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Original Article

Acinetobacter baumannii and methicillin-resistant *Staphylococcus aureus* in long-term care facilities in eastern Taiwan

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Abstract

Objective: The prevention of infections is crucial in long-term care programs. Investigations of the occurrence and sources of pathogens in long-term care facilities (LTCFs) are still lacking, especially in eastern Taiwan. In this study, we conducted a surveillance of two common pathogens, *Acinetobacter baumannii* (AB) and methicillin-resistant *Staphylococcus aureus* (MRSA), in LTCFs in Hualien. **Materials and Methods:** Pathogenic assays including isolation, identification, and antimicrobial susceptibility tests were conducted for AB and MRSA at LTCFs in Eastern Taiwan. Staphylococcal cassette chromosome mec typing assays were done to understand the relatedness of clonal strains of MRSA. **Results:** All AB-positive samples in the LTCFs were mainly from water-rich samples and were drug susceptible. Our data indicated that the AB strains from LTCFs were similar to those from Puzi River watersheds in Taiwan, which were not drug resistant to commonly used antibiotics. On the other hand, the drug resistance analysis of MRSA indicated that the genotypes from the LTCFs were similar to those from nearby hospitals. Eight strains of MRSA were isolated from four LTCFs, of which five were identified as hospital-acquired strains according to SSCmed typing assays. **Conclusion:** These findings suggest that MRSA in LTCFs might propagate from hospitals and could be transmitted between hospitals and LTCFs. Health authorities should be aware of this risk. The long-term follow-up of MRSA is recommended in local medical institutions as well as in LTCFs for correlative analysis.

Keywords: *Acinetobacter baumannii, Long-term care facilities, Methicillin-resistant Staphylococcus aureus*

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Introduction

Long-term care facilities (LTCFs) are defined as institutions that provide services for patients who do not need acute treatment in a hospital but require long-term daily assistance [1-4]. Many private and government institutions offering long-term health-care services have opened in Taiwan to serve the rapidly aging population. The number of older people using the services of LTCFs in the USA is projected to increase from 15 million in 2000 to 27 million in 2050 [5]. Nosocomial infection is defined as an illness which is not present at the time of admission to the hospital but develops 48 h after admission or within 48 h after being discharged [6-8]. Since residents in LTCFs need to see physicians at both hospitals and LTCFs, the risks of nosocomial infections of multidrug-resistant bacteria in LTCFs may increase [9-11].

There were 4,012 deaths from nosocomial infections in 2008, the tenth leading cause of death that year in Taiwan [12]. A few nosocomial pathogens, known as "ESKAPE," which is an acronym for *Enterococcus faecium*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Acinetobacter baumannii* (AB), *Pseudomonas aeruginosa,* and *Enterobacter* spp., have been found to associate with antibacterial resistance and are responsible for serious infections [13,14]. Multidrug-resistant bacteria are life-threatening to severely ill and immunocompromised individuals [15]. *S. aureus* is commonly isolated from premature neonates and patients on dialysis [16]. In the United States, methicillin-resistant *S. aureus* (MRSA) infections are normally treated with parenteral vancomycin [17,18]. AB is a nosocomial pathogen which leads to opportunistic infection in immunocompromised individuals [19]. It is well documented that the majority of AB isolated from hospitals are multiple drug-resistant strains [20-23].

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Studies have shown a possible transmission pathway between hospitals, LTCFs, and local communities [8,24-28]. Healthcare-associated infections resulting from MRSA and antimicrobial-resistant AB have been documented, indicating a potential crisis for the spread of drug-resistant bacteria to patients living in nearby LTCFs [25]. There are several reports of cluster infections among residents caused by long-term social contact with patients and personnel in LTCFs [25,26,29]. In a series of studies on the genotyping of MRSA in hospitals and LTCFs pulsed-field gel electrophoresis patterns showed a strong correlation between genetics and geography, suggesting a spreading effect to adjacent institutions [30-32]. Heterogeneous typing of the mobile genetic element, staphylococcal cassette chromosome mec (SCCmec) of MRSA acquired from hospital, community, and others environments is a convenient alternative typing method for discriminating the origins of the pathogen [33-35]. In general, speaking, hospital-acquired MRSA are dominated by Type I, II, and III, while Type IV and V are prevalent in community-acquired strains with the majority containing genes that encode the panton-valentine leukocidin (PVL) toxin. Recent studies have shown MRSA can be isolated from environments other than hospitals and communities and the pathogen is associated with the usage of antibiotics in the husbandry industry in the raising of livestock. Jayaweera and Kumbukgolla have confirmed that livestock-associated MRSA resembles the Type IV and V strains except for the existence of the PVL gene [36]. Owing to concerns about cross infection from hospital-acquired infections, many LTCFs impose quarantine policies on patients returning from hospitals [37-40].

