

Original Article

Molecular Characterization of Carbapenemase-Nonproducing Clinical Isolates of *Escherichia coli* (from a Thai University Hospital) with Reduced Carbapenem Susceptibility

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SUMMARY: Twelve nonreplicate carbapenemase-negative ertapenem (ETP)-nonsusceptible (CNENS) *Escherichia coli* isolates obtained at a Thai university hospital between 2010 and 2014 were characterized and compared with 2 carbapenemase-producing *E. coli* isolates from the same hospital. Eight unique pulsed-field gel electrophoresis patterns were obtained. All the isolates produced CTX-M-15 β -lactamase and 2 either coexpressed CMY-2 cephalosporinase or showed increased efflux pump activity. Amino acid sequence analysis revealed that an OmpF defect (in 7 isolates) due to mutations generating truncated proteins or an IS1 insertion was more prevalent than a defect in OmpC was (no truncated proteins detected). Seven out of 10 isolates possessing OmpC variants with any OmpF defect were weakly ETP-resistant (minimum inhibitory concentrations [MICs] of 1–4 μ g/mL) and imipenem (IPM)- and meropenem (MEM)-susceptible (MICs 0.125–0.5 μ g/mL). Two isolates with *ompC* PCR-negative results and an OmpF defect showed higher carbapenem MICs (8–32, 1–8, and 1–4 μ g/mL for ETP, IPM, and MEM, respectively) with the highest MICs associated with the additional efflux pump activity. Both carbapenemase producers possessing CTX-M-15 and a porin background identical to that in the CNENS isolates showed ETP, IPM, and MEM MICs of 128–256, 8, and 2–32 μ g/mL, respectively. These findings suggest that a porin defect combined with CTX-M-15 production is the major mechanism of low carbapenem susceptibility among our CNENS isolates, which have potential to become strongly carbapenem-resistant because of additional carbapenemase or efflux pump activities.

INTRODUCTION

Escherichia coli is a major pathogen causing both community-acquired and nosocomial infections particularly urinary tract infections, intra-abdominal infections, and bacteremia. Currently, it is the dominant species of Enterobacteriaceae that produces emerging CTX-M-type extended-spectrum β -lactamases (ESBLs) (1). Ertapenem (ETP), a long-acting carbapenem, is one of antimicrobial therapeutic options against severe infections with ESBL-producing Enterobacteriaceae including *E. coli*. Nonetheless, ETP-nonsusceptible clinical isolates of *E. coli* through acquisition of plasmid-borne carbapenemases have now become a major global threat (2).

Our previous hospital-based surveillance of

carbapenemase-producing Enterobacteriaceae in 2010 yielded the first report of NDM-1 produced by *Klebsiella pneumoniae*, *E. coli*, and *Citrobacter freundii* isolates and IMP-14a produced by *K. pneumoniae* isolates in Thailand (3). In addition, carbapenemase-negative ETP-nonsusceptible (CNENS) clinical isolates of *E. coli* were detected. Other mechanisms such as decreased drug uptake because of a porin defect or loss, in combination with ESBL or plasmid-mediated AmpC β -lactamase (pAmpC) production or efflux pump overexpression, may be involved in reduced susceptibility or resistance to ETP among our CNENS *E. coli* isolates as described elsewhere (4–8). We therefore investigated the molecular resistance mechanisms and relatedness of 12 clinical isolates of CNENS *E. coli* obtained at a Thai university hospital between 2010 and 2014.

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MATERIALS AND METHODS

Bacterial isolates and clinical data: Twelve non-duplicate CNENS *E. coli* isolates were obtained from different patients in Srinagarind Hospital, Thailand, between 2010 and 2014 (Table 1). Species identification

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was performed by the conventional biochemical tests. None of the isolates produced carbapenemases as confirmed by the updated Carba NP test (9), phenylboronic acid- and EDTA-carbapenem combined disk tests (10,11), and multiplex PCR (*bla*_{NDM}, *bla*_{KPC}, *bla*_{OXA-48}, *bla*_{IMP}, and *bla*_{VIM}) (12,13). Clinical data on all the patients except for patient 12 are summarized in Table 1. In addition, one isolate each of NDM-1- and OXA-48-producing *E. coli* collected during the same period was included for comparative analysis.

Strain typing: Clonality among the CNENS *E. coli* isolates was determined by pulsed-field gel electrophoresis (PFGE) according to the CDC PulseNet protocol (14) with some modifications as described previously (15). *Salmonella enterica* serovar Braenderup H9812 served as a standard DNA marker. A dendrogram for PFGE analysis was generated using BioNumerics version 5.0 (Applied Maths, Austin, TX, USA). Strains were defined as related isolates if they possessed at least 80% similarity of the PFGE fingerprints (16).

