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Original Article

# Predominance of methicillin-resistant *Staphylococcus aureus* in the residents and environments of long-term care facilities in Taiwan



Chia-Ying Liu <sup>a,p</sup>, Chih-Cheng Lai <sup>b,p</sup>, Hsiu-Tzy Chiang <sup>c</sup>,  
Min-Chi Lu <sup>d</sup>, Ling-Fang Wang <sup>e</sup>, Tsai-Ling Tsai <sup>f</sup>, Mei-Yu Kang <sup>g</sup>,  
Yi-Ni Jan <sup>h</sup>, Yi-Ting Lo <sup>i</sup>, Wen-Chien Ko <sup>j</sup>, Shu-Hui Tseng <sup>n</sup>,  
Chun-Ming Lee <sup>c,l,m,\*\*</sup>, Po-Ren Hsueh <sup>n,o,k,\*</sup>, Infection Control  
Society of Taiwan

<sup>a</sup> Department of Internal Medicine, Far Eastern Memorial Hospital, New Taipei City, Taiwan

<sup>b</sup> Department of Intensive Care Medicine, Chi Mei Medical Center, Liouying, Tainan, Taiwan

<sup>c</sup> Infection Control Center, MacKay Memorial Hospital, Taipei, Taiwan

<sup>d</sup> Department of Internal Medicine, Chung Shan Medical University, Taichung, Taiwan

<sup>e</sup> Yongen Nursing Home, Taichung, Taiwan

<sup>f</sup> Lukang Christian Nursing Home, Lukang, Taiwan

<sup>g</sup> Changhua Christian Hospital Erlin Nursing Home, Changhua, Taiwan

<sup>h</sup> Thanksgiving Nursing Home, Taichung, Taiwan

<sup>i</sup> Feng-Fung Nursing Home, Taichung, Taiwan

<sup>j</sup> Department of Internal Medicine, National Cheng Kung University Hospital, Medical College, Tainan, Taiwan

<sup>k</sup> Centers for Disease Control, Ministry of Health and Welfare, Taiwan

<sup>l</sup> Department of Internal Medicine, St. Joseph's Hospital, Yunlin County, Taiwan

<sup>m</sup> MacKay Junior College of Medicine, Nursing, and Management, Taipei, Taiwan

<sup>n</sup> Department of Laboratory Medicine, National Taiwan University Hospital, National Taiwan University College of Medicine, Taipei, Taiwan

<sup>o</sup> Department of Internal Medicine, National Taiwan University Hospital, National Taiwan University College of Medicine, Taipei, Taiwan

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\* Corresponding author. Departments of Laboratory Medicine and Internal Medicine, National Taiwan University Hospital, National Taiwan University College of Medicine, No. 7 Chung-Shan South Road, Taipei, 100, Taiwan.

\*\* Corresponding author. Department of Internal Medicine, St. Joseph's Hospital, Yunlin County, Taiwan.

E-mail addresses: [leecm4014@yahoo.com.tw](mailto:leecm4014@yahoo.com.tw) (C.-M. Lee), [hsporen@ntu.edu.tw](mailto:hsporen@ntu.edu.tw) (P.-R. Hsueh).

<sup>p</sup> These authors contributed equally to this work.

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**KEYWORDS**

Multidrug-resistant organisms;  
 Long-term care facility;  
 Methicillin-resistant *Staphylococcus aureus*;  
 Sequence type

**Abstract** *Background/purpose:* This study investigated the distribution and persistence of multidrug resistant organisms (MDROs) including methicillin-resistant *Staphylococcus aureus* (MRSA), carbapenem-resistant Enterobacteriaceae (CRE), carbapenem-resistant *Pseudomonas aeruginosa* (CRPA), and multidrug-resistant *Acinetobacter baumannii* (MDRAB) in six long-term care facilities (LTCFs).

*Methods:* We investigated the distribution of MDROs in residents of six LTCFs and their environments from January to December 2016 (intervention period). Active surveillance of colonization of MDROs was performed by culturing rectal and nasal swab samples from the residents every three months. Multilocus sequence typing (MLST) was conducted, and genes for panton-valentine leukocidin (PVL) from MRSA isolates were determined.

*Results:* A total of 521 samples were positive for MDROs, and MRSA was the most common organism (65.1%), followed by MDRAB (11.3%), carbapenem-resistant *Klebsiella pneumoniae* (11.1%), carbapenem-resistant *Escherichia coli* (4.6%), and carbapenem-resistant *P. aeruginosa* (2.1%,  $n = 11$ ). By a linear regression model, positive MRSA isolates from the environment were found to be statistically significant and associated with the number of colonized LTCF residents ( $p = 0.01$ ), while the timing of the surveillance culture was not ( $p = 0.227$ ). The main MLST types associated with PVL-production were sequence type (ST) 59, (40.0%, 24/60), ST30 (21.4%, 3/14), ST8 (87.5%, 14/16), and ST45 (3.6%, 1/28). The susceptibility rates of tetracycline (96.7%), trimethoprim-sulfamethoxazole (96.7%), and ciprofloxacin (81.7%) were statistically significant and higher in MRSA ST59, compared to the rates in MRSA ST45 isolates.

*Conclusions:* MRSA was the most commonly colonized MDRO, both in the LTCF residents and in the environment, followed by MDRAB and carbapenem-resistant *K. pneumoniae*.

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**Introduction**

Infection or colonization with multidrug-resistant organisms (MDROs) have become public health threats for the residents of long-term care facilities (LTCFs).<sup>1–14</sup> Most of the previous investigations<sup>3,5,10,12,14–16</sup> focused on methicillin-resistant *Staphylococcus aureus* (MRSA). However, an increasing amount of reports<sup>6,9,11,13,17</sup> have shown the emergence of multidrug-resistant Gram-negative bacillus (MDRGNB) such as multidrug-resistant *Acinetobacter baumannii* (MDRAB), extended spectrum beta-lactamase (ESBL)-producing Enterobacteriaceae, carbapenem-resistant Enterobacteriaceae (CRE) and carbapenem-resistant *Pseudomonas aeruginosa* (CRPA) in LTCFs. However, the microbiological distribution and characteristics of MDROs may be different in different regions and LTCFs.<sup>6</sup> Therefore, every site should conduct its own surveillance to establish epidemiologic data for further intervention to eradicate these life-threatening MDROs.

In this prospective study, we investigated the distribution and persistence of MDROs including MRSA, CRE, CRPA, and MDRAB in six LTCFs in northern and southern Taiwan. Once the presence of MDROs was documented in the LTCFs, several infection control measures were implemented. Infection densities of these LTCFs before and during intervention were also evaluated.

