



The prevalence and diversity of carbapenem-hydrolyzing bacteria and β -lactamases in the aquatic environment of the Han River, South Korea

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ABSTRACT

Antimicrobial resistance poses a critical global health threat, and carbapenem-resistant bacteria (CRB) are of a particular concern. Aquatic environments are increasingly recognized as reservoirs for CRB and associated resistance determinants. This study investigated the diversity, distribution, and functional properties of carbapenem-hydrolyzing β -lactamases (CH β Ls) in environmental isolates from the Han River, South Korea. A multi-step screening of approximately 3,200 environmental isolates identified 64 carbapenem-hydrolyzing bacteria, predominantly from the genera *Chryseobacterium*, *Stenotrophomonas*, and *Aeromonas*. Using 41 carbapenemase-specific primer pairs and multiplex PCR, 62 distinct CH β L sequences were detected, with Ambler class B metallo- β -lactamases—particularly subclasses B1 (CGB and IND families) and B3 (L1 and GOB families)—being most prevalent. Notably, 46 sequences represented novel variants, each differing from previously reported CH β Ls by at least one amino acid substitution. Two clinically relevant class D β -lactamase genes, *bla*_{OXA-334} and *bla*_{OXA-652}, were identified in *Acinetobacter johnsonii* isolates, and their heterologous expression confirmed their functionality by conferring resistance against carbapenems. Genomic analysis further revealed that *bla*_{OXA-652} gene was flanked by transposases associated with the insertion sequence (IS) 200/605, IS4, and IS1 families, suggesting potential for transmission via horizontal gene transfer. These results demonstrate the substantial diversity of carbapenemases and their producers of environmental origin in the Han River during the investigation period, suggesting the riverine environment as a reservoir of various CH β Ls, including clinically important enzymes. Our findings underscore the potential role of aquatic ecosystems in the dissemination of clinically relevant carbapenemase genes and highlight the necessity for continuous surveillance of the environmental resistome.

1. Introduction

Antimicrobial resistance (AMR) represents a serious global health crisis, jeopardizing the effectiveness of vital medicines. β -Lactam antibiotics remain fundamental to antibacterial therapy; however, their effectiveness is increasingly undermined by resistance mechanisms, primarily the production of β -lactamases (Bush and Bradford, 2016). Carbapenems—often reserved as last-resort antibiotics for treating multidrug-resistant (MDR) infections (WHO, 2023)—are particularly impacted. The emergence and global dissemination of carbapenem-resistant *Enterobacterales* (CRE) exemplify this crisis; designated as 'critical group' pathogens by the WHO (WHO, 2024), CRE infections are associated with limited treatment options and high mortality rates. Resistance in CRE is primarily driven by carbapenemases,

representing a diverse group of β -lactamases that hydrolyze most β -lactams, including carbapenems (Queenan and Bush, 2007). These enzymes are predominantly classified into Ambler classes A, B, and D, with a smaller subset categorized as class C (Hammoudi Halat and Ayoub Moubareck, 2020). While classes A, C, and D carbapenemases function as serine hydrolases, class B enzymes are zinc-dependent metallo- β -lactamases (MBLs) (Queenan and Bush, 2007). Five major carbapenemase families—KPC, NDM, OXA, VIM, and IMP—are of critical clinical importance due to their global prevalence and substantial impact on treatment efficacy (Alvisi et al., 2025; Bonomo et al., 2018).

Carbapenem-resistant bacteria (CRB), initially associated with nosocomial infections, are now increasingly detected in aquatic environments such as sewage (Kehl et al., 2022), freshwater (Zurfluh et al., 2013a), and coastal waters (Paschoal et al., 2017). This environmental

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dissemination raises significant public health concerns regarding potential transmission routes back to human populations. Indeed, carbapenemase-encoding genes have been identified in various bacterial genera isolated worldwide from aquatic environments, including *Acinetobacter*, *Citrobacter*, *Enterobacter*, *Klebsiella*, and *Pseudomonas* (Cherak et al., 2021). These findings indicate that carbapenemase-producing bacteria are prevalent in aquatic settings. The emergence of CRB in aquatic environments—once considered primarily a hospital-associated problem—has heightened concerns about their potential transmission from environmental reservoirs back into clinical settings (Cho et al., 2023; Larsson and Flach, 2022; Mollenkopf et al., 2025).

Aquatic environments serve as intricate microbial ecosystems, facilitating the transmission of antibiotic resistance genes (ARGs) (Marti et al., 2014). Addressing this environmental dimension of AMR requires a One Health approach—integrating human, animal, and environmental perspectives—to develop comprehensive mitigation strategies (Kim and Cha, 2021). Through characterization of β -lactamase diversity, particularly carbapenemases, within environmental microbial communities is critical for deciphering resistance dynamics and guiding targeted intervention to curb ARG transmission. Although the presence of CRB in aquatic environments is well-documented, detailed analyses of the diversity, distribution, and specific variants of carbapenem-hydrolyzing β -lactamases (CH β LS) in localized river ecosystems remain scarce. Understanding this environmental resistome is vital for crafting region-specific risk assessment and containment measures.