Antibiotic sensitivity testing: Minimum inhibitory concentrations (MICs) were determined by an agar dilution method. Antimicrobials were acquired as follows: amikacin (AK) and MEM from Siam Bheasach, Bangkok, Thailand; cefotaxime (CTX), ceftazidime (CAZ), ciprofloxacin (CIP), colistin (CT), and gentamicin (GN) from Sigma-Aldrich, St. Louis, MO,

USA; ETP from MSD, Paris, France; imipenem (IPM) from MSD, Whitehouse Station, NJ, USA; fosfomycin (FOS) from Meiji Seika Pharma, Tokyo, Japan; and tigecycline (TG) from Pfizer Inc., Philadelphia, PA, USA. The MICs were interpreted according to the CLSI guidelines (9) except for CT and TG: we used those approved by the EUCAST (17). *E. coli* ATCC 25922 served as an antibiotic-sensitive control strain.

ESBL and pAmpC detection: ESBL was detected by double-disk synergy and PCR assays for *bla*_{CTX-M}, *bla*_{SHV}, and *bla*_{VEB} as described elsewhere (18), whereas pAmpC production was examined by a phenylboronic acid combined disk test (11) and a multiplex PCR assay for *bla*_{ACC}, *bla*_{FOX}, *bla*_{DHA}, *bla*_{CMY}, and *bla*_{ACT} (19). Nucleotide sequences of *bla*_{ESBL} and *bla*_{pAmpC} were then determined (Applied Biosystems 3730XL, Foster City, CA, USA).

Efflux pump inhibitor-based test: Carbonyl cyanide-*m*-chlorophenylhydrazone (CCCP; Sigma Chemical Co., St. Louis, MO, USA) and phenylalanine arginine β -naphthylamide (PA β N; Sigma Chemical Co.) served as efflux pump inhibitors. MICs of ETP (final concentrations ranging from 0.125 to 128 μ g/mL), IPM (0.0625 to 64 μ g/mL), and MEM (0.0625 to 64 μ g/mL) in the presence or absence of either 50 mM CCCP (20) or 20 μ g/mL PA β N (21) were determined by the agar dilution method. Efflux activity was considered significant when

Table 1. Clinical detail of patients with the CNENS *E. coli* isolates

Patient no.	Isolate no.	Collection time	Specimen	Diagnosis	Underlying disease	Prior antimicrobial use	Antimicrobial therapy	Outcome
1	Ec-1	Oct 2010	Wound	Status post appendectomy with abdominal collection and enterocutaneous fistula	None	Meropenem, metronidazole	Colistin, imipenem	Improved
2	Ec-2	Dec 2010	Urine	Status post AP resection with UTI	Diabetes, CA rectum	Ceftriaxone, metronidazole	Ceftazidime, metronidazole	Improved
3	Ec-3	Mar 2011	Urine	Complicated UTI	Diabetes, hypertension, Parkinson's disease, cancer of prostate and bed ridden	Cephalexin	Meropenem	Improved
4	Ec-4	Apr 2011	Urine	Neurogenic bladder with UTI	Lipomyelomeningocele	None	Amikacin	Improved
5	Ec-5	Aug 2011	Urine	UTI	Myelomeningocele with neurogenic bladder	Ofloxacin	Ceftriaxone	Improved
6	Ec-6	Aug 2011	Urine	Adenocarcinoma of appendix	Diabetes, hypertension, ischemic heart disease	None	None	Died from acute myocardial infarction
7	Ec-7	Jan 2013	Drain	CA colon with diverticulosis status post right half colectomy with abdominal collection	Diabetes, ischemic heart disease, CA colon	Meropenem, vancomycin	Colistin, tigecycline	Died from <i>A. baumannii</i> septicemia
8	Ec-8	Mar 2013	Sputum	Hospital acquired pneumonia	Leiomyosarcoma of duodenum status post hemicolectomy, subtotal gastrectomy with partial gut obstruction	Ceftriaxone, metronidazole, ceftazidime, vancomycin	Piperacillin/tazobactam	Died from pneumonia septic shock
9	Ec-9	Mar 2013	Urine	Status post AP resection, partial colectomy with UTI	CA rectosigmoid colon status post AP resection with rectosigmoid fistula	Ceftriaxone, metronidazole	Imipenem	Improved
10	Ec-10	May 2013	Bile	Cholangitis	Common bile duct obstruction	None	Ceftriaxone, metronidazole	Improved
11	Ec-11	Jul 2013	Bile	Cholangitis	Cholangiocarcinoma	Ceftriaxone, metronidazole	Piperacillin/tazobactam	Improved
12	Ec-12	Oct 2013	Sputum	-	-	-	-	-

-, not available; AP, abdominoperineal; CA, cancer; UTI, urinary tract infection.

the carbapenem MIC decreased at least 4-fold in the presence of either CCCP or PA β N.

