Journal of Microbiology, Immunology and Infection (2017) 50, 133—144

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ORIGINAL ARTICLE

Presence of multidrug-resistant organisms in the residents and environments of long-term care facilities in Taiwan

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Received 3 December 2016; accepted 14 December 2016
Available online 20 February 2017

KEYWORDS
environment;
infection control;

Abstract *Objectives:* This study investigated the prevalence of multidrug-resistant organisms (MDROs) in the residents and environments of long-term care facilities (LTCFs) in Taiwan. *Methods:* We prospectively investigated the distribution of MDROs in residents of six LTCFs and

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http://dx.doi.org/10.1016/j.jmii.2016.12.001

long-term care facility;
multidrug-resistant organisms;
residents

their environments from January 2015 to December 2015 (intervention period). Active surveillance of colonization of MDROs was performed by culturing rectal and nasal swab samples every 3 months for the residents: 63, 79, and 73 in the first, second, and third surveillance investigations, respectively. If MDROs, including methicillin-resistant *Staphylococcus aureus*, carbapenem-resistant Enterobacteriaceae, carbapenem-resistant *Pseudomonas aeruginosa*, and MDR *Acinetobacter baumannii* were identified, then swab specimens from environmental sources were also collected and cultured. During the study period, several infection control measures were also implemented.

Results: The overall infection density decreased significantly from 2.69 per 1000 patient—days in the preintervention (January 2014 to December 2014) to 2.39 per 1000 patient—days during the intervention period ($p < 0.001$). A total of 154 samples from residents and environmental sources were positive for MDROs. Methicillin-resistant *S. aureus* ($n = 83$, 53.9%) was the predominant organism, followed by carbapenem-resistant Enterobacteriaceae ($n = 35$, 22.7%), MDR *A. baumannii* ($n = 30$, 19.5%), and carbapenem-resistant *P. aeruginosa* ($n = 6$, 3.9%). The rates of detection of MDROs were 27.9% (60/215) in nasal swabs, 15.8% (34/215) in rectal swabs, and 11.1% (60/542) in the environmental sources.

Conclusions: The distribution and persistence of MDROs varied among the different LTCFs and time periods.

Introduction

Long-term care facilities (LTCFs), including nursing homes, rehabilitation facilities, and long-term chronic care hospitals, provide rehabilitative, restorative, and/or ongoing skilled nursing care for patients with significant disabilities.[1] These institutions are also the last medical resource for patients who have survived acute illnesses in hospitals. Infection or colonization with multidrug-resistant organisms (MDROs), such as methicillin-resistant *Staphylococcus aureus* (MRSA), vancomycin-resistant enterococci (VRE), carbapenem-resistant Enterobacteriaceae (CRE), carbapenem-resistant *Pseudomonas aeruginosa* (CRPA), and multidrug-resistant *Acinetobacter baumannii* (MDRAB) have become an important issue not only for hospitals but also for LTCFs.[2–9] In a recent study, Liu et al[10] found that clinical isolates obtained from 215 (45.5%) of 473 nursing home residents harbored extended-spectrum β-lactamase-producing Enterobacteriaceae.

In this prospective study, we investigated the distribution and persistence of MDROs in six LTCFs in Northern and Central Taiwan. Once the presence of MDROs was documented in the LTCFs, several infection control measures were also 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 2015 to December 2015 and included six LTCFs (A—F) located in Northern ($n = 1$, LTCF-A) and Central ($n = 5$, LTCF-B—F) Taiwan. The total bed number of the six LTCFs was 621, ranging from 45 (LTCF-E) to 153 beds (LTCF-B; Table 1). A total of 313 residents staying 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 this study, including age, sex, daily activities, underlying conditions, and devices used, were collected by infection control nurses. 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 (15MMHISO016e), and written informed consent was obtained from each enrolled resident of the participating LTCFs.

Infection control interventions

During the study period (January 2015 to December 2015, intervention period), several infection control measures were implemented. Adenosine triphosphate 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 hand hygiene adherence and active enhancing 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 LTCFs were conducted every 3 months to monitor the progress of the intervention programs. All of the above intervention measures were conducted in six LTCFs during the intervention period. We also calculated the number of episodes of infection per 1000 patient—days in each LTCF in the

Table 1 Characteristics of the 313 residents in six long-term care facilities.

Variable	No. (%) of residents in each indicated long-term care facility						Total
	A	B	C	D	E	F	
No. of beds	110	153	68	117	45	128	621
No. of residents initially designated (screened) for this study	35	21	66	39	45	107	313
Male (%)	17 (48.6)	8 (38.1)	28 (42.4)	14 (35.9)	31 (68.9)	50 (46.4)	148 (47.3)
Mean age (y)	80.9	84.0	77.5	80.0	72.4	84.6	80.3
Activity of daily living							
Fully dependent	34 (97.0)	21 (100.0)	37 (56.0)	14 (35.0)	11 (24.0)	71 (66.0)	187 (59.7)
Partly dependent	1 (3.0)	0 (0.0)	7 (10.0)	2 (4.0)	7 (16.0)	31 (29.4)	48 (15.3)
Underlying condition							
Hypertension	20 (31.4)	14 (66.7)	47 (71.2)	23 (27.3)	9 (20.0)	55 (51.4)	168 (53.7)
Diabetes mellitus	11 (31.4)	5 (23.8)	27 (40.9)	12 (30.8)	6 (13.3)	37 (34.6)	98 (31.3)
Dementia	6 (17.1)	0 (0.0)	7 (10.6)	9 (23.1)	6 (13.3)	39 (36.4)	67 (21.4)
Heart failure	10 (28.6)	0 (0.0)	18 (27.3)	7 (17.9)	3 (6.7)	16 (15.0)	54 (17.3)
Asthma	2 (5.7)	0 (0.0)	10 (15.2)	2 (5.1)	1 (2.2)	4 (3.7)	19 (6.1)
Parkinsonism	2 (5.7)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	2 (0.6)
Device							
Nasogastric tube	29 (82.9)	20 (95.2)	37 (56.1)	32 (82.1)	10 (22.2)	43 (40.2)	171 (54.6)
Foley catheter	17 (48.6)	0 (0.0)	14 (21.2)	13 (33.3)	7 (15.6)	8 (7.5)	59 (18.8)
Tracheostomy tube	19 (54.3)	0 (0.0)	0 (0.0)	8 (20.5)	0 (0.0)	6 (5.6)	33 (10.5)
Gastrostomy	2 (5.7)	0 (0.0)	0 (0.0)	2 (2.6)	0 (0.0)	0 (0.0)	3 (1.0)
Cystostomy	3 (8.6)	1 (4.8)	1 (1.5)	1 (2.6)	0 (0.0)	1 (0.9)	7 (2.2)
Ileostomy	1 (2.9)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	1 (0.3)

preintervetion period (January 2014 to December 2014) and intervention period.