In this study, we investigated the diversity of CH β LS in environmental bacteria isolated from the Han River in South Korea. By analyzing the distribution and characteristics of carbapenemase-producing bacteria, we aimed to enhance understanding of AMR dissemination within this aquatic ecosystem. Our research provides valuable insights through characterizing β -lactamase diversity, with a particular focus on carbapenemase producers in a major urban river system. Ultimately, our findings aim to contribute to the development of effective AMR management strategies based on the One Health framework.

2. Materials and methods

2.1. Isolation and identification of carbapenem-resistant bacteria

From 2016 to 2017 every May, surface water samples were collected from 15 sampling sites of the Han River (Fig. S1a). Antibiotic-resistant bacteria were isolated from the water samples using Mueller Hinton agar (BD Difco, USA) supplemented with each of the following antibiotics: gentamicin (8 mg/L), amoxicillin (16 mg/L), cephalexin (32 mg/L), tetracycline (4 mg/L), erythromycin (2 mg/L), chloramphenicol (16 mg/L), ciprofloxacin (2 mg/L), lincomycin (2 mg/L), vancomycin (4 mg/L), and sulfamethoxazole (40 mg/L). Antimicrobial susceptibility testing was conducted using the disk diffusion assay according to Clinical and Laboratory Standards Institute (CLSI) guidelines. Carbapenem-resistant bacteria were selected based on the inhibition zone diameter values against meropenem (10 μ g; Liofilchem, Italy) of less than 10 mm. While this cutoff is more stringent than the standard CLSI and EUCAST susceptibility breakpoints (typically 15–23 mm for resistance) (CLSI, 2025; EUCAST, 2025), we intentionally employed this conservative threshold to prioritize the selection of isolates exhibiting high-level resistance and to increase the probability of identifying carbapenemase-producing strains. Taxonomic identification of CRB was performed by 16S rRNA gene sequencing (Macrogen, Korea). The 16S rRNA gene sequences of CRB were aligned with the nearest sequences from the EzBioCloud server (<https://www.ezbiocloud.net>) (Chalita et al., 2024).

2.2. Carbapenem biotransformation assay

CRB were cultivated in R2A broth (MB cell, Korea) at 30 °C for 12

hours, after which meropenem trihydrate (JW Pharmaceutical, Korea) was added at a concentration of 100 mg/L. The culture supernatant was obtained by centrifugation at 13,000 $\times$ g for 10 min. Biotransformation of meropenem was analyzed by high-performance liquid chromatography (HPLC) using Kinetex C-18 column (Phenomenex, USA). The mobile phase consisted of an isocratic solution containing 30 % acetonitrile (J.T. Baker) and 0.1 % formic acid, with a flow rate of 1.0 mL/min for 10 min. Detection was performed using a UV-Vis detector set at a wavelength of 300 nm. For the crude extract assay, CRB cultured in R2A broth at 30 °C for 24 h were disrupted by sonication in sodium phosphate buffer (pH 7.0) with 50 μ M ZnSO₄. Cell debris was removed by centrifugation at 13,000 $\times$ g at 4 °C for 30 min, followed by filtration through a 0.2 μ m membrane to obtain cell-free crude extracts. The reaction mixture contained 100 μ L of crude extract (approximately 0.2–1 mg protein), 0.2 mM meropenem, 50 mM phosphate buffer (pH 7.0), and 50 μ M ZnSO₄ in a total volume of 2 mL (Nordmann et al., 2012). Samples from the reaction mixtures were tested at time interval using UV spectrophotometry (Cary 100 UV-Vis spectrophotometer, Agilent, USA) at 298 nm.

2.3. Design of carbapenemase-targeted primers

CH β LS were systematically classified into 41 distinct families or types following previously reported classification frameworks (Lee et al., 2015). Gene sequences corresponding to each designated CH β L type were retrieved from three databases: NCBI GenBank, the Comprehensive Antibiotic Resistance Database (CARD) (Alcock et al., 2023), and the β -lactamase database (BLDB) (Naas et al., 2017). Multiple sequence alignments for each gene family were conducted using MAFFT (Katoh and Standley, 2013). CH β L-specific primer pairs were designed based on published sequences to amplify all alleles of each carbapenemase gene (Lee et al., 2015).

2.4. DNA extraction and PCR amplification

Genomic DNA was extracted from carbapenem-hydrolyzing isolates using the DNeasy Blood and Tissue kit (Qiagen), following the manufacturer's protocol. DNA concentrations were normalized to 20 ng/ μ L. PCR amplification was performed using AccuPower PCR Premix (Bio-ner, Korea). The thermal cycling conditions were as follows: initial denaturation at 95 °C for 5 min; 30 cycles of denaturation at 95 °C for 30 s, annealing at 50–64 °C for 40 s (annealing temperature optimized for each primer pair), and elongation at 72 °C for 50 s; followed by a final extension at 72 °C for 10 min.

