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Original Article

Clonal spread of carbapenem-resistant *Acinetobacter baumannii* across a community hospital and its affiliated long-term care facilities: A cross sectional study

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Abstract *Background:* The global spread of carbapenem-resistant *Acinetobacter baumannii* (CRAB) is now a public health problem. In Taiwan, the relationship of the CRAB circulation between long-term care facilities (LTCFs) and acute care hospitals remains unclear. Here, we use molecular epidemiologic methods to describe the transmission of CRAB isolates between a community hospital and its affiliated LTCFs.

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A community hospital;
Long-term care facilities

Methods: Subjects localized in eight LTCFs who were not admitted acute care hospitals in recent a year were enrolled in this study. CRAB isolates were collected during June 1, 2015 and December 31, 2015. DNA fingerprinting was performed by repetitive extragenic palindromic sequence-based polymerase chain reaction (Rep-PCR) and multilocus sequence typing (MLST). Multiplex-PCR amplification for the detection of bla_{OXA} genes and beta-lactamase genes was performed.
Results: Twenty one subjects were enrolled. The major hospital admission diagnoses among the 21 subjects were pneumonia (71.4%). Genotyping of CRAB isolates by Rep-PCR revealed that a major clone, designated as type III, comprised fifteen of 21 (71.4%) isolates taken from 5 LTCFs and one study hospital. The isolates with type III were subtyped by PubMLST into 4 ST types. The most prevalent bla_{OXA} genes in these isolates were bla_{OXA-23}-like (85.70%, 18/21). Twenty isolates carried bla_{SHV}.
Conclusion: Clonal spread of bla_{OxA-23}-carrying CRABs was found around LTCFs and the affiliated hospital. In Taiwan, it is important for the government to focus attention on the importance of identifying and tracing CRAB infections in LTCFs.

Introduction

An increasingly disabled and aging population has become a major concern worldwide.[1] It is an increasing demand for long-term care services because of the dwindling function of family care and low-quality of patients care for some foreign caregivers. However, acute care is limited at long-term care facilities (LTCFs), and it is a trend which prompts LTCFs to cooperate with community hospitals to continue to provide elderly quality care and acute care. The increasing use of oral antibiotics, crowded LTCFs' environment, and colonized or infected LTCFs' residents may act as vectors for the transfer of multi-drug resistant micro-organisms (MDRO) into other vulnerable patients.[2] Analyzing the dissemination of MDRO in LTCFs, at least four possible reasons were related. First is previous antibiotics exposure. It is possible that frequent use of oral antibiotics in LTCFs may select for MDRO. Second is poor compliance of hand hygiene. Because hand-washing rates during patient care often are low among LTCFs' personnel. Third is cross-transmission. Even if LTCFs' residents have not been hospitalized recently, they are likely to be exposed to the flora of other LTCFs' residents who shuttle between LTCFs and a hospital. Colonized or infected LTCFs' residents may serve as MDRO reservoirs. Lastly is from LTCFs' environments. The ubiquity of MDROs in their environments is another potential threat to weaker residents and patients among hospitals, LTCFs and the community.[3] However, the direction of transmission in the overall epidemiology of MDRO is not still known.

Among MDROs, *Acinetobacter baumannii* (*A. baumannii*) has emerged as a major pathogen causing a multitude of infections, and the prevalence of multidrug resistant *A baumannii* (MDRAB) has risen in the past few decades in Taiwan.[4] The distribution of *A. baumannii* carrying these different acquired carbapenemase-hydrolyzing class D β-lactamases (CHDLs) genes varies among different regions and hospitals.[5] The most common acquired CHDL gene of carbapenem-resistant *A. baumannii* (CRAB) in many Asia—Pacific countries is bla_{OxA-23}.[6] To date, at least 13 variants of bla_{OXA-23} have been described in more than 50 countries.[7] Despite several studies related to the prevalence of CRABs in acute care hospitals have been reported, studies on the CRAB spread between LTCFs and acute care hospital are scarce.[8]

This study hospital signed cooperative agreements with 24 nearby LTCFs, and the number of collaborated LTCFs is the second largest in Taiwan. This is a representative institute to evaluate how CRAB spread between LTCFs and a community hospital. Here, we designed a cross-sectional study to describe the relationship to CRAB colonization/infection between a community hospital and the collaborated LTCFs. We also propose possible directions of the spreading of CRAB between a community hospital and the collaborated LTCFs.

