Analysis

To investigate the relationships of the $bla_{\text{OXA-48-like}}$ genes, the maximum-likelihood phylogenetic tree of $bla_{\text{OXA-48-like}}$ genes on *Shewanella* species available on Beta-Lactamase DataBase (BLDB, http://bldb.eu/) was constructed using MEGA 7.0.[19]

To describe the phylogenetic relationships of *Shewanella xiamenensis* strains, we collected all accessible genomes of *Shewanella xiamenensis* on NCBI to perform phylogenetic analysis. Based on recombination-free core genome, single-nucleotide polymorphisms (SNPs) was conducted with MUMmer 3.0, ClonalFrameML and MEGA7.0 as described previously.[20] The sequence of *Shewanella xiamenensis* HD6416 (GenBank accession no. CP079717) was used as the reference, and the sequence of *Shewanella putrefaciens* CN-32 (GenBank accession no. NC_021505) was used as the outgroup. And the phylogenetic tree was further improved on iTOL and Inkscape v1.0 (https://inkscape.org/).[21]

## Bioinformatic Analysis

Species identification was performed by calculating the pairwise average nucleotide identity (ANI) value between the genome sequence of the query strain and type strain of *Shewanella xiamenensis* (Accession number: BMOP01000000) by using OAT 0.93.1 (cutoff value of 96%).[22]

Open reading frames (ORFs) and pseudogenes were predicted using RAST 2.0[23] combined with BLASTP/BLASTN searches against the UniProtKB/Swiss-Prot database and the RefSeq database.[23] Annotation of resistance genes, mobile elements, and other features were carried out using the online databases including CARD,[24] ResFinder 4.0,[25] ISfinder,[26] and Tn Number Registry. Gene organization diagram was drawn in Inkscape v1.0 (https://inkscape.org/). BLAST Ring Image Generator (BRIG)[27] was used to compare the differences between the plasmids.

# Results

## Isolate Characterization

Seven *Shewanella putrefaciens* were identified by MALDI-TOF-MS, but they were further determined as *Shewanella xiamenensis* in WGS analysis. The *Shewanella xiamenensis* were isolated from three different spots of the sewage outlets (Depth A, B and C) (Table 2). PCR testing and Sanger sequencing showed that all isolates co-harbored $bla_{\text{NDM-1}}$ and $bla_{\text{OXA-48-like}}$ carbapenemase genes (including $bla_{\text{OXA-48}}$, $bla_{\text{OXA-181}}$ and $bla_{\text{OXA-252}}$, Table 2). Antimicrobial susceptibility testing (Table 3) confirmed that all seven *Shewanella* strains were resistant to cefotaxime, ceftazidime, imipenem, meropenem, gentamycin and trimethoprim-sulfamethoxazole. And all of them were determined as multidrug-resistant. Only amikacin (100%), aztreonam (85.7%), tetracycline (71.4%) and levofloxacin (85.7%) retained high antibiotic activity to them. Additionally, just part of the strains were susceptible to piperacillin (14.3%), cefepime (14.3%), piperacillin-tazobactam (42.9%) and ciprofloxacin (28.6%), respectively.

## Genome Analysis

### Genetic Characterization

Whole genome sequencing (Illumina Hiseq) (Bioproject PRJNA755833) and analysis showed that $bla_{\text{OXA-48-like}}$ genes were on chromosomes while $bla_{\text{NDM-1}}$ were likely on plasmids. Sequence analysis showed that HD6416, HD6424, HD6449 and HD6452 carry $bla_{\text{OXA-48}}$, and HD6443 harbors $bla_{\text{OXA-252}}$, while HD6420 and HD6446 contain $bla_{\text{OXA-181}}$. The phylogenetic analysis of $bla_{\text{OXA-48-like}}$ of *Shewanella spp*. from NCBI showed the $bla_{\text{OXA-48}}$ genes have 3 genotypes (different nucleotide sequences) despite being the same amino acid sequences while $bla_{\text{OXA-181}}$ gene only has one genotype. In addition, plasmid-borne and chromosome-borne $bla_{\text{OXA-48}}$ displayed some nucleotide variations but $bla_{\text{OXA-181}}$ were identical (Figure 1A).

