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Original Paper

Genotypic characterization *Escherichia coli* strains from Japan producing AmpC beta-lactamase

Tadanori Yamochi*[1], Katsuhiko Yoshida[2], Yasuyuki Ohira[1], Hirotaka Ota[1], Toshiko Yamochi[1], Masafumi Takimoto[1] and Issei Tokimatsu[3]

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Abstract

AmpC is a class C Ambler β-lactamase that confers resistance to cephamycins. Globally reported AmpC-producing Enterobacterales are clinically important due to their therapeutic restrictions and epidemiology. Between April 2021 and May 2021, an outbreak of AmpC beta-lactamase-producing *Escherichia coli* occurred at Showa University Hospital. Because of this outbreak, plasmid typing, enterobacterial repetitive intergenic consensus-polymerase chain reaction (ERIC-PCR) analysis, conjugation experiments, and pulsed-field gel electrophoresis (PFGE) were performed to study the molecular epidemiological characteristics of these *E. coli* strains. ERIC-PCR and conjugation experiments detected IncFrepB+, IncFIA+, and IncFIB+ multiple-replicon conjugative plasmids carrying the *AmpC* gene (bla_{DHA-1}). Five of eleven isolates showed the same PFGE banding pattern in the XbaI-digested genomic DNA, whereas the remaining six strains displayed minor variants. These data highlight the need for improvements to infection control and surveillance measures to prevent the spread of bla_{DHA-1}-carrying *E. coli* strains.

Key words: *E. coli*, AmpC beta-lactamase, bla_{DHA-1}, Plasmid

Introduction

Enterobacteriaceae produce cephalosporinases and can show resistance to various beta (β)-lactam antibiotics including 7-alpha-methoxy cephalosporins (cefoxitin or cefotetan), oxyimino cephalosporins (cefotaxime, ceftazidime, and ceftriaxone), and monobactams (aztreonam)[1,2]. However, these bacteria may be susceptible to cefepime due to the formation of a stable acyl-enzyme complex[3]. AmpC β-lactamases are typically encoded on chromosomes of many gram-negative bacteria, including *Citrobacter*, *Serratia*, and *Enterobacter* species, and usually show an inducible expression. *Escherichia coli* may also possess AmpC β-lactamase genes that are not typically inducible but can be hyper-expressed[4]. Hyper-production of chromosomal AmpC β-lactamase genes by *E. coli* is caused by mutations in their promoter / attenuator regions between positions −42 to +81 of the *AmpC* open reading frame[5,6].

Regulation of AmpC β-lactamase expression is closely linked to cell wall recycling and at least three genes as follows: *ampR* (which encodes a transcriptional regulator of the LysR family), *ampG* (which encodes a transmembrane permease), and *ampD* (which encodes a cytosolic *N*-acetyl-anhydromuramyl-L-alanine amidase)[7,8]. AmpR can be a transcriptional repressor or activator of AmpC β-lactamase synthesis depending on precursor muropeptide concentrations. When this occurs, high precursor concentrations lead to increased enzyme production in the presence of β-lactam antibiotics[9].

Regulation of AmpC production in *E. coli* through the chromosomal *AmpC* gene is distinctly characterized by a lack of AmpR. But unlike most other Enterobacteriaceae members, *E. coli* exhibit a non-inducible AmpC phenotype and wild-type strains

* Corresponding author
✉ Tadanori Yamochi
tyamochi@med.showa-u.ac.jp

[1] Department of Pathology, Showa University, School of Medicine, 1-5-8 Hatanodai, Shinagawa-ku, Tokyo 142-8555, Japan.
[2] Clinical Laboratory, Showa University Hospital.
[3] Department of Medicine, Division of Clinical Infectious Diseases, Showa University School of Medicine.

constitutively produce low enzyme levels[6].

However, bacteria with chromosomes that lack genes inducing β-lactamases acquire necessary genes through plasmid transmission in order to produce enzymes[9]. For example, cephamycin-resistant *E. coli* occasionally carry plasmid-mediated AmpC (pAmpC) β-lactamases[1].

