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ORIGINAL RESEARCH

Resistance mechanisms and molecular epidemiology of carbapenem-nonsusceptible *Escherichia coli* in Taiwan, 2012-2015

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Purpose: This study aimed to investigate the resistance mechanisms and molecular epidemiology of carbapenem-nonsusceptible *Escherichia coli* (CnsEC) in Taiwan.

Patients and methods: From 2012 to 2015, 237 *E. coli* isolates with minimum inhibitory concentrations of imipenem or meropenem >1 μg/mL were collected in a nationwide surveillance and subjected to polymerase chain reaction (PCR) for carbapenemase, AmpC-type β-lactamase, and extended spectrum β-lactamase (ESBL) genes. We evaluated outer membrane proteins (OmpF and OmpC) loss and conducted multilocus sequence typing and pulsed-field gel electrophoresis (PFGE). Isolates that were resistant to all carbapenems were designated as pan-carbapenem-resistant *E. coli* (pCREC) in this study.

Results: The predominant resistance mechanism of CnsEC in Taiwan was the CMY-2 β-lactamase in combination with OmpF and OmpC loss. Sequence type 131 was the most prevalent type (29.2%). Among 237 CnsEC isolates, 106 (44.7%) isolates were pCREC and 18 (7.59%) produced carbapenemase. The prevalence of carbapenemases increased from 6% in 2012 to 11.36% in 2015. Various carbapenemases including KPC-2, IMP-8, NDM-1, NDM-5, VIM-1, OXA-48, and OXA-181 were identified, with NDM-1 being the most common (38.9%) carbapenemase. Comparison between pCREC and non-pCREC among the non-carbapenemase-producing CnsEC isolates revealed SHV, CMY, co-carriage of SHV and CTX-M and concurrent loss of both OmpF and OmpC were more commonly detected in the pCREC group. PFGE revealed no nationwide clonal spread of carbapenemase-producing *E. coli*.

Conclusion: NDM-1 was the most common carbapenemase and combination of CMY-2 and concurrent OmpF and OmpC porin loss was the most prevalent resistance mechanism in CnsEC in Taiwan.

Keywords: multidrug resistance, carbapenemase, Enterobacteriaceae, epidemiology

Introduction

Escherichia coli is one of the most common human pathogen, the major etiology of community-acquired urinary tract infection and a major nosocomial Gram negative bacteria.[1,2] In the late 1990s, extended spectrum β-lactamase (ESBL)-producing *E. coli* infections emerged.[3] Since the worldwide propagation of ESBL-producing *Enterobacteriaceae*, carbapenems have been the prevailing treatment of such infections. Carbapenem resistance in *Enterobacteriaceae* was relatively uncommon before 2000. Nevertheless, the prevalence of carbapenem-resistant *Enterobacteriaceae* (CRE) rose markedly in the following decade.[4] The United States Centers for Disease Control and Prevention (CDC) listed CRE as an urgent threat that requires intensive monitoring

and prevention (https://www.cdc.gov/drugresistance/biggest_threats.html). There are two primary mechanisms of carbapenem resistance: (1) carbapenemase production and (2) a combination of β-lactamases, ESBLs, or AmpC cephalosporinases with structural mutations such as outer membrane protein (OMP) deficiency or efflux pump overproduction.[5] Carbapenemases are generally considered more hazardous, because the genes are carried mostly on plasmids, which can transmit between *Enterobacteriaceae* species and facilitate spread.[6]

Most surveillance reports have shown lower prevalence rates of carbapenam resistance and carbapenemase-producing *Enterobacteriaceae* (CPE) for *E. coli*, when compared with the rates of *Klebsiella pneumoniae*.[7–9] Seeing the global dissemination of CTX-M β-lactamase-producing *E. coli* clones in community-onset infections,[3,10] the carbapenem resistant *E. coli* should be a serious public health concern and its spread may potentially change the status quo. CTM-X 15 is the most common type of ESBL among *E. coli* and has been associated with *E. coli* sequence type 131 (ST131). ST131 correlates with extraintestinal infections, fluoroquinolone resistance, and community-onset infections.[11] The carbapenemase genes of bla_{NDM}, bla_{VIM}, bla_{KPC}, bla_{IMP}, and bla_{OXA-48} have all been identified from ST131.[11] A global epidemic caused by carbapenemase-producing ST131 *E. coli* in nosocomial and community-onset infections would be a nightmare. Because less is known about the carbapenem-nonsusceptible *E. coli* (CnsEC) in Taiwan, we conducted a nationwide surveillance to investigate the characteristics, resistance mechanisms, and molecular typing of CnsEC in Taiwan.