An environmental survey addressing the common pathogens AB and MRSA in LTCFs in Taiwan focused on western Taiwan where the majority of the population resides [4]. There are numerous of medical centers and regional hospitals in western Taiwan, and residents have access to more hospitals than residents in eastern Taiwan, making it difficult to analyze the evolutionary connection of bacteria between LTCFs and hospitals. In the Hualien area, the single medical center, Tzu Chi Hospital, is surrounded by several LTCFs. The aim of this study is to investigate the occurrence of nosocomial-acquired pathogens in LTCFs in eastern Taiwan and track microbial sources associated with Hualien Medical Center.

Materials and methods

Sample collection

In total, 154 environmental samples (including moist and arid samples) from four LTCFs in Eastern Taiwan were subjected to the detection of AB and MRSA [Figure 1]. The approximate geographical coordinates (latitude/longitude) of the LTCF B, C, D, and E are (23.970045, 121.571641), (23.958705, 121.547541), (24.126644, 121.651398), and (23.960602, 121.599818), respectively. For each LTCF, the sampled areas comprised private areas and appliances (including doorknobs, living room floors, bedding, bed rails, curtains, bathroom floors, sinks, toilet bowls, toilet seats, drinking fountains, nasogastric tubes, and washbasins) and public areas (including hall railings, public sinks, and drinking fountains). Sterile cotton swabs were used to wipe the surface areas

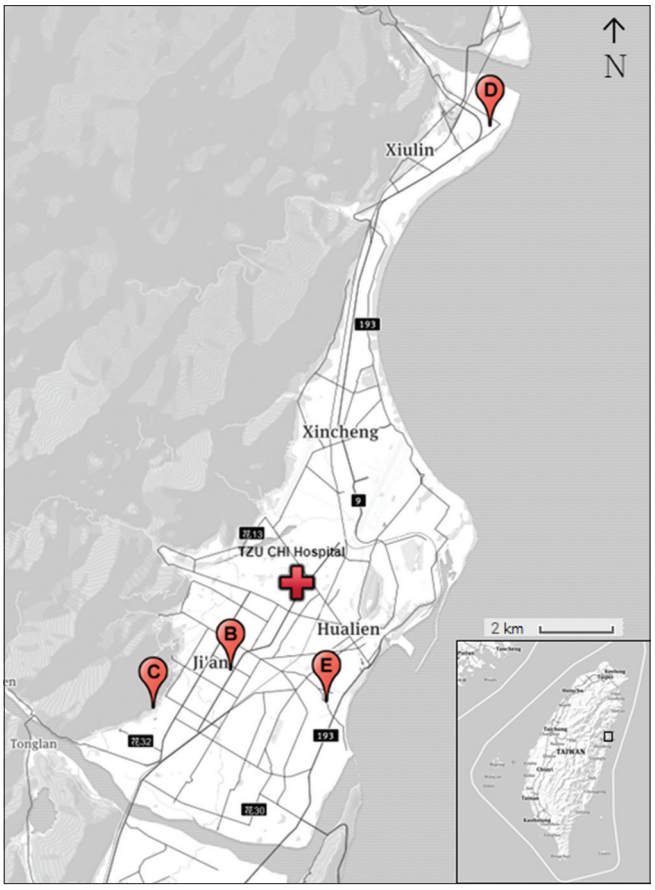

Figure 1: Sampling locations of the four long-term care facilities in eastern Taiwan

of sampled spots. Water from private bathrooms, public bathrooms, and used nasogastric tubes were collected for pathogen detection. For the water samples, 300 mL was filtered through 45-mm-diameter cellulose nitrate membranes (Pall, Michigan, USA) with a pore size of 0.45 μm. After filtration, the membranes were incubated with MacConkey Broth (Merck, Darmstadt, Germany) and Trypticase Soy Broth supplemented with 6.5% NaCl (TPM ready-to-use media, Taipei, Taiwan) for the enrichment of AB and MRSA, respectively. To collect bacteria, the cotton swabs were immersed in 2 mL of 1X phosphate buffer saline (PBS) and vortexed thoroughly for subsequent enrichment. Nasogastric tubes were washed with 5 mL of PBS. One-tenth of the elution buffer was transferred to the specific enrichment media for growth and detection of AB and MRSA. A series of steps for specific pathogen selection and enrichment were carried out to harvest these pathogens.