2.5. Whole genome sequencing and annotation

Whole genome sequencing and assembly of *bla*_{OXA}-harboring strains were performed using the PacBio RS II (Pacific Bioscience, USA) platform at CJ Bioscience (Seoul, Korea). Functional annotation of coding sequences—including ARGs and mobile genetic elements (MGEs)—was conducted using Bakta (Schwengers et al., 2021). The genome sequences of *Acinetobacter johnsonii* strains 16H1E3 and 16H4P8 were deposited in the NCBI BioProject under the accession numbers PRJNA1260445 and PRJNA1260447, respectively.

2.6. Heterologous expression of OXA family β -lactamases and antimicrobial susceptibility testing of recombinants

The cloning and expression of OXA family β -lactamase genes were conducted using pET28-(a) vector in *E. coli* BL21 (DE3). The genes encoding OXA family β -lactamases were amplified by PCR using specific primers as summarized in Table S1. *E. coli* BL21 (DE3) harboring recombinant plasmid constructs were cultivated at 37 °C in LB medium. When cultures reached an OD₆₀₀ of 0.5, protein expression was induced with IPTG at a final concentration of 0.1 mM. The minimum inhibitory

concentrations (MIC) of ampicillin (LPS solution, Korea), cefotaxime, cefoxitin, imipenem, oxacillin (Sigma-Aldrich, USA), and meropenem (TCI, Japan) were determined using the broth microdilution method in accordance with CLSI guidelines. MICs were assessed for IPTG-induced *E. coli* BL21 (DE3) clones cultured in Mueller-Hinton broth (Difco, USA).

2.7. Phylogenetic and sequence analyses of carbapenemases

Phylogenetic analysis was conducted using sequences from amplified PCR products and carbapenemase-encoding genes obtained from genome sequencing data, together with representative β -lactamase sequences retrieved from the BLDB (Naas et al., 2017). Amino acid sequences were aligned using MAFFT (Katoh and Standley, 2013). Phylogenetic tree was constructed using FastTree2 (Price et al., 2010) and visualized using Interactive Tree of Life (Letunic and Bork, 2024). Amino acid sequences of carbapenemase variants were analyzed using BLASTP search in the BLDB (Naas et al., 2017).

2.8. In silico plasmid prediction

To determine whether putative *bla*_{OXA} genes are located on plasmids or chromosomes, *in silico* plasmid prediction was performed using multiple tools, including geNomad (Camargo et al., 2024), PlasFlow (Krawczyk et al., 2018), PlasClass (Pellow et al., 2020), MOB-suite (Robertson and Nash, 2018), and PlasForest (Pradier et al., 2021).

3. Results

3.1. Screening of environmental bacteria producing carbapenem-hydrolyzing β -lactamases (CH β Ls)

From an initial collection of approximately 3,200 antibiotic-resistant bacteria isolated from the Han River water samples, 220 isolates were selected as potential CRB based on meropenem inhibition zone diameters (≤ 10 mm) measured by disk diffusion assay (Fig. S1b). These 220 isolates were further evaluated for their ability to degrade meropenem using HPLC analysis, enabling their categorization into four profiles: 'complete degradation', 'partial degradation', 'transformation to one metabolite', and 'transformation to two metabolites' (Fig. S2). Subsequent UV spectrophotometry assays confirmed meropenem hydrolysis activity, resulting in the identification of 78 carbapenemase-producing isolates. Molecular screening using a panel of 41 primer pairs targeting previously known carbapenemase gene families or types (Table S1) detected a total of 62 unique CH β L gene sequences from 64 isolates. Based on 16S rRNA gene sequencing, these 64 CH β L-positive isolates were assigned to nine distinct genera spanning five bacterial classes (Fig. S3). Carbapenemase-producing isolates were recovered from all 15 sampling sites along the Han River (Fig. S3). *Chryseobacterium* was the most frequently identified genus harboring CH β L genes ($n = 31$ isolates), followed by *Stenotrophomonas* ($n = 15$) and *Aeromonas* ($n = 6$).