MDRO surveillance and environmental sampling

After informed consent was obtained, we collected rectal and nasal surveillance cultures for MRDOs, and the surveillance cultures were conducted every 3 months by infection control nurses. If cultures were positive for MDROs, including MRSA, VRE, 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 (Becton Dickinson Microbiology Systems, Sparks, MD, USA). All inoculated plates were sent to the Microbiology Laboratory at National Taiwan University Hospital 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_2 atmosphere for 18—24 hours. Single colonies that grew on the initial or subcultured blood-agar plates were identified to the species level using the Bruker Biotyper matrix-assisted laser desorption/ionization time-of-flight mass spectrometry system (Bruker Biotyper; Bruker Daltonics GmbH, Bremen, Germany). Antimicrobial susceptibilities were determined by the Phoenix PMIC/ID-62 and PMIC/ID-72 systems (Becton Dickinson Microbiology Systems) as previously reported.[11,12] The minimum inhibitory concentration (MIC) values of the tested antibiotics against the collected isolates were interpreted according to Clinical and Laboratory Standards Institute guidelines.[13] 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, and amikacin.

Molecular typing

The genetic relationships of the isolates of MRSA, carbapenem-resistant *Klebsiella pneumoniae* (CRKP), and MDRAB were determined by pulsed-field gel electrophoresis (PFGE) as described in our previous study.[14] DNA was digested by the restriction enzyme *Sma*I for MRSA, *Apa*I for MDRAB, and *Xba*I for CRKP, and the restriction fragments were separated in a CHEF-DR III unit (Bio-Rad Laboratories, Hercules, CA, USA). The pulsotypes were analyzed using the Bio-Rad CHEF-Mapper apparatus (Bio-Rad Laboratories). Cluster analysis was performed using BioNumerics version 5.0 (Applied Maths, Sint-Martens-Latem, Belgium) and the unweighted pair-group method with arithmetic averages. The Dice correlation coefficient was used with a tolerance of 1% in order to analyze any similarities between banding patterns. Isolates showing identical PFGE patterns were considered to be the same strain (same pulsotypes) and

isolates exhibiting PFGE patterns with a similarity of >80% were considered to represent closely related strains.

Statistical analysis

Categorical variables are presented as counts and percentages. Differences in infection density in each LTCF and in all six LTCFs between the preintervention and intervention periods were evaluated using the Pearson χ^2 test. A p value < 0.05 was considered to represent statistical significance. All statistical analyses were conducted using the statistical package SPSS for Windows version 11.0 (SPSS Inc., Chicago, IL, USA).

Results

Settings and residents

Table 1 summarizes the characteristics of 313 residents in the six LTCFs. The average age of residents was 80.3 years, and 47.3% of the residents were men. The majority (59.7%) of residents were totally dependent, which was defined as the resident needing full staff support to perform all of their daily living activities, and 15.3% were partially dependent, which was defined as the resident performing some of their activities. Hypertension was the most common underlying disease (53.7%), followed by diabetes mellitus (31.3%) and dementia (21.4%). About 55% of the residents had nasogastric tubes, 18.8% had Foley catheters, and 10.5% had tracheostomy tubes. The actual number of residents participating in three MDRO surveillance studies during intervention period (informed consents were signed) was 63 (first surveillance), 79 (second), and 73 (third), respectively. The number of resident who participated all the three surveillance studies was 52. The number of residents joined the first and second surveillance, second and third, and one and third surveillance investigations was 60, 70, and 53, respectively.

Infection types and density before and after intervention

Prior to the intervention, urinary tract infection (1.63 per 1000 patient—days) was the most common type of infection, followed by respiratory tract infection (1.52 per 1000 patient—days) and bloodstream infection (0.52 per 1000 patient—days) in the LTCF located in Northern Taiwan (LTCF-A). In the five LTCFs in Central Taiwan, respiratory tract infection (1.51 per 1000 patient—days) was the most common type of infection, followed by urinary tract infection (0.84 per 1000 patient—days) and skin/soft tissue infection (0.11 per 1000 patient—days). After the intervention, the incidence densities per 1000 patient—days were 1.45 for respiratory tract infection, 1.50 for urinary tract infection, and 0.31 for bloodstream infection in the LTCF in Northern Taiwan. In the five LTCFs in Central Taiwan (LTCF-B—F) the incidence densities per 1000 patient—days were 1.22 for respiratory tract infection, 0.77 for urinary tract infection, and 0.06 for skin/soft tissue infection. The overall incidence density was highest for respiratory tract infection (1.22 per 1000 patient—days), followed by urinary tract infection (0.77 per 1000 patient—days), and skin/soft tissue infection (0.06 per 1000 patient—days). The overall infection density decreased significantly from 2.69 per 1000 patient—days before the intervention to 2.39 per 1000 patient—days after the intervention ($p < 0.001$; Fig. 1). Infection density

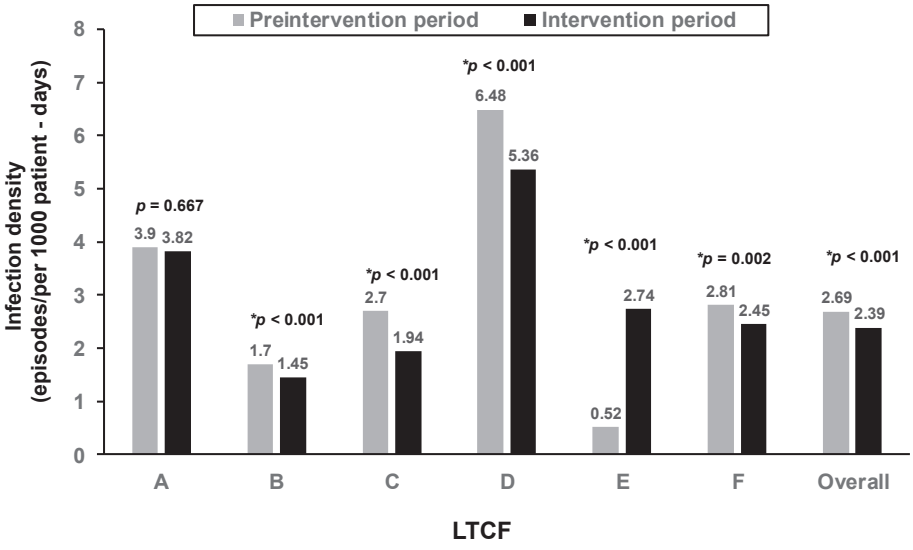


Figure 1. Infection density (episode/per 1000 patient—days) among residents of six LTCFs in the preintervetion period (January 2014 to December 2014) and the intervention period (January 2015 to December 2015). Differences in infection density in each LTCF and all six LTCFs between the preintervention and intervention periods were evaluated using the Pearson χ^2 test. *$p < 0.05$ was considered to represent statistical significance. LTCF = long-term care facility.

decreased significantly in all LTCFs with the exception of LTCF-E after the intervention (Fig. 1).