Porin gene analysis: PCR amplification of *ompC* and *ompF* followed by nucleotide sequencing was carried out as described previously (15). In addition, primer omp-IS1 (5'-ACGGCAATGAGAGCAGAGAT-3') was used for amplification of IS1. The nucleotide sequences were analyzed online (<http://www.ncbi.nlm.nih.gov>) and compared with wild-type *ompC* and *ompF* (GenBank accession No. CP001637, nucleotide positions 1564223 to 1565326 and 2894137 to 2895225 for *ompC* and *ompF*, respectively). The nucleotide positions where mutations occur and the number of amino acid residues in a truncated protein were counted from a start codon, whereas mutated amino acid positions were numbered according to Bornet et al. (22).

Ethics statement: The study was conducted in accordance with the Declaration of Helsinki and Good Clinical Practices, and the protocol was approved by the Khon Kaen University Ethics Committee on Human Research, Faculty of Medicine, Khon Kaen University (Project ID: HE571290).

RESULTS

Clinical information: There were 12 cases of infection with CNENS *E. coli*; however, clinical information about one patient was missing. Five isolates (41.7%) were obtained from patients with urinary tract infection (Table 1). Only 2 patients received MEM prior to getting infected with CNENS *E. coli*. Of the 11 patients, 8 received either β -lactam monotherapy or a combination with other agents. In addition, 2 patients received either AK or CT plus TG, whereas no antimicrobial therapy was administered to one patient. Eight patients showed clinical improvement.

Strain typing: Eight different PFGE patterns were

obtained (Table 2). Three small clusters of 2 or 3 isolates were clonally related (pulsotypes A, G, and H).

Antibiotic sensitivity: All the CNENS isolates showed strong resistance to CTX and CAZ with MICs of ≥ 256 μ g/mL (Table 2). They were ETP-nonsusceptible with MICs of 1–32 μ g/mL (MIC₅₀ and MIC₉₀ of 4 and 16 μ g/mL, respectively) but remained susceptible or intermediately susceptible to IPM (0.125–1 μ g/mL) and MEM (0.125–2 μ g/mL) except for isolate Ec-7 (IPM and MEM MICs of 8 and 4 μ g/mL, respectively). Other non- β -lactam antimicrobials with the highest in vitro activity against these isolates were FOS (100% cases of susceptibility), AK (91.7%), CT (91.7%), and TG (75%). The 2 carbapenemase producers yielded MICs of 128–256, 8, and 2–32 μ g/mL for ETP, IPM, and MEM, respectively.

ESBL and pAmpC production: All the CNENS isolates produced CTX-M-15 ESBL and 2 isolates (Ec-3 and Ec-4) coexpressed CMY-2 pAmpC (Table 2). The 2 carbapenemase producers also coproduced CTX-M-15.

Increased efflux pump activity: Only 2 isolates (Ec-7 and Ec-8) showed increased efflux pump activity. Isolate Ec-7 revealed a 4-fold decrease in ETP MIC in the presence of PA β N, whereas isolate Ec-8 showed a significant decrease in ETP (4-fold) and IPM (≥ 4 -fold) MICs in the presence of CCCP.

Porin gene analysis: The expected DNA fragments of both *ompC* (1,117 bp) and *ompF* (1,072 bp) were detected in 9 isolates, whereas 2 isolates (Ec-2 and Ec-7) carried either *ompC* or *ompF* (Table 2). In addition, one isolate (Ec-3) yielded an unexpected 1.8-kb DNA fragment of *ompF* only, indicating an insertion of insertion sequence (IS) into this gene. Amino acid sequence analysis of OmpC from the 10 isolates compared with that of the wild type (GenBank accession No. CP001637) revealed 6 OmpC variants, designated as C-1 to C-6 (Table 2 and Fig. 1). Among the 6 variants,

Table 2. Phenotypic and genotypic characteristics of the 12 CNENS *E. coli* isolates

