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Original Article

A longitudinal survey of methicillin-resistant *Staphylococcus aureus* carriage in nursing homes and the long-term care facility in Taiwan

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Sequence type 30;
Nursing homes

Abstract *Background:* We conducted a longitudinal survey for methicillin-resistant *Staphylococcus aureus* (MRSA) carriage in nursing homes and long-term care facilities (LTCFs) in northern Taiwan.
Methods: From July 2016 to February 2017, healthcare workers and residents in four institutions were enrolled. One swab sample from nares and another swab sample from umbilicus were obtained from each participant for detection of MRSA at enrolment and then follow-up samples were collected every two months for additional three times if feasible.
Results: We enrolled a total of 194 participants, including 127 residents and 67 healthcare workers. MRSA colonization rates were 23.2%, 22.8%, 20.7% and 18.6% at enrolment, the 2-, 4-, and 6-month follow-up survey, respectively, and the cumulative colonization rate was 40.2%. The MRSA detection rate was significantly higher at Institution 2 (70.7%) than that at other three institutions (25.7% ~ 35%) ($p < 0.001$). Among 78 MRSA carriers, 45 were found to be colonized at enrolment, and other 33 were newly identified as MRSA colonization during follow-up. Of 172 MRSA isolates identified, there were two major clones, sequence types (ST) 45 (49.4%), and ST30 (25%). ST45 prevailed in three institutions and ST30 prevailed in two institutions.
Conclusions: Nearly one in five residents or healthcare workers in nursing homes and LTCFs harbored MRSA, mostly ST45 or ST30 strains, at any given time point in the study. The prevalence

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and molecular epidemiology of MRSA could vary in different institutions and molecular evidence for intra- and inter-institutional spread of MRSA was provided.

Introduction

Staphylococcus aureus (*S. aureus*) is a common cause of skin and soft tissue infection. It can also cause severe infections, such as myositis, bone/joint infection, pneumonia, endocarditis, bacteremia, septicemia, necrotizing fasciitis, and toxic shock syndrome.[1,2]

Long-term care facilities (LTCFs) and nursing homes provide care for individuals with mental diseases, those in need of convalescent care, and those who are disabled, etc.[3,4] A substantial proportion of residents in LTCFs and nursing homes are discharged from acute care hospitals and may be colonized with multi-resistant microorganisms, such as methicillin-resistant *S. aureus* (MRSA), carbapenem-resistant *Acinetobacter baumannii*, or *Enterobacteriaceae*. The spread of MRSA strains has become a major issue in these institutions. Since the transition of institution residents to and from the hospital is a common situation, it is relevant to determine the magnitude of MRSA colonization in LTCFs and nursing homes.[3,4]

In Taiwan, there are limited reports about the MRSA colonization in LTCFs and nursing homes.[5–8] Here, we conducted a longitudinal survey for MRSA carriage among residents and healthcare workers (HCWs) in nursing homes and LTCFs in northern Taiwan and investigated the molecular evidence for intra- and inter-institutional spread of MRSA in these institutions.

Methods

Ethical approval and consent to participate

This study was approved by the Institutional Reviewing Board of Chang Gung Memorial Hospital (201509728B0) and an informed consent was obtained from each participant or legal representative.

Study subjects

The study was conducted in three nursing homes (Institution 1, 2 and 4) and one LTCF (Institution 3) in northern part of Taiwan. These nursing homes and LTCF were private institutions. From August 2016 to February 2017, all the healthcare workers and residents in these four institutions were invited to participate in the study. We obtained one swab sample from nares and another swab sample from umbilicus from each participant at enrolment and then collected the follow-up samples bimonthly for additional three times if feasible.

Sample collection and microbiologic methods

The swabs were obtained with sterile cotton-top swabs for the detection of MRSA. The swab sample was placed into the transport medium (VenturiTransystem; Copan Innovation, Copan Diagnostics, Italy) immediately and sent to the laboratory of Chang Gung Memorial Hospital.

Swab samples were inoculated onto Trypticase soy agar with 5% sheep blood plates and plates were incubated at 37 °C overnight. *S. aureus* was identified according to the presentation of beta-hemolysis, the colony morphology, Gram stain and coagulase test. The cefoxitin disk-diffusion method was used to identify MRSA according to the recommendation of Clinical and Laboratory Standards Institutes.[9]

Those participants who were negative with MRSA at enrolment but were then colonized with MRSA in the follow-up survey were classified into the "New detection" group in the Table 1.

