Original

Analysis of amikacin resistance determinants in carbapenem-resistant *Escherichia coli* detected in Japan

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ABSTRACT

The global spread of antimicrobial-resistant pathogens remains a major challenge in clinical practice. Among these, carbapenem-resistant Enterobacterales (CRE) are of particular concern, with their prevalence increasing markedly across Asia. In Japan, most CRE isolates produce imipenemase (IMP)-type metallo-β-lactamases (MBLs). Moreover, many strains detected in western Japan carry pKPI-6–related plasmids and their derivatives harboring $bla_{\text{IMP-6}}$, which confer resistance to carbapenems but characteristically retain susceptibility to the aminoglycoside amikacin. We conducted a 13-year surveillance study (2012–2024) of *Escherichia coli* isolates at a mixed-care medical facility in northern Osaka Prefecture, primarily serving patients requiring chronic care. Among 59 carbapenem-resistant *E. coli* strains identified, two exhibited resistance to both carbapenems and amikacin. Both isolates carried $bla_{\text{IMP-6}}$ and the aminoglycoside 6′-N-acetyltransferase (*aac(6′)-Ib*) genes with an *aac(6′)-Ib* variant associated with reduced amikacin resistance, arranged in tandem on the same plasmid. Furthermore, one strain harbored the *aac(6′)-Iae*, which appears to represent a rarely documented occurrence in *E. coli,* whereas the other carried *aac(6′)-Ib-cr.* Cloning experiments and minimum inhibitory concentrations (MICs) analyses identified *aac(6′)-Iae* and *aac(6′)-Ib-cr* as the likely principal contributors to amikacin resistance in these isolates, whereas the contribution of the *aac(6′)-Ib*(L119S) variant appeared limited. Genome database analysis of IMP-type MBL-producing CRE prevalent in Japan suggested parallel dissemination of bla_{IMP} and the *aac(6′)-Ib*(L119S) variant, which may partly explain the high proportion of isolates that remain susceptible to amikacin. Continued surveillance is warranted to monitor potential shifts in resistance profiles, including the emergence of isolates with increased resistance to amikacin.

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Key Words
aac(6′)-Iae, *aac(6′)-Ib*, *aac(6′)-Ib-cr*, bla_{IMP}, CRE

I. Introduction

The increasing prevalence of antimicrobial-resistant bacteria represents a major global health challenge. In 2024, the World Health Organization (WHO) updated the "Bacterial Priority Pathogens List", highlighting carbapenem-resistant *Acinetobacter*, carbapenem-resistant and third-generation cephalosporin-resistant Enterobacterales, and rifampicin-resistant *Mycobacterium tuberculosis* as pathogens of particular concern [1]. Among these, car-

bapenem-resistant Enterobacterales (CRE) have shown a particularly marked increase in prevalence, especially in Asia[2].

Enterobacterales, including *Escherichia coli* and *Klebsiella* species, are major causes of clinical infections, such as urinary tract infections and sepsis. A subset of these organisms exhibits resistance to imipenem (IPM) and meropenem (MEPM) by producing carbapenemases. Globally, CRE strains producing carbapenemases such as New Delhi metallo-β-lactamase (NDM), *Klebsiella pneumoniae* carbapenemase (KPC), and oxacillinase (OXA) are frequently multidrug-resistant[3)4)]. These strains often display resistance to fluoroquinolones, including ciprofloxacin (CPFX) and levofloxacin (LVFX), due to chromosomal mutations in the quinolone resistance-determining region (QRDR), as well as resistance to aminoglycosides, such as amikacin (AMK), mediated by aminoglycoside acetyltransferases or 16S rRNA methyltransferases[3)5)-7)]. In contrast, in Japan, most CRE isolates harbor imipenemase (IMP)–type metallo- β -lactamases (MBLs) encoded on transmissible plasmids[8)]. In western Japan in particular, pKPI-6–related plasmids and their derivatives carrying $bla_{\text{IMP-6}}$, *aac(6′)-Ib*, and $bla_{\text{CTX-M-2}}$ have become predominant[9)10)]. Although these strains are typically resistant to fluoroquinolones, the vast majority remain clinically susceptible to the AMK[11)–13)].