% Similarity			Pulso type	Isolate no.	Collection time	β-lactamase	Porin variant ³⁾				MICs (μg/mL) ⁴⁾								
							OmpC	OmpF	ETP	IPM	MEM	CTX	CAZ	AK	GN	CIP	FOS	TG	CT
100	0		A-1	Ec-3	Mar 2011	CTX-M-15, CMY-2	—	F-IS1	8	1	1	> 256	256	4	2	32	2	0.25	0.5
98	A-2		Ec-6	Aug 2011	CTX-M-15	C-1	F-1	2	0.25	0.25	> 256	> 256	4	> 32	> 32	1	2	0.5	
96	B		Ec-8 ¹⁾	Mar 2013	CTX-M-15	C-2	F-1	8	0.25	1	> 256	> 256	4	2	16	1	0.5	0.5	
94	C		Ec-9	Mar 2013	CTX-M-15	C-2	F-P1	1	0.125	0.125	> 256	> 256	4	1	> 32	1	0.5	0.5	
92	D		Ec-1	Oct 2010	CTX-M-15	C-5	F-1	16	1	2	> 256	> 256	4	> 32	32	2	0.25	0.5	
90	E		Ec-4	Apr 2011	CTX-M-15, CMY-2	C-1	F-1	2	0.25	0.125	> 256	> 256	4	> 32	2	≤0.25	2	0.5	
88	F		Ec-2	Dec 2010	CTX-M-15	C-3	—	4	0.5	0.25	> 256	> 256	> 64	> 32	> 32	8	8	0.5	
86	G-1		Ec-7 ²⁾	Jan 2013	CTX-M-15	—	F-P2	32	8	4	> 256	> 256	16	> 32	> 32	4	0.5	0.5	
84	G-2		Ec-11	Jul 2013	CTX-M-15	C-4	F-P3	2	0.25	0.125	> 256	> 256	4	> 32	> 32	1	0.5	0.5	
82	H-1		Ec-10	May 2013	CTX-M-15	C-2	F-P1	16	0.125	1	> 256	> 256	4	> 32	> 32	1	0.5	0.5	
80	H-2		Ec-5	Aug 2011	CTX-M-15	C-6	F-P1	4	0.25	0.5	> 256	> 256	4	> 32	> 32	1	0.5	0.5	
78	H-3		Ec-12	Oct 2013	CTX-M-15	C-2	F-P1	1	0.25	0.125	> 256	> 256	4	> 32	> 32	1	0.5	4	
76			NDM	Nov 2013	NDM-1, CTX-M-15	C-2	F-P1	128	8	32	R	R	S	S	R	S	S	S	
74			OXA	Oct 2014	OXA-48, CTX-M-15	C-1	F-1	256	8	2	R	R	S	R	R	S	S	NT	

¹⁾ and ²⁾: isolates showing increased efflux pump activity when tested by using the CCCP and PA β N respectively.

³⁾: OmpC and OmpF variants are shown in Fig. 1 and Fig. 2 respectively.

⁴⁾: AK, amikacin; CTX, cefotaxime; CAZ, ceftazidime; CIP, ciprofloxacin; CT, colistin; ETP, ertapenem; FOS, fosfomycin; GN, gentamicin; IPM, imipenem; MEM, meropenem and TG, tigecycline. For the NDM-1 and OXA-48 producers, susceptibility to various antimicrobials except for carbapenems was performed by disk diffusion test (R, resistant; S, susceptible).

—, PCR-negative for either *ompC* or *ompF*; NT, not determined.

point mutations, amino acid (aa) deletion or insertion, were found, but neither a frameshift nor premature stop codon was seen in any variant. Four variants, C-1 (2 isolates), C-2 (4 isolates), C-3 (one isolate), and C-4 (one isolate), had amino acid sequences identical to those reported previously (GQ166996 and GQ166997 from Taiwan (8), HM565112 from Korea [unpublished], and JF694832 from China [unpublished], respectively; Fig. 1). Two novel variants, C-5 (KU866181) and C-6 (KU866182), were uncovered in this study. Variant C-5 differs from C-1 by one amino acid residue, Gly91Ser in the $\beta 5$ region, whereas variant C-6 had 2 amino acids different from those of C-2, Lys16Ile and Lys33Glu, in regions $\beta 1$ and $\beta 2$, respectively.