Isolation of *Acinetobacter baumannii* and methicillin-resistant *Staphylococcus aureus*

Two steps for selective culture were used to grow AB on inoculation agar using CHROMagar™ *Acinetobacter* (TPM ready-to-use media, Taipei, Taiwan) and 5% sheep blood agar (TPM ready-to-use media, TPM150M). CHROMagar™ *MRSA* (TPM ready-to-use media) and Baird-Parker agar (TPM ready-to-use media) were used for selective growth of MRSA. After inoculation, the agar plates were incubated at 30°C for 24 h. The colonies on the inoculation plates were transferred to sterile tubes containing brain-heart infusion broth. Bacterial

DNA in a volume of 300–600 μL in the broth was isolated for further species identification, molecular characterization, and drug resistance analysis.

Identification of *Acinetobacter baumannii* and methicillin-resistant *Staphylococcus aureus*

Bacterial DNA was extracted by commercial kits (MagPurix Viral DNA Extraction Kit ZP02006) under an automated DNA extraction system (MagPurix 12s Automated Nucleic Acid Purification System, Zinexts Life Science Corp., Taipei, Taiwan) according to the user manual. The total DNA eluate (2 μL) was mixed with the primers (1 μL each, 0.4 μM), Fast-Run Taq master mix with dye (5 μL), and deionized water (16 μL) to make a final reaction volume of 25 μL. The primers and thermal cycling used are summarized in Table 1.

For AB and MRSA detection [41-43], DNA extractions of positive controls (AB, ATCC 19606, and MRSA ATCC 29213) were also included in each run. Amplicons of polymerase chain reaction (PCR) products were electrophoresed on 2% agarose gel (AMRESCO, Solon, US). Gels were stained with a solution of ethidium bromide for visualization under ultraviolet (UV) light.

Enterobacterial repetitive intergenic consensus-polymerase chain reaction for *Acinetobacter baumannii* and methicillin-resistant *Staphylococcus aureus*

Enterobacterial repetitive intergenic consensus (ERIC) - PCR was performed as described in Rivera *et al*. with some modifications [44]. The primers ERIC-1R (5'-ATG TAA GCT CCT GGG GAT TCA C-3') and ERIC-2 (5'-AAG124 TAA GTG ACT GGG GTG AGC G-3') were employed to amplify the ERIC-PCR fingerprints of AB and MRSA [Table 1]. The mixture (25 μL) consisted of dNTP (200 μM), HiFi DNA polymerase (Yeastern Biotech, Tapei, Taiwan), $MgCl_2$ (3 mM), Tris-HCl (pH = 9.0, 10 mM), primers (1.0 μM each), and DNA templates (50 ng) with distilled sterile water to make the final volume of 50 μL [45]. The thermal cycling conditions for AB and MRSA are summarized in Table 1. Electrophoresis was carried out to separate the amplicons of the ERIC-PCR products on agarose gel 1.5% (Biobasic Inc.,) containing Tris-acetate-EDTA (TAE) buffer and 1 μg/mL ethidium bromide at 100 V for 30 min. Gels were visualized with a UV transilluminator.

ERIC-PCR patterns (PCR) were analyzed with the Bionumerics (Applied Maths, Inc., Austin, USA) software package. The relationship between two given isolates was scored by the Jaccard similarity coefficient, and isolates were clustered into groups of inter-isolation similarities based on the unweighted pair group method with arithmetic averages.

Antibiotic susceptibility of *Acinetobacter baumannii* and methicillin-resistant *Staphylococcus aureus*

All AB and MRSA isolates were tested for antibiotic susceptibility with Kirby–Bauer disk diffusion tests (BD BBL, Sparks, USA) on Mueller-Hinton agar plates (TPM ready-to-use media) according to the National Committee for Clinical Laboratory Standards. The antibiotics and dosages tested in this study were as follows: ciprofloxacin (5 μg), clindamycin (2 μg), erythromycin (15 μg) cefepime (30 μg), gentamicin (10 μg), imipenem (10 μg), ampicillin/sulbactam (20/10 μg), sulfamethoxazole/trimethoprim (23.75/1.75 μg), and tetracycline (30 μg).

Virulence Gene profile and staphylococcal cassette chromosome mec with PVL typing assay for methicillin-resistant *Staphylococcus aureus*

The DNA elution of MRSA was subjected to virulence gene and SCCmec PCR for toxin and SSCmec typing [46-49]. The primer information for virulence genes and SCCmec with PVL for MRSA is shown in Table 1. The mixture (25 μL) consisted of dNTP (200 μM), Taq polymerase (1.8 U, Biolabs), $MgCl_2$ (3 mM), Tris-HCl (pH = 9.0, 10 mM), primers (1.0 μM each), and DNA templates (50 ng) with distilled sterile water to make a final volume of 50 μL [46-48]. Electrophoresis was carried out to separate the amplicons of ERIC-PCR products on agarose gel 1.5% (Biobasic Inc.,) containing TAE buffer and 1 μg/mL ethidium bromide at 100 V for 30 min. Gels were visualized with a UV transilluminator.