Previously, we analyzed carbapenem-resistant *E. coli* isolates collected between 2012 and 2016 at a healthcare facility in northern Osaka Prefecture, Japan, and reported their molecular epidemiology and some resistance determinants[12)]. Continued surveillance through 2024 led to the identification of two extensively drug-resistant *E. coli* strains that exhibited resistance to carbapenems, fluoroquinolones, and AMK. The present study aims to elucidate the resistance mechanisms underlying this atypical phenotype and to explore potential factors contributing to the preserved amikacin susceptibility observed in most IMP-type MBL–producing CRE strains detected in Japan.

II. Subjects and Methods

Target strains

This study focused on clinical strains of *E. coli* isolated between 2012 to 2024 at a mixed-care medical facility in the northern part of Osaka Prefecture, Japan. This facility has approximately 900 beds and mostly serves patients requiring chronic care. The AUH-81 strain was the first carbapenem-resistant *E. coli* detected at this institution[12)]. It was therefore selected as a representative AMK-susceptible strain, because it reflects the susceptibility profile commonly observed among IMP-type CRE isolates in Japan. AUH-256 and AUH-310 were identified as extensively drug-resistant strains exhibiting resistance to MEPM, AMK, and LVFX. *Escherichia coli* DH5α was used as the reference laboratory strain (**Table 1**).

Antimicrobial susceptibility testing

Antimicrobial susceptibility testing for the target strains was performed using the MicroScan WalkAway plus System (Beckman Coulter). The drug panel used was Neg EN Combo 1J, which included MEPM, AMK, and LVFX. Testing was conducted in accordance with the Clinical and Laboratory Standards Institute (CLSI) guidelines[14)], and the results were interpreted as susceptible (S), intermediate (I), or resistant (R). Minimum inhibitory concentrations (MICs) for AMK and CPFX were determined using the broth microdilution according to the CLSI guidelines to further characterize resistance levels.

Whole-genome DNA sequencing and multilocus sequence typing (MLST) analysis

The target strains AUH-81, AUH-256, and AUH-310 were cultured in LB medium supplemented with 4 μg/mL of MEPM, and genomic DNA was extracted using the QIAamp DNA Mini Kit (QIAGEN). Long-read sequencing was performed using the MinION (Oxford Nanopore Technologies) with an R9.4.1 flow cell and the Ligation Sequencing Kit (SQK-LSK110), whereas short-read sequencing was conducted using the NovaSeq 6000 (Illumina). Hybrid assembly was carried out with Unicycler[15)], which integrates both long- and short-read data, to obtain complete genome sequences, including plasmids. Gene annotation was performed using DFAST[16)]. The sequences of the MLST target regions of these strains were extracted from the whole-genome sequencing data, and sequence types (STs) were determined using EnteroBase[17)].

Analysis of aminoglycoside acetyltransferase-harboring plasmids

Based on whole-genome DNA sequencing results, all isolates were screened for aminoglycoside acetyltransferase genes known to confer AMK resistance. For isolates carrying these genes, the corresponding plasmids were further characterized and compared with pKPI-6 (AB616660), the predominant plasmid reported in western Japan. Plasmid replicon types were identified using PlasmidFinder 2.0[18)]. Plasmid copy number was estimated by calculating average read depth using SAMtools[19)], and determining the ratio of mean plasmid coverage to

mean chromosomal coverage. Promoter variants were classified according to previously reported Pc promoter sequences [20], and the promoter regions were compared among the isolates (**Table 2**).