3.2. Diversity and distribution of CH β Ls in the Han River isolates

Phylogenetic analysis based on the amino acid sequences of 62

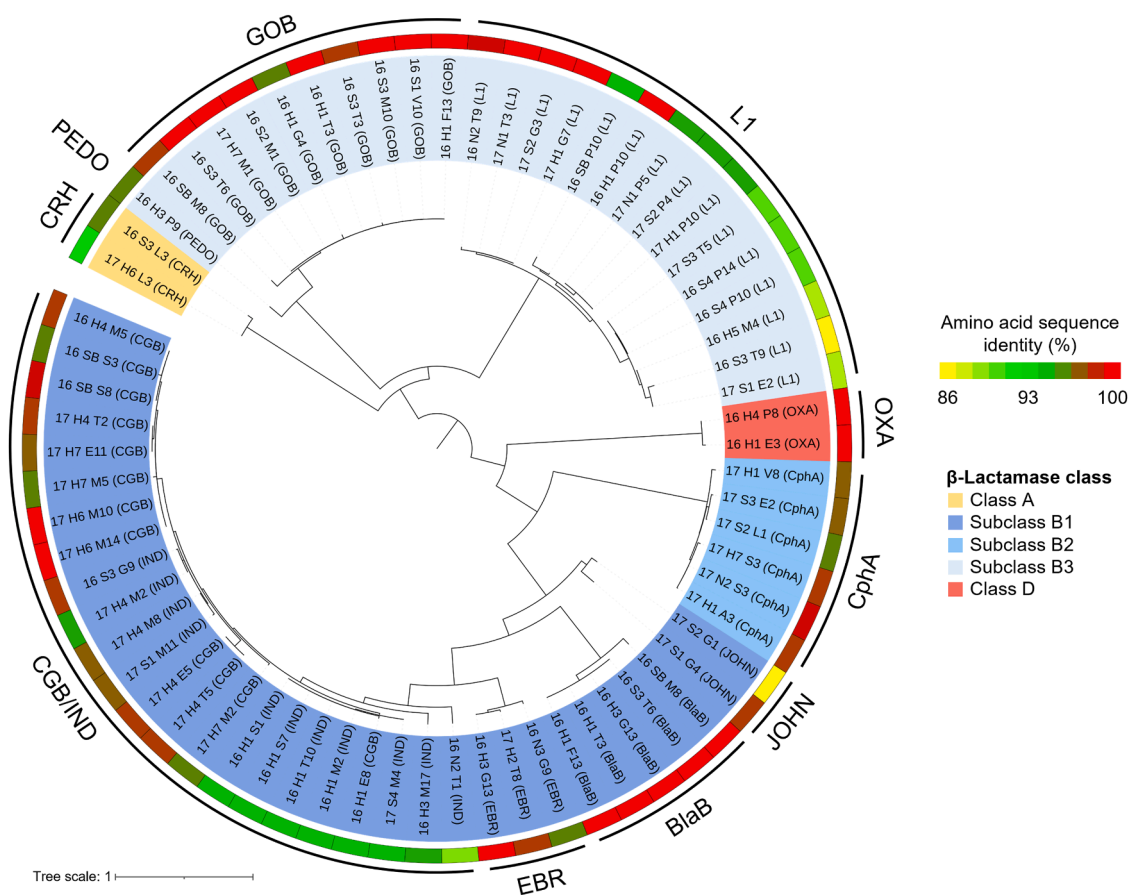


Fig. 1. Diversity and distribution of carbapenem-hydrolyzing β -lactamases (CH β Ls) in environmental isolates from the Han River. Phylogenetic tree of CH β L amino acid sequences identified from carbapenemase-producing isolates from the Han River. Colors above strain names indicate amino acid sequence identity to the most similar CH β L reference sequence in the BLDB. Shaded colors on strain names denote Ambler β -lactamase class.

CHβLs from 64 carbapenemase-producing isolates revealed significant genetic diversity among carbapenemases in the Han River (Fig. 1). The resulting phylogenetic tree demonstrated distinct clustering patterns consistent with both the isolates' taxonomic origins and the established β-lactamase classification system. Pairwise amino acid sequence identities between the CHβL sequences detected from the Han River isolates and the most similar reference sequences from each family in the BLDB ranged from 86 % to 100 %, with sequences being grouped into clades specific to carbapenemase classes (Fig. 1). The majority of carbapenemase sequences were classified as Ambler class B, primarily subclasses B1 and B3, while smaller proportions belonged to classes A and D (Fig. 1).

The distribution of specific carbapenemase families varied among the identified bacterial genera (Fig. 2a and b). *Chryseobacterium* isolates harbored the highest number of distinct carbapenemase sequences ($n = 33$), followed by *Stenotrophomonas* ($n = 15$) and *Aeromonas* ($n = 6$) (Fig. 2a). Analysis based on carbapenemase family revealed that L1 family enzymes (subclass B3) were the most prevalent overall and found exclusively in *Stenotrophomonas* isolates (Fig. 2b). Similarly, CGB and IND family enzymes (subclass B1) were detected only in *Chryseobacterium*, while GOB family enzymes (subclass B3) were present in both *Chryseobacterium* and *Elizabethkingia* isolates (Fig. 2b). Subclass B2 MBLs (CphA family) were identified exclusively in *Aeromonas* species (Fig. 2a and b). Except for BlaB (subclass B1), all other less frequently detected carbapenemase families were confined to individual bacterial genera among the isolates studied (Fig. 2b). Overall, the phylogenetic relationships among the carbapenemase sequences largely reflected the taxonomic relationships of their host bacteria, with sequences from the same genus typically clustering together.