Materials and methods

Bacterial isolates and definitions

A total of 21 hospitals in Taiwan, including 12 tertiary medical centers and 9 regional hospitals, participated in the surveillance program from January 2012 to September 2015. The study was approved by the institutional review boards (IRBs) of all participating hospitals, including Chang Gung Memorial Hospital (IRB No.: 1003399B), Taipei Veterans General Hospital (IRB No.: 2011-11-001IC), National Taiwan University Hospital (IRB No.: 201110043RB), Tri-Service General Hospital (IRB No.: 100-05-205), Kaohsiung Medical University Chung-Ho Memorial Hospital (IRB No.: KMUH-IRB-20110328), Chi- Mei Medical Center (IRB No.: 10012-001), China Medical University Hospital (IRB No.: DMR100-IRB-214), Kaohsiung Armed Forces General Hospital (IRB No.: 100–076), National Cheng Kung University Hospital (IRB No.: A-ER-101–304), Tzu Chi Hospital (IRB No.: ACT-IRB100-14), Chung Shan Medical University Hospital (IRB No.: CS12187) and Taichung Veterans General Hospital (IRB No.: SG14157). The IRBs waived the requirement for informed consents from participants because all bacterial isolates were obtained from clinical samples as part of standard care.

A total of 237 nonduplicate imipenem- or meropenem-nonsusceptible *E. coli* isolates (minimum inhibitory concentrations [MICs] of imipenem or meropenem >1 μg/mL) were collected. Isolates with imipenem or meropenem MICs of >1 μg/mL were defined as CnsEC. Isolates that were resistant to all carbapenems, including imipenem, meropenem, doripenem (MICs ≥4 μg/mL) and ertapenem (MICs ≥2 μg/mL), were designated as pan-carbapenem-resistant *E. coli* (pCREC) in this study. The preliminary identification of *E. coli* and testing of carbapenem susceptibility were performed at the participating hospitals as routine laboratory procedures. All isolates were sent to a reference laboratory at the National Health Research Institutes in Taiwan. Species identification was confirmed with a VITEK 2 automated system (bioMerieux, Marcy l'Etoile, France) and the Bruker Biotyper MALDI-TOF MS system (Bruker Daltonik GmbH, Leipzig, Germany).

Antimicrobial susceptibility testing

All isolates were tested for MICs of (1) β-lactam agents, including penicillin (piperacillin-tazobactam), cephalosporins (cefazolin, cefoxitin, cefuroxime, cefotaxime, ceftazidime, and cefepime), carbapenems (doripenem, ertapenem, imipenem, and meropenem), and a monobactam (aztreonam); and (2) non-β-lactams, including fluoroquinolones (ciprofloxacin and levofloxacin), aminoglycosides (gentamicin and amikacin), trimethoprim-sulfamethoxazole, colistin, and tigecycline. The MIC of tigecycline was determined through the E-test (AB Biodisk, Solna, Sweden) on Mueller-Hinton media, and the MICs of other agents were determined using the broth microdilution method (Sensititre, Trek Diagnostic Systems, Cleveland, OH, USA). Susceptibilities to colistin and tigecycline were determined based on the European Committee on Antimicrobial Susceptibility Testing (EUCAST) guidelines (http://www.eucast.org/clinical_breakpoints/), and susceptibilities to other agents were determined based on the updated guidelines from the Clinical and Laboratory Standards Institute (CLSI).[12]

Detection of genes encoding carbapenemases, AmpC, and ESBLs

All verified CnsEC isolates were subjected to polymerase chain reaction (PCR) for the detection of genes encoding carbapenemase (class B families IMP, VIM, NDM, GIM, SPM, and SIM; class A families NMC, IMI, SME, KPC, and GES; class D family OXA-48),[13] plasmidic AmpC (CMY, DHA, and ACT),[14] and ESBL genes (CTX-M, TEM, and SHV).[15]

Pulsed-field gel electrophoresis

Total DNA was prepared, and pulsed-field gel electrophoresis (PFGE) was performed as described.[16] The restriction enzyme, XbaI (New England Biolabs, Beverly, MA, USA), was used at the temperature suggested by the manufacturer. The Dice coefficient was used to calculate similarities, and the unweighted pair-group method with arithmetic mean was used for cluster analysis with BioNumerics software version 5.10 (Applied Maths, St-Martens-Latem, Belgium).

Isolation and analysis of OMPs

Bacterial OMPs were prepared as described.[17] The OMPs were then separated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis through 7.5% polyacrylamide-6 M urea gels and visualized using Coomassie Blue staining (Bio-Rad). A reference strain, *E. coli* ATCC25922, was included as a control.

Multilocus sequence typing

Multilocus sequence typing (MLST) with seven housekeeping genes[18] —adk (adenylate kinase), fumC (fumarate hydratase), gyrB (DNA gyrase), icd (isocitrate dehydrogenase), mdh (malate dehydrogenase), purA (adenylosuccinate synthetase), and recA (ATP/GTP motif)—was performed on all isolates according to the protocol described on the Enterobase website. (http://mlst.ucc.ie/mlst/dbs/Ecoli/documents/primersColi_html). The allele sequences and STs were verified by the website as well (http://mlst.ucc.ie/mlst/dbs/Ecoli/).