Materials and methods

Study population and data collection

The study was approved by the Ethics Committee of the Changhua Christian Hospital (CCH IRB No. 140318). This prospective cross-sectional study was performed a culture concerning CRAB colonization/infection in collaborated LTCFs and a study hospital located in central Taiwan between June 1, 2015 and December 31, 2015. Samples were collected for all symptomatic patients at time of admission to the study hospital from collaborated LTCFs. The LTCFs' residents, who had been admitted to acute care institutes in recent a year, were excluded. The geographic relationship is listed in Supplementary file 1. When LTCFs' residents admitted to the study hospital from the collaborated LTCFs, we performed a culture on if they had fevers or systemic inflammatory response syndrome. Physician screened the subjects from the collaborated LTCFs for using the definition of systemic inflammatory response syndrome.[9] After early microbiological survey, cases of CRAB were identified from microbiological databases

according to the clinical microbiologists' sentinel report. Cases in which CRAB was isolated from clinical specimens were selected for review. Each patient had a medical record which contained medical diagnoses, surgical interventions, and other key information from medical records. Risk factors for CRAB infection and patient demographic data were recorded as follows: living arrangements, patient age, gender, diagnosis at discharge, comorbidities, antibiotic prescriptions, microbiological data and outcome. The scoring system for risk assessment to get CRAB colonization/infection divided into three factors, including patient factor, disease factor at admission, and duration factor (Supplementary file 2). The outcome was defined as (1) expired group who was passed away in this admission, (2) survival group who was survived in this admission, and (3) referral group who was referred to medical center for further management during this admission.

Sample collection and identification of CRAB

Only *A. baumannii* from clinical microbiologists' sentinel report were included and all of CRAB were sent to reference laboratory. All imipenem-resistant *Acinetobacter* spp. in the study were stored at −70 °C in trypticase soy broth (Difco Laboratories, Detroit, MI, USA) supplemented with 20% glycerol until they were tested. The microbiological methods used for the identification of *A. baumannii* were as previously described.[10] In brief, the clinical CRAB strains were isolated and identified using the Vitek system (bioM áerieux Vitek, Hazelwood, Mo., USA), and characterization of these isolates as *A. baumannii* or non-*baumannii Acinetobacter* was performed by one-tube multiplex PCR on the basis of the method of Chen et al.[11] Susceptibilities to antimicrobial agents were determined by the disk diffusion method in accordance with the guidelines of the Clinical and Laboratory Standards Institute.[12] The agents tested included amikacin, ceftazidime, ciprofloxacin, ceftriaxone, gentamicin, imipenem-cilastatin, levofloxacin, ampicillin/sulbactam, trimethoprim/sulfamethoxazole, and piperacillin-tazobactam. The minimum inhibitory concentration (MIC) of imipenem in all 21 CRAB isolates were reconfirmed according to the guidelines of the Clinical Laboratory Standards Institutes.[13]

Detection of resistant genes and molecular typing of CRAB

Deoxyribonucleic acid (DNA) was extracted using a Wizard genomic DNA purification kit (Promega, Madison, WI) following the manufacturer's protocol and immediately stored at −20 °C. PCR amplification was performed using a GeneAmp 2720 thermal cycler (PE Applied Biosystems, Foster City, Calif., USA). The primers used in this study were listed in Supplementary File 3. DNA fingerprinting was performed by repetitive extragenic palindromic sequence-based polymerase chain reaction (Rep-PCR).[14] Briefly, the REP-PCR profiles of the *A. baumannii* strains were analyzed using Numerical Taxonomy and Multivariate Analysis System version 2.0 (Applied Biostatistics Inc., Setauket, New York). Cluster analysis for REP-PCR DNA fingerprints was performed by constructing a dendrogram using the bands-based dice coefficient method. A similarity matrix was generated and subsequently clustered using the unweighted pair group method with arithmetic means. The cut off value for group similarity was defined as 79%.

The *A. baumannii* isolates were grouped according to the REP-PCR fingerprinting patterns, and then the main group was analyzed by multilocus sequence typing (MLST). The MLST profiles were deduced as described in the protocols of the PubMLST (http://pubmlst.org/abaumannii/) databases. Clonal complexes (CCs) were defined as containing at least three STs sharing the same allele numbers in at least six of seven loci. The graphic results included different circles to illustrate the relationships between different STs. CCs were determined by eBURST version 3 (http://eburst.mlst.net).