To identify potential risk factors for MRSA colonization, clinical information was collected from each participant. For the residents, we collected demographic data, personal history, length of stay in the nursing home, underlying diseases, ambulatory status, dialysis status, the presence of chronic wounds, and insertion of medical devices. In addition, the information of antibiotic use, hospitalization, surgery, and infection events in recent three months were also collected. For HCWs, personal history, underlying diseases, and working information were collected.

Table 1 Detection of methicillin-resistant *Staphylococcus aureus* (MRSA) colonization among residents and healthcare workers in nursing homes and long-term care facility in northern Taiwan.

Characteristics	No. subjects	MRSA No. (%)		P value
		Positive	New detection	
Four surveys	194	78 (40.2)		0.716
At enrolment	194	45 (23.2)	—	
2-month follow-up	180	41 (22.8)	16 (8.9)	
4-month follow-up	174	36 (20.7)	8 (4.6)	
6-month follow-up	156	29 (18.6)	9 (5.8)	
All participants	194	78 (40.2)	33 (17.0)	0.166
Residents	127	56 (44.1)	24 (18.9)	
Healthcare workers	67	22 (32.8)	9 (13.4)	0.435
Taiwanese	30	8 (26.7)	3 (10)	
Non-Taiwanese	37	14 (37.8)	6 (16.2)	
Institution 1	35	9 (25.7)	3 (8.6)	<0.001
Institution 2	41	29 (70.7)	8 (19.5)	
Institution 3	78	26 (33.3)	16 (20.5)	
Institution 4	40	14 (35)	6 (15.0)	

Molecular typing

Chromosomal DNAs were extracted from MRSA isolates for molecular characterizations. The MRSA isolates were characterized by pulsed-field gel electrophoresis (PFGE) with *Sma*I digestion,[5] staphylococcal cassette chromosome (SCC*mec*) typing using the multiplex PCR method,[10] and the detection of Panton—Valentine leukocidin (PVL) genes using a PCR assay.[11] Representative isolates were further selected for multilocus sequence typing (MLST)[12] and *spa* typing.[13]

Pulsed-field gel electrophoresis (PFGE) with *Sma*I digestion was performed according to the previously published procedure.[5] The genotypes were designated in alphabetical order, as in our previous study; any new genotype, if identified, was designated consecutively.[5] PFGE patterns with minor band differences (fewer than four bands) from an existing genotype were defined as subtypes of that genotype. MRSA isolates sharing identical pulsotypes and subtypes were considered genetically indistinguishable, those having identical pulsotypes but distinct subtypes were considered genetically related, and those with distinct pulsotypes were considered genetically distinct.[5]

Statistical analysis

ANOVA was used to analyze significance of differences in MRSA carriage among residents and HCWs from different institutions. Student's t-test was used to compare two groups of continuous variables. Chi-square test or Fisher's exact test was used to compare categorical variables as appropriate. A p-value <0.05 was considered statistically significant difference. SPSS 22nd edition was used for statistical analysis.

Results

Nasal carriage of MRSA in nursing homes and long-term care facilities

A total of 208 subjects in four institutions were eligible and 194 participants (93%), including 127 residents and 67 HCWs (Taiwanese, 30; non-Taiwanese, 37), were enrolled. We enrolled 18 residents and 17 HCWs in Institution 1, 30 residents and 11 HCWs in Institution 2, 53 residents and 25 HCWs in Institution 3, and 26 residents and 14 HCWs in Institution 4 (Supplementary Table 1). All non-Taiwanese HCWs were from countries in Southeast Asia. 33 participants (13 HCWs and 20 residents) withdrew in the follow-up survey (Fig. 1).

The mean age of enrolled residents was 76.1 $\pm$ 23.3 years and 63 of them (49.6%) were female. The mean age of enrolled HCWs was 34.8 $\pm$ 23.3 years and 64 of them (95.5%) were female. Demographic data of participants in each institution is shown in Supplementary Table 1 in detail.

In the study, 704 specimens from umbilicus and 699 specimens from nares were obtained from enrolled participants, and 171 specimens, including 120 (17.2%) of nasal specimens and 51 (7.2%) umbilical specimens, were positive with MRSA (Supplementary Table 2). 32 carriers had isolates only identified from umbilicus. 172 MRSA isolates were identified, because one nasal specimen yields two MRSA isolates of distinct colony morphology.