Cloning of genes conferring resistance to AMK

The *aac(6′)-Ib* gene from AUH-81 was cloned by digesting genomic DNA using EcoRI and SalI and inserting a 3,453-bp DNA fragment into the corresponding sites of pUC118. This insert was subsequently transferred to the EcoRI and XhoI sites of the low-copy plasmid pNIT6011 (accession no. AB043475) [21][22] to construct pINN464. Similarly, a 1,677-bp SphI/EcoRI fragment containing the *aac(6′)-Iae* from AUH-256 was inserted into the SphI/EcoRI sites of pNIT6011 to generate pINN466. Moreover, a 1,314-bp SphI/HindIII fragment containing the *aac(6′)-Ib* from AUH-256 was cloned into the SphI/HindIII sites of pNIT6011 to construct pINN468. Furthermore, a 2,382-bp SphI/BglII fragment containing the *aac(6′)-Ib-cr* from AUH-310 was inserted into the SphI/BglII sites of pNIT6011 to obtain pINN472. In addition, the *aac(6′)-Ib* gene from AUH-310 was amplified by PCR using KOD-plus DNA polymerase (TAKARA) with primers sul1_R [23] and aac6Ib_R [24], yielding a 1,020-bp product that was cloned into the SphI/EcoRI sites of pNIT6011 to construct pINN474. All constructs were introduced into *E. coli* DH5α for MIC determination. Cloning procedures were performed as described previously [25], ensuring that each insert included the existing Pc promoter upstream of the target gene (**Table 1**, **Figure 1**).

Data availability

The whole-genome DNA sequencing data are deposited in DDBJ accession numbers as follows: *E. coli* AUH-81, genome (accession no. AP044703) and plasmids pAU81-1 (AP044704), pAU81-2 (AP044705), pAU81-

Table 1 *E. coli* strains and plasmids used in this study

Strains & plasmids	Relevant characteristics	Reference or sources
Strains		
DH5α	*F-/endA1 hsdR17 (rk-, mk+) supE44 thi-1 recA1 gyrA relA1D(lacIZYA-argF)U169 deoR (F80dlacD(lacZ)M15)*	Bethesda Research Laboratories
AUH-81	$MEPM^R$, $LVFX^R$	Maki et al. 2020
AUH-256	$MEPM^R$, AMK^R, $LVFX^R$	Maki et al. 2020
AUH-310	$MEPM^R$, AMK^R, $LVFX^R$	This study
Plasmids		
pUC118	Ap^R, ColE1 replicon	Vieira et al. 1987
pNIT6011	Tc^R, pACYC177 and pVS1 replicons	Itoh et al. 2001 (AB043475)
pKPI-6	$MEPM^R$, IncN replicon	Kayama et al. 2015 (AB616660)
pINN464	Tc^R, pNIT6011 derivative carrying *aac(6′)-Ib* of AUH-81	This study
pINN466	Tc^R, pNIT6011 derivative carrying *aac(6′)-Iae* of AUH-256	This study
pINN468	Tc^R, pNIT6011 derivative carrying *aac(6′)-Ib* of AUH-256	This study
pINN472	Tc^R, pNIT6011 derivative carrying *aac(6′)-Ib-cr* of AUH-310	This study
pINN474	Tc^R, pNIT6011 derivative carrying *aac(6′)-Ib* of AUH-310	This study

$MEPM^R$, meropenem; AMK^R, amikacin; $LVFX^R$, levofloxacin; Ap^R, ampicillin; Tc^R, tetracycline.

Table 2 Analysis of plasmids and acetyltransferase promoters and amikacin MICs in carbapenem-resistant *E. coli* isolates

Strains	Plasmids	Replicon types	Plasmid copy numbers	Target genes	Promoter variants	-35 sequence	Spacer region	-10 sequence	Amikacin MICs (μg/mL)
AUH-81	pAU81-1	IncFIA/IncFIB/IncFII/IncN †	1.81	*aac(6′)-Ib* ††	PcH1	TGGACA	17	TAAACT	4
AUH-256	pAU256-1	IncC	5.91	*aac(6′)-Iae*	PcH1	TGGACA	17	TAAACT	128
	pAU256-3	IncN	1.01	*aac(6′)-Ib* ††	PcH1	TGGACA	17	TAAACT	
AUH-310	pAU310-1	IncFIA/IncFIB/IncFII/IncN †	2.00	*aac(6′)-Ib* ††	PcH1	TGGACA	17	TAAACT	64
				aac(6′)-Ib-cr	P_A †††	TTGCAA	17	CATAAT	

MICs, minimum inhibitory concentrations

† Circular multireplicon plasmid (IncFIA/IncFIB/IncFII/IncN replicons).