**Materials and methods****Study design, setting, and participants**

This prospective study was conducted from January to December 2016 and included six LTCFs (A to F) located in

northern ( $n = 1$ , LTCF-A) and southern ( $n = 5$ , LTCF-B to -F) Taiwan. The total bed number of the six LTCFs was 491 and ranged from 32 (LTCF-B) to 152 beds (LTCF-C) (Table 1). A total of 200 residents at the same areas or floors of the six LTCFs were initially designated and screened for enrollment in this study (Table 1). Characteristics of each facility and clinical data of the residents designated for participating in this study were collected and included age, gender, daily activities, underlying conditions, and devices used. The study protocol was approved by the Institutional Review Boards of the Chung Shan Medical University Hospital (CS15022), National Taiwan University Hospital (201502026RINB), and Mackay Memorial Hospital (15MMHI-SO016e), and written informed consent was obtained from each enrolled resident of the participating LTCFs.

**Infection control interventions**

During the study period (January to December 2016, intervention period), several infection control measures were implemented. Adenosine triphosphate (ATP) testing of various environments was periodically conducted before and after cleaning to determine the degree of sterility of the facilities and whether a disinfection education program was needed. We also instituted an education program regarding MDRO control for healthcare workers and then periodically evaluated the effectiveness of the program. Monitoring of hand-hygiene adherence and actively increasing contact-precautions to interrupt transmission including hand washing and the use of disposable gloves and gowns were also conducted. Regular meetings between infection control experts and representatives of the six

**Table 1** Characteristics of the 200 residents in six long-term care facilities (LTCFs).

Variable	No. (%) of residents in each LTCF						Total
	A	B	C	D	E	F	
Bed no., total	110	32	152	95	53	49	491
No. of residents initially designated (screened) for this study	9	32	68	30	37	24	200
Gender and age							
Male (%)	8 (88.9)	17 (53.1)	29 (42.6)	17 (56.7)	13 (35.1)	9 (37.5)	93 (46.5)
Age range, year	58–96	48–100	64–106	55–97	49–99	64–96	49–106
Activity of daily living							
Fully dependent	9 (100.0)	13 (40.6)	53 (77.9)	27 (90.0)	26 (70.3)	17 (70.8)	145 (72.5)
Partially dependent	0 (0.0)	16 (50.0)	12 (17.6)	3 (10.0)	11 (29.7)	7 (29.2)	49 (24.5)
Underlying condition							
Hypertension	1 (11.1)	23 (71.9)	36 (52.9)	3 (10.0)	21 (56.8)	13 (54.2)	97 (48.5)
Diabetes mellitus	6 (66.7)	4 (12.5)	19 (27.9)	4 (13.3)	15 (40.5)	4 (16.7)	52 (26.0)
Dementia	4 (44.4)	14 (17.0)	17 (25.0)	2 (6.7)	10 (27.0)	9 (37.5)	56 (28.0)
Heart failure	4 (44.4)	2 (6.3)	11 (16.2)	1 (3.3)	2 (5.4)	4 (16.7)	24 (12.0)
Asthma	0 (0.0)	0 (0.0)	1 (1.5)	0 (0.0)	0 (0.0)	0 (0.0)	1 (0.5)
Device							
Nasogastric tube	9 (100.0)	10 (31.3)	16 (23.5)	17 (56.7)	26 (70.3)	13 (54.2)	91 (45.5)
Foley catheter	6 (66.7)	5 (15.6)	11 (16.2)	5 (16.7)	7 (18.9)	12 (50.0)	46 (23.0)
Tracheostomy tube	6 (66.7)	0 (0.0)	0 (0.0)	8 (26.7)	0 (0.0)	0 (0.0)	14 (7.0)
Cystostomy	0 (0.0)	0 (0.0)	0 (0.0)	1 (3.3)	0 (0.0)	0 (0.0)	1 (0.5)
Number of devices							
No device	0 (0.0)	19 (59.4)	47 (69.1)	12 (40.0)	11 (29.7)	6 (25.0)	95 (47.5)
One	1 (1.1)	11 (34.4)	15 (22.1)	7 (23.3)	19 (51.4)	11 (45.8)	64 (32.0)
Two	4 (44.4)	2 (96.3)	6 (8.8)	9 (30.0)	7 (18.9)	6 (25.0)	34 (17.0)
Three	4 (44.4)	0 (0.0)	0 (0.0)	2 (6.7)	0 (0.0)	4 (4.2)	7 (3.5)

LTCFs were conducted every three months to monitor the progress of the intervention programs. Not all of the above intervention measures were conducted at six LTCFs during the pre-intervention period. We also calculated the number of episodes of infection per 1000 patient-days in each LTCF in the pre-intervention period (January–December 2015) and the intervention period.

### MDRO surveillance and environmental sampling

After the informed consent was obtained, we collected rectal and nasal surveillance cultures for MRDOs. The surveillance cultures were conducted every three months by infection control nurses. If cultures were positive for MDROs including MRSA, CRE, CRPA, or MDRAB, then swab specimens from environmental sources (including beds, bedside tables, toilet door handles, ward door handles, and curtains) were also collected and cultured. Swab samples from all sources were inoculated onto Trypticase soy agar plates

supplemented with 5% sheep blood (BAP, Becton Dickinson Microbiology Systems, Sparks, MD, USA). All inoculated plates were sent to the Microbiology Laboratory at National Taiwan University Hospital (NTUH) on the same day for bacterial cultures, antimicrobial susceptibility testing, and molecular typing.

### Bacterial identification and antimicrobial susceptibility testing

The inoculated plates were incubated at 35 °C in a 5% CO<sub>2</sub> atmosphere for 18–24 h. Single colonies that grew on the initial or subcultured blood-agar plates were identified at the species level using the Bruker Biotyper matrix-assisted laser desorption/ionization time-of-flight mass spectrometry system (MALDI-TOF MS Bruker Biotyper) (Bruker Daltonics GmbH, Bremen, Germany). Antimicrobial susceptibilities were determined by the Phoenix PMIC/ID-62 and PMIC/ID-72 systems (Becton Dickinson Systems, Sparks,

MD, USA), as previously reported.<sup>11,12</sup> The minimum inhibitory concentration (MIC) values of the antibiotics tested against the collected isolates were interpreted according to the Clinical and Laboratory Standards Institute guidelines.<sup>13</sup> A carbapenem-resistant isolate was defined as an isolate resistant to imipenem, meropenem, or ertapenem. An MDR isolate was defined as an isolate resistant to at least three of the following antibiotics: ceftazidime, piperacillin-tazobactam, cefepime, ciprofloxacin, levofloxacin, imipenem, meropenem, gentamicin, or amikacin.