On the other hand, 5 *OmpF* variants were obtained when compared with the wild type (CP001637): one variant with point mutations, a 1-aa insertion and 6-aa deletion (designated as F-1), 3 variants with truncated proteins (F-P1 to F-P3), and one variant with the IS1 insertion (F-IS1; Table 2 and Fig. 2). Variants F-1 and

F-P1 (each detected in 4 isolates) had amino acid sequences identical to those of Taiwanese *E. coli* isolates (GQ465819 and GQ465794, respectively) (8). Three novel *OmpF* variants include F-IS1, F-P2, and F-P3 (KU866183 to KU866185, respectively; this study). Variants F-P1, F-P2, and F-P3 were truncated at positions 256 (a 4-bp insertion at nucleotide position 757), 142 (a 1-bp insertion at nucleotide position 426), and 57 (a 7-bp deletion at nucleotide position 115), respectively. In variant F-IS1, IS1 (768 bp) with 15-bp inverted repeat sequences (5'-GGTAATGACTCCAAC-3') at each end got inserted at nucleotide position 312 of *ompF* and was flanked by 8-bp direct repeat sequences (5'-GAAATTCG-3').

Of note, the NDM-1 producer carries the C-2 and F-P1 variants as found in isolates Ec-9, Ec-10, and Ec-12, whereas the OXA-48 producer carries C-1 and F-1 variants identical to those of isolates Ec-4 and Ec-6 (Table 2).