However, bacterial resistance to β-lactamases acquired through the production of pAmpC enzymes is less common than the production of extended-spectrum β-lactamases (ESBLs). Additionally, pAmpC has quickly become a leading clinical concern because of its self-transmissibility, which permits it to spread among different bacterial species[1]. Testing for pAmpC is not routinely conducted in laboratories and phenotypic tests can be ambiguous and unreliable, which results in misreporting and treatment failure[10]. Moreover, the coexistence of ESBLs may mask phenotypic pAmpC detection. No Clinical Laboratory Standards Institute guidelines are currently available for the optimal detection and confirmation of pAmpC, and phenotypic tests do not differentiate between chromosomal AmpC- and pAmpC-encoding genes, which is why genotypic characterization is the gold standard[10]. In this study, plasmid testing and polymerase chain reaction (PCR) were used to characterize several *E. coli* strains carrying pAmpC ($bla_{\text{DHA-1}}$) genes. Additionally, the clonality of isolates was studied, including those indistinguishable from or closely related to the outbreak clones, which suggests that plasmid transmission did not occur.

Materials and methods

Strains

We screened fecal samples from patients admitted to the neonatal intensive care unit at Showa University Hospital (Tokyo, Japan) as an ongoing survey. Between April 2021 and May 2021, eleven *E. coli* isolates resistant to ceftazidime and sensitive to cefepime were isolated from nine babies (Table 1). Two (of the eleven) isolates were isolated from the nasal and stool swabs of two individual babies. For the first baby, the nasal and stool samples were collected and simultaneously processed; for the second baby, processing was conducted separately for both samples. The remaining isolates were separated from stool samples for processing (Table 1).

Susceptibility to antibiotics

Antibiotic susceptibility of isolated strains was determined using a microdilution method and according to procedures implemented by the National Committee of the Clinical and Laboratory Standards Institute. All testing was conducted by Bio Medical Laboratories Inc. (Tokyo, Japan).

Identification of AmpC β-lactamase- and ESBL-encoding genes using PCR and DNA sequencing assays

PCR and DNA sequencing analyses were performed on strains resistant to ceftazidime (but sensitive to cefepime) to detect *AmpC* β-lactamase genes, ESBL enzymes, and the region encompassing the *DHA-1* gene following previously described methods[1, 11, 12]. Briefly, total DNA was extracted from selected strains that were grown on Luria-Bertani (LB) medium agar plates. A PCR assay was used to screen for the presence of six *AmpC* genes (using family-specific primer pairs for MOX, FOX, EBC, ACC, DHA, and CIT) and three ESBL-encoding genes (using family-specific primer pairs for CTX, TEM, and SHV) (Table 2). PCR amplicons were separated using electrophoresis on 1% agarose gels, purified using a GenElute™ column (Sigma-Aldrich, St. Louis, MO, USA), and verified by sequence analysis.

*Detection of the ampR gene and amplicons between ampR and bla*DHA-1

To study the structure surrounding the $bla_{\text{DHA-1}}$ gene, the adjacent *ampR* gene (using the ampR primer pair) and the structure between $bla_{\text{DHA-1}}$ and *ampR* (using the ampR-AmpC primer pair) were examined (Table 2). The PCR assay for the *ampR* gene amplicon and the region between $bla_{\text{DHA-1}}$ and *ampR* was performed as previously described[13].

Conjugation transfer experiments

Conjugation transfer experiments were performed as previously described[11, 12]. Rifampicin-resistant and cefotaxime-susceptible *E. coli* TUM2235 strains that were used as conjugation recipients were provided by Toho University (Tokyo, Japan). Seven *E. coli* strains carrying $bla_{\text{DHA-1}}$ were isolated from patients and used as donors. Strains were mixed at a 1∶5 donor∶recipient ratio and incubated for 3 h in 2× yeast extract-tryptone medium at 37°C. Then, transconjugant colonies were selected from LB agar plates supplemented with rifampicin (200 μg/ml) and ampicillin (100 μg/ml).