The positive rates of MRSA were 23.2%, 22.8%, 20.7% and 18.6% at enrolment, and the 2-, 4-, and 6-month follow-up survey, respectively (Table 1). No significant difference in the MRSA colonization rate was found among four surveys. Of 194 participants, MRSA was detected at least once at any site in 78 participants (40.2%). 56 (44.1%) of residents and 22 (32.8%) of HCWs (8 Taiwanese and 14 non-Taiwanese) were colonized with MRSA (Table 1). No significant difference in the MRSA colonization rate was found between residents and HCWs. Of 78 carriers, MRSA was detected for 45 participants at enrolment and new detection of MRSA occurred in 33 (42.3%) participants during the follow-up survey (Table 1). MRSA was detected once for 36 participants, twice for 19, three times for 15 and four times for eight (Supplementary Table 3).

Among four institutions, the MRSA detection rate was significantly higher at Institution 2 (70.7%) than that at other three institutions (25.7% $\sim$ 35%) ($p < 0.001$) (Table 1). As shown in Supplementary Table 4, no significant risk factors for MRSA colonization in residents was identified in terms of underlying diseases, current diseases, use of nasogastric tube or foley catheter, smoking, alcohol drinking, duration of nursing home stay, and ambulation or

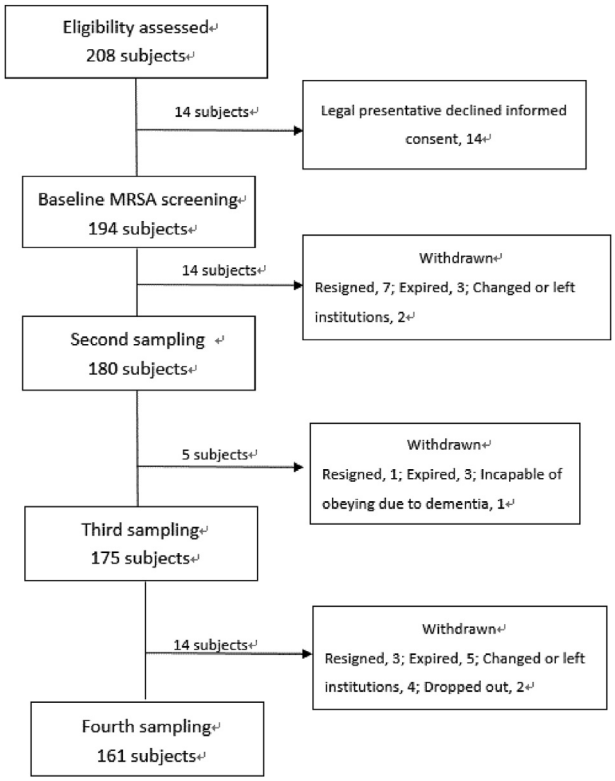

Figure. 1. The flow chart of a longitudinal survey of methicillin-resistant *Staphylococcus aureus* colonization among residents and healthcare workers in three nursing homes and one long-term care facility in Taiwan. A total of 33 participants withdrew, of whom 13 were healthcare workers (11 resigned and 2 dropped out) and other 20 were residents.

not. No significant risk factors for MRSA colonization were identified in HCWs either (Supplementary Table 5).

Molecular characterization of MRSA isolates

Within 172 MRSA isolates, six cannot be digested by *Sma*I and were classified as untypeable, and other 166 isolates were classified into eight pulsotypes with pulsotype BM and AG being dominant. All but three isolates possessed SCC*mec* type IV or V (including type V_T). PVL genes were detected in 18 isolates, of which 17 were pulsotype AI/ST8. The pulsotype BM/ST45 was the most prevalent clone, accounting for 49.4% of MRSA isolates, and was resistant to multiple antimicrobials. Other two major clones were ST30/SCC*mec* IV, accounting for 25% of isolates, and ST8/SCC*mec* IV/PVL-positive (USA 300), accounting for 9.9% of isolates. Details of molecular characterizations for these 172 isolates are shown in Table 2.

Of 78 carriers, multiple MRSA isolates were found from each of 44 carriers. Of 44 carriers with multiple isolates, 12 carriers had multiple isolates from different sampling sites at a single time point and ten of them had multiple isolates from different time points (Table 3). The colonized isolates were genetically indistinguishable in 19 (43.2%) of these 44 carriers, and were genetically identical or related in 13 (29.5%) of them. It was noted that seven isolates were identified from four different sampling time points from each of two carriers, and all isolates from one such carrier were genetically identical or related. By contrast, the colonized MRSA isolates from 12 (27.3%) carriers had at least two distinct genotypes (Table 3).