3.3. Diversity and distribution of class B carbapenemases

Phylogenetic analysis focused on class B metallo-β-lactamases (MBLs), the most abundant carbapenemase class identified among the Han River isolates, using closely related reference sequences from the BLDB. The environmental class B MBL sequences clustered within nine major β-lactamase families: IND, CGB, EBR, BlaB, and JOHN (subclass B1); CphA (subclass B2); and GOB, PEDO, and L1 (subclass B3). Subclass B1 MBLs identified from the Han River isolates exhibited the greatest diversity within class B, encompassing sequences from five families: IND, CGB, EBR, BlaB, and JOHN (Fig. S4). The most prevalent subclass B1 sequences, belonging to the IND and CGB families, formed clusters that included reference sequences previously identified in environmental samples, indicating phylogenetic relatedness within the Han River ecosystem (Fig. S4). The clustering patterns within these families suggest considerable genetic diversity among the variants. Sequences from the CphA family (subclass B2), primarily derived from *Aeromonas* isolates, clustered closely with reference CphA sequences from the BLDB

(Fig. S4). Subclass B3 MBLs detected in this study were mainly from the L1 and GOB families. Similar to the IND and CGB families, L1 family sequences—predominantly detected in *Stenotrophomonas*—formed diverse clusters interspersed with sequences from various environmental origins reported previously (Fig. S4).

3.4. Prevalence of novel carbapenemase variants

Of the 62 CHβL sequences identified, 45 (73 %) differed by at least one amino acid substitution from previously reported CHβLs in the BLDB (Table S2), while 17 sequences (27 %) were identical to previously reported CHβLs (Fig. 3a). These variant sequences are regarded as novel CHβL variants according to the standard β-lactamase nomenclature (Bradford et al., 2022). These novel variants were distributed across class A, B, and C β-lactamase, with the highest abundance and diversity in subclass B1 and B3, whereas the known variants belonged to subclass B1, B3, and class D (Fig. 3a). The degree of sequence divergence varied considerably among enzyme families. The L1 family exhibited the greatest variations, with an average of 14.9 amino acid substitutions per isolate (Fig. 3b and Table S2). High levels of substitution were also observed in the PEDO (7.0 substitutions) and IND (6.3 substitutions) families (Fig. 3b and Table S2). These findings suggest the widespread prevalence of diverse and novel CHβL variants in the riverine ecosystem. All carbapenemase variants retained the conserved amino acid motifs at the catalytic sites characteristic of their respective β-lactamase classes (Bahr et al., 2021; Tooke et al., 2019) (Table S2).

3.5. Identification and functional validation of clinically relevant carbapenemases

Since the novel carbapenemase variants were all partial sequences revealed by PCR amplification, further characterization was limited. However, the complete sequences of genes encoding class D β-lactamases OXA-334 and OXA-652, both belonging to the OXA-211-like subfamily, were recovered from the genome sequences of *Acinetobacter johnsonii* isolates from the Han River. The OXA-652 sequence was identical to a previously known variant, whereas the OXA-334 sequence represented a novel variant with a single amino acid substitution after its complete sequence was analyzed (Table S2). While *A. johnsonii* is known to inhabit diverse environments, including aquatic ecosystems (Guardabassi et al., 1999), its association with nosocomial infections has also been documented (Montana et al., 2016; Seifert et al., 1993). This context underscores the significance of detecting clinically relevant OXA family members (Diebold et al., 2023) in environmental isolates. Whole genome sequencing analysis confirmed that the *bla*_{OXA-334} and *bla*_{OXA-652} genes identified in these environmental isolates exhibited 99.9 % and 100 % nucleotide sequence identity, respectively, to sequences previously reported in *A. johnsonii* strains (Figueiredo et al., 2012).

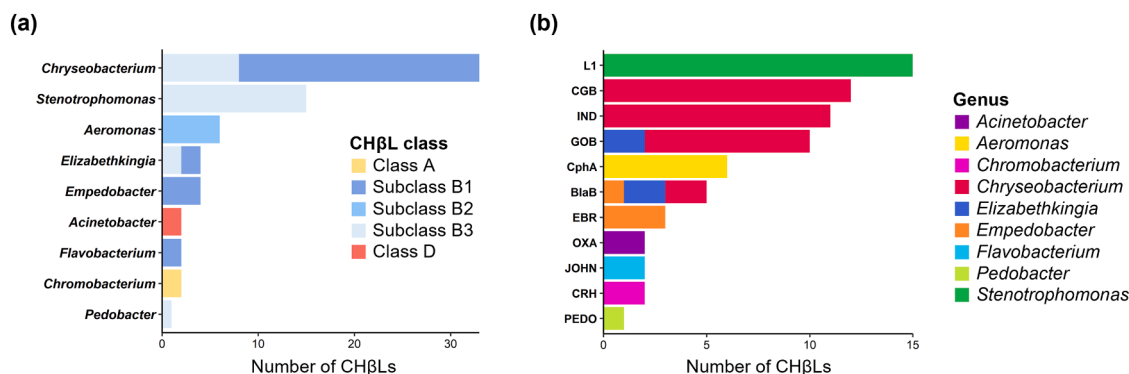


Fig. 2. Profile of carbapenemase classes and their gene families across bacterial genera identified from the Han River isolates. (a) Distribution of Ambler classes of identified carbapenemase sequences across different bacterial genera. (b) Distribution of specific carbapenemase families among the identified bacterial genera.