Statistical analyses

SPSS version 17.0 (SPSS, Chicago, IL, USA) was used to perform statistical analyses. Categorical variables were compared using the chi-square test or Fisher's exact test. A p-value of <0.05 was considered statistically significant.

Results

Of the 237 CnsEC isolates, most were collected from urine (35.0%), followed by abscess, drainage, pus, or wound cultures (22.4%), and blood (11.4%). Among all CnsEC, 18 isolates (7.59%) produced carbapenemase. We identified 44.7% (106 isolates) of the CnsEC to be pCREC, including 17 carbapenemase-producing isolates.

Antimicrobial susceptibilities

Table 1 shows the resistance rates of CnsEC to antimicrobial agents. With the exception of piperacillin-tazobactam and cefepime, the resistance rates to all noncarbapenem β-lactams were 95–100%. The resistance rates were higher for ertapenem (96.2%), followed by imipenem (74.3%), meropenem(61.6%) and doripenem (47.3%). Figure 1 shows the MIC distribution of carbapenems in CnsEC. The MIC is higher for imipenem than meropenem and doripenem, with 60% of isolates presenting MIC >4 mg/L for imipenem and only 36.3% and 18.1% for meropenem and doripenem,

Table 1 Antimicrobial resistance rates of carbapenem-nonsusceptible *E. coli* isolates in Taiwan

	2012	2013	2014	2015	Total
	(N=50)	(N=62)	(N=81)	(N=44)	(N=237)
ETP	96.00	96.77	95.06	97.73	96.2
IPM	74.00	77.42	72.84	72.73	74.26
MEM	62.00	62.90	56.79	68.18	61.6
ATM	94.00	98.39	96.30	93.18	95.78
DOR	50.00	50.00	44.44	45.45	47.26
TZP	78.00	91.94	90.12	90.91	88.19
CFZ	100	100	100	100	100
FOX	98.00	100	97.53	97.73	98.31
CTX	96.00	96.77	100	100	98.31
CAZ	100	98.39	98.77	97.73	98.73
FEP	60.00	79.03	85.19	81.82	77.64
CIP	80.00	75.81	79.01	86.36	79.75
LVX	74.00	67.74	76.54	86.36	75.53
GEN	42.00	54.84	48.15	38.64	46.84
AMK	0.00	4.84	8.64	6.82	5.49
SXT	70.00	72.58	65.43	72.73	69.62
COL	2.00	1.61	4.94	4.55	3.38
TGC	6.00	0.00	0.00	2.27	1.69

Abbreviations: ETP, ertapenem; IPM, imipenem; MEM, meropenem; ATM, aztreonam; DOR, doripenem; TZP, piperacillin-tazobactam; CFZ, cefazolin; FOX, cefoxitin; CTX, cefotaxime; CAZ, ceftazidime; FEP, cefepime; CIP, ciprofloxacin; LVX, levofloxacin; GEN, gentamicin; AMK, amikacin; SXT, trimethoprim-sulfamethoxazole; COL, colistin; TGC, tigecycline.

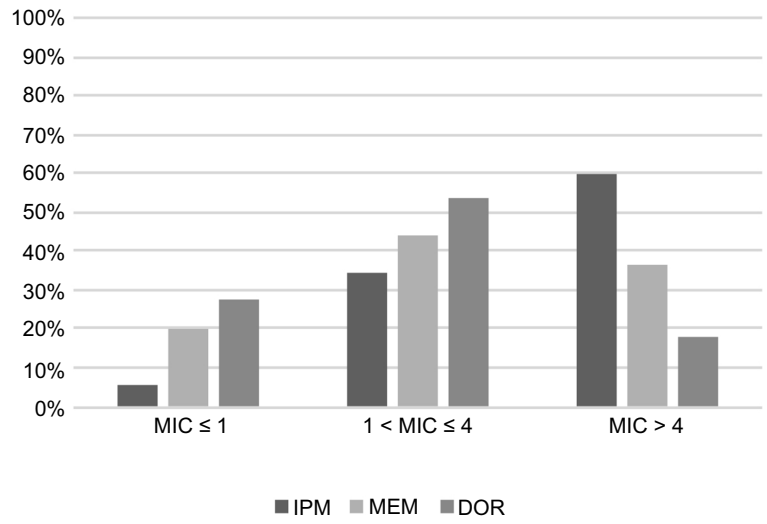

Figure 1 The MIC distribution for three carbapenems for CnsEC isolates in Taiwan.
Abbreviations: IPM, imipenem; MEM, meropenem; DOR, doripenem; MIC, minimal inhibitory concentration; CnsEC, carbapenem-nonsusceptible *E. coli*.

respectively. Amikacin, colistin, and tigecycline were the most effective agents among all antimicrobials. Trends of increasing nonsusceptibility were observed for amikacin (2.69% in 2012–2013 and 8% in 2014–2015) and colistin (1.79% and 4.8% in two consecutive 2-year periods) without statistical significance.