### SNP Analysis

Core genome single-nucleotide polymorphisms (SNPs) maximum-likelihood phylogenetic tree result (Figure 1B) showed that *S. xiamenensis* were classified into multiple clades with different subtree lengths. For the strains from our study, HD6416 and HD6424, HD6420 and HD6446, HD6449 and HD6452 were respectively grouped together, while HD6443 was located on a different branch. In addition, the seven genomes were distributed into different clades of the tree, indicating genomic diversity of the seven *S. xiamenensis* isolates, despite isolating from the same hospital. The *S. xiamenensis* phylogenetic clades in general correlated with the $bla_{\text{OXA-48-like}}$ gene sequences, but interestingly the

**Table 2** Isolation Spot and Resistance Genes of Seven *Shewanella xiamenensis*

| Isolates | Source | Carbapenemase Genes |
|---|---|---|
| HD6416 | Depth A | $bla_{\text{OXA-48}}$, $bla_{\text{NDM-1}}$ |
| HD6420 | Depth B | $bla_{\text{OXA-181}}$, $bla_{\text{NDM-1}}$ |
| HD6424 | Depth A | $bla_{\text{OXA-48}}$, $bla_{\text{NDM-1}}$ |
| HD6443 | Depth C | $bla_{\text{OXA-252}}$, $bla_{\text{NDM-1}}$ |
| HD6446 | Depth B | $bla_{\text{OXA-181}}$, $bla_{\text{NDM-1}}$ |
| HD6449 | Depth A | $bla_{\text{OXA-48}}$, $bla_{\text{NDM-1}}$ |
| HD6452 | Depth A | $bla_{\text{OXA-48}}$, $bla_{\text{NDM-1}}$ |

**Table 3** Antimicrobial Drug Susceptibility of Seven *Shewanella xiamenensis**

| Isolates | MIC (mg/L) and Interpretation | | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | AMP | PIP | AMC | SAM | TZP | IPM | MEM | CZ | FEP | CTX | CAZ | ATM | CL | C | TE | SXT | AMK | GEN | CIP | LEV | MXF |
| HD6416 | >16 | >64 | 16/8 | >16/8 | >64/4 | >8 | >8 | >16 | >16 | >32 | >16 | ≤2 | ≤0.5 | >16 | ≤2 | >2/38 | ≤8 | >8 | ≤0.5 | ≤1 | ≤1 |
| HD6420 | >16 | >64 | 16/8 | >16/8 | >64/4 | >8 | >8 | >16 | >16 | >32 | >16 | ≤2 | ≤0.5 | ≤4 | 4 | >2/38 | ≤8 | >8 | >2 | ≤1 | ≤1 |
| HD6424 | >16 | >64 | 16/8 | >16/8 | 16/4 | >8 | >8 | >16 | 16 | >32 | >16 | ≤2 | ≤0.5 | 16 | ≤2 | >2/38 | ≤8 | >8 | ≤0.5 | ≤1 | ≤1 |
| HD6443 | >16 | >64 | >16/8 | >16/8 | >64/4 | >8 | >8 | >16 | >16 | >32 | >16 | >16 | ≤0.5 | >16 | ≤2 | >2/38 | ≤8 | >8 | >2 | 2 | ≤1 |
| HD6446 | >16 | 8 | 16/8 | >16/8 | 64/4 | >8 | >8 | >16 | >16 | >32 | >16 | ≤2 | ≤0.5 | ≤4 | 4 | >2/38 | ≤8 | >8 | >2 | 4 | 2 |
| HD6449 | >16 | 64 | 16/8 | >16/8 | 8/4 | >8 | >8 | >16 | >16 | >32 | >16 | ≤2 | ≤0.5 | ≤4 | 8 | >2/38 | ≤8 | >8 | >2 | 2 | 2 |
| HD6452 | >16 | >64 | 16/8 | >16/8 | 8/4 | >8 | >8 | >16 | 8 | >32 | >16 | ≤2 | 1 | ≤4 | 8 | >2/38 | ≤8 | >8 | >2 | 2 | 2 |

**Notes**: *Antimicrobial susceptibility testing was performed on Phoenix M50 Automated System (BD, USA). And the breakpoint was interpreted by the "Other Non-Enterobacterales" criteria: blank: no reference breakpoint; light gray: susceptible; medium gray: intermediate; dark gray: resistant.