†† *aac(6′)-Ib* identified in this study was the L119S variant.

††† The sequence TTGCAA–N_{17}–CATAAT was designated as P_A in this study.

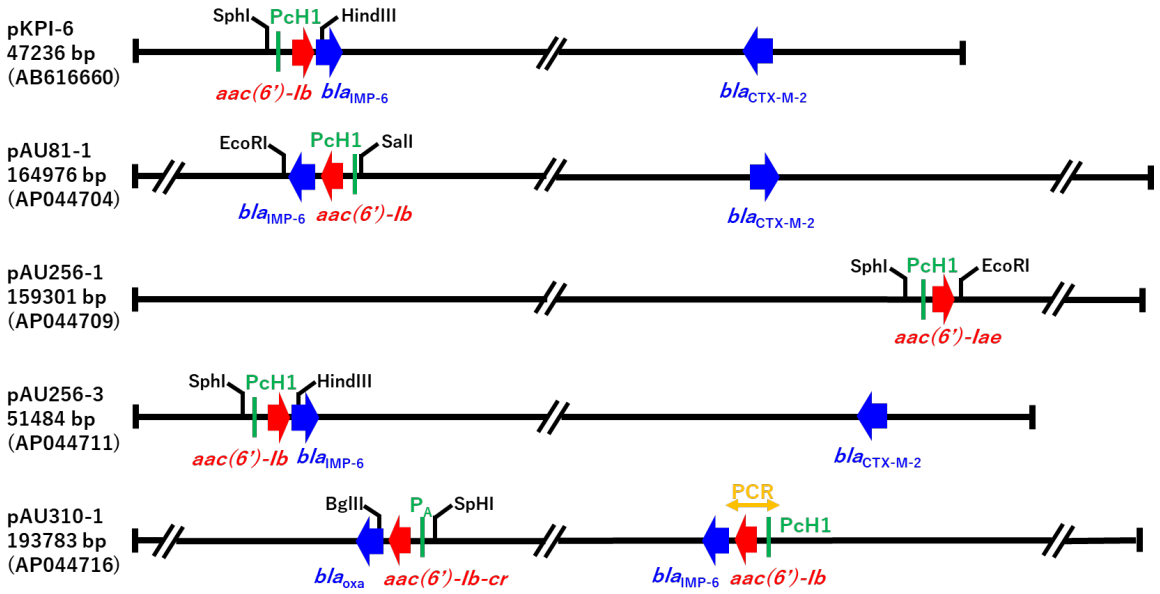

Figure 1 Comparison of plasmids harboring antimicrobial resistance genes and their cloning sites.
Plasmids were obtained from *E. coli* strains AUH-81 (pAU81-1), AUH-256 (pAU256-1 and pAU256-3), and AUH-310 (pAU310-1). In addition, pKPI-6 is shown as a representative plasmid predominantly detected in CRE isolates in western Japan.
β -lactamase genes are indicated by blue arrows, aminoglycoside acetyltransferase genes by red arrows, PCR-amplified regions by yellow double-headed arrows, promoter regions by green lines, and restriction enzyme sites by black bent lines. *aac(6´)-Ib* identified in this study corresponds to the L119S variant. DNA fragments were inserted into pNIT6011 as appropriate.