PFGE patterns, MLST profiles, and PCR analyses for carbapenemases

During the study period, eight pulsotypes were found consecutively in three or four of the years. Of the eight pulsotypes, seven corresponded to a specific ST, and four of these belonged to ST131. MLST analysis revealed ST131 to be

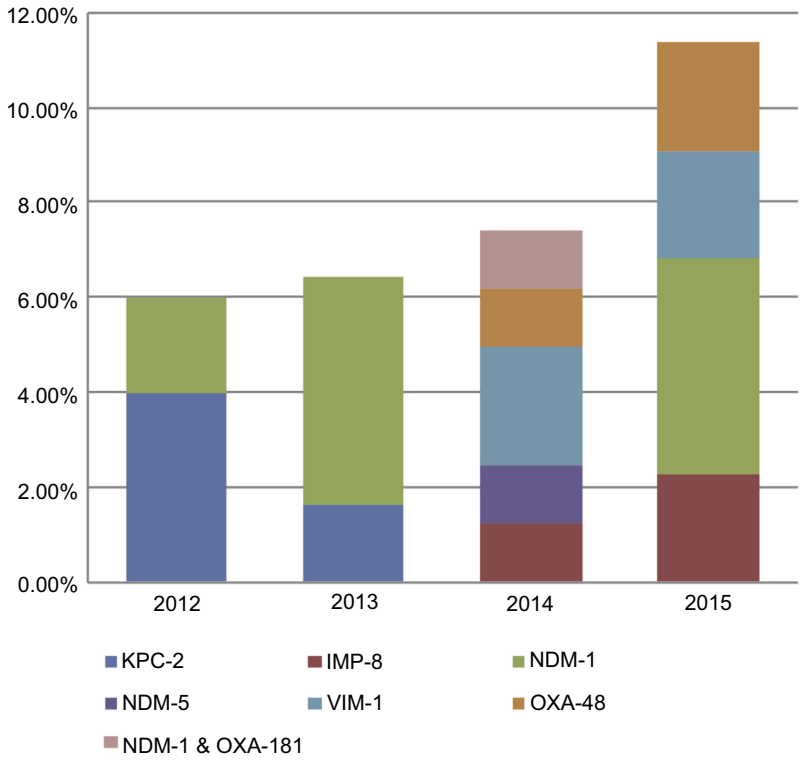

Figure 2 Distribution of carbapenemases among carbapenem-nonsusceptible *E. coli* isolates in Taiwan.

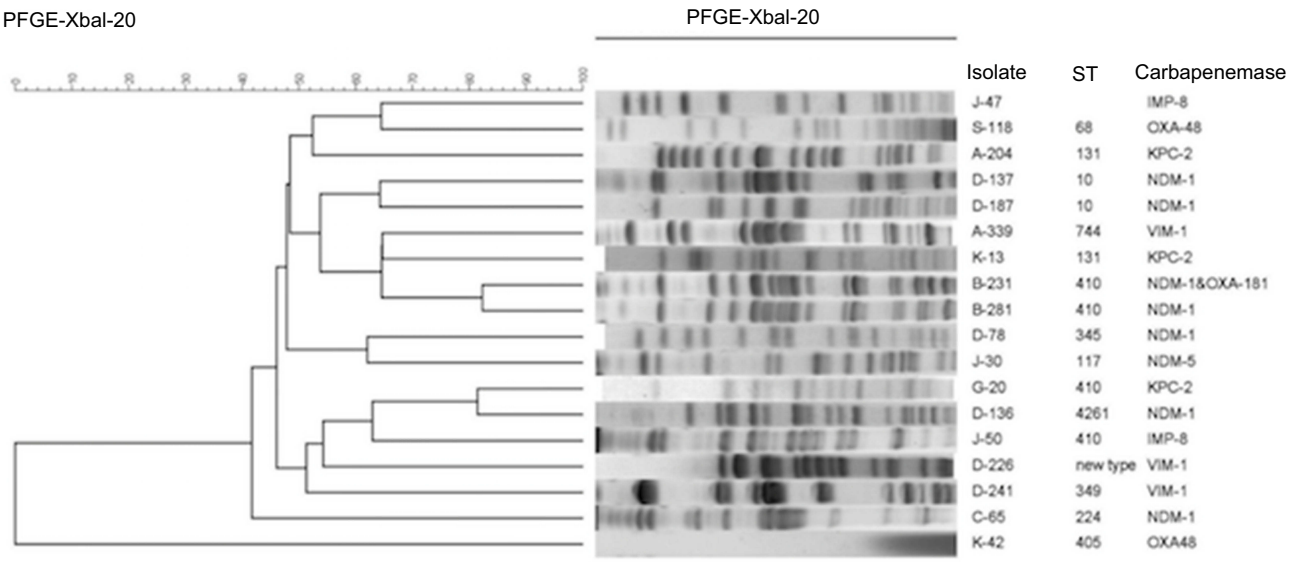

Figure 3 Dendrogram generated by pulsed-field gel electrophoresis (PFGE) patterns of the 18 isolates of carbapenemase-producing carbapenem-nonsusceptible *E. coli* using the BioNumerics software.

the most prevalent ST, accounting for 29.2% of all isolates, followed by ST410 (13 isolates, 5.6%) and ST457 (13 isolates, 5.6%). A total of 11, 10, 9, 8, and 6 isolates were designated as ST405, ST2003, ST38, ST68, and ST354, respectively. Moreover, 10 isolates were found to be new STs (4.3%), and the remaining isolates were of diverse STs.