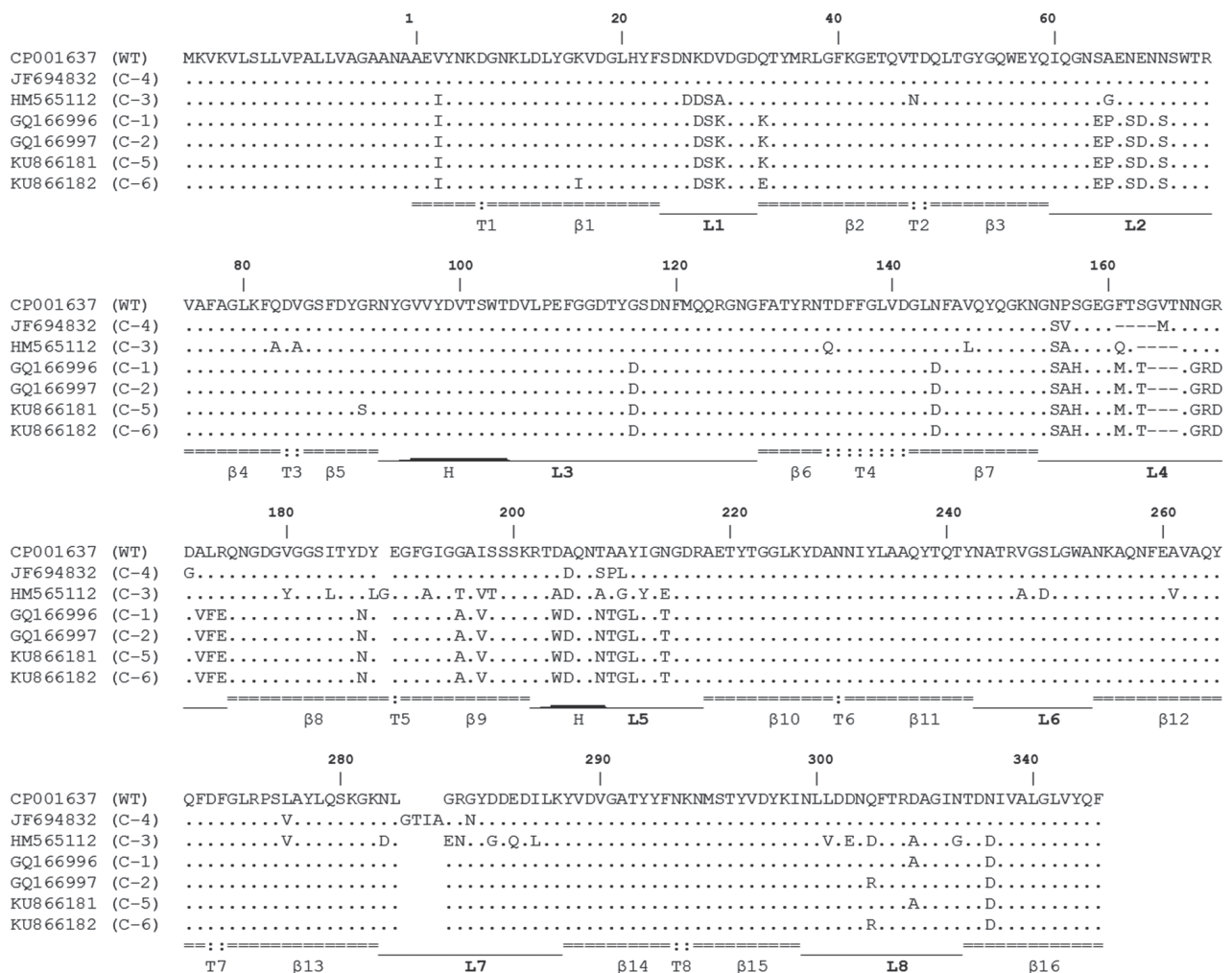


Fig. 1. Amino acid sequences of the *OmpC* variants (designated as C-1 to C-6) from the CNENS *E. coli* isolates compared with the wild type (WT) (GenBank accession no. CP001637). C-1 and C-2 previously reported from the Taiwanese *E. coli* isolates (GQ166996 and GQ166997 respectively) (8); C-3 from the Korean *E. cloacae* strain YMC 05/10/5303 (HM565112) [unpublished]; C-4 from the Chinese *E. coli* strain EcWH098 (JF694832) [unpublished]; C-5 (KU866181), and C-6 (KU866182) from this study. Identical amino acid (dot), amino acid deletion (hyphen) and amino acid insertion (space) are indicated. The bottom lines showing secondary structural motifs are described based on the crystal structure of *OmpC* variants (36). =, β strands ($\beta 1$ – $\beta 16$); :, periplasmic turns (T1–T8); __, internal/external loop (L1–L8); ==, α helix (H).

DISCUSSION

The phenotypic carbapenemase tests followed by the PCR assays revealed that low ETP susceptibility in the 12 *E. coli* isolates was not mediated by true carbapenemases. All of them produced CTX-M-15 ESBL, whereas only 2 isolates coexpressed CMY-2 pAmpC. Enzymes CTX-M-15 and CMY-2 were first detected in this hospital in 2003 and 2005, respectively (18,23). These results suggest that CTX-M-15 ESBL is the major β -lactamase responsible for reduced ETP susceptibility among our CNENS *E. coli* isolates, in line with reports from Hong Kong and Lebanon (4,5). In contrast to this study, CMY-2 expression is mostly associated with Taiwanese CNENS *E. coli* isolates (8). Genetic diversity of our isolates supports the findings of Yan et al. (8), i.e., that the emergence of CNENS *E. coli* isolates is due to the inability to control the spread of either ESBL producers or pAmpC producers rather than the consequent spread of certain resistant strains in the hospital.

Carbapenem resistance can develop via porin loss or mutations in genes encoding porins, particularly in their constriction L3 loop region (24,25). In the present study, the frequency of an OmpF defect manifesting itself as

a truncated protein (7 out of 11 isolates) is higher than that of OmpC defects (no truncated proteins in any isolate), in agreement with the in vitro study by Adler et al. (26). Furthermore, to the best of our knowledge, this is the first report of disruption of the *ompF* gene by the insertion of IS1 (F-IS1) in a clinical isolate of *E. coli*, as previously reported for experimental *E. coli* mutants (26) and clinical isolates of *Serratia marcescens* (27). In our study, neither OmpC nor OmpF variants in any isolate had mutations in the key L3 residues (PEFGG motif at positions 108–112 and 116–120 for OmpC and OmpF, respectively). Among the 7 isolates (Ec-2, Ec-4, Ec-5, Ec-6, Ec-9, Ec-11, and Ec-12) carrying OmpC variants (C-1 to C-6 except for C-5) with *ompF*-PCR negativity or truncated (F-P1 or F-P3) or full-length OmpF (F-1), all yielded similar carbapenem MICs with weak ETP resistance and susceptibility to both IPM and MEM (1–4, 0.125–0.5, and 0.125–0.5 $\mu\text{g/mL}$, respectively), as in the *E. coli* clinical isolate with OmpC expression but a lack of *ompF* reported by Oteo et al. (28) (MICs 0.12, 0.25, and 0.06 $\mu\text{g/mL}$, respectively). These findings suggest that reduced ETP susceptibility is initially mediated by the loss of or defect in OmpF. In addition, a porin defect combined with β -lactamase expression had a stronger