Multiplex-PCR amplification for the detection of bla_{OXA} genes was performed as previously described.[15] The genetic elements carrying the $bla_{OXA\text{-}51\text{-}like}$ genes were investigated by PCR mapping using IS*Aba1* forward/OXA-51-like reverse primers, while the genetic elements carrying the $bla_{OXA\text{-}23\text{-}like}$ genes were investigated using IS*Aba1* forward/OXA-23-like reverse primers.[16] PCR amplification of 19 beta-lactamase genes, including extended-spectrum β-lactamase genes, *ampC* β-lactamase genes and carbapenemase genes, were following the directions of Dallenne et al.[17] To completely identify the PCR amplicons, the amplicons were subjected to DNA sequencing using an ABI 3730 XL DNA Analyzer (Applied Biosystems, Foster City, CA, USA). DNA sequences were compared to those registered in the National Center for Biotechnology Information (NCBI) database.

Statistical analysis

We used descriptive statistics to describe the demographic characteristics, risk assessment and outcome.

Results

Description of enrolled subjects

Flow chart of study subjects enrolled in this study is listed at Fig. 1. In brief, a total of 592 visits of resident-time from 8 collaborated LTCFs were admitted to the study hospital during June 1, 2015 and December 31, 2015. Cultures collected for 498 visits of resident-time who had severe inflammation response symptoms and impressions of infection by physician at time of admission to an affiliated community hospital (study hospital) from 8 collaborated LTCFs. A total of 21 subjects, whose microbiological report showed CRAB and who had not been hospitalized in recent one year, were enrolled according to the protocol. The general information of 8 LTCFs and one study hospital are listed in Supplementary file 4. The bed capacity of 8 LTCFs ranted from 30 to 498 beds. The number of cared residence by each registered nursing and by each nursing assistant are 1:15 and 1:5, respectively. Total number of admission to the study hospital ranged from 4 to 22 person times during the study period. In Table 1, 14 out of 21 (66.7%) patients were male, and the mean age (±stand deviation, SD) was