Table 3 Comparison of carbapenem-resistant *E. coli* isolates AUH-81, AUH-256, and AUH-310

Strains	MLSTs	Breakpoints			MBLs	AACs	mutations		
		MEPM	AMK	LVFX			*aac(6')-Ib*	*gyrA*	*parC*
AUH-81	ST131	R	S	R	bla_{IMP-6}	*aac(6´)-Ib*	L119S	S83L D87N	S80I
AUH-256	ST131	R	R	R	bla_{IMP-6}	*aac(6´)-Ib* *aac(6´)-Iae*	L119S	S83L D87N	S80I
AUH-310	ST648	R	R	R	bla_{IMP-6}	*aac(6´)-Ib* *aac(6´)-Ib-cr*	L119S	S83L D87N	S80I

MLST, multilocus sequence typing; MBL, metallo-β-Lactamase; AAC, aminoglycoside acetyltransferase; MEPM, meropenem; AMK, amikacin; LVFX, levofloxacin.

3 (AP044706), pAU81-4 (AP044707); *E. coli* AUH-256, genome (AP044708) and plasmids pAU256-1 (AP044709), pAU256-2 (AP044710), pAU256-3 (AP044711), pAU256-4 (AP044712), pAU256-5 (AP044713), pAU256-6 (AP044714); *E. coli* AUH-310, genome (AP044715) and plasmids pAU310-1 (AP044716), pAU310-2 (AP044717), pAU310-3 (AP044718), pAU310-4 (AP044719), pAU310-5 (AP044720), pAU310-6 (AP044721).

Statistical analysis

All data were analyzed using descriptive statistics. No further statistical hypothesis testing was performed.

III. Results

Antimicrobial susceptibility testing

Antimicrobial susceptibility testing was conducted on a total of 5,675 *E. coli* isolates collected over a 13-year period (2012–2024), mostly from patients receiving long-term chronic care. Fifty-nine carbapenem-resistant *E. coli* strains were identified. Among these, two extensively drug-resistant strains (AUH-256 and AUH-310) were detected and exhibited resistance to all tested carbapenems (MEPM), fluoroquinolones (LVFX), and aminoglycosides (AMK) (**Table 3**).

Whole-genome DNA sequencing and MLST analysis

Hybrid assembly confirmed that AUH-81 harbored a 5,186,478-bp chromosome and four circular plasmids. Among these, a 164,976-bp circular multireplicon plasmid (IncFIA/IncFIB/IncFII/IncN) designated pAU81-1, carried bla_{IMP-6}, *aac(6′)-Ib*, and $bla_{CTX-M-2}$. AUH-256 harbored a 5,034,660-bp chromosome and six plasmids, including a 159,301-bp IncC plasmid pAU256-1 and a 51,484-bp IncN plasmid pAU256-3. Comparative analysis demonstrated that pKPI-6 and pAU256-3 shared 99.9% average nucleotide identity (ANI) across 98.8% of their aligned regions, indicating that pAU256-3 is a pKPI-6–related plasmid. Moreover, the 1,314-bp SphI/HindIII fragment harboring *aac(6′)-Ib* was identical in sequence between the two plasmids. AUH-310 contained a 5,098,348-bp chromosome and six plasmids, including a 193,783-bp circular multireplicon plasmid (IncFIA/IncFIB/IncFII/IncN) designated pAU310-1 (**Figure 1**, **Table 2**).

Multilocus sequence typing (MLST) analysis showed that AUH-81 and AUH-256 belonged to ST131, which is frequently associated with carbapenem-resistant *E. coli* in Japan [26], whereas AUH-310 was classified as ST648 (**Table 3**).

Analysis of carbapenem and fluoroquinolone resistance determinants

Whole-genome sequencing revealed that AUH-81, AUH-256, and AUH-310 each harbored plasmids encoding bla_{IMP-6} (**Figure 1**). In addition, all three strains harbored mutations in the chromosomal *gyrA* and *parC* genes [27,28], which are known to confer high-level fluoroquinolone resistance (**Table 3**). These findings indicate that the presence of plasmid-mediated bla_{IMP-6}, together with chromosomal mutations in *gyrA* and *parC*, represents the major mechanisms underlying resistance to carbapenems and fluoroquinolones in these strains.