## Molecular typing

The genetic relationships of the MRSA isolates were determined by multilocus sequence typing (MLST) as described in our previous study.<sup>18</sup> Seven housekeeping genes of *S. aureus* were used in MLST. These genes including carbamate kinase (*arcC*), shikimate dehydrogenase (*aroE*), glycerol kinase (*glpF*), guanylate kinase (*gmk*), phosphate acetyltransferase (*pta*), triosephosphate isomerase (*tpi*) and acetyl coenzyme A acetyltransferase (*yqiL*) were analyzed and typed based on the MLST database. MLST types of selected isolates was used to determine if clonal expansion developed in a single LTCF or across LTCFs. The sequences of *SCCmec* gene elements, including *mecA* and *ccr* genes were analyzed to determine the *SCCmec* typing of these selected isolates as well. The gene for panton-valentine leukocidin (PVL) of the isolates was also determined.

## Statistical analysis

Categorical variables are presented as counts and institutes. Differences in infection density in each LTCF and in all six LTCFs between the pre-intervention and intervention periods were evaluated using the Pearson Chi-square test. The surveillance spots were treated as categorical time-dependent variables. The associations between MRSA isolate counts and other independent variables were evaluated using linear regression models. A *p* value <0.05 was considered to represent statistical significance. All statistical analyses were conducted using the statistical package STATA for Windows (Version 12.1, STATA, College Station, TX, USA).

## Results

### Demographic characteristics of residents

The demographics and characteristics of the six LTCFs are shown in Table 1. Among 200 residents, females were predominant (*n* = 107, 53.5%), and their ages ranged from 49 to 106 years. Regarding daily activity, 72.5% of them were totally dependent, and 24.5% were partially dependent. Hypertension was the most common underlying disease (48.5%), followed by dementia (28.0%) and diabetes mellitus (26.0%). A nasogastric tube was the most common device (*n* = 91, 45.5%), followed by a Foley catheter (*n* = 46, 23.0%). Only 95 (47.5%) residents had no in situ device.

### Infection types and density before and after intervention

Prior to intervention, the overall infection density was 2.37 per 1000 patient-days; respiratory tract and urinary tract infections were the two most common infection types, with infection densities of 1.12 and 0.86 per 1000 patient-days, respectively. After intervention, the overall infection density increased to 3.59 per 1000 patient-days, and similar trends were observed for the two most common infection types, with infection densities of 1.57 per 1000 patient-days for respiratory tract infections and 1.32 per 1000 patient-days for urinary tract infections, respectively. The infection density increased in all LTCFs after the intervention (Fig. 1).

### Distribution of MDROs

A total of 521 samples were positive for MDROs, and MRSA was the most common organism (*n* = 339, 65.1%) (Table 2). In addition, a total of 182 samples from residents and environment sources were positive for MDRGNB. MDRAB was the most common organism (*n* = 59, 11.3%), followed by carbapenem-resistant *Klebsiella pneumoniae* (*n* = 58, 11.1%), carbapenem-resistant *Escherichia coli* (*n* = 27, 4.6%), and carbapenem-resistant *P. aeruginosa* (*n* = 11, 2.1%). Among 339 MRSA isolates, 133 specimens (39.2%) were identified from LTCF residents, and 206 specimens (60.8%) were identified from the environment. The distribution of MRSA varied markedly between different LTCFs (Table 3). In LTCF C, a total of 49 MRSA isolates were identified from the residents, including 20, 14, 4, and 11 isolates from four surveillance spots. In contrast, only 2 to 3 residents were colonized with MRSA in LTCF B in each surveillance spot. By linear regression models, positive MRSA isolates from the environment were found to be statistically significantly associated with the number of colonized LTCF residents (*p* = 0.01), while the timing of the surveillance culture was not (*p* = 0.227). Therefore, the MRSA isolates from the LTCF residents did not significantly change during the study period.

For 59 MDRAB isolates, 28 (47.5%) and 31 (52.5%) isolates were obtained from resident and environment specimens, respectively. In contrast, 84.5% (49/58) and 71.9% (23/32) of carbapenem-resistant *K. pneumoniae* and *E. coli* isolates, respectively, were grown from resident specimens. Only 15.5% (9/58) and 28.1% (9/32) of carbapenem-resistant *K. pneumoniae* and *E. coli* isolates, respectively, were grown from environmental specimens.

### Antimicrobial susceptibility patterns

The susceptibility results for 333 isolates of MRSA, 59 isolates of MDRAB, 58 isolates of carbapenem-resistant *K. pneumoniae*, and 27 isolates of carbapenem-resistant *E. coli* are shown in Table 4. For MRSA, all isolates were susceptible to vancomycin, nitrofurantoin, quinupristin-dalfopristin, linezolid, mupirocin, and teicoplanin, and a majority of the MRSA isolates were susceptible to daptomycin (99.7%), rifampin (97.9%), and fucidic acid (96.8%). In contrast, trimethoprim-sulfamethoxazole, tetracycline,

ciprofloxacin, and clindamycin were only moderately effective against the MRSA isolates, exhibiting susceptibility percentages of 77.6%, 68.6%, 56.8%, and 54.8%, respectively. Erythromycin and gentamycin exerted very limited in vitro activities. All beta-lactam agents were inactive against these 339 MRSA isolates, both from the LTCF residents or the environments (see Table 5).

For the 59 isolates of MDRAB, only colistin showed good in vitro activity with a susceptibility rate of 100% and MIC  $\leq 1$  mg/L. For ampicillin-sulbactam, the MIC<sub>50</sub> and MIC<sub>90</sub> values were 8 mg/L and 16 mg/L, respectively, and the overall susceptibility rate was 52.5%. For meropenem and imipenem, the MIC<sub>50</sub> and MIC<sub>90</sub> values were  $>8$  mg/L, and the overall non-susceptibility rates against both agents were greater than 60%. For amikacin, the MIC<sub>50</sub> and MIC<sub>90</sub> values were  $>8$  mg/L, and the overall non-susceptibility rate was 66.1%.