Results

Methicillin resistant *Staphylococcus aureus* and *Acinetobacter baumannii* detection rates at four long-term care facilities

The occurrence of AB and MRSA in moist and arid samples is summarized in Table 2. In this study, MRSA and AB were detected in 5.2% and 2.6% of all samples, respectively. MRSA was present in three of the five nasogastric tubes sampled, while AB was isolated from two types of aquatic samples, for example, drinking fountains and sinks. The hot spots for AB were high humidity environments such as drinking fountains and washbasins. MRSA was mostly associated with bathrooms, suggesting a connection with feces contamination.

Characterization of methicillin-resistant *Staphylococcus aureus* and *Acinetobacter baumannii* by enterobacterial repetitive intergenic consensus-polymerase chain reaction fingerprinting

The ERIC-PCR fingerprint analysis outcomes are shown in Table 3. Data from cluster analysis by ERIC-PCR fingerprinting showed that three strains of AB, classified into AB-1, AB-2, and AB-3 clusters were isolated from different LTCFs. Both AB-2 and AB-3 were isolated from drinking fountains and presented high similarity in ERIC PCR-based analysis. Eight strains of MRSA were divided into six subtypes in ERIC PCR-based analysis. The MRSA-1 type was found in three different LTCFs. AB was detected on bathroom floors, toilet seats, and nasogastric tubes. The MRSA strains from nasogastric tube samples comprised different subtypes in ERIC fingerprinting analysis, although they were all from the same LTCF.

Antimicrobial susceptibility of *Acinetobacter baumannii* and methicillin-resistant *Staphylococcus aureus*

The antimicrobial susceptibility outcomes are also shown in Table 3. All AB strains were sensitive to antibiotics listed in the table. In the MRSA resistance study, a total of five strains met the definition of multidrug-resistant strains. These five strains were isolated from three LTCFs, of which two were from bathroom floor samples. MRSA-6, with the most serious potential for drug resistance, was isolated from nasogastric

Table 1: Detail parameters in primers, reaction formula, and polymerase chain reaction conditions for identifying pathogens from environmental samples

Pathogens	Target gene	Analysis	Size	Sequence (5' to 3')	Reaction Materials Final Volume: 25 μL	PCR condition	Reference
A. baumannii	ITS region	Isolates identification	208	p-Ab-ITSF: 5'-CATTATCACGGTAATTAGTG-3' p-AbI-TSB: 5'-AGAGCACTGTGCACTTAAG-3'	DNA: 100-300 ng Primer: 400 nM Master mix: 5 μL	Predenaturation: 94°C 5 min Denaturation: 94°C 30s Annealing: 55°C 30s Extension: 72°C 30s DAE cycles: 30 cycles Final extension: 72°C 7 min	[41]
A. baumannii	ERIC	Isolates typing		ERIC1R: 5′-ATGTAAGCTCCTGGGGATTCAC-3′ ERIC2: 5′-AAGTAAGTGACTGGGGTGAGCG-3′	DNA: 100-300 ng Primer: 1000 nM Master mix: 5 μL	Predenaturation: 95°C 7 min Denaturation: 95°C 60s Annealing: 52°C 60s Extension: 65°C 8 min DAE cycles: 30 cycles Final extension: 65°C 10 min	[44]
Methicillin-resistant *S. aureus*	*nuc* *mecA*	Isolates identification	270 448	nuc-F 5′-GCGATTGATGGTGATACGGTT-3′ nuc-R 5′-AGCCAAGCCTTGACGAACTAAAGC-3′ mecA-F 5′-CTCAGGTACTGCTATCCACC-3′ mecA-R 5′-CACTTGGTATATCTTCACC-3′	DNA: 100-300 ng Primer: 400 nM nuc FR and mecA FR Master mix: 5 μL	Predenaturation: 95°C 5 min Denaturation: 94°C 60s Annealing: 55°C 60s Extension: 72°C 60s DAE cycles: 30 cycles Final extension: 72°C 10 min	[42,43]
Methicillin-resistant *S. aureus*	ERIC	Isolates typing	-	ERIC1R: 5′-ATGTAAGCTCCTGGGGATTCAC-3′ ERIC2: 5′-AAGTAAGTGACTGGGGTGAGCG-3′	DNA: 100-300 ng Primer: 500 nM Master mix: 5 μL	Denaturation-1: 95°C 5 min Annealing-1: 36°C 1 min Extension-1: 72°C 4 min DAE-1 cycles: 1 cycles Denaturation-2: 95°C 1 min Annealing-2: 36°C 1 min Extension-2: 72°C 4 min DAE-2 cycles: 35 cycles Final extension: 72°C 8 min	[44]
Methicillin-resistant *S. aureus*	*entA* *entB* *entC* *entD* *entE* *tsst-1*	Virulence gene detection	121 478 459 384 495 271	entA-F: 5′-TTGGAACGGTTAAAACGAA-3′ entA-R: 5′-GAACCTTCCCATCAAAAACA-3′ entB-F: 5′-TCGCATCAAACTGACAAACG-3′ entB-R: 5′-GCAGGTACTCTATAAGTGCC-3′ entC-F: 5′-GGAGGAATAACAAAACATGAAGG-3′ entC-R: 5′-AAAGGCAAGCACCGAAGTAC-3′	DNA: 100-300 ng Primer: 400 nM Primer FR Master mix: 5 μL	Predenaturation: 94°C 5 min Denaturation: 94°C 1 min Annealing: 2 min Extension: 72°C 1 min DAE cycles: 35 cycles Final extension: 72°C 5 min	[49]