Of note, different clones of MRSA prevailed in different institutions (Table 4). The major clone pulsotype BM/SCC*mec* V/ST45 was prevalent in three institutions but the clone pulsotype AG/ST30 was prevalent at Institution 1. Besides, clones ST30 and ST8 were also frequently identified at Institution 2 and Institution 3, respectively. The molecular characteristics and prevalence of MRSA clones indicated intra- and inter-institutional transmission of MRSA in the study.

Of 37 non-Taiwanese HCWs, MRSA was detected in 14 (37.8%) of them, including seven of 16 subjects from Vietnam, four of 11 subjects from the Philippines and three of ten subjects from Indonesia. All 10 Indonesian HCWs worked at Institution 3, three of them had MRSA carriage and all MRSA isolates belonged to ST45/pulsotype BM. Three of six Vietnamese HCWs at Institution 3 were MRSA carriers and all MRSA isolates belonged toST8/pulsotype AI. The detailed information of MRSA carriage among 37 non-Taiwanese HCWs is shown in Table 5.

Table 2 Molecular characteristics of 172 MRSA isolates, categorized by pulsed-field gel electrophoresis (PFGE) patterns.

PFGE pattern	No. isolates (%)	SCC*mec* type	PVL-positive	MLST type	*Spa* typing
A	2 (1.16)	III	—	239	t037
B	1 (0.58)	III	—	239	t037
AG	43 (25.0)	IV	—	30	t019, t1826
AI	17 (9.9)	IV	17	8	t008
BM	81 (47.1)	V	—	45	t1081, t4981
	4 (2.32)	IV	—	45	t7513
C	6 (3.49)	IV	—	59	t437
	4 (2.32)	V_T	—	59	t441
D	1 (0.58)	V_T	1	59	t437
U	7 (4.07)	IV	—	573, 3372[a]	t3525, t16260
UT	6 (3.49)	V_T	—	398	t034

[a] A single locus variant of ST573.
SCC*mec*: staphylococcal cassette chromosome; MLST: multilocus sequence type.
PVL: Panton-Valentine leukocidin; UT: untypeable.

Discussion

This longitudinal study revealed that approximately one in five residents or healthcare workers was colonized with MRSA in the nursing homes and LTCFs in northern Taiwan at any given time point in the study. The cumulative rate of MRSA colonization could be up to two in five residents or healthcare workers when they have stayed or worked in the institutions for up to six months. A few studies have investigated the rate of MRSA colonization in nursing homes and LTCFs in Taiwan.[5–8] The nasal MRSA colonization rate was reported 20% among 523 participants (including 360 residents and 163 healthcare workers) in 14 nursing homes in a cross-sectional study in Taiwan in 2012.[5] Two other studies investigated multidrug-resistant organisms (MDROs) colonization among residents and in the environments in six LTCFs of Taiwan in 2015 and 2016, and revealed that MRSA was the most frequently identified pathogen among MDROs.[6–8] In 2015, the nasal MRSA colonization rate ranged from 11.4% to 27.4%,[6,7] and in 2016, the nasal MRSA colonization rate ranged from 10.5% to 23% among LTCF residents.[8] The MRSA colonization rate among LTCF residents in Taiwan was similar to that in UK,[14,15] Ireland,[16] Hawaii,[17] California[18,19] or Singapore,[20] but was higher than that in Germany[21] or Italy.[22,23] These results showed that the MRSA colonization rate in LTCFs may vary by region and study setting. Besides, the MRSA colonization could be transient or persistent among LTCF residents, as shown in the present study.

In the previous study,[5] age over 60 years and the presence of chronic wound were risk factors associated with MRSA colonization among nursing home residents; for healthcare workers, foreign nationality and chronic wound were significant risk factors for the MRSA carriage but none was significant in multivariate analysis. In the present study, there were no significant risk factor of MRSA colonization among residents as well as among healthcare workers. Such difference may be due to different designs (cross sectional vs. longitudinal study), different populations and sample sizes. In the study we found that MRSA colonization rate was significantly higher at Institution 2 (70.7%) than that at other three institutions (25.7% ~ 35%) ($p < 0.001$). This was noted at enrollment and persisted throughout the study period. We found that the staffing level is low at Institution 2 (1:3 HCW per resident ratio) in comparison to that at other institutions (1:1, 1:2 and 1:2

Table 3 Genetic relatedness of MRSA isolates in 44 carriers with multiple isolates by pulsotypes, stratified by the number of MRSA-positive samplings (two sites per one sampling) and isolates.