Figure 2 depicts the percentages and distributions of carbapenemases among CnsEC isolates in each year. Increasing prevalence was found with more diverse carbapenemase genes over the 4-year study period. The prevalence of carbapenemases rose from 6% in 2012 to 11.36% in 2015; however, no statistical significance was found. The isolate number of each carbapenemase-producing *E. coli* and the associated MLST types were as follows: three KPC-2 (two ST131 and one ST410), two IMP-8 (ST410 and a new type), seven NDM-1(two ST10, two ST410, and one of each ST224, ST345, ST4261), one NDM-5 (ST117), three VIM-1 (ST349, ST744, and a new ST type), and two OXA-48 (ST68 and ST405) isolates. An ST410 NDM-1-producing isolate also harbored OXA-181. The diverse STs of these carbapenemase-producing *E. coli* isolates indicate the absence of epidemic clones. The PFGE patterns of carbapenemase-producing isolates are shown in Figure 3. All isolates demonstrated different PFGE patterns.

AmpC, ESBL, and porin loss

86.5% of CnsEC isolates produced AmpC β-lactamase, and most of them (96.1%) carried CMY-2 gene. CMY-4, CYM-42, DHA-1, and CMY-2/DHA-1 co-carriage were detected in one, three, four, and eight isolates, respectively. CTX-M-type ESBL was detected in 103 isolates (43.5%), whereas only 12 isolates harbored SHV genes (5.1%). Among the 219 CnsEC isolates without carbapenemases, the most common pattern of porin deficiency was the loss of both OmpC and OmpF (157/219, 71.7%), followed by the loss of OmpF alone (42/219, 19.2%). The patterns of coexistence of β-lactamases and Omp loss in non-carbapenemases-producing CnsEC were shown in Table 2. For the 18 isolates of carbapenemase-producing *E. coli*, there were coexistence of DHA, CMY, SHV and CTX-M genes in 1, 8, 3, 10 isolates, respectively (data not shown). The prevailing type of porin loss in carbapenemase-harboring isolates was the loss of OmpF only (11/18, 61.1%) (Table 2). Furthermore, in the non-carbapenemase-producing subgroup, resistance rates to seven antimicrobial agents were shown to be significantly related to the loss of OmpC or OmpF (Table 3). In order to evaluate the contribution to resistance by each Omp, a categorical comparison was performed between isolated loss of OmpC or OmpF and concurrent loss of both OMPs. Compared to OmpF, the loss of OmpC was more significantly associated with resistance to all carbapenems (Table 3).

Comparisons between pan-carbapenem-resistant *E. coli* (pCREC) and non-pCREC among the non-carbapenemase producing CnsEC isolates

With the exclusion of 18 carbapenemase-producing *E. coli* isolates, subgroup analyses were performed between pCREC

Table 2 Outer membrane profiles for carbapenem-nonsusceptible *E. coli* isolates

β-lactamases	Outer membrane profile																				
	2012 (n=50)				2013 (n=62)				2014 (n=81)				2015 (n=44)				Total				
	F/C	ΔC	ΔF	ΔC/F	F/C	ΔC	ΔF	ΔC/F	F/C	ΔC	ΔF	ΔC/F	F/C	ΔC	ΔF	ΔC/F	F/C	ΔC	ΔF	ΔC/F	All
Carbapenemase [a]	1	1	1	0	1	1	2	0	0	1	4	1	0	0	4	1	2	3	11	2	18
KPC-2	1	1	0	0	0	0	1	0	0	0	0	0	0	0	0	0	1	1	1	0	3
NDM-1,5	0	0	1	0	1	1	1	0	0	1	1[b]	0	0	0	1	1	1	2	4	1	8
IMP-8	0	0	0	0	0	0	0	0	0	0	0	1	0	0	1	0	0	0	1	1	2
VIM-1	0	0	0	0	0	0	0	0	0	0	2	0	0	0	1	0	0	0	3	0	3
OXA-48	0	0	0	0	0	0	0	0	0	0	1	0	0	0	1	0	0	0	2	0	2
AmpC[c]																					195
CMY	4	7	6	30	0	4	12	36	1	4	12	49	0	0	9	21	4	15	38	136	193
DHA	0	0	0	1	0	0	0	1	0	0	0	0	0	0	0	1	0	0	0	2	2
ESBL[c]																					102
CTX-M	2	1	0	9	0	2	7	17	0	0	7	31	0	0	6	10	2	3	20	67	92
SHV	0	0	1	3	0	0	2	2	0	0	0	0	0	0	0	2	0	0	3	7	10