Contd...

Table 1: Contd...

Pathogens	Target gene	Analysis	Size	Sequence (5' to 3')	Reaction Materials Final Volume: 25 µL	PCR condition	Reference
	eta *etb*		464 200	entD-F: 5′-TGGTGGTGAAATAGATAGGAC-3′ entD-R: 5′-TGAAGGTGCTCTGTGGATAAT-3′ entE-F: 5′-TGGTAGCGAGAAAAGCGAAG-3′ entE-R: 5′-TGTAAATAATGCCTTGCCTGAA-3′ tsst-1-F: 5′-CTGGTATAGTAGTGGGTCTG-3′ tsst-1-R: 5′-AGGTAGTTCTATTGGAGTAGG-3′ eta-F: 5′-TTTGCTTTCTTGATTTGGATTC-3′ eta-R: 5′-GATGTGTTCGGTTTGATTGAC-3′ etb-F: 5′-ACGGCTATATACATTCAATT-3′ etb-R: 5′-TCCATCGATAATATACCTAA-3′		AnnealingTemp. *entA*: 50°C *entB*: 55°C *entC*: 59°C *entD*: 51°C *entE*: 55.5°C *tsst-1*: 54°C *eta*: 54°C *etb*: 50.9°C	
Methicillin-resistant *S. aureus*	SCCmec I SCCmec II SCCmec III SCCmec V	SCCmec typing	613 398 280 325	Type I-F: 5'-GCTTTAAAGAGTGTCGTTACAGG-3' Type I-R: 5'-GTTCTCTCATAGTATGACGTCC-3' Type II-F: 5'-CGTTGAAGATGATGAAGCG-3' Type II-R: 5'-CGAAATCAATGGTTAATGGACC-3' Type III-F: 5'-CCATATTGTGTACGATGCG-3' Type III-R: 5'-CCTTAGTTGTCGTAACAGATCG-3' Type V-F: 5'-GAACATTGTTACTTAAATGAGCG-3' Type V-R: 5'-TGAAAGTTGTACCCTTGACACC-3'	DNA: 100-300 ng Primer: 48 nM I-FR, 32 nM II-FR, 40 nM III-FR, 104 nM IVa-FR, 92 nM IVb-FR, 78 nM IVc-FR, 280 nM IVd-FR, 60 nM V-FR Master mix: 5 µL	Predenaturation: 94°C 5 min Denaturation-1: 94°C 45s Annealing-1: 65°C 45s Extension-1: 72°C 1.5 min DAE-1 Cycles: 10 cycles Denaturation-2: 94°C 45s Annealing-2: 55°C 45s Extension-2: 72°C 1.5 min DAE-2 cycles: 25 cycles Final extension: 72°C 10 min	[47]
Methicillin-resistant *S. aureus*	SCCmec I SCCmec II SCCmec II, III SCCmec III SCCmec III SCCmec I, II, IV	SCCmec typing	495 284 209 243 414 342	CIF2 F2: 5'-TTCGAGTTGCTGATGAAGAAGG-3' CIF2 R2: 5'-ATTTACCACAAGGACTACCAGC-3' KDP F1: 5'-AATCATCTGCCATTGGTGATGC-3' KDP R1: 5'-CGAATGAAGTGAAAGAAAGTGG-3' MECI P2: 5'-ATCAAGACTTGCATTCAGGC-3' MECI P3: 5'-GCGGTTTCAATTCACTTGTC-3' RIF F3: 5'-GTGATTGTTCGAGATATGTGG-3' RIF R9: 5'-CGCTTTATCTGTATCTATCGC-3' RIF F10: 5'-TTCTTAAGTACACGCTGAATCG-3' RIF R13: 5'-GTCACAGTAATTCCATCAATGC-3' DCS F2: 5'-CATCCTATGATAGCTTGGTC-3' DCS R1: 5'-CTAAATCATAGCCATGACCG-3'	DNA: 100-300 ng Primer: 400 nM CIF-FR, 200 nM KDP-FR, 200 nM RIFF3R9 400 nM MECI-FR, 400 nM RIFF10R13, 800 nM DCS-FR Master mix: 5 µL	Predenaturation: 94°C 4 min Denaturation: 94°C 30s Annealing: 53°C 30s Extension: 72°C 1 min DAE cycles: 30 cycles Final extension: 72°C 4 min	[46]

