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ORIGINAL RESEARCH

Genetic and Molecular Epidemiological Characterization of ESBL-Producing *Escherichia coli* Isolated from University Students in Japan

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Background: Extended-spectrum β-lactamase (ESBL)-producing *Escherichia coli* are major contributors to global antimicrobial resistance. Colonization in healthy individuals represents a silent reservoir that links community and healthcare settings. Determining the prevalence and molecular characteristics of ESBL-producing strains in community populations is essential for effective infection control.

Methods: We enrolled 96 healthy third-year students and collected perianal swabs to detect ESBL-producing *E. coli*. Antimicrobial susceptibility testing, ESBL genotyping, phylogrouping, and POT-based molecular epidemiological typing were performed to characterize the isolates and identify ST131 and its H30 subclone.

Results: ESBL-producing *E. coli* were detected in 7.3% of students (7/96; 95% CI: 3.0–14.4%). All isolates harbored CTX-M–type ESBL genes, and the high-risk clone ST131/H30 was frequently identified. POT-based molecular typing revealed distinct patterns for all isolates, indicating independent acquisition rather than clonal transmission within the cohort.

Conclusion: The detection of multidrug-resistant ST131-H30 in healthy young adults highlights the potential role of community carriers in introducing high-risk clones into healthcare settings. Strengthened surveillance and integrated One Health–based approaches are needed to curb the spread of antimicrobial resistance.

Keywords: ESBL-producing *Escherichia coli*, CTX-M, phylogroup, ST131/H30 subclone, POT typing, antimicrobial resistance

Introduction

Extended-spectrum β-lactamase (ESBL)-producing Enterobacteriaceae are resistant to third-generation cephalosporins and monobactams and are recognized globally as major causes of healthcare- and community-associated infections. Their increasing detection in community settings raises concern that healthy individuals may serve as silent reservoirs for dissemination. In Japan, fecal carriage among healthy adults has been estimated at 6–9%, with CTX-M-type enzymes predominating. Potential sources include companion animals, food products, and environmental water, emphasizing the importance of a One Health perspective.

Among *Escherichia coli* lineages, sequence type 131 (ST131) combines multidrug resistance with enhanced virulence and is a leading cause of extraintestinal infections such as urinary tract infection and sepsis. The H30 subclone is notable for high-level fluoroquinolone resistance and its frequent association with CTX-M-15, and it has emerged as a globally disseminated pandemic lineage. Although ST131 has been extensively studied in clinical isolates, its prevalence and characteristics among healthy community members remain incompletely understood.

University students represent a socially active cohort of healthy young adults who may facilitate transmission between community and healthcare environments. Previous studies from Europe and Asia have reported variable ESBL carriage rates in this population, but comparative data from Japan are limited. To address this gap, we investigated ESBL-producing *E. coli* among healthy university students in Japan. Our objectives were to determine carriage

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prevalence, characterize ESBL genotypes, define phylogenetic groups, assess clonal relatedness by POT-based typing, and identify the distribution of ST131 and its H30 subclone. By focusing on this community cohort, our study aims to support risk assessment and prevention strategies within a One Health framework.[1–9]

Methods

Participants

We enrolled 96 healthy third-year students in the Department of Clinical Laboratory Science at Teikyo University during the 2023 academic year. Perianal swab specimens were collected using Cary–Blair transport medium (BD).

Ethics Statement

This study was conducted in accordance with the Declaration of Helsinki.

Participation was entirely voluntary, and the purpose of the study was explained verbally to all participants prior to sample collection.

Written informed consent was obtained from all participants.

No personal identifiers or clinical information were collected, and all samples were anonymized.

According to the Ethics Committee of Teikyo University, formal institutional review board approval was not required for this study, as confirmed by the Chair of the Ethics Committee.

Culture, Species Identification, and Antimicrobial Susceptibility Testing

Presumptive ESBL-producing Enterobacterales were isolated on CHROMagar ESBL (Kanto Chemical) after incubation at 35 °C for 18 hours. Seven isolates confirmed as ESBL producers were included for analysis. Species identification was performed using the MALDI Biotyper Sirius system (Bruker Daltonics).