**Abbreviations**: AMP, ampicillin; PIP, piperacillin; AMC, amoxicillin-clavulanate; SAM, ampicillin-sulbactam; TZP, piperacillin-tazobactam; IPM, imipenem; MEM, meropenem; CZ, cefazolin; FEP, cefepime; CTX, cefotaxime; CAZ, ceftazidime; ATM, aztreonam; C, chloramphenicol; CL, colistin; TE, tetracycline; SXT, trimethoprim-sulfamethoxazole; AN, amikacin; GM, gentamycin; CIP, ciprofloxacin; LVX, levofloxacin; MXF, moxifloxacin.



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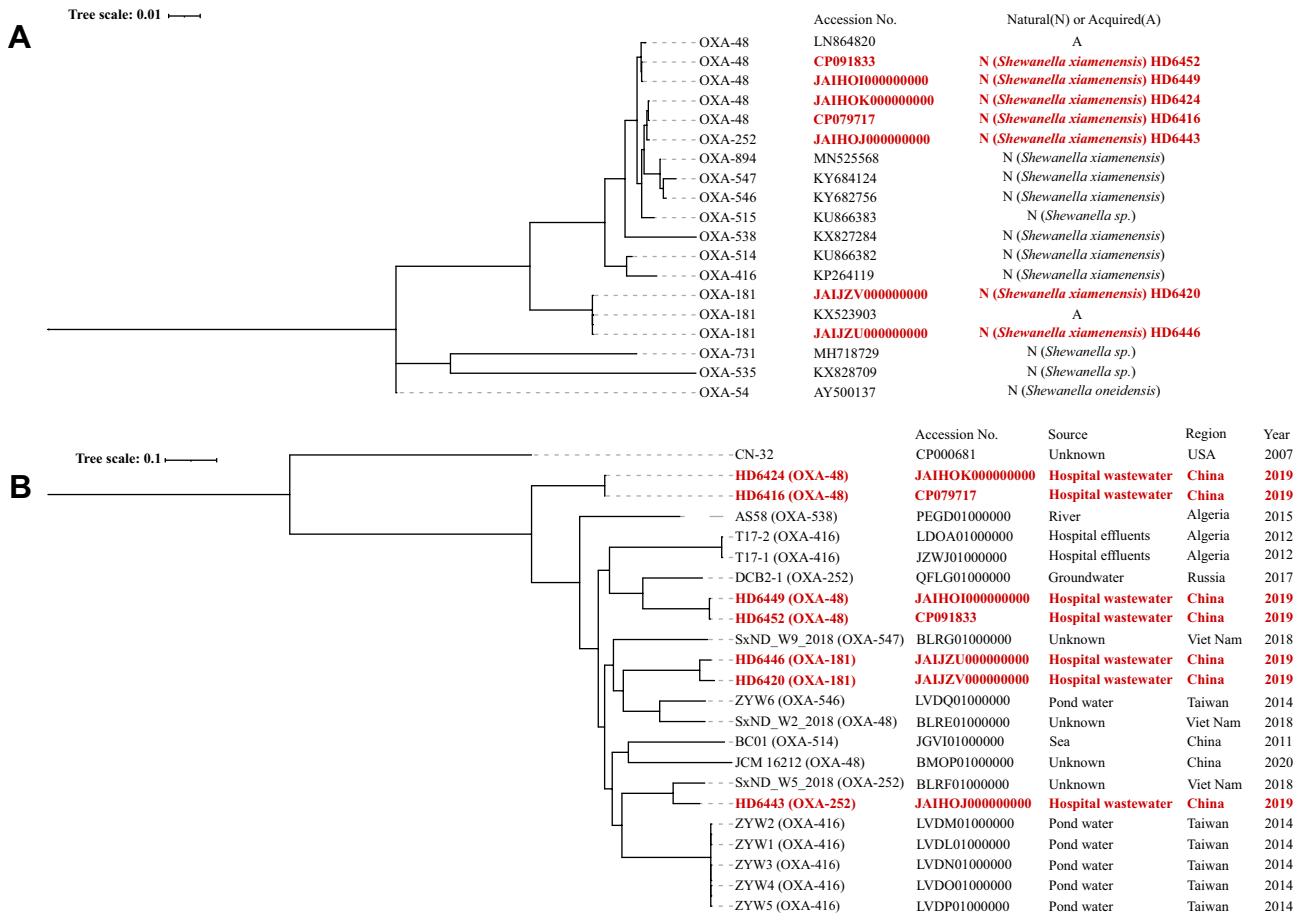