Plasmid extraction and DNA sequencing

Plasmid extraction and subsequent DNA sequencing

were performed as previously described[11, 12]. Briefly, large plasmids harboring the AmpC-lactamase-encoding gene were purified from transconjugants derived from clinical isolates using a Plasmid Midiprep kit (Qiagen, Hilden, Germany) and sequenced using DHAMF, DHAMR, DHA1+76R, and AmpR 338F primers as shown in Table 2.

Pulsed-field gel electrophoresis

Pulsed-field gel electrophoresis (PFGE) was performed as previously described[12]. Briefly, genomic DNA was isolated from bacterial cells and digested using the *Xba*I restriction enzyme. Pulsing was performed using the lamp-pulse method and the CHEF DR III system (Bio-Rad, Hercules, CA, USA) at 14°C with 60–90 s of switching for 20 h at 6 V/cm.

Plasmid profiling

S1 nuclease-PFGE and Southern blotting assays were performed as previously described[11]. Briefly, *E. coli* suspensions were mixed and dispensed into 100 µl plug molds. After solidification, plugs were lysed and digested with proteinase K buffer. Then, S1 nuclease was added at 37°C for 25 min to nick

Table 1. Genotypic or biological characterization and antibiotic susceptibility of eleven isolated *E.coli* strains

Sample Number	Isolation Site	DHA-1	AMPR	ESBL	Plasmid Type	MIC (µg/ml)															
						ABPC	PIPC	CEZ	CTM	CTX	CAZ	CTRX	CFPM	CCL	CMZ	FMOX	P/T	IPM	MEPM	GM	AMK	LVFX
2021-175	stool	+	+	-	IncFrepb (FII) / IncFIA / IncFIB	>16	>64	>16	>16	>2	>8	>2	≤2	>16	>32	>32	64	≤1	≤1	≤2	≤4	>4
2021-188	stool	+	+	-	IncFrepb (FII) / IncFIA / IncFIB	>16	>64	>16	>16	>2	>8	>2	≤2	>16	>32	>32	>64	≤1	≤1	≤2	≤4	>4
2021-189	stool	+	+	-	IncFrepb (FII) / IncFIA / IncFIB	>16	>64	>16	>16	>2	>8	>2	≤2	>16	>32	>32	64	≤1	≤1	≤2	≤4	>4
2021-202	stool	+	+	-	IncFrepb (FII) / IncFIA / IncFIB	>16	>64	>16	>16	>2	>8	>2	≤2	>16	>32	>32	64	≤1	≤1	≤2	≤4	>4
2021-203	stool	+	+	-	IncFrepb (FII) / IncFIA / IncFIB	>16	>64	>16	>16	>2	>8	>2	≤2	>16	>32	>32	>64	≤1	≤1	≤2	≤4	>4
2021-205	stool	+	+	-	IncFrepb (FII) / IncFIA / IncFIB	>16	>64	>16	>16	>2	>8	>2	≤2	>16	>32	>32	64	≤1	≤1	≤2	≤4	>4
2021-214	nasal swabs	+	+	-	IncFrepb (FII) / IncFIA / IncFIB	>16	64	>16	>16	>2	8	≤1	≤2	>16	32	≤8	≤16	≤1	≤1	≤2	≤4	>4
2021-219	stool	+	+	-	IncFrepb (FII) / IncFIA / IncFIB	>16	>64	>16	>16	>2	>8	>2	≤2	>16	>32	>32	>64	≤1	≤1	≤2	≤4	>4
2021-223	nasal swabs	+	+	-	IncFrepb (FII) / IncFIA / IncFIB	>16	<16	>16	>16	>2	8	≤1	≤2	>16	32	≤8	≤16	≤1	≤1	≤2	≤4	>4
2021-231	stool	+	+	-	IncFrepb (FII) / IncFIA / IncFIB	>16	>64	>16	>16	>2	>8	>2	≤2	>16	>32	>32	64	≤1	≤1	≤2	≤4	>4
2021-247	stool	+	+	-	IncFrepb (FII) / IncFIA / IncFIB	>16	>64	>16	>16	>2	>8	>2	≤2	>16	>32	>32	>64	≤1	≤1	≤2	≤4	>4