For the 58 isolates of CRKP, meropenem showed good in vitro activity, the MIC<sub>50</sub> and MIC<sub>90</sub> values were  $\leq 1$  mg/L, and the overall susceptibility rate was 94.8%. For amikacin, the overall susceptibility rate was 81.0%. For cefepime, the MIC<sub>50</sub> and MIC<sub>90</sub> values were  $\leq 2$  mg/L, and the overall non-susceptibility rate was 41.4%.

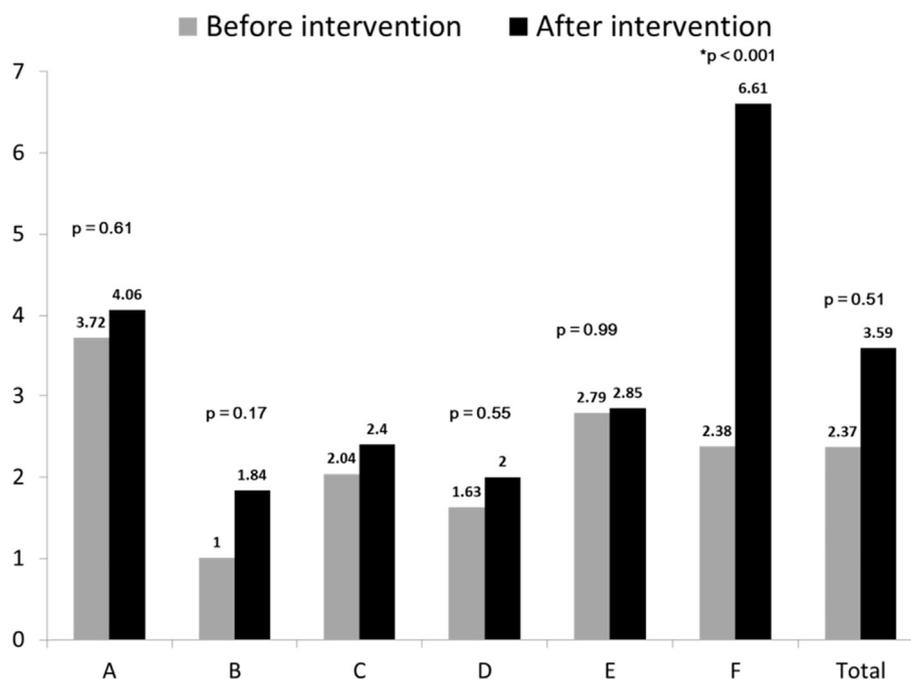
For the 27 isolates of CRKP, amikacin showed good in vitro activity, the MIC<sub>50</sub> and MIC<sub>90</sub> values were  $\leq 8$  mg/L, and the overall susceptibility rate was 100.0%. For ertapenem, meropenem, and imipenem, the overall susceptibility rates were 77.8%, 85.2%, and 14.8%, respectively. For cefepime, the MIC<sub>50</sub> and MIC<sub>90</sub> values were  $\leq 2$  mg/L, and the overall non-susceptibility rate was 92.6%. Colistin exhibited good in vitro activities against all isolates of CRKP and CREC, and all MIC values were  $\leq 1$  mg/L.

**Table 2** Distribution of 521 multidrug-resistant organisms collected from residents and environmental sources in six long-term care facilities.

Organism	No. (%) of isolates (n = 521)
MRSA	339 (65.1)
Multidrug-resistant <i>A. baumannii</i>	59 (11.3)
Carbapenem-resistant <i>Enterobacteriaceae</i>	115 (22.1)
<i>K. pneumoniae</i>	58 (11.1)
<i>E. coli</i>	27 (4.6)
<i>Providencia stuartii</i>	8 (1.5)
<i>E. aerogenes</i>	4 (0.8)
<i>S. cloacae</i>	4 (0.8)
<i>S. marcescens</i>	4 (0.8)
<i>M. morgani</i>	3 (0.6)
<i>P. mirabilis</i>	2 (0.4)
<i>Providencia rettgeri</i>	2 (0.4)
<i>C. freund</i>	1 (0.2)
<i>C. koseri</i>	1 (0.2)
<i>P. vulgaris</i>	1 (0.2)
Carbapenem-resistant <i>P. aeruginosa</i>	11 (2.1)

## Molecular investigation

The SCCmec genotypes and PVL toxin-encoding gene of 339 isolates of MRSA are shown in Table 4. MLST of 124 isolates of MRSA collected from LTCF residents and 9 selected isolates of MRSA collected from the environment are also



**Figure 1.** Infection density (episodes/per 1000 patient-days) among residents of six long-term care facilities (LTCFs) in the pre-intervention period (January–December 2015) and the intervention period (January–December 2016). Differences in infection density in each LTCF and all six LTCFs between the pre-intervention and intervention periods were evaluated using the Pearson chi-square test. \*A p value  $< 0.05$  was considered to represent a statistical significance.

**Table 3** Distribution of 339 isolates of methicillin-resistant *Staphylococcus aureus* collected from residents and environmental sources in six long-term care facilities (LTCF).

Facility	Surveillance	Sampling targets	No. of samplings	Facility	Surveillance	Sampling targets	No. of samplings		
A	1st	Residents	2	D	1st	Residents	7		
		Environment	5			Environment	35		
	2nd	Residents	2		2nd	Residents	7		
		Environment	1			Environment	10		
	3rd	Residents	3		3rd	Residents	4		
		Environment	3			Environment	27		
	4th	Residents	3		4th	Residents	5		
		Environment	2			Environment	5		
	B	1st	Residents		2	E	1st	Residents	10
			Environment		2			Environment	28
		2nd	Residents		3		2nd	Residents	8
			Environment		0			Environment	5
3rd		Residents	2	3rd	Residents		6		
		Environment	0		Environment		0		
4th		Residents	2	4th	Residents		3		
		Environment	0		Environment		5		
C		1st	Residents	20	F		1st	Residents	5
			Environment	15				Environment	6
		2nd	Residents	14			2nd	Residents	6
			Environment	19				Environment	2
	3rd	Residents	4	3rd		Residents	2		
		Environment	3			Environment	1		
	4th	Residents	11	4th		Residents	2		
		Environment	32			Environment	5		

shown. *SCCmec* III was rarely identified (9 isolates, 2.7%), while only 1 isolate belonged to *SCCmec* II. *SCCmec* type III isolates were sparsely obtained from LTCF A, C, D, and E and were obtained primarily from the residents. Thus, clonal expansion or outbreak of *SCCmec* type III was not observed from these four surveillance spots. *SCCmec* typing disclosed that types IV and V were the predominant types in LTCFs, and they accounted for 32.2% (109 isolates) and 64.9% (220 isolates), respectively. The MRSA isolates were distributed unevenly between different LTCFs. For example, LTCF C was associated with the majority of *SCCmec* type V isolates (96 isolates) and with the second most abundant number of isolates of *SCCmec* type IV (21 isolates). In contrast, in LTCF D, we identified the greatest number of isolates of *SCCmec* IV (51 isolates), but only 47 isolates of *SCCmec* V. Notably, not all of the LTCFs were heavily infected with MRSA. For example, in LTCF B, a total of 11 isolates of MRSA were identified during the study period; *SCCmec* IV and V accounted for 7 and 4 isolates, respectively.