Analysis of AMK resistance determinants

Whole-genome sequencing revealed that AUH-81, AUH-256, and AUH-310 each carried the *aac(6′)-Ib* gene on their plasmids (**Figure 1**). These plasmids were low-copy-number and shared the integron-associated PcH1 promoter variant, which is associated with moderate promoter strength (**Table 2**). All three isolates also carried the previously reported *aac(6′)-Ib* (L119S) substitution [29]. This mutation has been shown to reduce susceptibility to AMK, whereas it increases resistance to gentamicin. MIC testing of the cloned strains carrying *aac(6′)-Ib* (L119S) (DH5α/pINN464, DH5α/pINN468, and DH5α/pINN474) demonstrated only a modest increase in AMK MICs. These findings indicate that this gene alone does not confer clinically relevant resistance (MIC ≥ 32 μg/mL) according to the CLSI standards (**Table 4**).

Conversely, AUH-256, which was resistant to AMK, carried both *aac(6′)-Ib* (L119S) and *aac(6′)-Iae*. The cloned strain harboring *aac(6′)-Iae* (DH5α/pINN466) exhibited a marked increase in AMK MIC. Considering the estimated plasmid copy number and the strength of the PcH1 promoter, these findings suggest that *aac(6′)-Iae* is likely the principal determinant of AMK resistance in AUH-256. Similarly, AUH-310 carried both *aac(6′)-Ib* (L119S) and *aac(6′)-Ib-cr*. The cloned strain harboring *aac(6′)-Ib-cr* (DH5α/pINN472) exhibited elevated AMK MICs, suggesting that *aac(6′)-Ib-cr* is the likely primary determinant of AMK resistance in AUH-310. Moreover, *aac(6′)-Ib-cr* is also known to increase resistance to CPFX [30], and in this study, the cloned strain DH5α/pINN472 exhibited a slight increase in CPFX resistance as well (**Table 4**).

Table 4 Minimum inhibitory concentrations of target resistant gene clones

Strains	Target genes			MICs (μg/mL)	
	aac(6')-Ib †	*aac(6')-Ib-cr*	*aac(6')-Iae*	AMK	CPFX
DH5α	−	−	−	0.5	0.025
DH5α/pNIT6011	−	−	−	0.5	0.025
AUH-81	+	−	−	4	64
DH5α/pINN464	+	−	−	4	n.d.
AUH-256	+	−	+	128	128
DH5α/pINN466	−	−	+	64	0.025
DH5α/pINN468	+	−	−	2	n.d.
AUH-310	+	+	−	64	512
DH5α/pINN472	−	+	−	16	0.05
DH5α/pINN474	+	−	−	2	n.d.

MICs, minimum inhibitory concentrations; AMK, amikacin; CPFX, ciprofloxacin; n.d., not done.
† *aac(6')-Ib* identified in this study was the L119S variant.

IV. Discussion

At a medical facility in Osaka City—the site of the first reported healthcare-associated infections caused by CRE in Japan—the causative organisms were *E. coli* and *Klebsiella* spp. harboring pKPI-6–related plasmids and their derivative plasmids carrying the IMP-6–type MBL gene and the CTX-M-2 group extended-spectrum beta-lactamase (ESBL) gene [10]. Subsequent reports have shown that CRE isolates detected in western Japan share similar characteristics [8], a pattern also observed in the present study. These IMP-6–type MBLs belong to the IMP-1 group, in which part of the amino acid sequence of IMP-1 is substituted, and are known to hydrolyze MEPM more efficiently than IPM [31]. Consequently, strains producing IMP-6–type MBL may appear susceptible to IPM, potentially leading to delayed detection and implementation of infection control measures. Careful interpretation of susceptibility profiles is therefore essential.