**Figure 1** Phylogenetic trees of OXA-48-like genes and *Shewanella xiamenensis* strains. (**A**) Phylogenetic trees of OXA-48-like genes. $bla_{OXA-48}$ genes have three genotypes despite being the same amino acid sequences while $bla_{OXA-181}$ gene only has one genotype. Plasmid-borne and chromosome-borne $bla_{OXA-48}$ displayed some nucleotide variations but $bla_{OXA-181}$ were identical; (**B**) Phylogenetic trees of *Shewanella xiamenensis* strains. Seven *S. xiamenensis* genomes were distributed into different clades of the tree, and the phylogenetic clades in general correlated with the $bla_{OXA-48-like}$ gene sequences. Bootstrap values for each cluster of associated taxa are shown next to each branch. Bar corresponds to the scale of sequence divergence. *Shewanella xiamenensis* from our study were characterized in boldly red.

OXA-48 producing HD6416/HD6424 and HD6420/HD6446 were located on different clusters. And a pairwise comparison of SNPs and similarities for all strains have been shown in Table S1.

## Plasmid Analysis

Nanopore sequencing was further conducted in strain HD6416 and HD6452 (Bioproject PRJNA755833) to obtain completely closed chromosome and plasmid sequences. HD6416 and HD6452 both contain one circular chromosome and a plasmid. The PlasmidFinder analysis showed no known plasmid type were matched to pHD6416 and pHD6452, indicating that both plasmids belong to novel plasmid type.[28,29] And plasmid comparison was further conducted using BRIG and result (Figure 2) indicated some variation between the backbone region of $bla_{NDM-1}$-harboring plasmids. The backbone region of pHD6420, pHD6424 and pHD6443 share 99% identity with pHD6416, and pHD6446 has about 90% similarity with pHD6416 backbone, while pHD6449 and pHD6452 contain a different backbone. Notably, the multidrug-resistant regions of the seven plasmids were highly comparable with just a few mobile elements different. We then depicted gene diagrams of MDR region of two closed plasmids from our study (pHD6416 and pHD6452) for further comparison (Figure 3). The MDR region of both plasmids replaced In*834* on Tn*6358a*, but with slight difference in resistance gene organization, so we designated them as Tn*6358b* (on pHD6416) and Tn*6358c* (on pHD6452), respectively. On Tn*6358c*, An ISCR-$ble_{MBL}$-$bla_{NDM-1}$ unit and an ISCR-*qnrV6*-$bla_{CMY-10}$ unit were inserted in the In469 on Tn*6358b*. And the ΔIn1357 on Tn*6358b* was replaced by Tn*6309* and part of ΔIn2145. The MDR region of Tn*6358b* and Tn*6358c* encode resistance to various antibiotics, such as aminoglycoside (*dfrA27,*