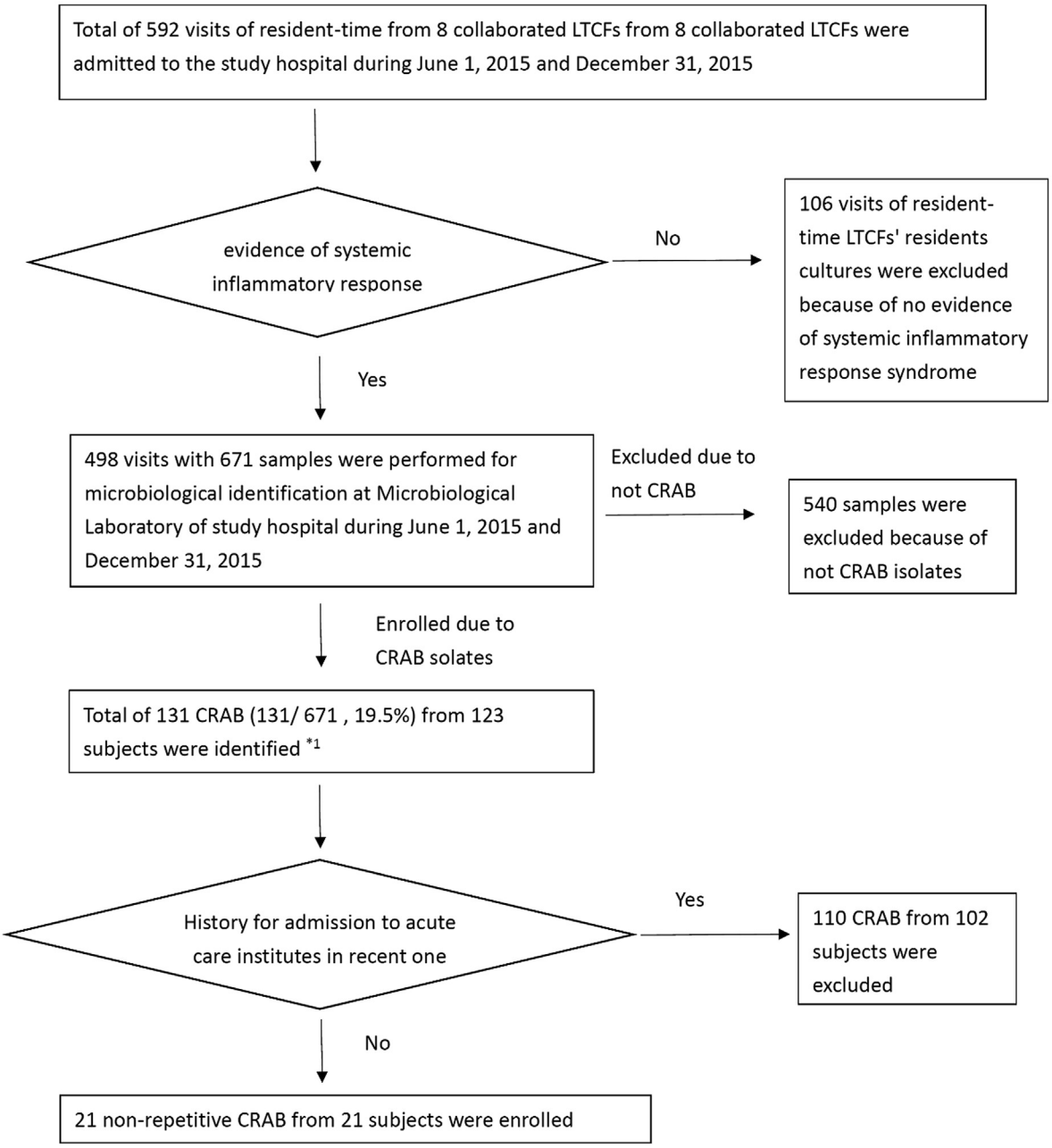

Figure 1. Flow chart of study subjects enrolled in this study. *1 The clinical CRAB strains were isolated and identified using the Vitek system. The screening method for antimicrobial susceptibilities to antimicrobial agents were determined by the disk diffusion method,[12] and the result was shown at Supplementary Table 4. Abbreviation: CRAB: Carbapenem-resistant *Acinetobacter baumannii;* LTCF: long-term care facilities.

76.1 ± 9.0 years. The mean (±stand deviation, SD) of the risk score for each patient was 9.0 ± 1.3 years. The top three major diagnoses at admission were pneumonia (15 patients), urinary tract infection (7 patients), and bacteremia (6 patients). The mean number (±SD) of comorbidities was 6.5 ± 1.4 diseases, and most of the patients showed chronic obstructive pulmonary disease, and type 2 diabetes mellitus (DM) (Supplementary file 5). There was also an incident of an old cerebrovascular accident. The mean number (±SD) of antibiotics used during the hospitalization period was 2.6 ± 1.2, and the majority was β-lactams. The three most common clinical isolates from 21 patients during the hospitalization period were *Pseudomonas aeruginosa*, *Staphylococcus aureus*, and *Escherichia coli*. The all-cause mortality rate is 42.1% (8/19). The risk assessment showed no strong statistical significance because the risk assessment score from each of 19 subjects is high and the sample size is small (Table 1).

Distribution of CRAB

A total of 21 *A. baumannii* isolates were collected during the study period. These isolates were found to be co-resistant to most of the commonly used antimicrobial

Table 1 Risk assessment of the 21 patients with CRAB infections.