Distribution of MDROs

A total of 154 samples from residents and environment sources were positive for MDROs. MRSA was the most common organism (n = 83, 53.9%), followed by CRE (n = 35, 22.7%), MDRAB (n = 30, 19.5%), and CRPA (n = 6, 3.9%; Table 2). No VRE was detected in this study. Table 3 summarizes the distribution of MDROs isolated from three surveillance cultures from residents and environmental sources during the study period. The distribution of MDROs differed markedly in each LTCF and in different time periods. Overall all, the positive rates of MDROs in all six LTCFs was 15.8% (154/972): 10.5% in the first surveillance, 20.1% in the second surveillance, and 17.3% in the third surveillance cultures, but the positive rates of MDRO isolates were also different, ranging from 6.5% (4/62, LTCF-C) to 25.7% (37/144, LTCF-B) in different LTCFs (Fig. 2). The highest rates of MDRO isolation were found in second surveillance cultures in LTCF-A (23/56, 41.1%) and LTCF-B (17/50, 34.0%; Fig. 2). In the first surveillance survey, 22.2% of the MDRO isolates were isolated from nasal swabs (14/63), 11.1% (7/63) were from rectal swabs, and 6.8% (14/206) were isolated from swabs of environmental sources. In the second surveillance survey, 27.8% (22/79) were isolated from nasal swabs, 22.8% (18/79) from rectal swabs, and 14.0% (19/136) from swabs of environmental sources. In the third surveillance survey, 32.9% (24/73) were isolated from nasal swabs, 12.3% (9/73) from rectal swabs, and 13.5% (27/200) from environmental specimens. Overall, 27.9% (60/215) of MDRO isolates were obtained from nasal swabs, 15.8% (34/215) from rectal swabs, and 11.1% (60/542) from environmental specimens.

Table 2 Distribution of 154 multidrug-resistant organisms collected from residents and environmental sources in six long-term care facilities.

Organism	No. (%) of isolates (n = 154)
Methicillin-resistant *Staphylococcus aureus*	83 (53.9)
Carbapenem-resistant *Enterobacteriaceae*	35 (22.7)
Klebsiella pneumoniae	18 (11.7)
Escherichia coli	8 (5.2)
Serratia marcescens	3 (1.9)
Proteus mirabilis	2 (1.3)
Enterobacter aerogenes	2 (1.3)
Citrobacter freundii	1 (0.6)
Providencia stuartii	1 (0.6)
Carbapenem-resistant *Pseudomonas aeruginosa*	6 (3.9)
Multidrug-resistant *Acinetobacter baumannii*	30 (19.5)

Antimicrobial susceptibility patterns

The susceptibility results for 80 isolates of MRSA, 18 isolates of CRKP, and 24 isolates of MDRAB are shown in Table 4. For MRSA, six isolates (7.5%) had intermediate resistance to vancomycin (vancomycin-intermediate *S. aureus*) and teicoplanin; two isolates (2.5%) were resistant to linezolid (linezolid-resistant *S. aureus*); and one isolate (1.3%) was resistant to daptomycin (daptomycin-resistant *S. aureus*). For the 18 isolates of CRKP, amikacin and gentamicin showed good *in vitro* activity with a susceptibility rate of 100%. The MIC required to inhibit growth of 50% of organisms (MIC_{50}) and MIC required to inhibit growth of 90% of organisms (MIC_{90}) were < 8 mg/L and 2 mg/L, respectively.

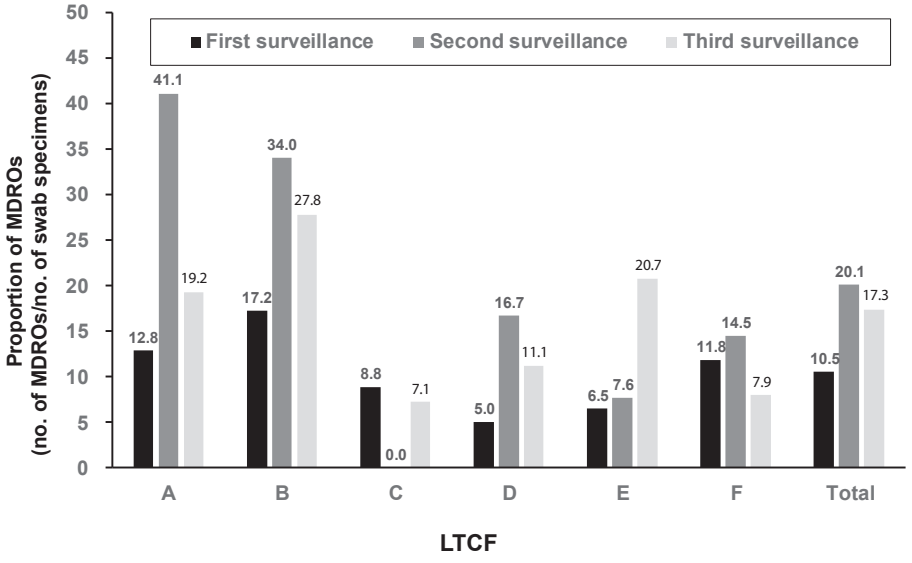


Figure 2. Positive rates of MDROs presented as number of MDROs/number of all swab specimens (nasal, anal, and environmental sources) of the three different surveillance cultures in the six LTCFs. LTCF = long-term care facility; MDRO = multidrug-resistant organism.

Table 3 Distribution of 154 MDROs from six LTCFs.