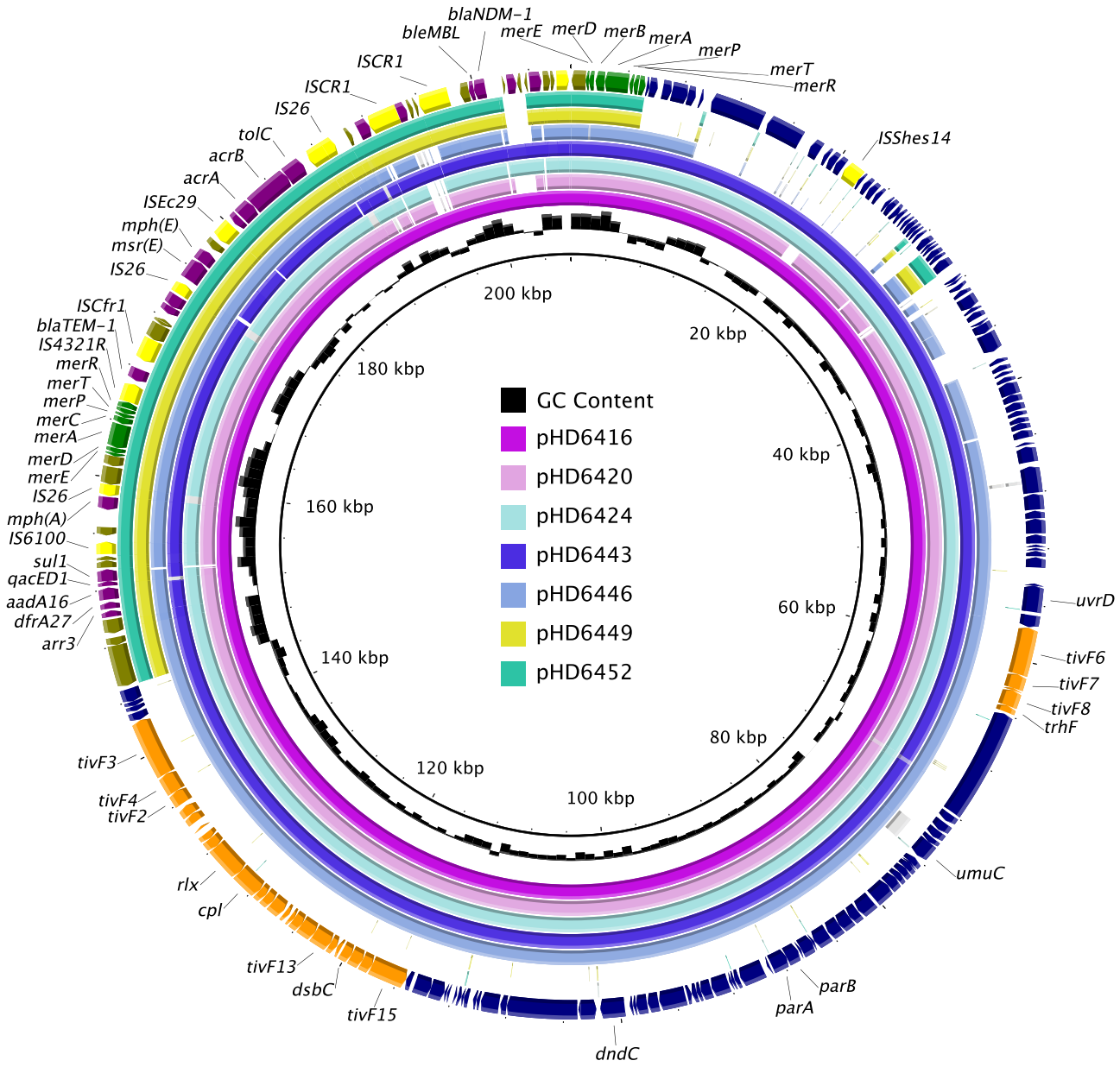


**Figure 2** Plasmid structure comparison between *bla*$_{NDM-1}$-harbouring pHD6416 and the other strains. The backbone region of pHD6420, pHD6424, pHD6443, pHD6446 was highly comparable with pHD6416 backbone, respectively, while pHD6449 and pHD6452 contain a different backbone. The multidrug-resistant regions of the seven plasmids were also highly comparable with just a few mobile elements different. Colored arrows indicate ORFs, with navy, yellow, Orange, olive, purple and green arrows representing plasmid backbone genes, mobile elements, plasmid transfer genes, Tn*6358b/c* backbone genes, the antimicrobial and heavy metal resistance genes, respectively.

*aadA16*), disinfectant (*qacE*), macrolides (*msr(E)*), rifampin (*arr-3*), sulfonamide (*sul1*), β-lactam, carbapenem (*bla*-$_{NDM-1}$), as well as efflux pump, such as acrABC-tolC. This suggests there were two backbone types between seven *bla*$_{NDM-1}$ plasmids. The MDR regions of seven *bla*$_{NDM-1}$ plasmids were highly similar with minor differences on resistance elements and arrangement, and they likely evolve from the same Tn element.

Besides, oriTfinder analysis (https://tool-mml.sjtu.edu.cn/oriTfinder) failed to identify transfer origin regions (oriT) and relaxases sequences in all seven *S. xiamenensis*, suggesting the plasmid is non-transferable. Further conjugation assay also failed to transfer *bla*$_{NDM-1}$-bearing plasmids to recipients of *E. coli* EC600 despite multiple attempts, which is consistent with the sequence analysis results. And *bla*$_{OXA-48}$ genes were also not detected in recipients.