Contd...

Table 1: Contd...

Pathogens	Target gene	Analysis	Size	Sequence (5' to 3')	Reaction Materials Final Volume: 25 µL	PCR condition	Reference
Methicillin-resistant *S. aureus*	PVL	PVL detection	433	PVL-1: 5'-ATCATTAGGTAAAATGTCTGGACATGATCCA-3' PVL-2: 5'-GCATCAAGTGTATTGGATAGCAAAAGC-3'	DNA: 100-300 ng Primer: 400 nM FR Master mix: 5 µL	Predenaturation: 94°C 5 min Denaturation: 94°C 40s Annealing: 53°C 40s Extension: 72°C 1 min DAE cycles: 35 cycles Final extension: 72°C 10 min	[48]

PCR: Polymerase chain reaction, PVL: Panton valentine leukocidin, *A. baumannii*: *Acinetobacter baumannii*, *S. aureus*: *Staphylococcus aureus*, SCCmec: Staphylococcal cassette chromosome mec

tubes. Some strains of MASA showed moderate resistance to antibiotics.

Staphylococcal cassette chromosome mec typing and virulence gene assays of methicillin-resistant *Staphylococcus aureus*

In the virulence gene study, our results indicated that all strains of MRSA comprised eta genes, which belong to a wide range of virulence factors and are associated with exfoliative toxins [Table 4]. The other two strains (106HT-NH-MRSA14111) and (106HT-NH-MRSA13911) that possess entC genes were found in LTCF-D, while the 106HT-NH-MRSA20211 strain from nasogastric tube samples comprised three virulence genes (entA, entE, eta). Using the SCCmec typing method [Table 4], five out of eight strains were classified as SCCmec Type I (1/5), and III (4/5), which are all hospital-acquired MRSA. The other three strains resemble SCCmec Type IV except for the PVL genes, suggesting the involvement of livestock-associated MRSA in LTCFs. Samples of the hospital-acquired strains (Type I and III) were taken from bathroom floors, toilet seats, nasogastric tubes, and bedding.

Discussion

A previous study conducted in western Taiwan has shown MRSA which was not the predominant species in the hospital was the most common pathogen in LTCFs. That study also indicated that MRSA and AB occur significantly less frequently in LTCFs than hospitals [4]. Our data from the occurrence of both pathogens in LTCFs were consistent with the previous study. The identification of nosocomial pathogens in LTCFs compared with those in hospitals is necessary to reveal the relationship between LTCF-acquired and nosocomial pathogens. It has been documented that *S. aureus* is capable of surviving for days to weeks on dry inanimate surfaces [11,40]. AB was only found in high moisture environments in this study. Most sampling sites in this study were low moisture, which can explain why the incidence of *S. aureus* was two-fold higher than that of AB.

In the virulence gene study, three-eighths of MRSA contented ent genes. The ent genes are mainly associated with enterotoxins, which are made of antigens constructed from polypeptide chains. They can bind to MHC-II on macrophages, and interact with T-cell receptor β, resulting in the release of T-cell proliferation cytokines and causing systemic disease [50,51]. When comparing typing results from Tables 3 and 4, there was no association between the types of virulence genes and the subtypes in ERIC fingerprint analysis. However, it is worth noting that the MRSA-1 was detected in many LTCFs, which could be one of the main epidemic bacteria in the region. Further surveillance of these potential pathogens in local hospitals is suggested for the understanding of their transmission pathways and co-evolution.