Many of the CRE strains detected in Japan remain susceptible to AMK despite harboring *aac(6′)-Ib*. Analysis of genome database entries of CRE isolates detected and registered in Japan identified several strains, including IMP-6–type MBL-producing *E. coli* (accession no. AP022350) [32], IMP-6–type MBL-producing *K. pneumoniae* (accession no. AP022358) [32], and IMP-1–type MBL-producing *E. coli* (accession no. LC720959) [33], which carry the *aac(6′)-Ib* (L119S) variant, which carries a mutation located immediately upstream of the IMP-type MBL gene. This variant is considered one of the factors contributing to the continued susceptibility of many IMP-type MBL–producing CRE isolates to AMK in Japan. Consistent with these findings, a previous study reported that AMK MIC of a strain harboring *aac(6′)-Ib* (L119S) was approximately one-eighth of that observed in a strain carrying the wild-type gene [29], with the MIC remaining below the CLSI breakpoint. However, despite MIC values below the CLSI breakpoint, the potential for residual enzymatic activity suggests that caution may be warranted in clinical practice, for example, by avoiding AMK monotherapy and considering its use in combination with other antimicrobial agents [34][35].

In the AUH-256 strain, which was resistant to AMK, both *aac(6′)-Ib* (L119S) and *aac(6′)-Iae* were detected, and *aac(6′)-Iae* has previously been reported primarily in *Pseudomonas aeruginosa* isolates in Japan [36]. Its identification in *E. coli* in this study appears to represent a rare occurrence. This gene was located on an IncC plasmid [37], a large multidrug resistance plasmid, suggesting the possibility of interspecies transmission from *P. aeruginosa*. Furthermore, the AUH-310 strain, which also exhibited resistance to AMK, carried both *aac(6′)-Ib* (L119S) and *aac(6′)-Ib-cr*. The *aac(6′)-Ib-cr* is known to confer resistance to both AMK and CPFX, and when combined with *gyrA* and *parC* mutations, it can substantially enhance fluoroquinolone resistance [30]. Collectively, these findings suggest that in CRE carrying IMP-type MBL—frequently detected in Japan—clinically relevant AMK resistance may arise through acquisition of additional determinants, such as *aac(6′)-Iae* or *aac(6′)-Ib-cr*, whereas *aac(6′)-Ib* (L119S) alone appears insufficient to confer clinically relevant AMK resistance in the isolates examined in this study.

The AUH-81 and AUH-256 strains, analyzed in this study, belonged to ST131, which has been reported as the dominant lineage among clinical *E. coli* isolates [8]. In contrast, the AUH-310 strain belonged to ST648, a lineage reported in the European Economic Area to carry NDM-type MBL and OXA-type ESBL [38]. Although ST648 has been detected only rarely in Japan, one case was reported in a patient with a history of hospitalization in India [39]. Moreover, the AUH-310 strain carried OXA-type ESBL genes, representing resistance determinants distinct from those identified in the AUH-81 and AUH-256 strains. With the increase in international travel, the potential exists for regionally prevalent plasmids to disseminate into strains with diverse genetic backgrounds, potentially contributing to the emergence of new multidrug-resistant lineages.

This study has several limitations. The isolates were obtained from a single medical facility in northern Osaka, and therefore, the findings may not be generalizable to the entire Japanese healthcare setting. Furthermore, only two aminoglycoside-resistant isolates were identified, limiting the strength of conclusions regarding the relative contribution of individual *aac(6′)* genes to AMK resistance. Further studies involving a larger number of isolates, as well as experimental assessments of plasmid transmissibility and functional analyses, are warranted. In addition, for the extensively drug-resistant strains identified in this study, assessment of plasmid transferability and exploration of effective therapeutic options remain necessary. Nevertheless, the identification of distinct genetic mechanisms underlying AMK resistance in IMP-type MBL–producing isolates highlights the ongoing diversification of resistance determinants. Continuous surveillance and molecular characterization of these determinants will therefore be important for improving our understanding of emerging resistance patterns and for

informing appropriate antimicrobial treatment strategies.