Abbreviations: MIC, minimum inhibitory concentration; ABPC, ampicillin; PIPC, piperacillin; CEZ, cefazolin; CTM, cefotiam; CTX, cefotaxime; CAZ, ceftazidime; CTRX, ceftriaxone; CFPM, cefepime; CCL, cefaclor; CMZ, cefmetazole; FMOX, flomoxef; P/T, piperacillin/tazobactam; IPM, imipenem; MEPM, meropenem; GM, gentamicin; LVFX, levofloxacin; ESBL, extended-spectrum beta-lactamase

AMPR, bla_{DHA-1}, ESBL, and plasmid types were tested using specific primers for multiplex or simplex PCR. MIC values (µg/ml) were divided into three categories, namely, susceptible (S), Intermediate (I), and Resistant (R) as follows: ABPC S; <8 I; 16, R; >16, PIPC S; ≤16, I; 32–64, R; >64, CEZ S; ≤2, I; 4, R; >4, CTM S; ≤8, I; 16, R; >16, CTX S; ≤1, I; 2, R; >2, CAZ S; ≤4, I; 8, R; >8, CTRX S; ≤1, I; 2, R; >2, CFPM S; ≤8 I;16, R; >16, CCL S; ≤8 I; 16, R; >16, CMZ S; ≤16, I; 32, R; >32, FMOX S; ≤8 I; 16–32, R; >32 P/T S; ≤16 I; 32–64, R; >64, IPM S; ≤1 I; 2, R; >2, MPEM S; ≤1 I; 2, R; >2, GM S; ≤4 I; 8, R>8, AMK S; ≤16, I; 32, R; >32, LVFX S; ≤2I; 4, R; >4

the plasmid DNA. Then, the digested DNA was separated by PFGE using the CHEF DR III drive module (Bio-Rad) as previously described. Finally, the electrophoretically separated fragments were transferred to a nylon membrane and visualized using digoxigenin-labeled bla_{DHA1}, IncFIA, IncFIB, or IncFrepB as specific probes using a PCR DIG Probe Synthesis Kit (Merck, Darmstadt, Germany)[12].

Stripping for reprobing

The membrane probes were stripped as described in the Roche Applied Science DIG application manual (Merck) for Southern blots. Briefly, membranes were incubated with dimethylformamide for 1 h at 60ºC. Membrane probes were then removed using a stripping buffer consisting of 0.2 M NaOH and 0.1% SDS.

PCR-based plasmid replicon typing

Plasmids were classified into incompatibility groups using a PCR-based replicon typing (PBRT) method as previously described[11].

Table 2. Primers used for PCR or sequence analysis

Primers	Sequence (5′-3′)
CTX Univ F	TTTGCGATGTGCAGTACCAGTAA
CTX Univ R	CTCCGCTGCCGGTTTTATC
TEM A F	GATCTCAACAGCGGTAAGATCC
TEM A R	TCACTCATGGTTATGGCAGC
SHVF	GGAGCGAAAGATCCACTATCG
SHVR	ACAATGCGCTCTGCTTTGTT
CITM F	TGGCCAGAACTG ACAGGCAAA
CITM R	TTTCTCCTGAACGTGGCTGGC
MOXMF	GCTGCTCAAGGAGCACAGGAT
MOXMR	CACATTGACATAGGTGTGGTGC
DHAMF	AACTTTCACAGGTGTGCTGGGT
DHAMR	CCGTACGCATACTGGCTTTGC
ACCMF	AACAGCCTCAGCAGCCGGTTA
ACCMR	TTCGCCGCAATCATCCCTAGC
EBCMF	TCGGTAAAGCCGATGTTGCGG
EBCMR	CTTCCACTGCGGCTGCCAGTT
FOXMF	AACATGGGGTATCAGGGAGATG
FOXMR	CAAAGCGCGTAACCGGATTGG
DHA-1+76R	CCATCCCGGGAATATCCTGC
AmpR 338F	TGGCCGGATTCTATGACAGC
AmpR F	GTGAGTTTTACGCCGCCG
AmpR R	ATGGTCAGACGTTATCTCCCC
AmpR-AmpC F	GGTAAAACTGAGATGACGGGC
AmpR-AmpC R	AGGTGTGAGATAATCCCAGCG