PVL toxin production was identified in 84 isolates of MRSA, and all of them belonged to either *SCCmec* type IV or V. In *SCCmec* type IV isolates, 23.4% (26/109) were PVL toxin-producers, while 26.4% (58/220) of *SCCmec* type V isolates produced PVL toxins. There was no statistically significant difference in the percentage of PVL toxin production between these two different *SCCmec* types.

MLST was conducted in 142 selected isolates of MRSA, including 133 isolates from LTCF residents and 9 isolates from the environment; 29.6% (42/142) of them were PVL

toxin-producers. The primary MLST types associated with PVL production were ST59 (40.0%, 24/60), ST30 (21.4%, 3/14), ST8 (87.5%, 14/16), and ST45 (3.6%, 1/28). None of the ST5, ST239, ST398, ST573, ST610, ST900, and ST965 isolates were PVL producers.

The distribution of MLST types is shown in Fig. 2, including PVL producers and non-producers. ST59 was the predominant MLST type (42.3%, 60 isolates), which was followed by ST45 (19.7%, 28 isolates), ST8 (16 isolates, 11.3%), ST30 (14 isolates, 9.9%), ST573 (9 isolates, 6.3%), and ST239 (4 isolates, 2.8%). Other minor MLST types included ST5 (3 isolates), ST398 (3 isolates), ST900 (3 isolates), ST965 (1 isolate), and ST610 (1 isolate). All 60 ST59 isolates and all 3 ST398 isolates had *SCCmec* type V genes, while most of the ST45 isolates also had *SCCmec* type V genes. In contrast, all of the ST573 isolates (9/9, 100%) and most of the ST8 (15/16, 93.8%) and ST30 (9/14, 64.3%) isolates were *SCCmec* type IV. All 4 ST239 isolates were *SCCmec* type III. The three isolates of ST5 MRSA belonged to *SCCmec* type III, IV, and V. ST59 was the predominant strain in residents living in LTCF C. During each surveillance period, approximately 50%–75% of MRSA isolates from residents belonged to ST59, indicating an endemic status. In other facilities, the MRSA isolates were more heterogeneous without a single predominant endemic ST type.

The in vitro antimicrobial susceptibilities between ST59 and ST45 are shown in Fig. 3. A total of 60 isolates of ST59 and 28 isolates of ST45 were identified. All of these isolates were fully susceptible to vancomycin, teicoplanin, linezolid, fucidic acid, mupirocin, nitrofurantoin, and

**Table 4** Production of panton-valentine leukocidin (PVL) and SCCmec typings of 339 isolates of methicillin-resistant *Staphylococcus aureus* and MLST of 142 selected isolates collected from residents and environmental sources in six long-term care facilities.

Facility	Surveillance	Source of isolates	SCCmec				PVL	MLST											
			II	III	IV	V		ST59	ST45	ST8	ST30	ST573	ST239	ST5	ST398	ST900	ST965	ST610	
A	1st	Residents	1	1		1				1		1							
		Environment			5		3												
	2nd	Residents			2		2				2								
		Environment		1															
	3rd	Residents			1	2	1		2		1								
		Environment			1	2													
	4th	Residents		1		2			2				1						
		Environment			1	1	1												
B	1st	Residents			2		1				2								
		Environment			2		1												
	2nd	Residents			1	2	1	1	1	1									
		Environment																	
	3rd	Residents			1	1	1	1			1								
		Environment																	
	4th	Residents			1	1	1			1									
		Environment																	
C	1st	Residents			3	17	1	15	3				2						
		Environment			3	12	2	6	2	1									
	2nd	Residents		1	2	11		7	2	2		1	1		1				
		Environment			3	16	2												
	3rd	Residents			2	2	3	2			2								
		Environment			2	1	3												
	4th	Residents			2	9	2	6	1				1		2				1
		Environment			4	28	9												
D	1st	Residents	1		4	2	2	1	1		4				1				
		Environment			20	15	9												
	2nd	Residents		1	4	2	2	1			3	2		1					
		Environment			9	1													
	3rd	Residents			3	1	1	1				1	2						
		Environment			10	17	3												
	4th	Residents			1	4	2	2				2	1						
		Environment					5	1											
E	1st	Residents			1	9	5	5	4	1									
		Environment			6	22	3												
	2nd	Residents		1	3	4	3	2	1	1	1			1		1		1	
		Environment			2	3	2												
	3rd	Residents		3	1	2				2		1		1		2			
		Environment																	

F	4th	Residents	3	1	2
		Environment			
	1st	Residents	5	4	5
		Environment	6	4	
	2nd	Residents	6	5	4
		Environment	2	1	2
	3rd	Residents	2	2	2
		Environment	1		
	4th	Residents	2	1	1
		Environment	5		

quinupristin-dalfopristin, though the MIC<sub>50</sub> of linezolid in ST 59 is lower than the MIC<sub>50</sub> in ST45. The antimicrobial susceptibility rates of tetracycline (96.7%), trimethoprim-sulfamethoxazole (96.7%), and ciprofloxacin (81.7%) were statistically significantly higher in ST59, compared to the rates in ST45. In contrast, the susceptibility of erythromycin and clindamycin in ST 59 was statistically significantly lower than ST45. In ST59, the susceptibility rate of gentamicin is 1.7% compared to 10.7% in ST45, though the difference did not reach a statistically significant difference.