Antimicrobial susceptibility testing was conducted with the MicroScan WalkAway system (Beckman Coulter; panel NMIC3.31E) and interpreted according to CLSI guidelines. The following antimicrobial agents were evaluated: piperacillin (PIPC), cefmetazole (CMZ), ceftriaxone (CTRX), ceftazidime (CAZ), cefotaxime (CTX), cefepime (CFPM), meropenem (MEPM), aztreonam (AZT), cefpodoxime (CPDX), levofloxacin (LVFX), and ciprofloxacin (CPFX).

ESBL production was confirmed by a ≥3 twofold dilution decrease in the MIC of CAZ or CTX in the presence of clavulanic acid compared with the MIC of the respective agent alone.

ESBL Genotyping and Phylogrouping

ESBL genotyping was performed using the Cica Geneus ESBL Genotype Detection Kit II (Kanto Chemical, Japan), according to the manufacturer's validated protocol.

Phylogrouping was conducted using the triplex PCR method described by Clermont et al,[10] classifying isolates into phylogenetic groups A, B1, B2, or D. The primer sets used were as follows:

- *ChuA* (279 bp): *ChuA*.1 (5′-GACGAACCAACGGTCAGGAT-3′) / *ChuA*.2 (5′-TGCCGCCAGTACCAAAGACA-3′)
- *YjaA* (211 bp): *YjaA*.1 (5′-TGAAGTGTCAGGAGACGCTG-3′) / *YjaA*.2 (5′-ATGGAGAATGCGTTCCTCAAC-3′)
- *TsE4C2* (152 bp): *TspE4C2*.1 (5′-GAGTAATGTCGGGGCATTCA-3′) / *TspE4C2*.2 (5′-CGCGCCAACAAAGTAT TACG-3′).

PCR conditions were as follows: initial denaturation at 94 °C for 4 min; 30 cycles of 94 °C for 5 s and 59 °C for 10 min; and a final extension at 72 °C for 5 min. Three microliters of bacterial suspension or a small portion of a colony was used as template DNA.

POT-Based Typing and Clone Identification

Clonal relatedness and ST131 identification were assessed using the Cica Geneus POT1 Molecular Epidemiology Kit for *E. coli* (Kanto Chemical, Japan), with all procedures performed according to the manufacturer's validated protocol. The

H30 subclone within ST131 was further confirmed by PCR following the method described by Colpan et al,[11] using the primer sets and cycling conditions reported in the original publication.

Results

Detection of ESBL-Producing *E. coli* and Antimicrobial Susceptibility

Among 96 specimens, seven (7.3%) yielded ESBL-producing *Escherichia coli*. In antimicrobial susceptibility testing, all isolates were resistant to piperacillin (PIPC), ceftriaxone (CTRX), cefotaxime (CTX), and cefpodoxime (CPDX). Susceptibility to ceftazidime (CAZ) varied, consistent with a CTX-M phenotype. Four isolates were susceptible to levofloxacin (LVFX), whereas three were resistant. All isolates remained susceptible to meropenem (MEPM) and cefmetazole (CMZ) (Table 1).

ESBL production was confirmed in all isolates by a ≥3 twofold dilution decrease in the MIC of CTX in the presence of clavulanic acid compared with CTX alone.

ESBL Genotypes and Phylogroups

Multiplex PCR detected $bla_{\text{CTX-M}}$ in all isolates, yielding four patterns: CTX-M-1 group in 2 isolates (28.6%); CTX-M-1 group plus bla_{TEM} in 1 (14.3%); CTX-M-9 group in 3 (42.9%); and CTX-M-9 group plus bla_{TEM} in 1 (14.3%) (Table 2).