Ethics approval and consent to participate

This was a retrospective study where consent was obtained using an opt-out method. This study was approved by the Ethics Committee of the Showa University School of Health Sciences (Kanagawa, Japan ; Approval No. 371).

Results

A total of eleven *E. coli* strains isolated from hospital fecal samples in 2021 showed resistance to third-generation cephalosporins (e.g., cefotaxime, ceftazidime, and ceftriaxone) but they were susceptible to cefepime (a fourth-generation cephalosporin) and carbapenems (Table 1). To confirm that clinical isolates produced β-lactamases via a plasmid or chromosome, assays involving PBRT, conjugation, and enterobacterial repetitive intergenic consensus-polymerase chain reaction (ERIC-PCR) were performed[1]. A PBRT test and conjugative plasmid analysis showed that isolates carried IncFrepB, IncFIA, and IncFIB, which are replicon-type conjugative plasmids harboring bla_{DHA-1} (Table 1).

S1 nuclease-PFGE analysis of ten representative clinical isolates was performed using a bla_{DHA-1} probe and detected a single plasmid band of approximately 100 kb (Fig. 1). However, seven representative

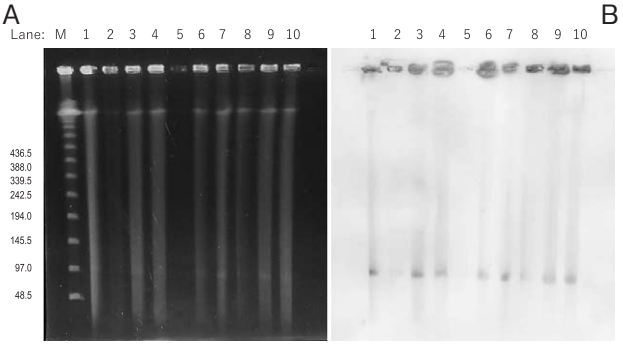

Fig. 1. (A) Pulsed-field gel electrophoresis of S1 nuclease-digested total DNA from ten isolated *Escherichia coli* strains
Lane M : phage λ DNA ladder (concatemers of 48.5 kb fragments). Lane 1 : strain 2021-175 ; Lane 2 : strain 2021-188 ; Lane 3 : strain 2021-189 ; Lane 4 : strain 2021-202 ; Lane 5 : strain 2021-203 ; Lane 6 : strain 2021-205 ; Lane 7 : strain 2021-214 ; Lane 8 : strain 2021-219 ; Lane 9 : strain 2021-223 ; and Lane 10 : strain 2021-231
(B) Hybridization with a bla_{DHA-1}-specific probe in ten isolated *Escherichia coli* strains
The lane number configuration is the same as that in Figure 1A.

transconjugants carried multiple plasmids based on conjugation experiments (Fig. 2A). Furthermore, Southern blot analysis of the S1 nuclease bands using digoxigenin-labeled IncFIA, IncFIB, and IncFrepB probes showed a ~100 kb plasmid, which was observed using the $bla_{\text{DHA-1}}$ probe (Fig. 2B–D). Additionally, PCR analysis of the sequence and surrounding $bla_{\text{DHA-1}}$ structure (using the AMPR and AMPR-AmpC primer pair) revealed the presence of the ampR gene (Table 1). A genotyping analysis using PFGE also revealed that five isolates belonged to one major profile (designated A), whereas the remaining six had minor variants that differed by less than four bands (which were designated as A1, A2, A3, and A4) (Fig. 3).