Notes: [a]For carbapenemase-producing *E. coli* strains, there was no duplicate calculation for each isolate. [b]The isolate harbored both NDM-1 and OXA-181. [c]For the columns that show the numbers of AmpC and ESBL genes, only non-carbabapenemase-producing isolates were included (n=219). Isolates that harbored multiple β-lactamases were duplicately calculated. [d]ΔC, ΔF, ΔC/F represent loss of OmpC, loss of OmpF, and loss of both OmpC and F, respectively.

Table 3 Association of outer membrane proteins loss and antimicrobial resistance rates (%) among the 219 carbapenem-nonsusceptible *E. coli* isolates without carbapenemase

	OmpF+	**OmpF-**		**OmpC+**	**OmpC-**	
	(N=20)	**(N=199)**	***p*-value**	**(N=47)**	**(N=172)**	***p*-value**
EPT	80.00	97.49	0.0002*	89.36	97.67	0.011*
IPM	60.00	73.37	0.2037	46.81	79.07	<0.0001*
MEM	45.00	60.30	0.1849	36.17	65.12	0.0004*
DOR	30.00	44.72	0.2053	17.02	50.58	<0.0001*
ATM	90.00	97.99	0.0369*	97.87	97.09	<0.0001*
FEP	55.00	78.39	0.0191*	74.47	76.74	0.74519
TZP	90.00	86.93	0.6956	78.72	89.53	0.0492*
	OmpF+C-	OmpF-C-		OmpF-C+	OmpF-C-	
	(N=15)	(N=157)	*p*-value	(N=42)	(N=157)	*p*-value
EPT	86.67	99.36	0.0203*	88.10	99.36	0.0017*
IPM	66.67	80.25	0.2164	50.00	80.25	<0.0001*
MEM	46.67	68.15	0.0926	35.71	68.15	0.0001*
DOR	26.67	54.14	0.0574	14.29	54.14	<0.0001*
ATM	93.33	98.73	0.3081	100.00	98.73	1
FEP	46.67	79.61	0.0039*	73.81	79.61	0.4165
TZP	93.33	89.17	1.0000	78.57	89.17	0.0702

Note: *Statistical significance.

(89 isolates) and non-pCREC (130 isolates) in order to compare the differences of resistance mechanisms and patterns. Regarding the presence of ESBL and AmpC genes, Figure 4A demonstrates that SHV, SHV and CTX-M co-carriage, and CMY were more commonly detected in the pCREC group with statistical significance. Figure 4B depicts the resistance rates of the three most effective antibiotics for CnsEC, namely colistin, tigecycline and amikacin. A trend of higher resistance rates to colistin and amikacin were noticed in pCREC isolates, but with no statistical significance. For the other antimicrobial agents, the resistance rates were similar between pCREC and non-pCREC except for piperacillin-tazobactam (89.9% vs 66.9%, $p<0.0001$) and cefepime (97.8% vs 80%, $p<0.0001$). Isolated loss of OmpF was more likely to be found among non-pCREC isolates (28.5% vs 5.6%, $p<0.0001$) while concurrent loss of both OmpF and OmpC was more commonly detected in pCREC isolates (87.6% vs 60.8%, $p<0.0001$) (Figure 4C).

Discussion

The predominant mechanism of CnsEC in Taiwan in 2012–2015 was observed to be AmpC β-lactamase CMY-2, in combination with OmpF and OmpC porin loss, similar to a previous report for 2010 and 2012 isolates in Taiwan.[19] The most prevalent carbapenemase was the NDM type (44.4%), among which NDM-1 type was the most common (7/8). In Taiwan, the first KPC-2- and NDM-1-harboring *E. coli* were identified in 2012.[19] Herein we report the first NDM-5 and OXA-181 in *Enterobacteriaceae* in Taiwan in this surveillance study.

There were 106 (44.7%) isolates of CnsEC to be pCREC. Among the 18 carbapenemase-producing *E. coli*, 17 isolates were pCREC except one that harbored OXA-48 type carbapenemase. The result is compatible with previous reports that OXA-48 producers are more frequent to be susceptible to carbapenems.[20] Aside from carbapenemase, the major resistance mechanisms that contributed to CnsEC were the CMY-type AmpC β-lactamase and the concurrent porin loss of OmpF and OmpC (Table 2).