Number	Living arrangements	Age	Gender	Major final diagnosis	Risk assessment for score of patient factor—disease factor—duration factor	Sample	CRAB type	Outcome
1	H2-2	74	M	UTI	4-3-3	Urine	III	Sur
2	H5-1	59	F	UTI	3-3-3	Urine	III	Exp
3	H5-2	84	M	UTI	4-3-3	Sputum	III	Sur
4	H7-1	76	M	Pn	4-3-3	Sputum	III	Sur
5	H8-1	85	F	BacT, Pn, UTI, PleuEm	3-3-3	Sputum	III	Sur
6	N-1	69	F	BacT, Pn	3-3-3	Blood	III	Ref
7	N-2	86	M	Pn	4-3-3	Sputum	III	Sur
8	N-3	78	M	BacT,UTI	4-3-3	Urine	III	Exp
9	N-4	83	F	Pn		Sputum	III	Ref
10	N-5	66	M	Pn, UTI, Fasc	4-3-3	Urine	III	Exp
11	N-6	84	M	Pn	4-3-3	Sputum	III	Exp
12	H6-1	78	M	Pn	4-3-3	Sputum	III	Sur
13	H6-2	60	M	DecU	4-3-3	Abscess	III	Sur
14	H6-3	78	F	BacT, UTI	3-3-3	Sputum	III	Exp
15	H6-4	76	M	BacT, Pn	4-3-3	Sputum	III	Exp
16	H2-5	78	M	Pn	4-3-2	Sputum	IV	Sur
17	H2-6	58	M	Pn	4-3-2	Sputum	V	Sur
18	H3-1	84	M	Pn	4-3-2	Urine	VI	Sur
19	H4-1	72	M	Pn	4-3-2	Sputum	VI	Sur
20	H1-1	91	M	Pn	4-3-2	Sputum	VII	Exp
21	H2-1	80	M	BacT, PleuEM, Pn	4-3-2	Sputum	VIII	Exp

Notes: This score is according to the risk assessment by Supplementary file 2. The expired group is defined as all-cause mortality.
Abbreviations: BacT, bacteremia; CRAB: carbapenem-resistant *Acinetobacter baumannii;* Exp: expired; Fasc: fasciitis; DecU, decubitus ulcer; PleuEm: pleural empyema; Pn, Pneumonia; Ref: referral to another institute; Sur: survival; UTI, urinary tract infection.

agents (Supplementary file 6). The $bla_{\text{OXA-51}}$-like gene (100%, 21/21) was present in all of the isolates. Other than the $bla_{\text{OXA-51}}$-like gene, the most prevalent bla_{OXA} genes in these isolates were $bla_{\text{OXA-23}}$-like (85.70%, 18/21), and $bla_{\text{OXA-24}}$-like (14.3.0%, 3/21) genes (Fig. 2).

Genotyping by Rep-PCR revealed that eight types (III-X) were present in all the isolates. Fifteen of 21 (71.4%) isolates taken from 5 LTCFs (H2, H5, H6, H7, H8) and one study hospital (N), belonged to type III. Moreover, all of the 21 isolates were not clonally related to TYTH-1,[18] which was previously regarded as an endemic clone in northern Taiwan. Type III group was further subtyped by PubMLST into four sequence types (ST), including ST 729, ST 687, ST 473 and ST 550. ST 473, carrying $bla_{\text{OXA-23}}$ and $bla_{\text{OXA-51}}$ in the H6 LTCF, belonged to clonal complex 92 (Supplementary file 7). ST 729 and ST550 had appeared in the N hospital, whereas ST687 had appeared in the H5, H7, and H8 LTCFs. Nineteen beta-lactamase genes was analyzed in all the isolates and only four genes were detected. In total, 20 (95%) CRAB isolates contained bla_{SHV}, one carried $bla_{\text{CTX-M}}$ and $bla_{\text{DHA-1}}$, and one carried $bla_{\text{CTX-}}$

Discussion

This is the first cross sectional study to evaluate the CRAB spread in LTCFs and an affiliated community hospital. The inter-hospital and intra-hospital dissemination of MDR *A. baumannii* was supported by several studies in Taiwan.[19] However, the direction of CRAB spread between residents of LTCFs and acute care hospitals is limited reported. This study confirmed previous findings of CRAB in both kinds of institutes[20] and provides a clearer context of the CRAB profile observed from LTCFs and a collaborative community hospital. The emergence of CRAB is a major public health concern.[4] Chen CH et al.'s reports showed the important risk factors contributing to become susceptible CRAB infections,[4] and our preliminary data of risk assessment showed no strong statistical significance because of all high scores and a small sample size (Table 1). Recent studies have shown that the outcomes of infections with these bacteria are worse than those caused by susceptible organisms, and LTCFs' residents may be a source of transmission in acute hospital settings.[21,22] The aging population is rapidly increasing in the next 10 years in Taiwan. Therefore, specific monitoring and infection control measures in LCTFs and a collaborative community hospital have significant implications for health services.