### Discussion

In this study, MRSA was the predominant strain in LTCFs, accounting for approximately two-thirds of all isolated organisms. MDRAB and CR-K *pneumoniae* were the two most common MDRGNB. This result is consistent with our previous study of other regions of Taiwan<sup>9</sup>; this study indicated that MRSA is the most prevalent MDRO in LTCFs in Taiwan and suggested that effective infection control measures should be implemented to eradicate MRSA. However, the colonization status varied in six LTCFs. By using the linear regression model, we noted that the number of MRSA-positive cultures from the environment was significantly correlated with the number of colonized LTCF residents. The associations were highly plausible clinically, because MRSA can be transmitted easily through the daily activities of household members, either through person-to-person contact or person-to-surface contact of fomites in the LTCFs. It is possible that LTCFs without prior colonization with MRSA could acquire new MRSA colonization through contact with fomites that were contaminated with MRSA. Effective isolation of MRSA-colonized residents may reduce the likelihood of environment contamination.<sup>19–22</sup>

In this study, the predominant SCCmec types were SCCmec type IV and V, as shown by other prior surveillance in LTCFs in Taiwan.<sup>14</sup> In Hong Kong, a study of 949 residents from LTCFs reported that 2.4% were colonized with SCCmec type IV or V MRSA isolates.<sup>23</sup> In northern Germany, Pfingsten-Würzburg et al. reported that more than 70% of MRSA isolates from nursing home residents belonged to the Barnim strain (ST-22), which was the typical hospital-acquired strain in northern Germany.<sup>10</sup> Although infrequent, we identified 1 isolate of SCCmec type II and 9 isolates of SCCmec type III. However, these 9 isolates were distributed among 4 different LTCFs during different surveillance periods. Therefore, clonal expansion of SCCmec III isolates was not observed in our studies.

PVL toxin production was identified in 84 MRSA isolates, and all of them belonged to either SCCmec type IV or V. For SCCmec type IV isolates, 23.4% (26/109) were PVL toxin producers, while 26.4% (58/220) of SCCmec type V isolates produced PVL toxins. In China, one study reported that 28.6% (74/259) of HA-MRSA isolates harbored PVL genes, and the PVL gene was identified in 60.9% of SCCmec IV isolates and 50.0% of SCCmec V isolates.<sup>24</sup> In the United States, Mody et al. reported that only 9% of MRSA isolates were SCCmec IV isolates, and all of the PVL-positive isolates were SCCmec IV.<sup>25</sup> Another study in Germany reported that 15/197 (7.6%) residents and 6/104 (5.8%) staff members

**Table 5** Antimicrobial susceptibilities of methicillin-resistant *S. aureus* (MRSA), multidrug-resistant (MDR) *A. baumannii*, carbapenem-resistant *K. pneumoniae*, and carbapenem-resistant *E. coli* collected from six long-term care facilities using the Phoenix susceptibility system.

Agent	Minimum inhibitory concentrations (MICs, mg/L)			No. (%) of isolates with indicated susceptibility		
	Range	MIC50	MIC90	Susceptible	Intermediate	Resistant
<b>MRSA (n = 339)</b>						
Ampicillin	0.5–>1	>4	>4			
Oxacillin	0.5–>4	>4	>4			
Cefazolin	≤2–>16	8	>16			
Cefoxitin	8–>16	>16	>16			
Erythromycin	≤0.25–>4	>4	>4	112 (33.0)	0 (0)	227 (67.0)
Clindamycin	≤0.5–>2	≤0.5	>2	125 (54.8)	0 (0)	103 (45.2)
Tetracycline	≤0.5–>8	≤0.5	>8	232 (68.6)	0 (0)	106 (31.4)
Gentamicin	≤2–>8	>8	>8	42 (12.4)	0 (0)	297 (87.6)
Trimethoprim-sulfamethoxazole	≤0.5–>2	≤0.5	≤0.5	263 (77.6)	–	76 (22.4)
Ciprofloxacin	≤0.5–>2	>2	>2	192 (56.8)	0 (0)	146 (43.2)
Vancomycin	≤1–2	≤1	≤1	339 (100)	0 (0)	0 (0)
Teicoplanin	≤1–>2	≤1	≤1	339 (100)	0 (0)	0 (0)
Linezolid	≤1–>2	≤1	2	339 (100)	0 (0)	0 (0)
Fusidic acid	≤1–8	≤1	≤1			
Mupirocin	≤256	≤256	≤256			
Nitrofurantoin	≤16	≤16	≤16	339 (100)	0 (0)	0 (0)
Quinupristin-dalfopristin	≤0.5–>1	≤0.5	≤0.5	339 (100)	0 (0)	0 (0)
Rifampin	≤0.5–>2	≤0.5	≤0.5	331 (97.9)	0 (0)	7 (2.1)
Daptomycin	≤1–>2	≤1	≤1	338 (99.7)	–	1 (0.3)
<b>MDR <i>A. baumannii</i> (n = 59)</b>						
Ampicillin-sulbactam	≤4–>16	8	>16	31 (52.5)	18 (30.5)	10 (16.9)
Piperacillin-tazobactam	≤4–>64	>64	>64	4 (6.8)	9 (15.3)	46 (78)
Cefametazole	16–>32	>32	>32			
Cefotaxime	4–>16	>16	>16	3 (5.1)	6 (10.2)	50 (84.7)
Ceftriaxone	≤4–>32	>32	>32	4 (6.8)	12 (20.3)	43 (72.9)
Cefopodoxime	8–>32	>32	>32			
Ceftazidime	4–>16	>16	>16	9 (15.3)	8 (13.6)	42 (71.2)
Cefepime	8–>16	>16	>16	3 (5.1)	7 (11.9)	49 (83.1)
Aztreonam	8–>16	>16	>16			
Ertapenem	4–>4	>4	>4			
Meropenem	≤1–8	>8	>8	21 (38.2)	3 (5.5)	31 (56.4)
Imipenem	≤1–8	>8	>8	20 (33.9)	1 (1.7)	38 (64.4)
Moxifloxacin	≤1–>4	>4	>4			
Levofloxacin	≤1–>8	>4	>4	9 (15.3)	4 (6.8)	46 (78)
Ciprofloxacin	≤0.5–>2	>2	>2	8 (13.6)	0 (0)	51 (86.4)
Gentamicin	8–>8	>8	>8	0 (0)	4 (6.8)	55 (93.2)
Amikacin	≤8–>32	>32	>32	20 (33.9)	2 (3.4)	37 (62.7)
Trimethoprim-sulfamethoxazole	≤0.5–>2	>2	>2	11 (18.6)	–	48 (81.4)
Colistin	≤1	≤1	≤1	59 (100)	–	0 (0)
<b>Carbapenem-resistant <i>K. pneumoniae</i> (n = 58)</b>						
Ampicillin	>16	>16	>16	0 (0)	0 (0)	58 (100)
Ampicillin-sulbactam	8–>16	>16	>16	1 (1.7)	2 (3.4)	55 (94.8)
Piperacillin-tazobactam	≤4–>64	8	>64	32 (55.2)	5 (8.6)	21 (36.2)
Cefazolin	≤4–>16	>16	>16	2 (3.4)	–	56 (96.6)
Cefmetazole	≤8–>32	>32	>32	1 (1.7)	3 (5.2)	54 (93.1)
Ceftriaxone	≤4–>32	≤4	>32			
Cefopodoxime	≤2–>32	≤2	>32			
Cefotaxime	≤2–>16	16	>16	–	–	36 (32.1)
Ceftazidime	≤0.5–>16	>16	>16	4 (6.9)	4 (6.9)	50 (86.2)
Cefepime	≤2–16	≤2	≤2	34 (58.6)	3 (5.2)	21 (36.2)
Aztreonam	≤2–>16	8	>16	27 (46.6)	8 (13.8)	23 (39.7)
Ertapenem	≤0.5–>4	≤0.5	1	31 (53.4)	21 (36.2)	6 (10.3)