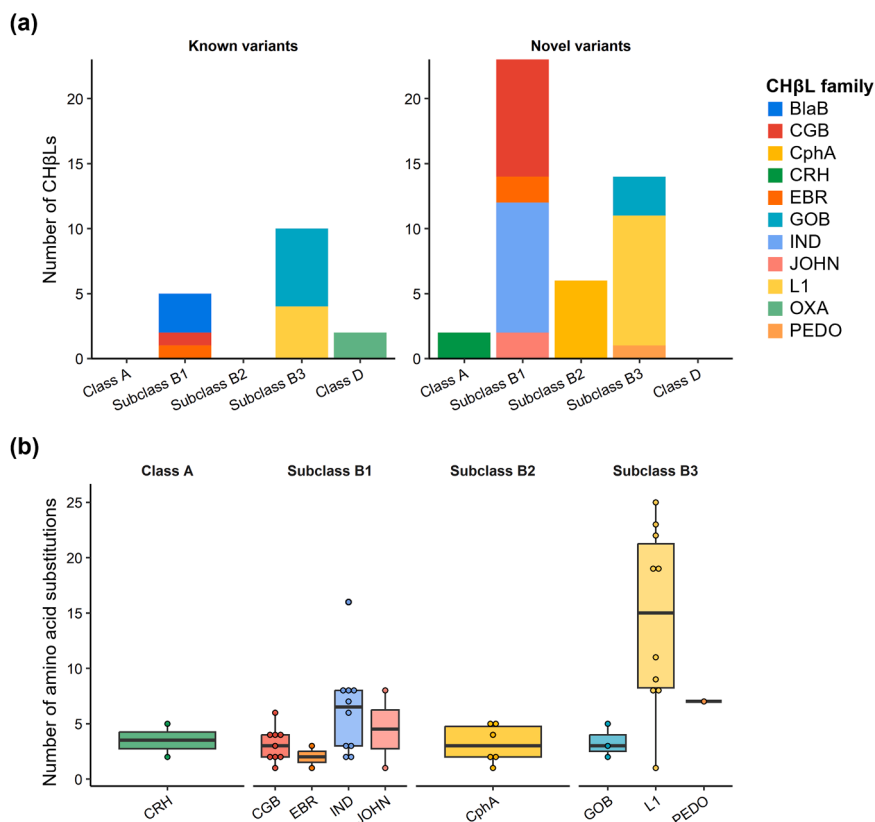


Fig. 3. Distribution and diversity of environmental CHβL variants. (a) Proportional distribution of CHβL families among known and novel variant sequences according to CHβL classes. (b) Number of amino acid substitutions observed in novel CHβL variant sequences across different CHβL families.

Phylogenetic analysis using known OXA-211-like amino acid sequences retrieved from the BLDB confirmed that the sequences from the Han River isolates clustered within the established OXA-211-like clade (Fig. S5).

To evaluate functionality, the identified *bla*_{OXA} genes were heterologously expressed in a meropenem-susceptible *E. coli* host strain. Functional assays demonstrated that these OXA-211-like subfamily enzymes degraded ampicillin, imipenem, meropenem, and oxacillin. Consequently, the recombinant *E. coli* strains expressing these OXA

enzymes exhibited 2- to 512-fold increases in MICs for these antibiotics compared to the control strain harboring the empty vector (Fig. 4). In contrast, no significant changes in MICs were observed for cefotaxime and ceftazidime (second- and third-generation cephalosporins) (Fig. 4). These results indicate a substrate profile characteristic of carbapenemase activity with limited activity against the tested cephalosporins. When these *bla*_{OXA} genes were analyzed using several *in silico* plasmid prediction methods, both genes were predicted to be located on chromosomal contigs. Notably, genomic analysis of the region surrounding the *bla*_{OXA-652} gene in the *A. johnsonii* isolate revealed the presence of transposase genes associated with insertion sequence (IS) 200/605, IS4, and IS1 families (Fig. 5), suggesting potential for transmission via horizontal gene transfer (HGT).

4. Discussion

Carbapenems, often referred to as ‘last resort antibiotics’, are critical therapeutic agents for treating infections caused by MDR Gram-negative bacteria. However, the global emergence and dissemination of CRE, frequently mediated by carbapenemases, constitute a significant public health threat (Gamal et al., 2016; Jeon et al., 2015; Nordmann et al., 2009; WHO, 2024). Several studies have reported the presence of carbapenemase-encoding genes in aquatic environments worldwide (Cherak et al., 2021; Cho et al., 2023; Zurfluh et al., 2013b), including documented cases in South Korea (Di et al., 2017; Yoon et al., 2018), highlighting these environments as potential reservoirs of carbapenem resistance.