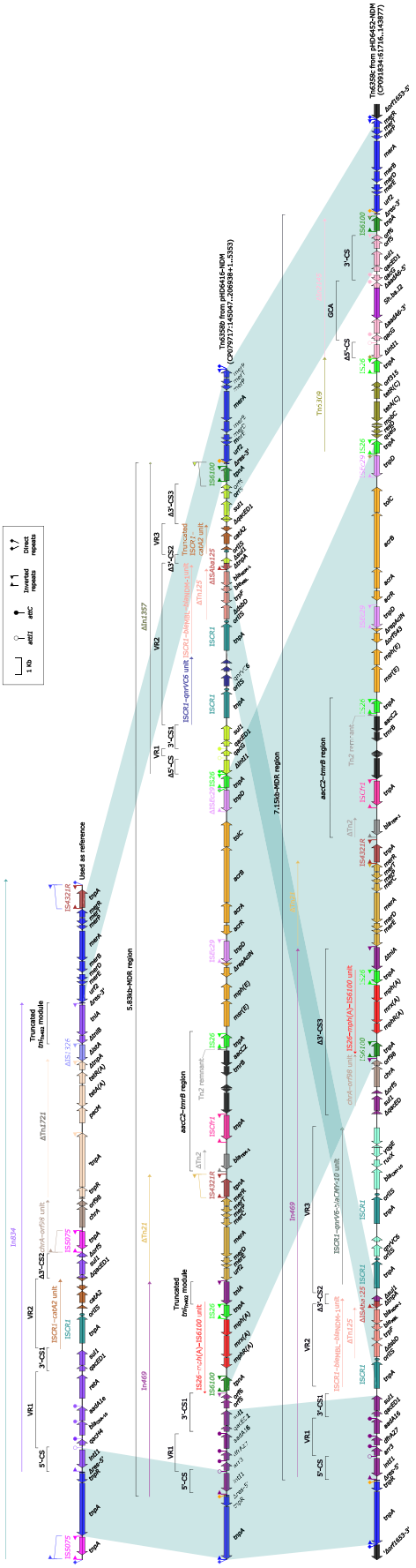

**Figure 3** Linear comparison of Tn*6358a*, Tn*6358b*, Tn*6358c*. The MDR regions from pHD6416 and pHD6452 were highly similar and could evolve from the same Tn element. Genes are denoted by arrows. Gene, mobile elements, and other features are colored based on their functional classification. Shading denotes regions of homology (nucleotide identity ≥95%). Numbers in brackets indicate nucleotide positions within the plasmid pHD6416-NDM and pHD6452-NDM. Tn*6358a* was used as reference and its accession number was JX141473.

## Discussion

*Shewanella* is an opportunistic pathogen and generally invades the immunocompromised and those who are exposed to marine environment. And the *Shewanella* genus has been frequently isolated from clinical specimens in recent years. According to a latest review, bloodstream infections, SSTIs and ENT disorders are most dominant *Shewanella*-related diseases, accounting for 37.0%, 35.9% and 27.5% of all cases, respectively. Unlike the former three, less *Shewanella* strains were isolated from patients of intra-abdominal infection (9.2%), bone arthropathy (6.6%), chest infection (4.4%), CNS disease (2.2%), cardiovascular infection (1.8%). And patients who suffer the infections are distributed in all generations, ranging from newborn to 92-years-old.[13] Besides being pathogenic to human, *Shewanella* have been considered as a reservoir and vehicle of multiple antimicrobial resistance since the description of OXA-54 on *S. oneidensis* as the progenitor of OXA-48 in *K. pneumoniae*.[30] Studies have found a variety of resistance genes from *Shewanella* genus, such as *qnrA* variants, *dfrA1, aadA1, sul1, sul2*, and $bla_{\text{OXA-48-like}}$ variants, conferring them resistance to many antibiotics, including β-lactams, quinolones, trimethoprim, aminoglycoside, sulfonamides, and carbapenems.[10,31–33] And the occurrence of ARBs and ARGs in environment raises worries due to the potential hazard to public health. In our study, we reported three types of OXA-48-like carbapenemases (OXA-48, OXA-181 and OXA-252) from hospital wastewater, where $bla_{\text{OXA-48-like}}$ has not been detected before. Although the occurrence of waterborne species harboring NDM-1-encoding plasmids in China has been frequently reported,[34,35] we describe a novel type $bla_{\text{NDM-1}}$ plasmid of complicated structure in our study. We then further searched for similar $bla_{\text{NDM-1}}$ plasmids in our isolate's storage, but no similar plasmid was found. This suggested that the $bla_{\text{NDM-1}}$ plasmids were assembled in wastewater, which could reflect the resistance gene diversity and complex genetic interaction in hospital wastewater.