Table 5 (continued)

Agent	Minimum inhibitory concentrations (MICs, mg/L)			No. (%) of isolates with indicated susceptibility		
	Range	MIC50	MIC90	Susceptible	Intermediate	Resistant
Meropenem	≤1–4	≤1	≤1	55 (94.8)	1 (1.7)	2 (3.4)
Imipenem	≤1–4	2	4	2 (3.4)	32 (55.2)	24 (41.4)
Moxifloxacin	≤1–>4	>4	>4			
Levofloxacin (57)	≤1–>4	2	>4	26 (44.8)	3 (5.2)	28 (48.3)
Ciprofloxacin	≤0.5–>2	>2	>2	19 (32.8)	9 (15.5)	30 (51.7)
Gentamicin	≤2–>8	≤2	>8	33 (56.9)	1 (1.7)	24 (41.4)
Amikacin	≤8–>32	≤8	>32	47 (81.0)	1 (1.7)	10 (17.2)
Trimethoprim-sulfamethoxazole	≤0.5–>2	>2	>2	19 (32.8)	–	39 (67.2)
Colistin	≤1–>4	≤1	≤1			
<b>Carbapenem-resistant <i>E. coli</i> (n = 27)</b>						
Ampicillin	≤4–>16	>16	>16	2 (7.4)	0 (0)	25 (92.6)
Ampicillin-sulbactam	≤4–>16	>16	>16	6 (22.2)	5 (18.5)	16 (59.3)
Piperacillin-tazobactam	≤4–>64	8	32	22 (81.5)	3 (11.1)	2 (7.4)
Cefazolin	≤4–>16	>16	>16	6 (22.2)	–	21 (77.8)
Cefmetazole	≤8–>32	>32	>32	9 (33.3)	3 (11.1)	15 (55.6)
Ceftriaxone	≤4–>32	32	>32			
Cefopodoxime	≤2–>32	≤2	≤2			
Cefotaxime	≤2–>16	16	>16			16 (59.3)
Ceftazidime	≤0.5–>16	>16	>16	11 (40.7)	0 (0)	16 (59.3)
Cefepime	≤2–>16	≤2	≤2	25 (92.6)	0 (0)	2 (7.4)
Aztreonam	≤2–>16	8	16	12 (44.4)	2 (7.4)	13 (48.1)
Ertapenem	≤0.5–>4	≤0.5	2	21 (77.8)	2 (7.4)	4 (14.8)
Meropenem	≤1–8	≤1	2	23 (85.2)	3 (11.1)	1 (3.7)
Imipenem	≤1–4	2	2	4 (14.8)	20 (74.1)	3 (11.1)
Moxifloxacin	≤1–>4	>4	>4			
Levofloxacin	≤1–>4	>4	>4	8 (29.6)	0 (0)	19 (70.4)
Ciprofloxacin	≤0.5–>2	>2	>2	7 (25.9)	1 (3.7)	19 (70.4)
Gentamicin	≤2–>8	≤2	>8	18 (66.7)	0 (0)	9 (33.3)
Amikacin	≤8	≤8	≤8	27 (100)	0 (0)	0 (0)
Trimethoprim-sulfamethoxazole	≤0.5–>2	1	>2	18 (66.7)	–	9 (33.3)
Colistin	≤1	≤1	≤1			

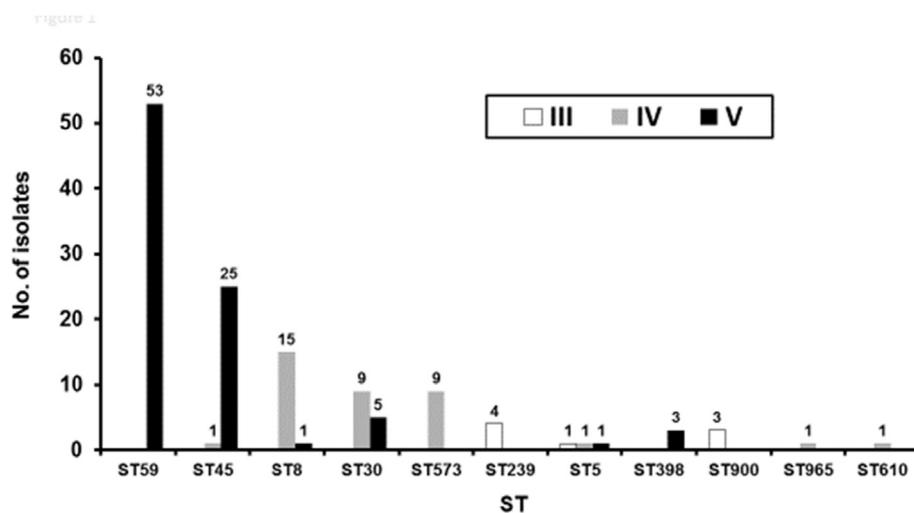
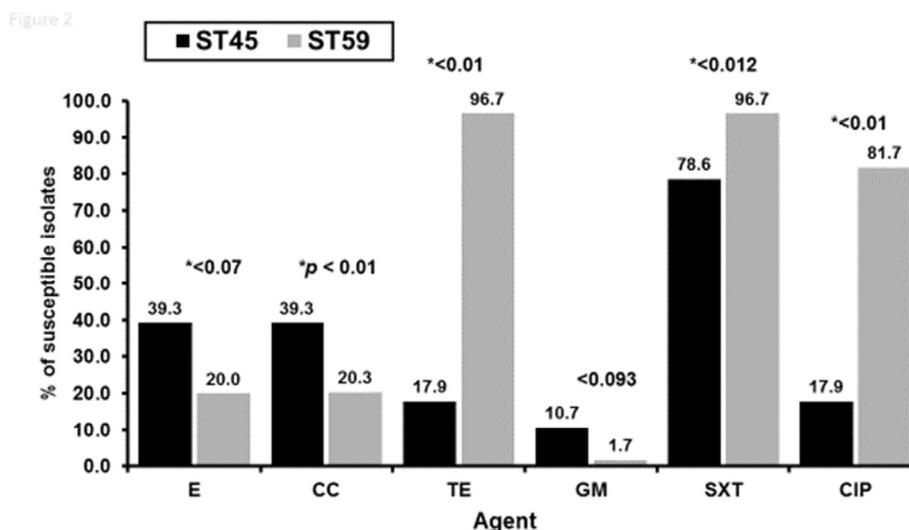