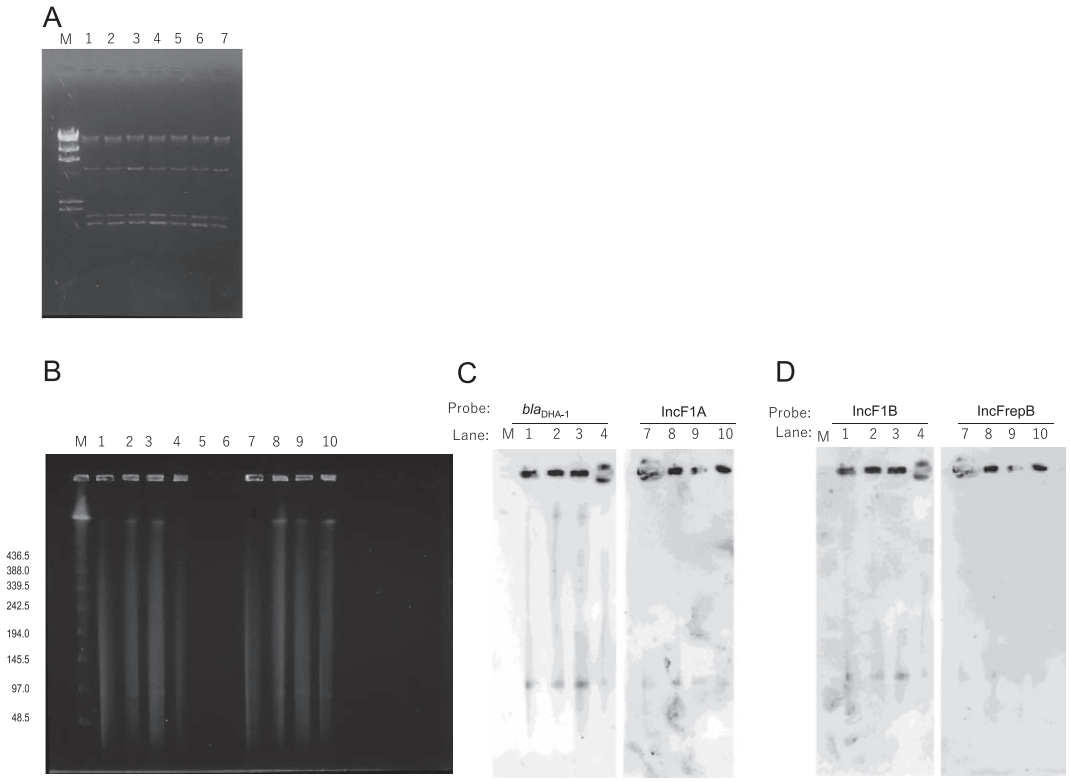

Fig. 2.

(A) S1 nuclease-digested multiple plasmids in transconjugants derived from clinical isolates

Lane M: phage λ HindIII-digested DNA ladder. Lane 1: Transconjugants from 2021-175; Lane 2: Transconjugants from 2021-189; Lane 3: Transconjugants from 2021-202; Lane 4: Transconjugants from 2021-205; Lane 5: Transconjugants from 2021-214; Lane 6: Transconjugants from 2021-219; and Lane 7: Transconjugants from 2021-223

(B) Pulsed-field gel electrophoresis of S1 nuclease-digested plasmid DNA isolated from four representative clinical isolates.