No. positive samplings (No. carriers)	No. MRSA isolates	No. carriers			
		Total	[a]Indistinguishable for all isolates	Indistinguishable or [a]related for all isolates	≥2 distinct pulsotypes
1 (n = 2)	2	2[b]	1	1	–
2 (n = 19)	2	17	7	6	4
	3	1[b]	1	–	–
	4	1[b]	–	–	1
3 (n = 15)	3	11	5	2	4
	4	1[b]	–	–	1
	5	2[b]	1	–	1
	6	1[b]	–	1	–
4 (n = 8)	4	4	4	–	–
	5	2[b]	–	1	1
	7	2[b]	–	2	–

[a] Two isolates sharing an identical pulsotype-subtype, or identical pulsotype but distinct subtypes were regarded as genetically indistinguishable or related, respectively.
[b] These 12 carriers had positive sampling for MRSA isolates from both nasal and umbilical sites at ≥ one sampling timepoint(s).

Table 4 Molecular characterizations of MRSA isolates identified from four institutions.

Characteristics/NHs (No. isolates)	Type A (n = 2)	Type C (n = 10)		Type U (n = 7)	Type AG (n = 43)	Type AI (n = 17)	Type BM (n = 85)		Untypeable (n = 6)
SCC*mec*	III	IV (n = 6)	V_T (n = 4)	IV	IV	IV	IV (n = 4)	V (n = 81)	V_T
MLST	239	59		573[b]	30	8	45		398
Spa typing	t037	t437	t441	t3525[b]	t019[b]	t008	t1081[b]	t7513	t034
Institution 1 (n = 12)[a]	2	–	–	1	7	1	–	–	–
Residents	1	–	–	1	4	–	–	–	–
HCWs	1	–	–	–	3	1	–	–	–
Taiwanese	–	–	–	–	–	–	–	–	–
Non-Taiwanese	1	–	–	–	3	1	–	–	–
Institution 2 (n = 79)	–	–	4	6	36	–	–	27	6
Residents	–	–	3	2	26	–	–	25	3
HCWs	–	–	1	4	10	–	–	2	3
Taiwanese	–	–	–	4	3	–	–	1	–
Non-Taiwanese	–	–	1	–	7	–	–	1	3
Institution 3 (n = 58)[a]	–	6	–	–	–	15	4	32	–
Residents	–	4	–	–	–	8	4	25	–
HCWs	–	2	–	–	–	7	–	7	–
Taiwanese	–	2	–	–	–	1	–	2	–
Non-Taiwanese	–	–	–	–	–	6	–	5	–
Institution 4 (n = 23)	–	–	–	–	–	1	–	22	–
Residents	–	–	–	–	–	–	–	18	–
HCWs	–	–	–	–	–	1	–	4	–
Taiwanese	–	–	–	–	–	1	–	3	–
Non-Taiwanese	–	–	–	–	–	–	–	1	–

[a] One of the isolate was singleton (pulsotype B/SCC III/ST239/t037 and pulsotype D/SCC VT/PVL-positive/ST59/t437, respectively).
[b] Included clonal complex variants of designated genotypes, respectively.

HCW per resident ratio for Institute 1, 3 and 4, respectively) and it remains unclear whether the staffing level would affect the MRSA colonization rate in the nursing home and LTCF. Besides, further studies are needed to investigate whether demographics and underlying diseases of participants, infection control measures and policies would affect the MRSA prevalence in the institution.

MRSA strains are traditionally classified as community-associated MRSA (CA-MRSA) and healthcare-associated MRSA (HA-MRSA) based on epidemiological and molecular characteristics.[2] However, such classification system has been challenged in recent years by a substantial spread of CA-MRSA into the health care settings.[2] In Taiwan, most HA-MRSA strains were ST239 and ST5 in the past two decades.

Table 5 Distribution of methicillin-resistant *Staphylococcus aureus* (MRSA) carriage among 37 non-Taiwanese health care workers in four long-term care facilities/nursing homes in Taiwan by countries.