Table 1 Antimicrobial Susceptibility results of ESBL-Producing *Escherichia coli* Isolates

Isolate No.	Species	MIC (μg/mL)												
		PIPC	CMZ	CTRX	CAZ	CAZ/CVA	CTX	CTX/CVA	CFPM	MEPM	AZT	CPDX	LVFX	CPFX
17	*E. coli*	>64	≦0.5	>64	16	≦0.12	>128	≦0.12	>32	≦0.5	16	>64	>4	>2
25	*E. coli*	>64	≦0.5	>64	16	≦0.12	>128	≦0.12	>32	≦0.5	32	>64	≦0.5	≦0.25
35	*E. coli*	>64	≦0.5	>64	32	≦0.12	>128	≦0.12	>32	≦0.5	64	>64	>4	>2
41	*E. coli*	>64	≦0.5	>64	8	≦0.12	>128	≦0.12	>32	≦0.5	32	>64	>4	>2
48	*E. coli*	>64	≦0.5	>64	128	≦0.12	>128	≦0.12	>32	≦0.5	>64	>64	2	>2
64	*E. coli*	>64	≦0.5	>64	1	≦0.12	>128	≦0.12	4	≦0.5	4	>64	≦0.5	≦0.25
101	*E. coli*	>64	≦0.5	>64	16	≦0.12	>128	≦0.12	>32	≦0.5	64	>64	≦0.5	≦0.25

Notes: All isolates remained susceptible to meropenem and cefmetazole. AST interpretation follows CLSI M100-22. Phylogroup assignments follow Clermont et al.
Abbreviations: PIPC, piperacillin; CMZ, cefmetazole; CTRX, ceftriaxone; CAZ, ceftazidime; CTX, cefotaxime; CFPM, cefepime; MEPM, meropenem; AZT, aztreonam; CPDX, cefpodoxime; LVFX, levofloxacin; CPFX, ciprofloxacin.

Table 2 Summary of ESBL Genotypes, Phylogroups, POT Types, and ST131/H30 Subclone Status of *Escherichia coli* Isolates

Isolate No.	ESBL Genotype	Phylogroup	POT	ST131	H30
35	CTX-M-1	B2	49-85-38	Positive	Positive
48	CTX-M-1	B2	49-39-34	Positive	Positive
17	CTX-M-9	B2	49-50-83	Positive	Positive
41	CTX-M-9	B2	49-122-83	Positive	Positive
64	CTX-M-9	D	17-16-7	Negative	Negative
101	CTX-M-1, TEM	D	24-25-14	Negative	Negative
25	CTX-M-9, TEM	B2	49-16-41	Positive	Negative

Note: POT1 value of 49 is interpreted as indicative of ST131.

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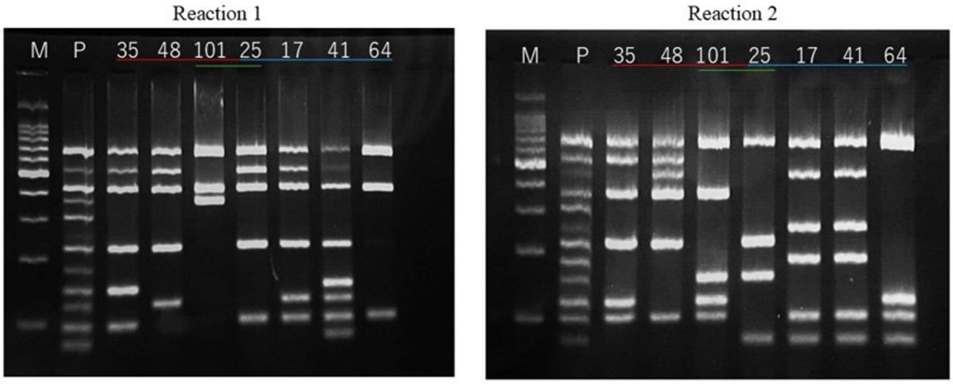

Figure 1 POT electrophoresis profiles of *Escherichia coli* isolates. The left (Reaction 1) and right (Reaction 2) panels show the POT banding patterns of the seven ESBL-producing *E. coli* isolates. M: 100 bp DNA ladder; P: positive control. Each lane represents a distinct isolate, and all isolates exhibited different POT profiles, indicating independent acquisition (non-clonal origin). Color bars indicate the detected ESBL genotypes: red, CTX-M-1 group; blue, CTX-M-9 group; green, TEM.