Our data revealed that all MRSA strains were resistant to gentamicin and many strains (more than 50%) showed resistance to erythromycin and ciprofloxacin, which is consistent with other findings in LTCFs from different countries [35,39,52]. Our test results provide valuable information for infection

Table 2: The occurrences of methicillin resistant *Staphylococcus aureus* and *Acinetobacter baumannii* by various methods from arid or moist samples in long-term care facilities of Eastern Taiwan

Sample types	MRSA (by medium cultivation method)	MRSA (by isolation method)	AB (by membrane filtration method)	AB (by isolation method)
Total	8/154	8/154	4/154	3/154
	5.2%	5.2%	2.6%	1.9%
Arid samples	1/75	1/75	0/75	0/75
	1.3%	1.3%	0%	0%
Moist samples	7/79	7/79	4/79	3/79
	8.9%	8.9%	5.1%	3.8%

Environmental moist and arid samples of residences were subjected for testing of pathogens. MRSA: Methicillin-resistant *Staphylococcus aureus*, AB: *Acinetobacter baumannii*

Table 3: The enterobacterial repetitive intergenic consensus typing and antibiotic susceptibility outcomes for *Acinetobacter baumannii* and methicillin resistant *Staphylococcus aureus*

n	Name	Location	ERIC typing	ERIC pattern MW	C	CIP	FEP	G	I	SAM	S/T	T	MDR
1	106HT-NH-AB05811	LTCF B- washbasins	AB-1	847, 912, 1481	-	S	S	S	S	S	S	S	X
2	106HT-NH-AB09211	LTCF C- drinking fountain	AB-2	560, 857, 1068, 1496	-	S	S	S	S	S	S	S	X
3	106HT-NH-AB16711	LTCF D- drinking fountain	AB-3	857, 1073, 1240, 1507	-	S	S	S	S	S	S	S	X
1	106HT-NH-MRSA03411	LTCF B- bathrooms	MRSA-1	166, 204, 384, 1032	S	R	S	S	R	S	S	R	V
2	106HT-NH-MRSA12411	LTCF D- stool seats	MRSA-2	154, 1035	S	S	S	R	R	S	S	I	X
3	106HT-NH-MRSA13911	LTCF D- bathrooms	MRSA-1	157, 200, 381, 1045	S	R	S	R	R	S	S	S	V
4	106HT-NH-MRSA14111	LTCF D- stool seats	MRSA-3	146, 193, 370, 549, 1062	S	R	I	R	R	S	S	R	V
5	106HT-NH-MRSA17411	LTCF E- bedding	MRSA-1	144, 185, 352, 1057	I	R	I	I	R	S	S	R	V
6	106HT-NH-MRSA19811	LTCF B- nasogastric tubes	MRSA-4	156, 197, 1022	S	S	S	S	R	S	S	I	X
7	106HT-NH-MRSA20011	LTCF B- nasogastric tubes	MRSA-5	151, 189, 776, 994	I	S	S	S	R	S	S	S	X
8	106HT-NH-MRSA20211	LTCF B- nasogastric tubes	MRSA-6	161, 981	S	R	R	R	R	S	R	R	V

C: Chloramphenicol, CIP: Ciprofloxacin, DA: Clindamycin, E: Erythromycin, FEP: Cefepime, G: Gentamicin, I: Imipenem, SAM: Ampicillin-sulbactam, S/T: Sulfamethoxazole-trimethoprim, T: Tetracycline, LTCF: Long-term care facilities, AB: *Acinetobacter baumannii*, MRSA: Methicillin resistant *Staphylococcus aureus*, ERIC: Enterobacterial repetitive intergenic consensus, MDR: Multidrug resistant

Table 4: The staphylococcal cassette chromosome mec typing and virulence gene assays of methicillin resistant *Staphylococcus aureus* strain isolated from long-term care facilities

n	Name	Location	Oliveira SSCmec	Asghar PVL	Virulence gene
1	106HT-NH-MRSA03411	LTCF B- bathrooms	III	-	*eta*
2	106HT-NH-MRSA12411	LTCF D- stool seats	IV	-	*eta*
3	106HT-NH-MRSA13911	LTCF D- bathrooms	I	-	*entC, eta*
4	106HT-NH-MRSA14111	LTCF D- stool seats	III	-	*entC, eta*
5	106HT-NH-MRSA17411	LTCF E- bedding	III	-	*eta*
6	106HT-NH-MRSA19811	LTCF B- nasogastric tubes	IV	-	*eta*
7	106HT-NH-MRSA20011	LTCF B- nasogastric tubes	IV	-	*eta*
8	106HT-NH-MRSA20211	LTCF B- nasogastric tubes	III	-	*entA, entE, eta*