Authorship Contributions

HY, AM, and YN conceived and designed the study, performed the experiments, and interpreted the data. SS, TN, and HI contributed experimental and analytical resources.

All authors critically reviewed the manuscript and approved the final version for publication.

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Disclosure

The authors declare no conflict of interest.

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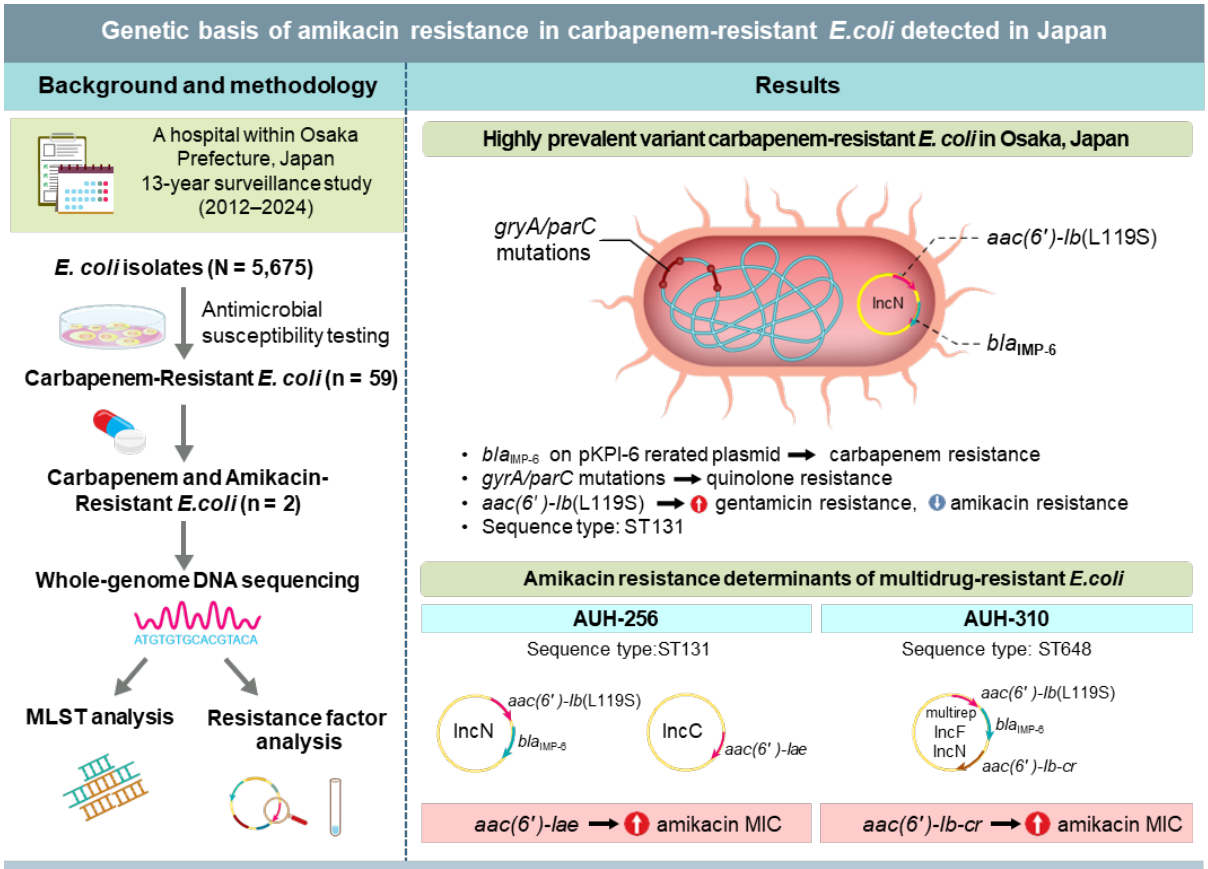
GRAPHICAL ABSTRACT



E. coli: *Escherichia coli*; MLST: multi-locus sequence typing; MIC: minimum inhibitory concentration

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