Phylogrouping assigned five isolates to phylogroup B2 and two to phylogroup D, with the predominance of virulent lineages among clinical strains.[12]

POT-Based Typing and ST131/H30 Subclone

POT-based typing showed distinct profiles for all seven isolates (Figure 1), indicating the absence of clonal transmission and suggesting individual acquisition routes. Of the seven isolates, five belonged to ST131, and four of those were classified as the H30 subclone.[11]

ST131 is widely recognized as a high-virulence, multidrug-resistant lineage, and H30 is associated with fluoroquinolone resistance.[11]

Details of ESBL genotypes, phylogroups, POT types, and ST131/H30 status are summarized in Table 2.[11,12]

Discussion

The present study revealed that 7.3% of healthy university students carried ESBL-producing *Escherichia coli*, with the majority belonging to the globally disseminated ST131 lineage, particularly the H30 subclone. This finding is of clinical importance because colonization among young, socially active individuals may serve as a hidden reservoir that facilitates the introduction of multidrug-resistant strains into healthcare environments.

The prevalence observed in this study is comparable to previous reports from Japan (6–9%)[1] and falls within the lower to mid-range of global estimates. In European cohorts, fecal carriage among healthy young adults has been reported at 5–12%[2,12] whereas rates in certain Asian countries exceed 20%.[3,4] The predominance of CTX-M-type enzymes and the frequent detection of ST131/H30 in our cohort are consistent with international trends. These findings underscore the global spread of high-risk clones and indicate that Japan is not exempt from these dynamics.

The detection of high-virulence, multidrug-resistant ST131-H30 in community carriers raises important infection control. University students frequently engage in social, educational, and healthcare-related activities, increasing the potential for dissemination. The introduction of such strains into clinical settings may compromise treatment options, particularly for urinary tract infections and sepsis. The observation that each isolate exhibited a distinct POT type further suggests independent acquisition events rather than clonal transmission, highlighting the complexity of community reservoirs.

ESBL-producing *E. coli* have been reported in companion animals, livestock, food products, and environmental water sources.[1,5] Genetic similarities between isolates from humans and animals support the possibility of interspecies exchange. Our findings contribute to growing evidence that human carriers form part of a broader One Health network facilitating the dissemination of antimicrobial resistance. Future studies should incorporate human, animal, and environmental surveillance to more precisely identify transmission pathways.

Several limitations should be acknowledged. First, the sample size was small and derived from a single university cohort, which may restrict the generalizability of the findings. Second, epidemiological information such as recent antibiotic exposure,

international travel, dietary habits, and contact with animals or healthcare environments was not collected, limiting assessment of potential risk factors for ESBL carriage. Third, although PCR-based genotyping was performed, higher-resolution approaches such as whole-genome sequencing (WGS) were not utilized, limiting detailed analysis of resistance determinants, plasmid content, and clonal relationships. Finally, the cross-sectional study design does not allow evaluation of persistence, acquisition dynamics, or temporal variation in ESBL colonization.

Despite these limitations, this study provides novel insights into the prevalence and molecular characteristics of ESBL-producing *E. coli* among healthy young adults in Japan. Future research should include multi-institutional and nationwide surveillance with larger sample sizes, combined with advanced genomic approaches such as WGS to track clonal dissemination and plasmid transfer. Linking community carriage data with clinical infection databases will further clarify the contribution of asymptomatic carriers to hospital outbreaks. International collaborations will also be essential to compare trends across regions and to develop coordinated strategies. At the policy level, our findings reinforce the importance of antimicrobial stewardship, infection prevention measures in both community and hospital settings, and integrated One Health frameworks to curb the spread of antimicrobial resistance.[5–11,13]

Conclusion

ESBL-producing *Escherichia coli* were identified in 7.3% of healthy university students, with most isolates belonging to high-virulence, multidrug-resistant lineages such as ST131, particularly the H30 subclone. POT genotyping indicated independent acquisition rather than clonal transmission.

These findings highlight the diversity and clinical relevance of ESBL-producing *E. coli* carriage in the community and underscore the importance of strengthening surveillance and risk assessment to mitigate the dissemination of antimicrobial resistance.

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Disclosure

The authors declare no conflicts of interest related to this study.

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