Characteristics		Vietnam No. (%)	Philippines No. (%)	Indonesia No. (%)	Total No. (%)
Institution 1	No. Subject	7	3	0	10
	No. MRSA (+)	3	1	0	4
Institution 2	No. Subject	2	3	0	5
	No. MRSA (+)	0	3	0	3
Institution 3	No. Subject	6	0	10	16
	No. MRSA (+)	3	0	3	6
Institution 4	No. Subject	1	5	0	6
	No. MRSA (+)	1	0	0	1
Total	No. Subject	16	11	10	37
	No. MRSA (+)	7	4[a]	3	14
MRSA clones identified in carriers					
ST8/pulsotype AI		4	0	0	5
ST45/pulsotype BM		1	1	3	5
ST30/pulsotype AG		0	3	0	3
ST398/untypeable pulsotype		0	3	0	3
ST239/pulsotype A		1	0	0	1
ST59/pulsotype C		0	1	0	1
ST59/pulsotype D		1	0	0	1

[a] Two of these 4 subjects had MRSA multiple isolates of 2 distinct pulsotypes and one had MRSA multiple isolates of 3 distinct pulsotypes.

By contrast, the majority of CA-MRSA strains has been ST59 since 2000s, but the ST59 clone has also became a major clone in the hospital settings since 2010s.[2] The molecular epidemiology of MRSA in nursing homes and LTCFs was unclear before 2012. In a previous study,[5] ST45 accounted for half of MRSA isolates identified from residents and staffs in 14 nursing homes in 2012. Two other studies conducted in six LTCFs in 2015 and 2016 showed that ST59 (40%), ST45 (20—30%) and ST8 (11—22%) were three prevalent MRSA clones identified from residents and the environment.[7,8] In the present study, nearly half of MRSA isolates were ST45, followed by ST 30 (25%) and ST8 (USA300, ~10%), while previously endemic HA-MRSA ST239 and CA-MRSA ST59 clones accounted for less than 10% of MRSA isolates. These results suggest that molecular epidemiology of MRSA in LTCFs and nursing homes may be different from that in the hospital settings in Taiwan. However, a large-scale hospital-based nationwide study of molecular epidemiology of clinical MRSA isolates is lacking in Taiwan after 2010. A follow-up study to investigate potential impact of MRSA clones prevailing in LTCFs and nursing homes on the molecular epidemiology of MRSA in the hospital should be conducted in the near future.

In this study, two or more MRSA isolates were identified in more than half of MRSA carriers and 40% of them were positive with multiple MRSA isolates that shared indistinguishable pulsotypes. However, more than 25% of them had distinct pulsotypes of MRSA isolates during the study period, suggesting that some carriers might sequentially acquire another colonization of a new MRSA strain that replaces original strain. Moreover, over 40% of MRSA carriers in the study were tested negative for MRSA at enrollment but were subsequently colonized with MRSA during follow-up. These findings suggest that intra-institutional transmission of MRSA may exist in the nursing home and LTCF.

Our results showed that different MRSA clones prevailed in different institutions. The major clone ST45 prevailed in three institutions, ST30 prevailed in two institutions, and ST8 prevailed in one institution. We noted that two major clones could be identified in two institutions. These findings indicate that there may be intra- and inter-institutional spread of MRSA in the nursing home and LTCF. We found that a dominant MRSA clone could be found among most of non-Taiwanese HCW carriers from the same country (Table 5). This finding implies that MRSA may spread among HCWs from the same country, since they may work together in an institution or live or meet together during off-duty hours.

We showed that a considerable proportion of residents or healthcare workers in nursing homes and LTCFs were colonized with MRSA in Taiwan. Infection control measures should be implemented vigorously in these institutions. Hand hygiene and the usage of hand rubs cannot be overemphasized. Active surveillance for MRSA carriage may be conducted. Once the MRSA carrier is identified, contact precaution with cohort care and chlorhexidine bath, etc., could be implemented soon.

Limitations of this study included a relatively small number of study subjects (124 residents and 67 healthcare workers), the lack of whole genome sequence data for MRSA isolates, and the lack of interventions being applied and assessed during the study. Besides, the information on infection control measures and policies in each institution was lacking in the study, and such information would be helpful to clarify the variation in MRSA colonization rates among different institutions.

Conclusions

Nearly 20% of the residents and HCWs in nursing homes and LTCFs in northern Taiwan harbored MRSA, mostly ST45 or ST30 strains, at any given time point in 2016—2017; however, the cumulative colonization rate of MRSA was up to 40% during the study. Persistent carriage and new acquisition of MRSA colonization were present among carriers. The prevalence and molecular epidemiology of MRSA could vary among different institutions and also suggest that intra- and inter-institutional spread of MRSA may exist in nursing homes and LTCFs. Infection control measures should be implemented to control the spread of MRSA.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jmii.2021.10.005.