We summarize the prevalence rates and predominant resistance mechanisms of CnsEC reported in recent literatures in Table 4.[7–9,21,22] Compared with the global data, the carbapenemase prevalence of CnsEC in Taiwan was relatively low (Table 4). The predominant type of carbapenemase in CnsEC varies in different areas, with NDM being the major type detected in both China and Taiwan.[7,22] Several novel β-lactam/β-lactamase inhibitor combinations, such as ceftazidime/avibactam, meropenem/vaborbactam and imipenem/relebactam, have been developed to treat multidrug-resistant organisms but all

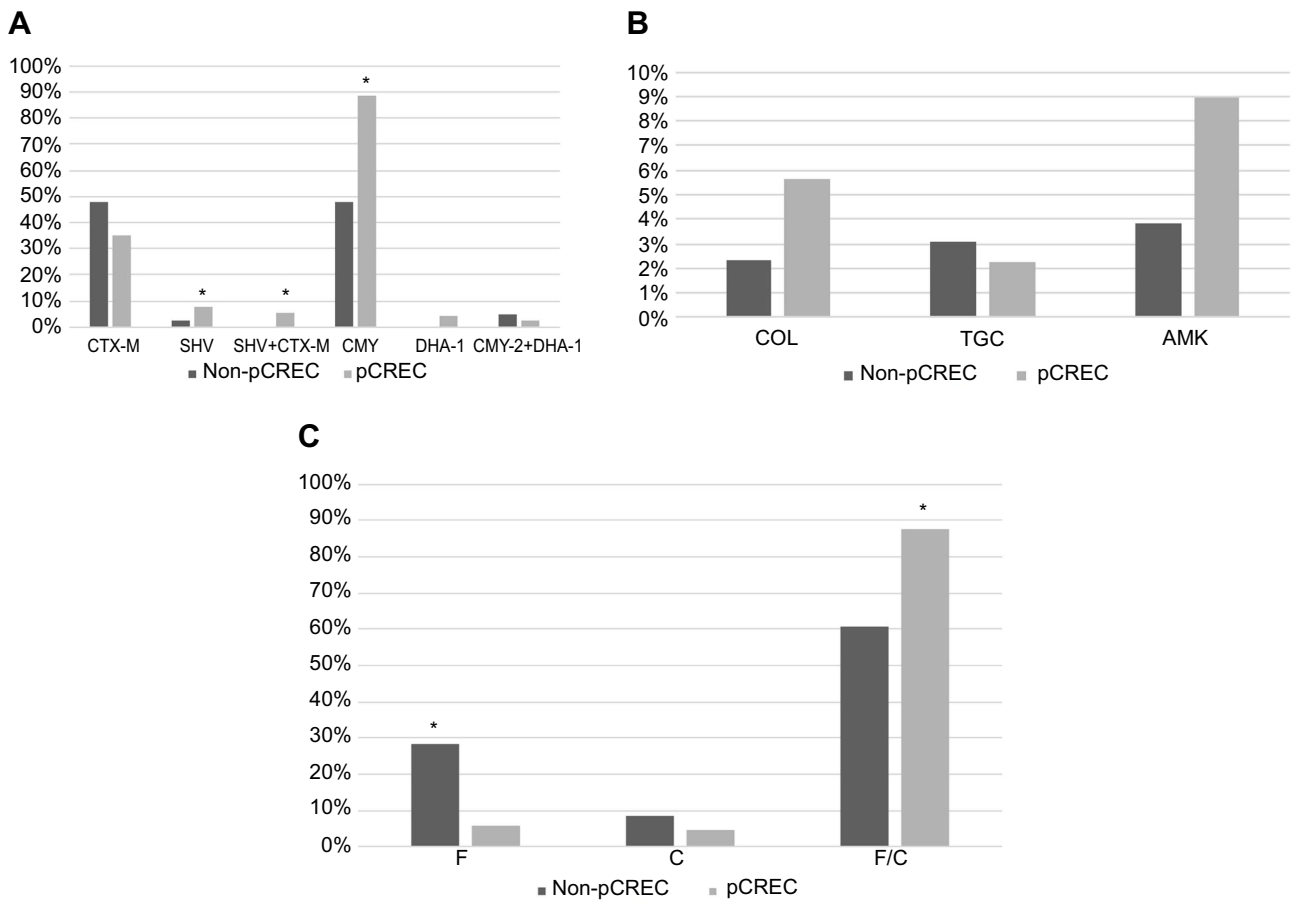

Figure 4 Comparisons of resistance mechanisms and antibiotics resistance rates between pan-carbapenem-resistant *E. coli* (pCREC) and non-pCREC. The 18 isolates of carbapenemase-producing *E. coli* are excluded. *Statistical significance. (**A**) The distribution of ESBL and AmpC genes. (**B**) The resistance rates of COL (colistin), TGC (tigecycline), and AMK (amikacin). (**C**) The percentages of outer membrane protein (Omp) loss: including the loss of only one Omp (OmpF or OmpC) and the loss of both OmpF and OmpC.