Surveillance cultures (no. of cultures positive for indicated MDRO/total no. of specimens submitted[a])	No. (%) of MDRO identified from indicated specimens in six LTCFs[b]						Total
	A	B	C	D	E	F	
1st surveillance							
Nasal swab (14/63)							
No. of specimens submitted	7	9	7	5	30	5	63
MRSA	1 (14.3)	4 (44.4)	1 (14.3)	1 (20.0)	4 (13.3)	0	11
CRKP	0	0	0	0	0	1 (20.0)	1
CREC	0	0	0	0	0	0	0
Other CRE	0	0	0	0	0	0	0
CRPA	0	0	0	0	0	1 (20.0)	1
MDRAB	0	0	0	0	0	1 (20.0)	1
Rectal swab (7/63)							
No. of specimens submitted	7	9	7	5	30	5	63
MRSA	0	0	0	0	0	0	0
CRKP	0	2 (22.2)	0	0	0	1 (20.0)	3
CREC	0	0	1 (14.3)	0	2 (6.7)	0	3
Other CRE	0	0	0	0	0	0	0
CRPA	0	0	0	0	0	0	0
MDRAB	0	1 (11.1)	0	0	0	0	1
Environment sources (14/206)							
No. of specimens submitted	64	40	20	10	48	24	206
MRSA	5 (7.8)	1 (2.5)	0	0	0	0	6
CRKP	1 (1.6)	0	0	0	0	0	1
CREC	0	0	0	0	0	0	0
Other CRE	0	0	0	0	0	0	0
CRPA	0	0	1 (5.0)	0	0	0	1
MDRAB	3 (4.7)	2 (5.0)	0	0	1 (2.1)	0	6
2nd surveillance							
Nasal swab (22/79)							
No. of specimens submitted	8	9	7	3	30	22	79
MRSA	3 (37.5)	3 (33.3)	0	1 (33.3)	1 (3.3)	1 (4.5)	9
CRKP	0	0	0	0	0	3 (13.6)	3
CREC	0	0	0	0	0	0	0
Other CRE	0	1 (11.1)	0	0	1 (3.3)	1 (4.5)	3
CRPA	3 (37.5)	0	0	0	0	0	3
MDRAB	3 (37.5)	1 (11.1)	0	0	0	0	4
Rectal swab (18/79)							
No. of specimens submitted	8	9	7	3	30	22	79
MRSA	0	1 (11.1)	0	0	0	0	1
CRKP	0	2 (22.2)	0	0	1 (3.3)	4 (18.2)	7
CREC	1 (12.5)	1 (11.1)	0	0	1 (3.3)	1 (4.5)	4
Other CRE	0	0	0	0	0	0	0
CRPA	1 (12.5)	0	0	0	0	0	1
MDRAB	2 (25.0)	3 (33.3)	0	0	0	0	5
Environment sources (19/136)							
No. of specimens submitted	40	32	0	0	32	32	136
MRSA	1 (2.5)	3 (9.4)	0	0	3 (9.4)	1 (3.1)	8
CRKP	0	0	0	0	0	0	0
CREC	0	0	0	0	0	0	0
Other CRE	3 (7.5)	0	0	0	0	0	3
CRPA	0	0	0	0	0	0	0
MDRAB	6 (15.0)	2 (6.3)	0	0	0	0	8
3rd surveillance							
Nasal swab (24/73)							
No. of specimens submitted	10	6	7	4	28	18	73
MRSA	2 (20.0)	2 (33.3)	0	1 (25.0)	10 (35.7)	5 (27.8)	20
CRKP	0	0	0	0	0	0	0
CREC	0	0	0	0	0	0	0

Table 3 (*continued*)

Surveillance cultures (no. of cultures positive for indicated MDRO/total no. of specimens submitted[a])	No. (%) of MDRO identified from indicated specimens in six LTCFs[b]						Total
	A	B	C	D	E	F	
Other CRE	1 (10.0)	0	0	0	0	0	1
CRPA	0	0	0	0	0	0	0
MDRAB	2 (20.0)	1 (16.7)	0	0	0	0	3
Rectal swab (9/73)							
No. of specimens submitted	10	6	7	4	28	18	73
MRSA	0	0	0	0	2 (7.1)	0	2
CRKP	0	3 (50.0)	0	0	0	0	3
CREC	0	0	0	0	1 (3.6)	0	1
Other CRE	0	0	1 (14.3)	0	1 (3.6)	0	2
CRPA	0	0	0	0	0	0	0
MDRAB	1 (10.0)	0	0	0	0	0	1
Environment sources (27/200)							
No. of specimens submitted	32	24	0	10	94	40	206
MRSA	4 (12.5)	4 (16.7)	0	1 (10.0)	16 (17.0)	1 (2.5)	26
CRKP	0	0	0	0	0	0	0
CREC	0	0	0	0	0	0	0
Other CRE	0	0	0	0	0	0	0
CRPA	0	0	0	0	0	0	0
MDRAB	0	0	0	0	1 (1.1)	0	1

[a] The number of nasal and rectal swabs obtained in each surveillance was the same as the number of residents enrolled in that surveillance.
[b] The positive rates of MDRO isolates presented as the total number of positive swab specimens for MDROs/total number of submitted swab specimens (nasal, rectal, and environmental swabs) in the six LTCFs are as follows: LTCF A (43/186, 23.1%), B (37/144, 25.7%), C (4/62, 6.5%), D (4/44, 9.1%), E (45/350, 12.9%), F (21/186, 11.3), total (154/972, 15.8%).
CREC = carbapenem-resistant *Escherichia coli*; CRKP = carbapenem-resistant *Klebsiella pneumoniae*; CRPA = carbapenem-resistant *Pseudomonas aeruginosa*; LTCF = long-term care facility; MDRAB, multidrug-resistant *Acinetobacter baumannii*; MDRO = multidrug-resistant organism; MRSA, methicillin-resistant *Staphylococcus aureus*; Other CRE, carbapenem-resistant Enterobacteriaceae other than *E. coli* or *K. pneumoniae*.

For meropenem, the MIC_{50} and MIC_{90} values were $\leq$ 1 mg/L, and the overall nonsusceptibility rate was 5.6%. For cefepime, the MIC_{50} and MIC_{90} values were $\leq$ 2 mg/L, and the overall nonsusceptibility rate was 11.2%. For amikacin, the MIC_{50} and MIC_{90} values were $\leq$ 8 mg/L and $>$ 32 mg/L, respectively, and the overall resistance rate was 41.7%. For ampicillin–sulbactam, the MIC_{50} and MIC_{90} values were 8 mg/L and $>$ 16 mg/L, respectively, and the overall nonsusceptibility rate was 50%. Colistin exhibited good *in vitro* activities against all isolates of CRKP and MDRAB with MIC values of $\leq$ 1 mg/L.