The contributing factor to the ARG horizontally transmission is usually mobile element, such as insertion sequence. Insertion sequence has been also suggested accountable for the mobilization of $bla_{\text{OXA-48-like}}$ genes from chromosome to plasmid in this situation. We compared the genetic environment of $bla_{\text{OXA-48}}$, $bla_{\text{OXA-181}}$ and $bla_{\text{OXA-252}}$ from our study with those on plasmids such as Tn*1999* and Tn*2013*, respectively (Figure 4). $bla_{\text{OXA-48}}$ and *lysR* on plasmid borne Tn*1999* shared 2.237 kb of and >95% similarity with those on chromosomes of *S. xiamenensis* (cHD6416, cHD6420, cHD6449 and cHD6452). The $bla_{\text{OXA-48}}$-*lysR* from Tn*1999* in *Enterobacterales* plasmids were flanked by two copies of IS*1999*, indicating Tn*1999* was likely responsible for the capture and mobilization of $bla_{\text{OXA-48}}$-*lysR* region from chromosomes of *S. xiamenensis* to plasmid. Similarly, $bla_{\text{OXA-181}}$-Δ*lysR* region on plasmid carried by Tn*2013* shared 1.299 kb and >95% similarity with chromosome of *S. xiamenensis* HD6424. And $bla_{\text{OXA-181}}$-Δ*lysR* region from Tn*2013* in *Enterobacterales* plasmids were adjacent to IS*Ecp1*, thus Tn*2013* was more than likely to catch $bla_{\text{OXA-181}}$-Δ*lysR* from chromosomes to plasmids.[36,37] This is consistent with previous study that chromosome-encoded β-lactamases of *Shewanella spp*., eg *S. xiamenensis*, are likely the progenitors of $bla_{\text{OXA-48-like}}$ genes in *Enterobacterale* members.[38] Different insertion sequences have been found to be linked

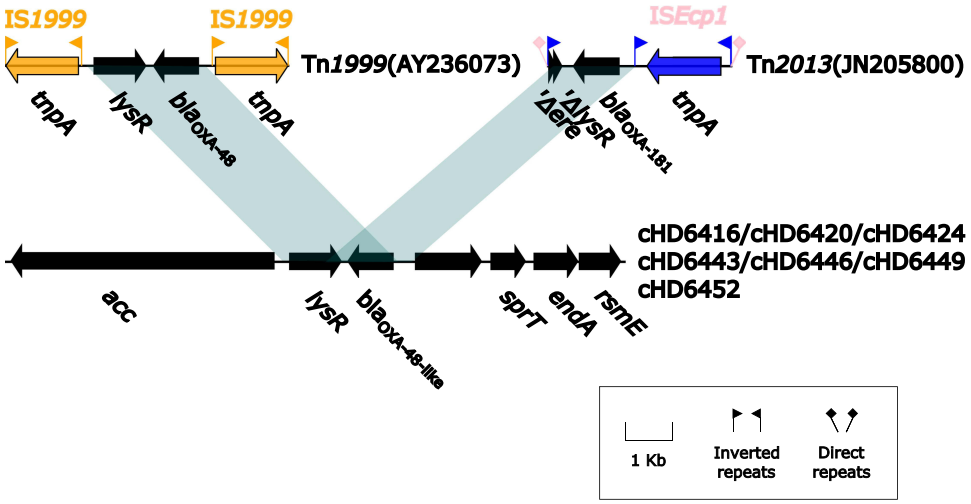


**Figure 4** Linear Comparison of blaOXA-48-like-related region and related regions. Genes are denoted by arrows. Shading denotes regions of homology (nucleotide identity ≥90%). Sequences of Tn*1999* and Tn*2013* are acquired from pOXA-48a plasmid and pKP3-A plasmid on NCBI, respectively, and accession numbers are noted in bracket.