To examine whether a conjugative plasmid contained multiple replicons, S1 nuclease Southern blotting was performed using $bla_{\text{DHA-1}}$, IncFIA, IncFIB, and IncFrepB probes. Because multiple probes were used, duplicate representative samples were separated using pulsed-field gel electrophoresis. Lane M: phage λ DNA ladder (concatemers of 48.5 kb fragments). Lane 1: strain 2021-175; Lane 2: strain 2021-189; Lane 3: strain 2021-202; Lane 4: strain 2021-205; Lane 5: Blank; Lane 6: Blank; Lane 7: strain 2021-175; Lane 8: strain 2021-189; Lane 9: 2021-202; and Lane 10: strain 2021-205. Transferred membranes were divided into two parts, membranes A and B, which correspond to lanes M to 4 and lanes 7 to 10, respectively.

(C) Hybridization with a specific probe for $bla_{\text{DHA-1}}$ and IncFIA in four isolated *Escherichia coli* strains.

Membranes A and B were used for $bla_{\text{DHA-1}}$ and IncFIA probes, respectively, as indicated at the top of the figure.

(D) Hybridization with a specific probe for IncFIB and IncFrepB in four isolated *Escherichia coli* strains.

Membranes A and B were used as reprobes for IncFIB and IncFrepB, respectively, as indicated at the top of the figure.

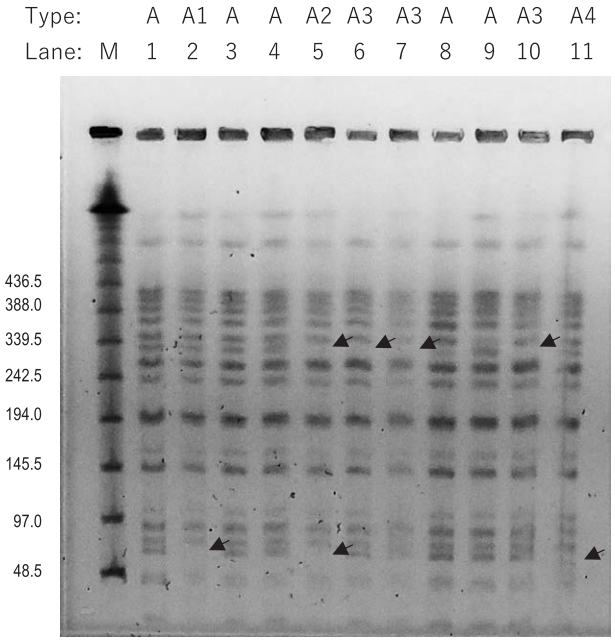

Fig. 3. Pulsed-field gel electrophoresis of XbaI-digested genomic DNA from 11 isolated *E.coli* strains
The upper and lower layers indicate PFGE profiles and lane number, respectively.
Lane M: phage λ DNA ladder (concatemers of 48.5 kb fragments). Lane 1: strain 2021-175; Lane 2: strain 2021-188; Lane 3: strain 2021-189; Lane 4: strain 2021-202; Lane 5: strain 2021-203; Lane 6: strain 2021-205; Lane 7: strain 2021-214; Lane 8: strain 2021-219; Lane 9: strain 2021-223; Lane 10: strain 2021-231; and Lane 11: strain 2021-247.

Discussion

In this study, we describe a 2021 hospital outbreak of *E. coli* that produced pAmpC β-lactamase (from $bla_{\text{DHA-1}}$). We predicted that these isolates were plasmid-carrying types using a PBRT test with IncFIA, IncFIB, and IncFrepB PCR amplicons (which correspond to IncFII using the PBRT kit)[14]. We then confirmed the $bla_{\text{DHA-1}}$ plasmid-mediated AmpC gene type using ERIC-PCR. After reporting these data, enhanced infection control measures were implemented by Showa University Hospital.