Molecular investigation

The PFGE patterns of MRSA, CRKP, and MDRAB are shown in Fig. 3. Closely related strains were demonstrated within MRSA, MDRAB, and CRKP isolates. Among 80 MRSA isolates, we found the same strains and closely related strains in different institutions, different patients, and different patient environments (Fig. 3A). For example, three isolates (485, 492, and 666) were closely related strains. Isolates 485 and 492 were isolated from residents at LTCF-F; however, 666 was recovered from one resident at LTCF-E. Two isolates (256 and 466) belonging to closely related strains were isolated from two different residents at LTCF-B. Among 16 CRKP isolates, seven closely related strains were identified (Fig. 3B). One closely related strain comprising nine isolates (184, 198, 202, 203, 204, 226, 227, 660, and 843) was present in four different residents (Isolates 198 and 843 in Patient 843; Isolates 202, 203, 226, and 227 in Patient 173; Isolates 184 and 660 in Resident 660; and Isolate 204 in Patient 204) and were detected in different periods (Isolates 660 and 843 in the 1st surveillance, and 184, 198, 202, 203, 204, 226, and 227 in the second surveillance) in LTCF-F. Among the 24 MDRAB isolates, seven closely related strains were identified (Fig. 3C). Eight isolates (586, 587, 590, 593, 596, 623, 636, and 640) belonging to a closely related strain were recovered from different residents (Isolates 636 and 640 in Resident 25118339, and Isolate 623 in Resident 4016807) and another patient's environments (Resident number 397214), including beds, bedside tables, and ward door handles (Isolates 586, 587, 590, 593, and 596) in LTCF-A during the third surveillance.

Discussion

During the 1-year surveillance period, five major MDROs (n = 145), including MRSA, MDRAB, CRKP, carbapenem-resistant *Escherichia coli*, and CRPA, and several rare CRE (n = 9), including carbapenem-resistant *Serratia*

Table 4 Antimicrobial susceptibilities of 80 isolates of MRSA, 18 of CRKP, and 24 of MDRA collected from six long-term care facilities using the Phoenix susceptibility system.

Agent	MICs (mg/L)			No. (%) of isolates with indicated susceptibility		
	Range	MIC_{50}	MIC_{90}	Susceptible (%)	Intermediate (%)	Resistant (%)
MRSA ($n = 80$)						
Ampicillin	≤ 1 to > 4	> 4	> 4			
Oxacillin	1 to > 4	> 4	> 4			
Cefazolin	≤ 2 to > 16	8	> 16			
Cefoxitin	8 to > 16	> 16	> 16			
Erythromycin	≤ 0.25 to > 4	> 4	> 4	15 (18.8)	0 (0)	65 (81.3)
Clindamycin	≤ 0.5 to > 2	≤ 0.5	> 2	40 (50)	0 (0)	40 (50)
Tetracycline	≤ 0.5 to > 8	≤ 0.5	> 8	55 (68.8)	1 (1.3)	24 (30)
Gentamicin	≤ 2 to > 8	> 8	> 8	27 (33.8)	0 (0)	53 (66.3)
Trimethoprim–sulfamethoxazole	≤ 0.5 to > 2	≤ 0.5	≤ 0.5	76 (95)	—	4 (5)
Ciprofloxacin	≤ 0.5 to > 2	> 2	> 2	38 (47.5)	0 (0)	42 (52.5)
Vancomycin	≤ 1 to > 16	≤ 1	≤ 1	74 (92.5)	0 (0)	6 (7.5)
Teicoplanin	≤ 1 to > 16	≤ 1	≤ 1	74 (92.5)	0 (0)	6 (7.5)
Linezolid	≤ 1 to > 4	≤ 1	2	78 (97.5)	—	2 (2.5)
Fusidic acid	≤ 1 to 8	≤ 1	≤ 1			
Mupirocin	≤ 256	≤ 256	≤ 256			
Nitrofurantoin	≤ 16 to > 64	≤ 16	≤ 16	76 (95)	3 (3.8)	1 (1.3)
Quinupristin–dalfopristin	≤ 0.5 to > 2	≤ 0.5	≤ 0.5	74 (92.5)	0 (0)	6 (7.5)
Rifampin	≤ 0.5 to > 2	≤ 0.5	≤ 0.5	79 (98.8)	0 (0)	1 (1.3)
Daptomycin	≤ 1 to > 4	≤ 1	≤ 1	79 (98.8)	—	—
CRKP ($n = 18$)						
Ampicillin	> 16	> 16	> 16	0 (0)	0 (0)	18 (100)
Ampicillin-sulbactam	8 to > 16	> 16	> 16	1 (5.6)	0 (0)	17 (94.4)
Piperacillin-tazobactam	≤ 4 to > 64	> 64	> 64	7 (38.9)	1 (5.6)	10 (55.6)
Cefazolin	8 to > 16	> 16	> 16	0 (0)	0 (0)	18 (100)
Cefmetazole	≤ 8 to > 32	> 32	> 32	1 (5.6)	3 (16.7)	14 (77.8)
Ceftriaxone	≤ 4 to > 32	32	> 32			
Cefopodoxime	≤ 2 to > 32	≤ 2	> 32			
Cefotaxime	≤ 2 to > 16	> 16	> 16	0 (0)	0 (0)	11 (61.1)
Ceftazidime	1 to > 16	> 16	> 16	3 (16.7)	2 (11.1)	13 (72.2)
Cefepime	≤ 2 to 16	≤ 2	≤ 2	16 (88.9)	1 (5.6)	1 (5.6)
Aztreonam	≤ 2 to > 16	> 16	> 16	8 (44.4)	0 (0)	10 (55.6)
Ertapenem	≤ 0.5 to > 4	≤ 0.5	> 4	11 (61.1)	2 (11.1)	5 (27.8)
Meropenem	≤ 1 to 2	≤ 1	≤ 1	17 (94.4)	1 (5.6)	0 (0)
Imipenem	≤ 1 to 8	2	4	2 (11.1)	8 (44.4)	8 (44.4)
Moxifloxacin	2 to > 4	> 4	> 4			
Levofloxacin	≤ 1 to > 4	> 4	> 4	6 (33.3)	0 (0)	12 (66.7)
Ciprofloxacin	≤ 0.5 to > 2	> 2	> 2	4 (22.2)	0 (0)	14 (77.8)
Gentamicin	≤ 2	≤ 2	≤ 2	18 (100)	0 (0)	0 (0)
Amikacin	≤ 8	≤ 8	≤ 8	18 (100)	0 (0)	0 (0)
Trimethoprim–sulfamethoxazole	≤ 0.5 to > 2	> 2	> 2	8 (44.4)	—	10 (55.6)
Colistin	≤ 1	≤ 1	≤ 1			
MDRA ($n = 24$)						
Ampicillin–sulbactam	≤ 4 to > 16	8	> 16	12 (50)	6 (25)	6 (25)
Piperacillin–tazobactam	16 to > 64	> 64	> 64	3 (12.5)	1 (4.2)	20 (83.3)
Cefametazole	32 to > 32	> 32	> 32			
Cefotaxime	8 to > 16	> 16	> 16			
Ceftriaxone	8 to > 32	> 32	> 32	1 (4.2)	6 (25)	17 (70.8)
Cefopodoxime	4 to > 32	> 32	> 32			
Ceftazidime	≤ 0.5 to > 16	> 16	> 16	5 (20.8)	3 (12.5)	16 (66.7)
Cefepime	16 to > 16	> 16	> 16	0 (0)	5 (20.8)	19 (79.2)
Aztreonam	> 16	> 16	> 16			
Ertapenem	> 4	> 4	> 4			
Meropenem	≤ 1 to > 8	> 8	> 8	6 (30)	0 (0)	14 (70)
Imipenem	≤ 1 to > 8	> 8	> 8	6 (25)	1 (4.2)	17 (70.8)