have limited activity against metallo-β-lactamases (MBL) producers (NDM or VIM).[23,24] These new antibiotics are expected to have a potential role for CnsEC treatment in Taiwan due to the low rate of carbapenemase production. However, NDM-1 as the major carbapenemase in carbapenemase-producing *E. coli* in Taiwan necessitates continuous monitoring of the molecular epidemiology. In addition, it is worth mentioning that different from *E. coli*, the predominant resistance gene of carbapenemase-producing *K. pneumoniae* (CPKP) in Taiwan during the same study period was KPC.[13,25]

The most commonly detected AmpC β-lactamase, ESBL type, and ST for CnsEC in Taiwan were CMY-2 (91.75%), CTX-M-type ESBLs (46.41%), and ST131 (29.18%), respectively. *E. coli* ST131 is a vehicle for the global dissemination of fluoroquinolone resistance and $bla_{\text{CTX-M-15}}$ among extraintestinal pathogenic *E. coli*. NDM, KPC, VIM, IMP, OXA-48 have all been identified in ST131 globally.[11] In our surveillance, we observed two ST131 strains among the carbapenemase-producing *E. coli*, and both harbored KPC-2 genes. The high prevalence of either nosocomial or community-onset ESBL-producing *E. coli* is a well-known issue in Taiwan, as in many Asia-Pacific countries.[26,27] The emergence of community-onset CRE infections worldwide, with reported prevalence rates of 0.04–29.5%,[28] further complicates the empiric treatment of community-onset *Enterobacteriaceae* infections. Continuous surveillance of carbapenemase-producing *E. coli* and epidemic clone such as ST131 is warranted in order to develop instantaneous containment strategies.

Amikacin, colistin, and tigecycline were the most effective antimicrobial agents for CnsEC in Taiwan. One review article reported that the treatment efficacy of carbapenems decreases from 69% for CPKP isolates with a MIC ≤4 mg/liter to 29% for isolates with MICs >8 mg/liter.[20] Some cohort studies

Table 4 The prevalence rates and genotypes of carbapenemase-producing *E. coli* among carbapenem-nonsusceptible *E. coli* isolates

Study period	Geographical area	Carbapenemase-producing *E. coli* (number of isolates)[a]	Genotypes of carbapenemases (number of isolates)	Reference
2008–2014	Global[b]	35.8% (149/408)	NDM (57) OXA-48-like (46) KPC (42) IMP (3), VIM (2)	9
2012–2013	USA	21.9% (7/32) [c]	KPC (7)	7
2013–2014	Europe[d]	40% (77/194)	OXA-48-like enzymes (43) NDM (20), KPC (14)	21
2012–2015	Taiwan	7.59% (18/237)	NDM-type (8) KPC-2 (3), VIM-1 (3) OXA-48 (2), IMP-8 (2)	Present study
2015	Korea	8.33% (3/36)	OXA-232 (1) KPC-2 (1) NDM-1 (1)	8
2015	China[e]	76.9%(30/39)[f]	NDM-type (29)	22

Notes: [a]The numbers represent: carbapenemase-producing *E. coli* isolates/carbapenem-nonsusceptible *E. coli* isolates. [b]The Study for Monitoring Antimicrobial Resistance Trends (SMART) global surveillance program: The data from Asian area excluded isolates from mainland China (all years) and India (most isolates from 2010 and all isolates from 2011 to 2014). [c]Among 89 isolates of carbapenemase-nonsusceptible *E. coli*, only 32 isolates were tested for carbapenemase. [d]The European Survey on Carbapenemase-producing Enterobacteriaceae (EuSCAPE): 455 sites from 36 countries. [e]China CRE Network. [f] Among 110 isolates of carbapenemase-nonsusceptible *E. coli*, only 39 isolates were tested for carbapenemase.

found that adding high dose meropenem (2g every 8 hrs by extended infusion) to another active drug was associated with lower mortality among patients with bloodstream infection caused by CPE with MIC≤8 mg/liter.[29,30] However, all the aforementioned studies contained predominantly KPC-producing *K. pneumoniae* and whether the "high-dose-extended-infusion meropenem" strategy also applies for other *Enterobacteriaceae* requires more research. According to our result, only 36.3% CnsEC isolates' MIC for meropenem is >4 mg/L. High-dose-extended-infusion meropenem for CnsEC might be applicable for some CnsEC infections in Taiwan, especially for infection sources not appropriate for tigecycline combination therapy (urinary tract infection or bloodstream infection) or for isolates with lower meropenem MIC.

Conclusion

The predominant mechanism of carbapenem nonsusceptibility in *E. coli* isolates in Taiwan was CMY-2-type AmpC β-lactamase in combination with OmpF and OmpC porin loss. The most common type of carbapenemase was NDM. About 45% of the CnsEC were resistant to all carbapenems-(pCREC). Besides carbapenemase, the major differences in resistant mechanisms between pCREC and non-pCREC were the significantly higher percentage of SHV and/or CMY and concurrent loss of both OmpF and OmpC in pCREC. Diverse PFGE patterns and ST analysis suggested that no nationally spread clones identified.

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