Some bacteria lacking genes required to induce β-lactamases can acquire necessary DNA fragments through plasmid transmission and still produce relevant enzymes[9]. Currently, six types of pAmpC β-lactamases have been reported and all of them have different genetic backgrounds including an ACT type from *Enterobacter* spp., a CIT type from *Citrobacter freundii*, a DHA type from *Morganella morganii*, an ACC type from *Hafnia alvei*, and MOX and FOX types from *Aeromonas* spp. Unlike bacteria with naturally occurring genes encoding AmpC β-lactamases, bacteria (except for DHA-producing species) lacking an *AmpR* constantly produce enzymes through pAmpC[9]. However, we detected $bla_{\text{DHA-1}}$-carrying conjugative plasmids that also carried *AmpR*, which suggests that β-lactamase might be inducible and easily transmitted between species. For example, if horizontal plasmid transmission occurs for any Enterobacteriaceae producing AmpC, either through the induction / derepression of a chromosomal *AmpC* gene or the presence of a *pAmpC* gene, the same organism may also produce additional β-lactamases. This results in complex susceptibility patterns and makes phenotypic interpretation challenging, which further complicates matters[3].

Because a high clinical failure rate occurred among patients infected with AmpC β-lactamase-producing *Klebsiella pneumoniae* and those who received initial antimicrobial therapy (especially cephalosporin), the detection of AmpC-producing organisms is important for effective therapeutic intervention and optimal clinical outcomes[15]. Although the coexistence of ESBLs with AmpC have been previously reported[16,17], ESBL-encoding genes were not detected in $bla_{\text{DHA-1}}$-carrying *E. coli* strains in this study (Table 1).

Southern blots of total DNA S1 nuclease-PFGE using the $bla_{\text{DHA-1}}$ probe revealed the $bla_{\text{DHA-1}}$ gene in nine of ten clinical isolates. However, the gene was not detected in lane 5, which corresponds to strain 2021-203 (Fig. 1). Therefore, evidence that strains 2021-203 and 2021-247 expressed DHA1 β-lactamase was only confirmed through ERIC-PCR.

Through conjugative analysis, we also confirmed that transconjugants could carry multiple plasmids (Fig. 2A). Using S1 nuclease Southern blotting, we confirmed the coexistence of a plasmid of approximately 100 kb that could hybridize with $bla_{\text{DHA-1}}$, IncFIA, IncFIB, and Inc FrepB probes, which suggests the multiple-replicon nature of the plasmid (Fig. 2B–D).

Furthermore, incompatibility could be caused by competition for replication systems. Two plasmids that compete for the same replication system cannot coexist within a cell line over multiple generations. Therefore, this characteristic was used to classify plasmids[18]. However, plasmids with a narrow host range can accomplish broad-host-range replication through multiple-replicon status[19]. IncF plasmids are usually low-copy-number plasmids of 100 kb that carry more than one replicon to promote replication initiation[19]. This supports our findings of approximately 100 kb IncFIA+, IncFIB+,

and IncFrepB+ multiple-replicon conjugative plasmids. Overall, the coexistence of multiple-replicon plasmids is more common in the IncF group[19]. Multiple-replicon plasmids carry resistance genes more efficiently than their non-multiple-replicon counterparts, which may be an important mechanism underlying bacterial response to environments with high antibiotic pressure[18]. Therefore, additional investigation is needed on this topic.

In the clonality test, PFGE genotyping of 11 isolates identified five strains belonging to a major profile (designated A) and six strains with minor variants (differing by <4 bands) (Fig. 1). Based on the PFGE interpretation criteria of Tenover *et al*.[20], five isolates were indistinguishable and six were closely related to outbreak clones, which suggests that these *E. coli* strains originated from a single clone and plasmid transmission did not occur.

In conclusion, using PBRT and ERIC-PCR analyses, we detected pAmpC ($bla_{\text{DHA-1}}$)-carrying *E. coli* isolates in our hospital. As a result, prompt action was implemented to enhance infection control measures, and $bla_{\text{DHA-1}}$-carrying *E. coli* has not been detected in the institution since.

Acknowledgments

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Conflicts of interest disclosure

I.T. received scholarship funding from Asahi Kasei Pharma (Tokyo, Japan), Astellas Pharma Inc, (Tokyo, Japan), Shionogi & CO., LTD (Osaka, Japan), and Daiichi Sankyo CO., LTD (Tokyo Japan). The other authors have no potential conflicts of interest to disclose.

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