Figure 2. Distribution of sequence types (STs) and SCCmec genotypes (III, IV, and V) in 142 selected isolates of methicillin-resistant *S. aureus* from six long-term care facilities.



**Figure 3.** Comparison of susceptible rates of six selected antimicrobial agents between two different sequence types (STs, ST 45 and ST59) of methicillin-resistant *S. aureus* from six long-term care facilities. E, erythromycin; CC, clindamycin; TE, tetracycline; GM, gentamycin; SXT, trimethoprim-sulfamethoxazole; CIP, ciprofloxacin. All ST45 and ST59 MRSA isolates were susceptible to fucidic acid, mupirocin, rifampin, nitrofurantoin, vancomycin, teicoplanin, linezolid, quinupristin-dalfopristin, and daptomycin. \* indicates that the difference in the rates of susceptibilities between ST45 and ST59 MRSA isolates is significant ( $p$  value < 0.05).

carried PVL-positive MRSA isolates.<sup>26</sup> In contrast, the MRSA isolates from residents in nursing homes in Italy, Ireland, and the United Kingdom reported no PVL genes.<sup>27–29</sup> In summary, the PVL gene carriage rate may vary in different geographical areas.

In 142 isolates with available MLST results, ST59 and ST45 were the predominant MLST types. ST-45 was first identified in Taiwan in 2011 in a respiratory care ward, gradually spreading to other facilities.<sup>30</sup> ST-45 isolates became one of the predominant isolates in LTCFs in Taiwan as well as in many other countries, including Germany, Hong Kong, and China.<sup>14,31–33</sup> Though ST59 remained the most prevalent strain in our study, further follow-up may be needed to monitor whether or not replacement with ST45 has developed.

The antimicrobial susceptibility of MRSA isolates disclosed high susceptibility rates of vancomycin, linezolid, and teicoplanin. Though nitrofurantoin and quinupristin-dalfopristin are not available in Taiwan, both agents were fully active according to our in vitro analysis. Furthermore, mupirocin, a topical agent which was commonly used in MRSA decolonization or topical therapy, also demonstrated excellent in vitro activity. This contrasts with the United States study, which showed mupirocin resistance in 12% of MRSA isolates.<sup>34</sup> Decolonization with mupirocin was not commonly carried out in Taiwan, which may explain the high susceptibility rates to mupirocin in the LTCF residents. Daptomycin, rifampin and fusidic acid were also associated with a very low MIC<sub>90</sub>, although some resistant isolates were obtained on rare occasions. Trimethoprim-sulfamethoxazole, tetracycline, ciprofloxacin, and clindamycin were only moderately effective against our MRSA isolates. Therefore, the probable emergence of isolates that are resistant to these last-resort agents should be closely monitored in Taiwan.

However, there were marked differences in antimicrobial susceptibilities of clindamycin, tetracycline, ciprofloxacin,

and trimethoprim-sulfamethoxazole among ST59 and ST 45 isolates. This finding is consistent with previous report in middle Taiwan's LTCFs.<sup>35</sup> In our study, ST59 MRSA isolates were associated with improved in vitro activities when treated with tetracycline, trimethoprim-sulfamethoxazole, and ciprofloxacin; the susceptibility rate was lower when treated with clindamycin, erythromycin, and gentamicin, though the latter two agents did not reach a statistically significant difference. However, both MLST types were fully susceptible to parental anti-MRSA agents, including vancomycin, teicoplanin, linezolid, and daptomycin. In a previous study in LTCFs in Taiwan,<sup>14</sup> ST45 isolates, including those carrying SCCmec V and un-typed SCCmec genes, were not susceptible to ciprofloxacin, but susceptible to trimethoprim-sulfamethoxazole. In contrast, we reported susceptibility rates of 17.9% to ciprofloxacin and 76.6% to trimethoprim-sulfamethoxazole, indicating an epidemiological change.

As for MDRGNB, only colistin showed good in vitro activity against MDR-A. *baumannii*, carbapenem-resistant *K. pneumoniae*, and carbapenem-resistant *E. coli*, with MIC<sub>50</sub> and MIC<sub>90</sub> less than 1 mg/L. No other antibiotic displayed good in vitro activity against MDR-A. *baumannii*. In contrast, meropenem retained good in vitro activity against more than 90% of carbapenem-resistant *K. pneumoniae*, and cefepime remained an effective agent, with a 92.6% susceptibility rate of carbapenem-resistant *E. coli*. For all of the other antibiotics, the non-susceptibility rates against MDR-A. *baumannii*, carbapenem-resistant *K. pneumoniae*, and carbapenem-resistant *E. coli* were greater than 10%. Therefore, our findings indicate that antibiotic resistance is a significant problem in LTCFs in Taiwan.

During the one-year surveillance period, there was a slight increase in rates of infections. In this surveillance study, using the ATP system and culture methods, we found that MDROs were often detected after the environment had been cleaned. The causes may be due to the lack of

appropriate infection control measures and lack of adequately trained infection prevention and control personnel. Therefore, more rigorous infection prevention and control measures are needed in LTCFs.

In conclusion, MRSA was the most commonly colonized MDRO both in the LTCF residents and the environment, though the colonization status varied markedly in different LTCFs. The number of MRSA colonizations in the environment correlated with the number of MRSA-colonized LTCF residents, indicating an association between the environment and the local residents. Although the majority of MRSA isolates were not susceptible to multiple antimicrobial agents, the susceptibility may vary significantly in different MLST types. More effective infection prevention and control measures are needed to reduce the prevalence of these multidrug-resistant pathogens in long-term care facilities.

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