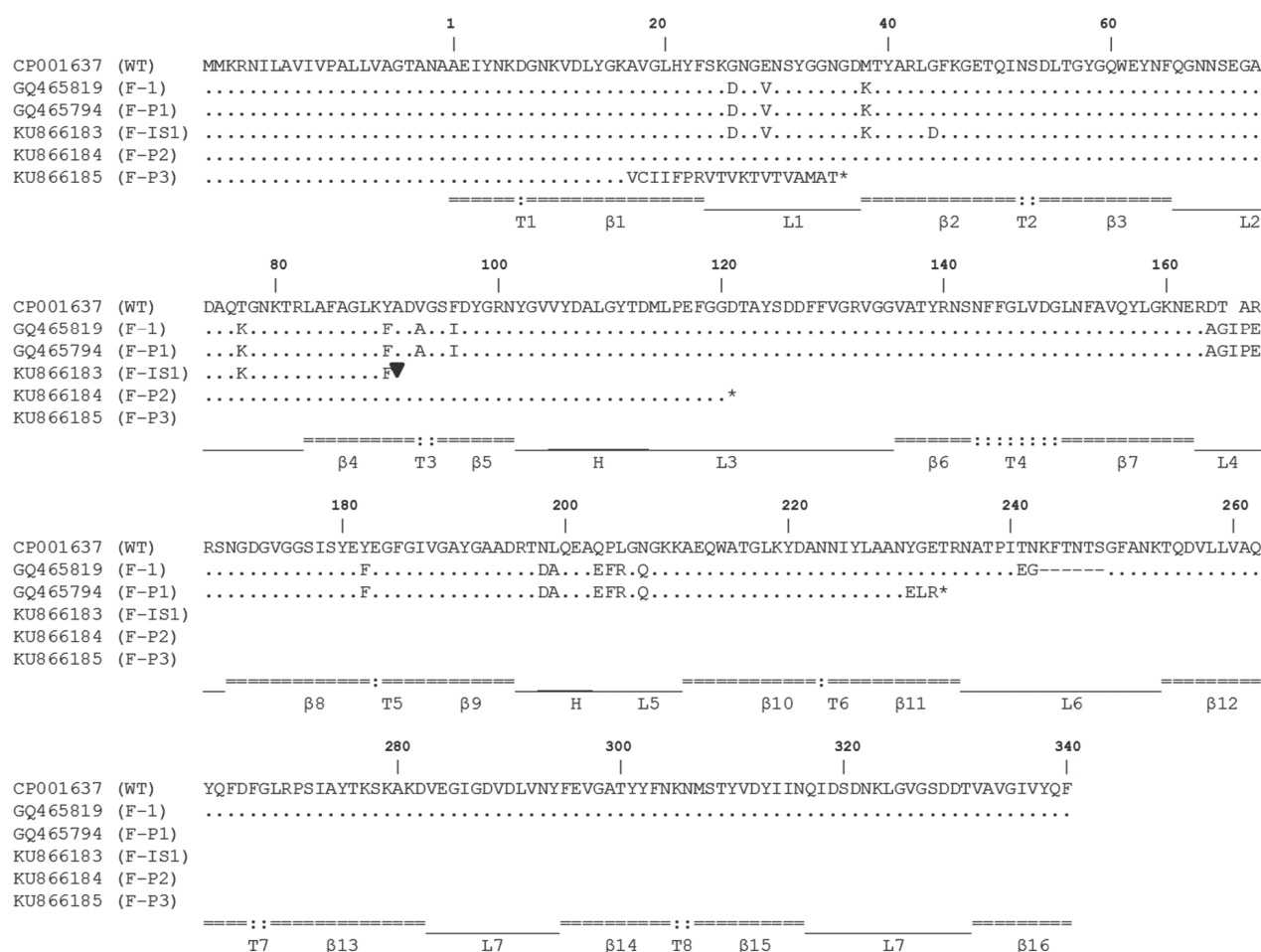


Fig. 2. Amino acid sequences of the OmpF variants (designated as F-1, F-P1 to F-P3, and F-IS1) from the CNENS *E. coli* isolates compared with the wild type (WT) (GenBank accession no. CP001637). F1 and F-P1 identical to those of the Taiwanese *E. coli* isolates (GQ465819 and GQ465794, respectively) (8); F-IS1 (KU866183), F-P2 (KU866184), and F-P3 (KU866185) from this study. Symbols are indicated in Fig. 1 except that asterisk and black triangle indicate premature stop codon and IS1 insertion, respectively.

effect on ETP than on other carbapenems. This is because of the larger size of ETP and its negative charge, resulting in slower penetration through the minor porins, which take over when major porins are lost (29,30).