Carbapenem resistance in *A. baumannii* is demonstrated by coexisting mechanisms including decrease in permeability of the outer membrane, efflux pumps, production of β-lactamases, and modification of penicillin-binding proteins.[23] Mostly, the resistance of *A. baumannii* to

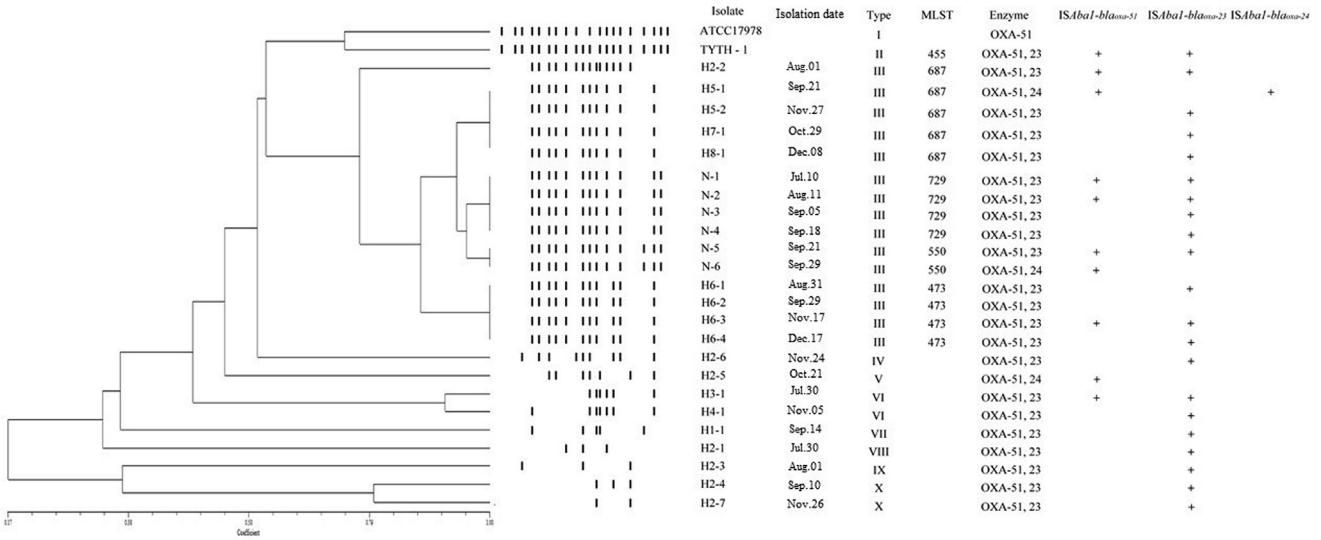

Figure 2. Dendrogram illustrating the genetic relatedness of the carbapenem-resistant Acinetobacter baumannii isolates. Results were obtained by Rep-PCR and MLST and the mobile insertion sequence ISAba1 for 21 isolates of CRAB from 8 LTCFs (H1—H8) and a community hospital (N) in the Nantou area of Taiwan and 2 reference strains (*A. baumannii* ATCC 17978 and *A. baumannii* TYTH-1 [§]). [§] The reference of *A. baumannii* TYTH-1 was cited from Liu CC.[16] Abbreviation: CRAB: carbapenem-resistant *Acinetobacter baumannii;* LTCF: long-term care facilities; MLST: multilocus sequence typing; Rep-PCR: repetitive-sequence-polymerase chain reaction.

carbapenem has been associated with CHDLs. Moreover, high-level carbapenem resistance attributable to the expression of genes encoding CHDLs requires a strong promoter such as that of the mobile insertion sequence IS*Aba1*. Except for the H6-2 strain, all the isolates carried IS*Aba1* ahead of CHDL genes. Eighteen of 21 (85.7%) isolates carried the IS*Aba1-bla*$_{OxA-23}$ element, and ten of 21 (47.6%) isolates carried the IS*Aba1-bla*$_{OxA-51}$ element (Fig. 2). *A baumannii* strains carrying *bla*$_{OxA-23}$ were the mostly common strains in our study, which was consistence with previous studies,[6,16,24] and *bla*$_{OXA-23}$ was also the most prevalent CHDL gene in all study hospitals. Although all CRAB isolates had carried *bla*$_{OxA-51}$ in this study, *IS*$_{Aba1}$ carrying the promoter was essential for its contribution to carbapenem resistance.[25]

The prevalence of MDROs in countries localized in Asia—Pacific areas, such as China and Taiwan, has become a serious problem.[2] In this study, we found that 95.2% (20/21) isolates contained *bla*$_{shv}$. And, the carrying rate of *bla*$_{shv}$ in our study was relatively high when compared to similar studies conducted in LTCFs worldwide.[22] In our study showed one CRAB carry *bla* $_{CTX-M}$ and *bla* $_{DHA-1}$ within the 8 LTCFs and one collaborative community hospital. None of our isolates carried *bla*$_{TEM}$.