to $bla_{\text{OXA-48-like}}$ genes on plasmids in *Enterobacterales*. The plasmid borne $bla_{\text{OXA-48}}$ and $bla_{\text{OXA-163}}$ are associated with IS*1999* and IS*Ecl4*, respectively, while $bla_{\text{OXA-181}}$, $bla_{\text{OXA-204}}$, and $bla_{\text{OXA-232}}$ genes are associated with IS*Ecp1* elements but each in slightly different arrangements.[38] These results suggested that $bla_{\text{OXA-48-like}}$ genes have been independently mobilized from their original location in *Shewanella* species by different insertion elements on multiple occasions. Under specific occasions, these $bla_{\text{OXA-48-like}}$ genes from the environmental *S. xiamenensis* may be recruited by additional mobile elements, followed by horizontal transfer and dissemination. In China, $bla_{\text{OXA-48-like}}$ producers are not as common as $bla_{\text{KPC}}$ producers, and so far they have been found in several provinces, including Shanghai, Jiangsu, Sichuan, Zhejiang, Guangdong, but few patients had a foreign stay before the $bla_{\text{OXA-48-like}}$ producers were isolated.[4,39–41] For example, the first $bla_{\text{OXA-181}}$ gene in China was found in a clinical ST410 *E. coli* strain WCHEC14828 from a Chinese patient without recent travel history.[5] In this case, the possibility that $bla_{\text{OXA-48-like}}$ genes in China may originate through the horizontal gene transfer from *S. xiamenensis to Enterobacterales* rather than import from other countries, cannot be ruled out. Therefore, detection of OXA-48-like-producing *S. xiamenensis* from hospital wastewater in non-epidemic region, such as China, is rather indicative of the possibility of further transmission, which could lead to local epidemic of $bla_{\text{OXA-48-like}}$ genes. According to the surveillance of CRE in our hospital, OXA-48-like-producing *Enterobacterales* have not been detected yet, however, $bla_{\text{OXA-48-like}}$ transmission in our hospital is still possible due to the emergence of *S. xiamenensis* harboring $bla_{\text{OXA-48-like}}$ in hospital environment. This observation raised the concern that the *S. xiamenensis* from the hospital wastewater may serve as a reservoir for the transmission of ARGs, suggesting the presence of naturally existing carbapenemases in the environment may be significantly overlooked. Hence, the hospital surveillance towards both clinical isolates and wastewater should afterward continue to detect $bla_{\text{OXA-48-like}}$ gene prevalence in real time.

Wastewater-based surveillance has been recommended as a low-cost methodology to monitor antimicrobial resistance prevalence, and could be useful in monitoring environmental resistance gene and helping decrease the health risks coming from the wastewater.[42] However, research about hospital wastewater remains rare in China because of a lack of understanding and underestimation of surveillance. Future work should be considered to establish wastewater surveillance system in China hospitals to assess antimicrobial resistance abundances and monitor the transmission of resistance between human and the environment.

## Conclusion

In our study, we reported seven *S. xiamenensis* co-producing NDM-1 and OXA-48-like carbapenemases (OXA-48, OXA-181 and OXA-252) from hospital wastewater in China and characterized them in some profiles. And to the best of our knowledge, this is the first report of detection of three types $bla_{\text{OXA-48-like}}$ genes in one hospital in China. This study proposes the possible origin of the $bla_{\text{OXA-48-like}}$ cases in China and warns the risk of $bla_{\text{OXA-48-like}}$ transmission in our hospital. Besides, an active antimicrobial resistance surveillance system in both clinical isolates and the hospital environment should be considered. And more attention is urgently needed on multi-drug resistant *Shewanella* genus.

## Institutional Review Board Statement

Ethical approval is not applicable in this study as the samples were derived from environment. And the study was conducted in accordance with the principles of the Declaration of Helsinki.

## Data Sharing Statement

The whole genome sequences of strains in this study were deposited in GenBank Bioproject PRJNA755833.

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## Disclosure

The authors declare no conflicts of interest in this work.

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