Table 4 (*continued*)

Agent	MICs (mg/L)			No. (%) of isolates with indicated susceptibility		
	Range	MIC_{50}	MIC_{90}	Susceptible (%)	Intermediate (%)	Resistant (%)
Moxifloxacin	≤ 1 to > 4	> 4	> 4			
Levofloxacin	≤ 1 to > 4	> 4	> 4	3 (12.5)	0 (0)	21 (87.5)
Ciprofloxacin	1 to > 2	> 2	> 2	1 (4.2)	2 (8.3)	21 (87.5)
Gentamicin	4 to > 8	> 8	> 8	1 (4.2)	9 (37.5)	14 (58.3)
Amikacin	≤ 8 to > 32	≤ 8	> 32	14 (58.3)	0 (0)	10 (41.7)
Trimethoprim—sulfamethoxazole	≤ 0.5 to > 2	> 2	> 2	9 (37.5)	—	15 (62.5)
Colistin	≤ 1	≤ 1	≤ 1	24 (100)	—	0 (0)

CRKP = carbapenem-resistant *Klebsiella pneumoniae*; MDRA = multidrug-resistant *Acinetobacter baumannii*; MIC_{50} = minimum inhibitory concentration required to inhibit growth of 50% of organisms; MIC_{90} = MIC required to inhibit growth of 90% of organisms; MRSA = methicillin-resistant *Staphylococcus aureus*.

marcescens, *Proteus mirabilis*, *Enterobacter aerogenes*, *Citrobacter freundii*, and *Providencia stuartii* were detected in six LTCF. The rates of detection of all MDROs were 27.9% (60/215) in nasal swabs, 15.8% (34/215) in rectal swabs, and 11.1% (60/542) in swabs of environmental sources. Of those organisms, MRSA was the most common pathogen, accounting for ~54% of the MDROs isolated from patients and environmental sources in LTCFs in this study. In contrast, MRSA is not commonly isolated in acute care hospitals in Taiwan.[15—17] For example, in a multicenter surveillance study of 56,830 episodes of healthcare-associated bloodstream infections during 2000—2011, only 3187 (5.6%) episodes were caused by MRSA and the incidence of MRSA associated healthcare-associated bloodstream infections decreased with time ($p < 0.001$).[15] Also, Lee et al[17] found that the incidence of healthcare-associated MRSA as well as the prevalence of MRSA decreased from 2001 to 2009 in a medical center in Northern Taiwan. The predominance of MRSA in LTCFs in our study has been reported in studies from other countries as well. Denis et al[18] conducted a cross-sectional study on the prevalence of MRSA carriage among 2953 residents in 60 nursing homes in Belgium and found that 587 (19.9%) residents were MRSA carriers. In another prevalence survey in Belgium, Jans et al[19] found that the weighted prevalence of MRSA carriage among 2791 screened residents in 60 participating nursing homes was 12.2% (range: 0—36%). In Italy, March et al[20] found that 35 (31.5%) of 111 LTCF residents were colonized with ≥ 2 MDROs and that MRSA was the second most common MDRO (38.7%). In contrast, in Swedish nursing homes, Andersson et al[21] reported that none of the 560 residents in nine nursing homes tested positive for MRSA. These findings suggest that LTCFs should conduct surveillance studies so that an LTCF-specific epidemiologic databank can be established. Moreover, the findings strongly suggest that more effective infection control measures are needed in LTCFs to decrease the rates of MRSA colonization and infection among LTCF residents.

In addition to MRSA, we found that CRE (22.7%) and MDRAB (19.5%) were also common MDROs in LTCFs in Taiwan. Among the 35 CRE isolates, CRKP ($n = 18$) was the most common organism, followed by carbapenem-resistant *E. coli* ($n = 8$). Cunha et al[22] conducted a surveillance study of fecal carriage of CRE in 404 patients admitted to acute care hospitals from nursing homes and found that the carriage rate was 4.6%. Dandachi et al[9] found that among 178 isolates of MDR *Enterobacteriaceae* from 68 nursing home residents in Northern Lebanon, only three isolates (1.7%) were nonsusceptible to carbapenems. For MDRAB, our finding is similar to that in a previous surveillance of 168 residents in four nursing homes in Michigan, in which 25 (15%) were colonized with MDRAB.[23] Overall, our finding indicates that CRE in LTCFs in Taiwan is a more serious problem than in other countries.