Rep-PCR and MLST has been successfully applied to analysis of molecular epidemiology.[14] It has been reported that bacteria isolated from different regions of the world can share a few common genetic characteristics. By Rep-PCR typing, our study showed that the cloneages of all of the isolates differed from an endemic clone (TYTH-1) in northern Taiwan, implied that clonal transmission of CRAB was still restricted to the local area. Most importantly, that clonal spread of CRAB among the 5 LTCFs and their associated hospital was observed from LTCFs to hospital. Based on MLST typing, our study showed that ST 473 is the most widespread strain in H6, and the results of eBURST indicated that ST 473 belongs to the CC 92, the largest and most widespread clonal complex in the world. ST 473 had been detected in China[26] and Taiwan.[27] That could be a clue of a predominant clone with ST 473 CRAB carrying *bla*$_{OXA-23}$ and *bla*$_{OXA-51}$ spreading among H6. In addition, H6 is very close to its collaborated hospital (N), and that would be a thread to become a clonal spread from LTCFs to hospital among the local areas. In Taiwan, infection control guidelines are difficult to perform in LTCFs due to the complexity of governance. High fecal carriage of extended-spectrum β-lactamases in the LTCFs' residents were disclosed in our previous report.[22] Thus, implementing effective infection control policies may be required to monitor and prevent the dissemination of CRAB in LTCFs and a collaborative community hospital.

Our study has several strong advantages. Most importantly, we accumulated a complete seven-month clinical and microbiological dataset to evaluate the CRAB spread from LTCFs to hospital. Firstly, we excluded the LTCFs' residents who had been hospitalized from acute care institutes in recent one year in order to differentiate isolates being LTCF-acquired from isolates being hospital-acquired because Zimmerman FC's study showed 39% of patients had positive culture for carbapenem-resistant Enterobacteriaceae after discharged at 1 year.[28] Secondly, only symptomatic patients at time of admission to the study hospital from LTCFs were enrolled for cultures. Our study population represented the status of CRAB infection rather than the status of CRAB colonization. Then, the study hospital signed cooperative agreements with 24 nearby LTCFs, and the number of collaborated LTCFs is the second largest in Taiwan. This is a representative model to evaluate how CRAB spread between LTCFs and a community hospital. In this study, we investigated CRAB spread by analysis of the

resistant gene spectra in individual subjects. Thus, our findings provided important epidemiological information about CRAB spread between LTCFs and a community hospital.

There are also some important limitations to this study. First, we had a small number of enrolled subjects (21 subjects) and no accurate denominator data were able to record. Hence, the prevalence of CRAB in LTCFs was not able to address in this study. Second, we performed Rep-PCR and MLST but we did not perform large-scaled molecular typing for CRAB, hence it's unable to confirm the outbreak event in the study hospital. Thirdly, the study is descriptive statistical analyses but no statistical analyses for correlations were conducted. Hence, it's unable to calculate the correlation accurately. Finally, there were several variables, including the timing of the enrolled subjects and the different healthcare workers with different clinical conditions between every institutes, which could have interfered with the results of the study. However, it is necessary to continue our study to evaluate the prevalence of CRAB profiles between LTCFs and a community hospital as well as to clarify the causality between different kinds of healthcare institutes.

Conclusion

In this study, we found a predominant clonal spread of bla_{OxA-23}-carrying CRAB among a community hospital (study hospital) and its collaborated LTCFs in central Taiwan. In addition, we disclosed a predominant CRAB with ST 473 in H6. In Taiwan, the government needs to focus more attention on the importance of identifying and tracing resistant pathogens and issuing notifications of CRAB infections in LTCFs.

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Competing interests

The authors declare that they have no competing interests.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.jmii.2017.08.001.