In this study, we employed a multi-step approach combining phenotypic screening for carbapenem susceptibility, biochemical characterization of carbapenem hydrolysis, and molecular detection of carbapenemase-encoding genes by multiplex PCR to investigate the diversity of carbapenemase-producing bacteria and carbapenemase genes

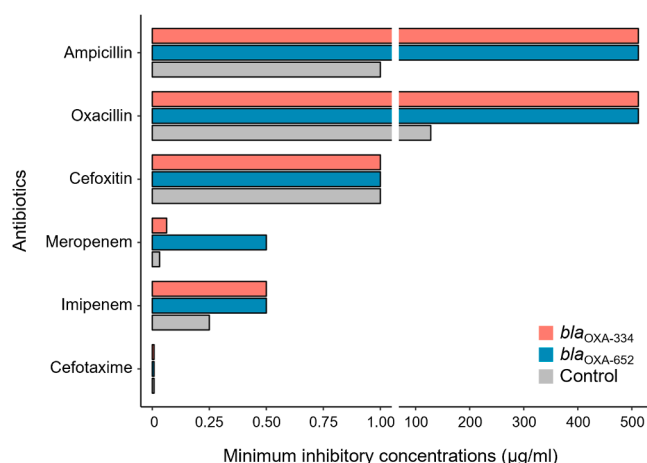


Fig. 4. Antimicrobial resistance profiles conferred by the heterologous expression of OXA-211-like β-lactamases (OXA-334 and OXA-652) in *E. coli*. MICs were determined for recombinant *E. coli* BL21 (DE3) strains expressing either *bla*_{OXA-334} (coral) or *bla*_{OXA-652} (teal), compared to a control strain harboring an empty vector (grey).

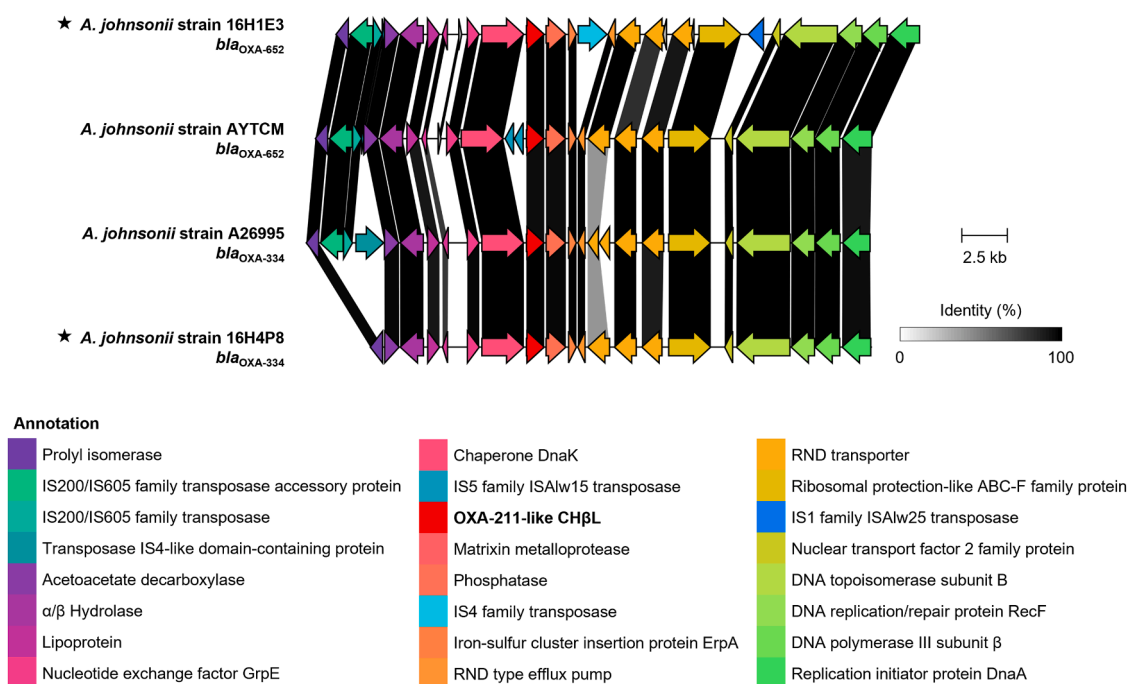


Fig. 5. Comparative analysis of the genomic context of *bla*_{OXA-334} and *bla*_{OXA-652} genes. Linear comparison of the genomic regions containing *bla*_{OXA-334} and *bla*_{OXA-652} from *A. johnsonii* strains isolated in this study (strains 16H1E3 and 16H4P8, indicated by star symbols) with publicly available *A. johnsonii* genomes from the NCBI RefSeq database (strains AYTCM and A26995). Pairwise alignment and visualization of gene clusters were conducted using Clinker.