Several studies have shown that functional status is a common risk factor for MDRO carriage among nursing home residents.[19,24,25] In our study, the vast majority (97—100%) of residents were not ambulatory and 17% of them had dementia. Both factors have been demonstrated to increase the risk of MDRO carriage.[24,25]

During the 1-year surveillance period there was a marked reduction in infections. This may be due to the infection control measures and education programs that were implemented in the LTCFs in this study. However, more needs to be done. We found that most residents with rectal or nasal swabs that tested positive for an MDRO had environmental swabs testing positive for the same MDRO. Moreover, the same MDRO was often detected at different surveillance periods. In addition, using the adenosine triphosphate 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.[26] Therefore, more rigorous infection prevention and control measures are needed in LTCFs.

In this study we also conducted an extensive investigation of antibiotic susceptibility patterns for each MDRO. For MRSA, commonly used antibiotics including vancomycin, teicoplanin, daptomycin, and linezolid still showed good *in vitro* activity with overall susceptibility rates > 90%.

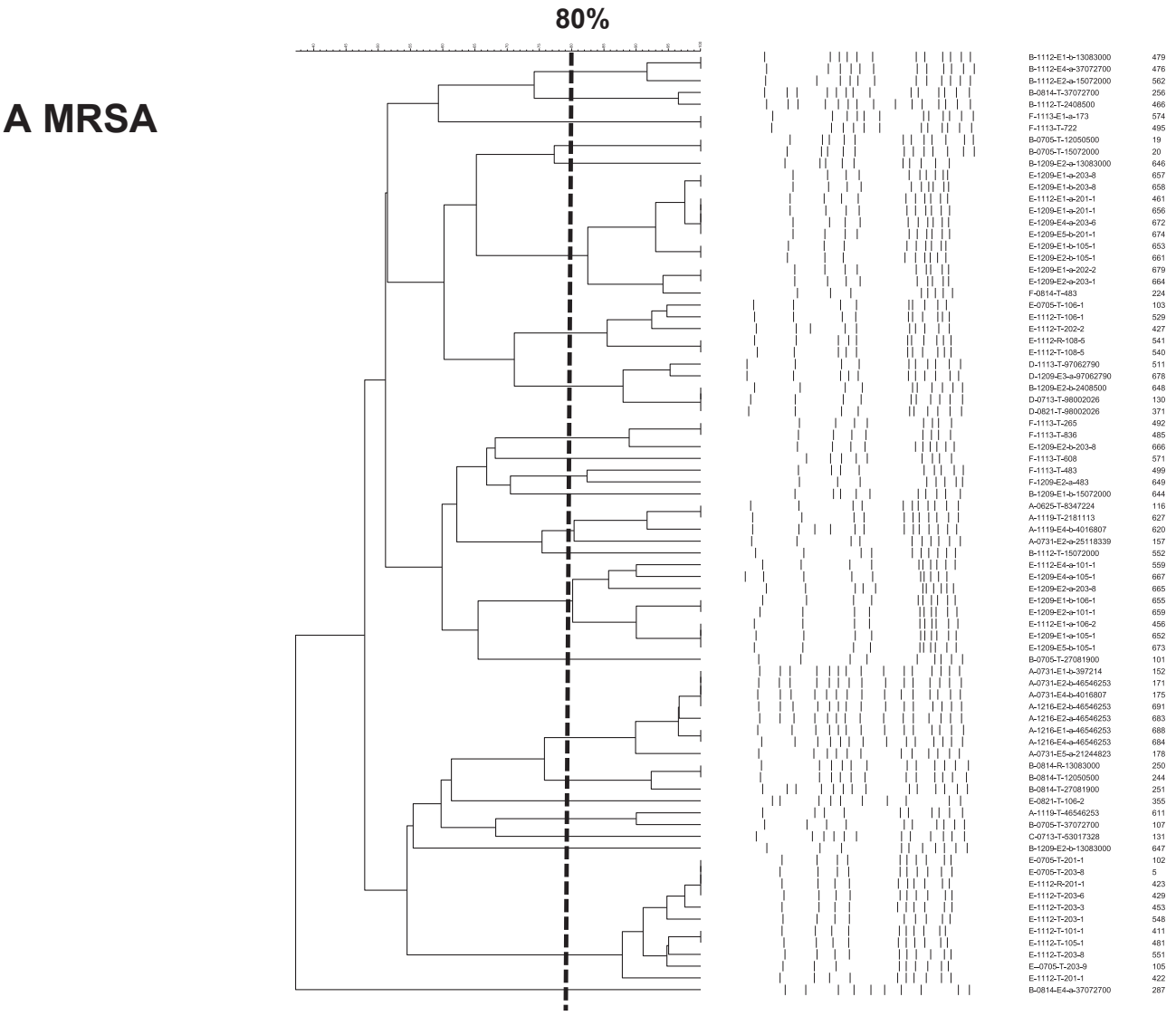
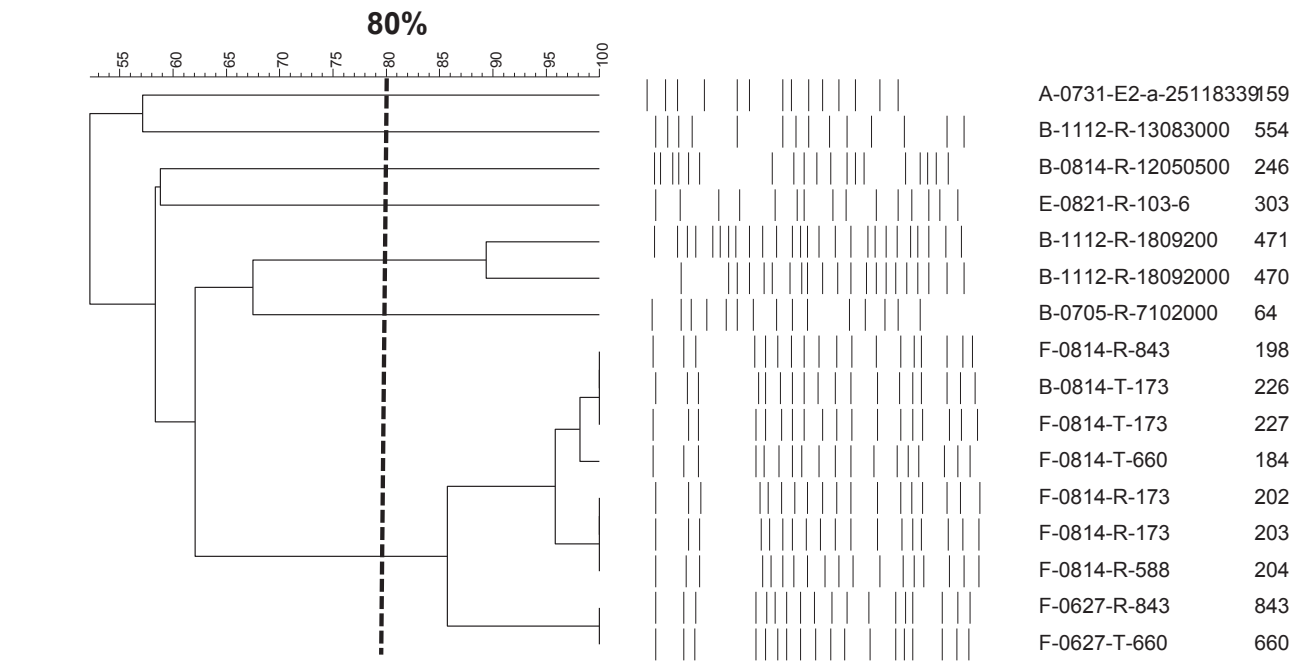