HA-MRSA: Oliveira SSCmec I, II, III without PVL genotype, CA-MRSA: Oliveira SSCmec IV, V with PVL genotype, LA-MRSA: Oliveira SSCmec IV, V without PVL genotype. PVL: Panton valentine leukocidin

control for LTCFs in eastern Taiwan. A long-term surveillance and clearance system is urgently needed to prevent the occurrence of drug-resistant pathogens, as residents of LTCFs are elderly and some are immunocompromised. Environmental studies of LTCFs and their personnel are limited in Taiwan. A recent investigation of nosocomial pathogens in LTCF personnel and environments in western Taiwan revealed that the occurrence of nosocomial pathogens was significantly higher in the personnel than in LTCF environments [4]. In the study, there was a quantitative parallel relationship between samples from the bodies of personnel and their residences, suggesting a close relationship between infection in a person and contamination from the environment. In addition, the majority of MRSA belong to strains in the multidrug-resistant category. Together with our finding, these results imply that antibiotic-resistant bacteria are the predominant species in LTCFs, possibly due to the abuse of antibiotics in our health-care system. The relationship between nosocomial and LTCF-associated infections is not entirely clear, and the role of LTCF environments in the preferential selection of bacterial growth needs to be determined. Future studies should compare the typing of pathogens in district hospitals and surrounding LTCFs.

MRSA was identified as a hospital-acquired infection but has developed into an endemic species and is now community-acquired. A rapid molecular beacon real-time PCR assay for SCCmec typing for MRSA has been developed [53]. This assay is able to discriminate community-acquired MRSA (Type IV) from hospital-acquired MRSA

(Types I–III) [47]. Descriptive research covering many countries in Asia has shown that Type III SCCmec was the dominant strain in hospital-acquired MRSA in most countries except for Japan and South Korea [54]. A later study from Taiwan confirmed this [55]. An investigation in medical centers in Taiwan, including Tzu Chi General Hospital, showed that 55% of a total of 561 isolates of MRS were SCCmec Type III [56]. Our results of SCCmec typing imply that the majority of MRSA in LTCFs in eastern Taiwan originate from local hospitals. Livestock-associated MRSA was also isolated in this study, suggesting contamination from the local husbandry industry. Further studies are needed to confirm the geographical relationship of transmission routes. Community-acquired MRSA was not detected in this study, further leading to an association of hospital-acquired MRSA with LTCFs in Eastern Taiwan.

Our antibiotic sensitivity tests showed most AB strains were sensitive to antibiotics. Our previous study showed that most environmental AB strains, which differ from nosocomial species, are drug sensitive. Only about 5% of them were tetracycline resistant. This study revealed that AB strains from LTCFs were sensitive to all antibiotics, a distinctive feature of drug resistance different from nosocomial species. The evidence implied that the AB bacteria isolated from LTCFs was not transferred from medical institutions by patients and may come from an outdoor environment. This study is consistent with our previous surveillance in an aquatic environment and is not consistent with other studies conducted in hospitals [57-59], which imply the AB isolates in this study may have come from contamination from a local aquatic environment. The MRSA isolates have the same features as hospital-acquired MRSA in antimicrobial susceptibility tests, such as resistance to ciprofloxacin and gentamicin; and in SCCmec typing [56,60], hospital-acquired MRSA-6 was isolated from nasogastric tubes, suggesting an association of drug-resistant strains in LTCF residents with frequent contact with nosocomial strains from hospitals. Some strains of MASA showed moderate resistance to antibiotics, similar to the strains isolated from hospitals, indicating a potential of MRSA propagation from local hospitals. Further investigations are necessary to identify the origins of multidrug-resistant strains of MRSA in LTCFs in eastern Taiwan. It is most likely that the MRSA isolates in this study originated from hospitals. Nevertheless, more studies are required to support this conclusion.

Conclusion

We conclude that of MRSA occurs at relatively higher rates than AB bacteria in LTFCs with about 5% of isolates in this study containing MRSA and 2% containing AB. The AB strains, which were detected mainly in aquatic environments, are more diverse, while MRSA-1 is more common in strains at LTCFs in Eastern Taiwan area.

There was no correlation between genotyping and drug resistance or toxicity genes of bacteria. All MRSA strains contained the eta gene in virulence genes analysis. This gene is mainly associated with exfoliative toxins. In the isolates for drug resistance, our data showed that three strains of AB bacteria and eight strains of MRSA were isolated from the environment in LTFCs. Among them, the MRSA isolates had more pronounced drug resistance while the AB strains were more sensitive to antibiotic treatment, suggesting the AB strains migrated from the local environment, whereas the MRSA strains originated from hospitals. Results of SCCmec typing assay of MRSA also favor a connection between LTCFs and hospitals. This study provides a theoretical basis for enforcement of quarantine policies and procedures in LTCFs for patients returning from hospitals.

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Conflicts of interest

There are no conflicts of interest.

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