Among the 3 isolates, Ec-9, Ec-10, and Ec-12, with identical porins (C-2 with F-P1 variants), isolate Ec-10 showed a 16-fold higher ETP MIC (16 µg/mL) as compared with isolates Ec-9 and Ec-12 (1 µg/mL). Similarly, of the 4 isolates (Ec-1, Ec-4, Ec-6, and Ec-8) possessing an identical F-1 variant with different OmpC variants (C-1, C-2, or C-5), isolates Ec-1 and Ec-8 showed ETP and MEM MICs (8–16 and 1–2 µg/mL, respectively) higher than those of isolates Ec-4 and Ec-6 (2 and 0.125–0.25 µg/mL, respectively). Increased efflux pump activity observed in isolate Ec-8 may be responsible for its increased carbapenem MICs. Adler et al. (26) reported that mutations in 2 domains of the OmpR protein, the N-terminal phosphorylation site and C-terminal DNA-binding domain, confer ETP resistance onto *E. coli* isolates. Isolates Ec-1 and Ec-10 carry *ompR* but no mutations in either domain were observed (data not shown), pointing to the existence of other resistance mechanisms in these isolates. Furthermore, isolate Ec-5, which carries an F-P1 variant identical to that in isolates Ec-9 and Ec-12 but possesses the C-6 variant instead of C-2, yielded ETP MIC (4 µg/mL) higher than those for isolates Ec-9 and Ec-12 (1 µg/mL). In the pore constriction region, the electrostatic field that governs the diffusion of charged molecules is formed by Gly112 and Tyr116 from the L3 loop as well as basic residues on the opposite barrel wall (Lys16, Asp106, Glu110, Arg37, Arg75, and Arg125) (31). One study on the role of anti-loop 3 in cefepime diffusion (32) revealed that the site-directed mutagenesis of Lys (positively charged) at amino acid position 16 to either Ala (nonpolar) or Asp (negatively charged) results in increased cation selectivity of OmpF. This result suggests that the increased ETP MIC for isolate Ec-5 may be due to the amino acid substitution in its OmpC at position 16 from Lys to Ile (nonpolar; Fig. 1), leading to reduced selectivity for the negatively charged ETP.

The clinical relevance of OmpC and OmpF to carbapenem susceptibility is controversial. An in vitro study by Adler et al. (26) revealed that an *E. coli* mutant with *ompF* deletion in the presence of an ESBL-encoding plasmid shows ETP and MEM MICs higher than MIC with *ompC* deletion, in agreement with the strong MEM resistance in *S. marcescens* isolates with intact OmpC but a loss of OmpF due to the *IS1* insertion reported by Suh et al. (27). In contrast, a carbapenem-nonsusceptible *E. coli* isolate with decreased OmpC and OmpF expression shows restored carbapenem susceptibility after introduction of cloned *ompC* but not cloned *ompF* (7). In our study, most of the isolates with full-length OmpC but defective OmpF showed reduced susceptibility to ETP only (1–4, 0.125–0.5, and 0.125–0.5 µg/mL for ETP, IPM, and MEM, respectively), whereas isolates Ec-3 and Ec-7 with an OmpF defect and *ompC* PCR negativity revealed higher carbapenem MICs (8–32, 1–8, and 1–4 µg/mL, respectively; Table 2), consistent with other reports (28,33). The highest carbapenem MIC seen in isolate Ec-7 may be due to additional efflux pump activity (4,34). These findings indicate that an additional OmpC loss is responsible for stronger resistance

to all carbapenems particularly ETP among our isolates (26,29).

In the present study, the NDM-1- or OXA-48-producing *E. coli* isolates from the same hospital coexpressing CTX-M-15 ESBL and both porins identical to those of the CNENS isolates showed higher carbapenem resistance. These findings support the contribution of acquired carbapenemases together with a porin defect, as a cofactor, to high resistance to carbapenems among clinical isolates of Enterobacteriaceae (15,35), thus limiting therapeutic options. Therefore, appropriate infection control measures to prevent the spread of these CNENS *E. coli* strains are still necessary.

The limitation of our study that should be noted is that the expression of both OmpC and OmpF was not examined by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE). Thus, alteration or diminished expression of OmpC or OmpF in these CNENS *E. coli* isolates was not demonstrated. In addition, the sample size in this study is small. In our hospital, the prevalence of the CNENS *E. coli* isolates between October 2010 and August 2011 was 0.28% (6 of 2,125 isolates; unpublished data). This low prevalence may be explained by the in vitro study by Adler et al. (26) showing that the carbapenem-nonsusceptible *E. coli* mutants with OmpC and/or OmpF loss in association with β-lactamase production have not emerged because they have limited fitness.

In conclusion, the low susceptibility to carbapenems in our carbapenemase-nonproducing *E. coli* isolates is mainly associated with the combination of an OmpF and/or OmpC defect with CTX-M-15 production. These isolates may manifest higher resistance to carbapenem by additional mechanisms such as increased efflux pump activity or carbapenemase production, leading to therapeutic difficulties. Therefore, effective infection control is still needed for prevention of the spread of these CNENS *E. coli* strains.

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Conflict of interest None to declare.

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