Figure 3. Pulsed-field gel electrophoresis patterns of (A) MRSA, (B) CRKP, and (C) MDRAB isolated from rectal swabs, nasal swabs and environmental sources in LTCFs. Column I indicates the LFCF (1—6, LTCF-A—F), date of specimen collection, specimen source (R, rectal swab; T, nasal swab; and E, environmental sources), resident number E1, beds; E2, bedside tables, E3 toilet door handles, E4, ward door handles, E5, curtain. Column II denotes the designation of the isolates (isolate number). CRKP = carbapenem-resistant *Klebsiella pneumoniae*; LTCF = long-term care facility; MDRAB = multidrug-resistant *Acinetobacter baumannii* MRSA = methicillin-resistant *Staphylococcus aureus*.

However, we also found the presence of vancomycin-intermediate *S. aureus*, linezolid-resistant *S. aureus*, and daptomycin-resistant *S. aureus* in the LTCFs. Fortunately, there was no colistin-resistant CRKP found in this study even though the *mcr*-1-mediated colistin resistance among Enterobacteriaceae isolates have been reported in many countries.[27,28] For MDRAB, only colistin was effective based on the *in vitro* susceptibility testing results. Overall, our findings suggest that antibiotic resistance is a remarkable problem in LTCFs in Taiwan.

In this study, there were several limitations. First, despite there were 621 beds in all LTCFs, we could only obtain the inform consent of 313 residents during the study period. Second, although urinary tract and respiratory tract infections are notoriously difficult to diagnose in this group of patients, all healthcare-associated infections were identified and confirmed by infection control nurses according to the definition of the US Centers for Disease Control guidelines.[29] Third, because all of residents could be freely admitted, discharged or even dead during the intervention period, we could not conduct the three surveillance investigations for the same cohorts in this study. Therefore, we may collect the samples from different populations among three surveillance studies, and only 52



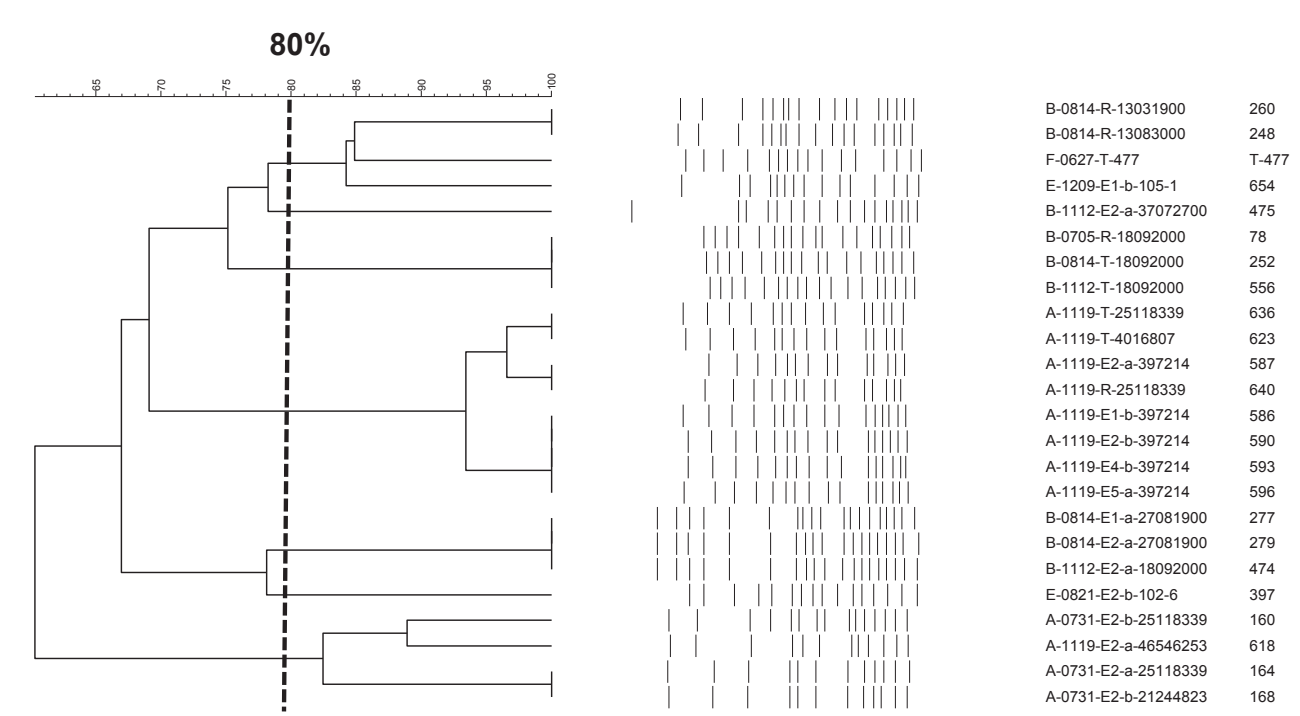


Figure 3. (*continued*).

residents participated all the three surveillance studies. Fourth, we did not assess the mechanism of resistance for MDROs in this study.

In conclusion, MRSA, CRE, and MDRAB were the most common MDROs among residents and environments in LTCFs in Taiwan, and the majority of those pathogens were resistant to most antibiotics. More effective infection prevention and control measures are needed to reduce the prevalence of these multidrug-resistant pathogens in long-term care facilities.

Conflicts of interest

All authors declare no conflict of interest.

Acknowledgments

This study was supported by a grant provided by the Centers for Diseases Control of Taiwan (MOHW104-CDC-C-114-112402).

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