in the Han River. This strategy led to the identification and characterization of 62 CHβL genes from 64 carbapenem-hydrolyzing bacterial isolates. Our results revealed that *Chryseobacterium*, *Stenotrophomonas*, and *Aeromonas* were the predominant genera exhibiting carbapenem-hydrolyzing activity in the Han River. Among the detected enzymes, the IND and CGB families belonging to subclass B1 MBLs were the most frequently identified. Furthermore, our analysis uncovered a diverse repertoire of carbapenemase genes, encompassing MBL subclasses B2 and B3, along with representatives from Ambler classes A and D.

The discovery of 46 novel variants characterized by extensive amino acid substitutions indicates the prevalence and diversity of carbapenemases in the anthropogenically influenced riverine environment, providing compelling evidence of ongoing diversification. These findings reinforce the recognized role of aquatic environments as significant reservoirs harboring a diverse array of carbapenem resistance determinants distributed across multiple bacterial genera (Baquero et al., 2008; Larsson and Flach, 2022). The prevalence of genera such as *Chryseobacterium* and *Stenotrophomonas* deserves particular attention, as these opportunistic pathogens are increasingly reported in nosocomial infections such as bacteremia and are known to carry various antibiotic resistance determinants (Koh et al., 2025). The predominance of class B MBLs found in this study, especially those from the CGB, IND, L1, and GOB families, underscores their ecological importance within the bacterial communities of the Han River and their potential contribution as environmental sources of clinically relevant carbapenem resistance genes, although clinically dominant carbapenemases, such as NDM, KPC, and VIM families, were not detected in this study.

While reports on OXA-211-like enzymes (class D) are relatively limited compared to other major OXA families, our identification of *bla*_{OXA-334} and *bla*_{OXA-652} in environmental *A. johnsonii* isolates represents a valuable addition to the overall carbapenem repertoire. Given that this subfamily remains relatively poorly characterized, our functional analysis via heterologous expression provides important insights into their properties and potential significance. Notably, we demonstrated hydrolytic activity against key β-lactams including ampicillin, oxacillin, meropenem and imipenem, but observed no activity with the

tested second- or third-generation cephalosporins (cefotaxime and cefoxitin). These results are consistent with previous findings that OXA-211 confers resistance to carbapenems, such as meropenem and imipenem, but not to cephalosporins like cefotaxime, ceftazidime, and cefepime (Figueiredo et al., 2012). Furthermore, the presence of transposase genes flanking the *bla*_{OXA-652} gene suggests its potential for mobilization via HGT. These observations raise concerns about the possible transmission pathways of such resistance determinants from environmental bacteria to clinically relevant pathogens. Members of the genus *Acinetobacter*, including *A. johnsonii*, are ubiquitous in the environment, including soil and water (Choi et al., 2012; Doughari et al., 2011). This widespread distribution potentially increases opportunities for ARG transfer through mobile genetic elements to pathogenic species, such as *A. baumannii*, a major cause of nosocomial infections. The detection of clinically relevant carbapenemase genes from environmental isolates, as demonstrated here with *bla*_{OXA-652}, underscores the potential for bidirectional transmission of ARGs between environmental reservoirs and clinical settings. This finding highlights the necessity of vigilant surveillance efforts informed by a One Health perspective.

5. Conclusion

The present study provides robust evidence for the prevalence and considerable diversity of carbapenem-hydrolyzing bacteria and β-lactamases in the Han River aquatic ecosystem, representing a valuable historical baseline of the riverine resistome during the study period. Although the environmental resistome is inherently dynamic and subject to temporal changes, these findings underscore the critical need for AMR surveillance in environmental reservoirs to effectively monitor the emergence, evolution, and dissemination pathways of clinically significant carbapenem resistance determinants. Furthermore, this work reinforces the indispensable role of an integrated One Health approach—encompassing human, animal, and environmental health sectors—in comprehensively understanding and effectively combating the global public health threat posed by AMR.

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CRediT authorship contribution statement

Do-Hoon Lee: Writing – review & editing, Writing – original draft, Visualization, Validation, Methodology, Investigation, Formal analysis. **Ji-Hye Bu:** Writing – original draft, Validation, Methodology, Investigation, Formal analysis. **Miryung Kim:** Writing – review & editing, Investigation. **Chang-Jun Cha:** Writing – review & editing, Writing – original draft, Validation, Supervision, Methodology, Funding acquisition, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at [doi:10.1016/j.hazadv.2026.101078](https://doi.org/10.1016/j.hazadv.2026.101078).

Data availability

The assembled genome sequences of the Han River isolates were deposited in the NCBI BioSample under the accession number SAMN48394712 and SAMN48394732. Novel variant sequence deposition to NCBI under the accession number WP_279665785.1.

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