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Repeated colonization of multidrug–resistant Acinetobacter calcoaceticus-Acinetobacter baumannii complex and changes in antimicrobial susceptibilities in surgical intensive care units

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Repeated colonization of multidrug–resistant *Acinetobacter calcoaceticus-Acinetobacter baumannii* complex and changes in antimicrobial susceptibilities in surgical intensive care units

Running title: Repeated colonization of *Acinetobacter* species

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Abstract

Background: A nosocomial outbreak of multidrug-resistant *Acinetobacter calcoaceticus-Acinetobacter baumannii* (MDR-Acb) complex infection occurred in a newly constructed building at a 2,500-bed tertiary medical centre in Taiwan.

Methods: An outbreak investigation was carried out by molecular approaches to trace the bacteria in samples. Antimicrobial susceptibilities, risk factors, and the occurrence for nosocomial MDR-Acb infections were further investigated. From January to December 2009, fifty-three patients were infected with MDR-Acb, and 23 environmental surveys were performed in two surgical intensive care units (ICUs) within the new building. Forty-two clinical isolates were obtained from patients and 22 samples were obtained from nine environmental surveys.

Results: Forty (95.2%) clinical isolates and 18 (81.8%) environmental samples were positive for type A MDR-Acb, indicating that patients and the environment harboured the predominant outbreak strain. The outbreak strain was identical to that isolated in a previous outbreak in the old hospital district in 2006, proved by repetitive extragenic palindromic-based polymerase chain reaction and pulsed-field gel electrophoresis. Although the outbreak isolates contained $bla_{\text{OXA-23-like}}$ and $bla_{\text{OXA-51-like}}$ genes, analysis of the antimicrobial susceptibilities demonstrated that increases of resistance rates of cefepime and imipenem were found in MDR-Acb isolated in different outbreaks.

Conclusions: These results suggest that not only patients or health care workers, but also medical equipments might have carried the predominant outbreak strain from the old district to the new building. Therefore, even in a new environment, infection control programs must be continually

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enforced and healthcare providers must be repeatedly educated to prevent recurrent outbreaks of MDR-Acb infection in the hospital setting.

Keywords: *Acinetobacter* species; antibiotic resistance; molecular epidemiology

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Introduction

Acinetobacter calcoaceticus-Acinetobacter baumannii (Acb) complex is an opportunistic pathogen that has been increasingly identified over the last decade as the source of recurring nosocomial infection [1]. *Acinetobacter* species has a ubiquitous ability to colonize in the environment and can be part of the bacterial flora in humans [2]. Infection of hospitalized patients with *Acinetobacter* species is thought to be associated with various nosocomial infections, including pneumonia, septicaemia, urinary tract infection, wound infection, and meningitis [3-5].

Multidrug-resistant *Acinetobacter calcoaceticus-Acinetobacter baumannii* (MDR-Acb) complex is an increasing threat to hospitalized patients, particularly those in intensive care units (ICUs), and it imposes therapeutic challenges [6, 7]. Carbapenems, including imipenem/cilastatin and meropenem, are the most commonly used treatment for infections caused by *Acinetobacter* species; however, an increase in the number of prescriptions for carbapenems has led to an increase in the resistance of *Acinetobacter* species to carbapenem [8, 9]. Among *Acinetobacter* species isolates, class D β–lactamase (OXA-23-, OXA-24-, and OXA-58-type carbapenemase) has been reported as the most prevalent mechanism for carbapenem resistance [10].

Hospitalized patients can acquire MDR-Acb infections through various modes of transmission including cross-transmission among patients, transmission by healthcare workers, invasive procedures, and contamination of the surrounding environment [11-14]. Comprehensive infection control programs can effectively prevent an outbreak of MDR-Acb dissemination in hospitals [11, 15]; thus, control of sporadic outbreaks of MDR-Acb infection is a critical policy issue in medical

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centers.

Throughout 2006, sporadic outbreaks of *Acinetobacter* species infection occurred in a district at China Medical University Hospital, a 2,500-bed university hospital in central Taiwan [14]. In October 2008, a newly constructed building was opened more than 500 meters from the older district. Beginning in February 2009, however, gradual increases of MDR-Acb clinical isolates were found in two surgical ICUs within the new building. The aim of this study was to investigate the antimicrobial susceptibilities and molecular epidemiology of MDR-Acb isolates in the new building and to determine whether there was a predominant strain among the outbreaks observed in both the new and old districts of the medical center. The relationship of risk factors between invasive medical procedures and the occurrence MDR-Acb infections were also investigated.

Materials and Methods

Study design and epidemiological surveillance in surgical ICUs

Retrospectively, we collected all isolates of MDR-Acb from the trauma and surgery intensive care unit (ICU-A) and the neurosurgical intensive care unit (ICU-B) from 1 January 2009 to 31 December 2009. These two ICUs located on consecutive floors in the newly constructed hospital building. In addition to positive cultures of MDR-Acb, all enrolled patients have corresponding clinical infection signs including: fever > 38^{o}C, pyuria for urinary tract infection, purulent sputum for pneumonia, pus discharge from operation wound, or erythema over central vascular catheter insertion sites. Demographic and clinical data were collected by medical chart review for each

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patient with a positive MDR-Acb culture. The periods of patient-days for the resident patients with MDR-Acb infection were the duration before acquisition of positive clinical isolations. The post-washing hands cultures of in-charge healthcare workers were also conducted to evaluate the accuracy of hand-washing procedures.

Bacterial culture and antimicrobial susceptibility test

All isolates from patients (except blood culture, which was processed initially with a Bactec 9000 system [Becton Dickinson, Sparks, MD, USA]) or environmental samples were streaked across Trypticase soy agar with 5% sheep blood (TSA II)/Levine EMB agar (Becton Dickinson) and incubated at 37°C. Bacterial isolates were identified as *Acinetobacter calcoaceticus-Acinetobacter baumannii* (Acb) complex, and the antibiotic susceptibility of the bacterial isolates to various antimicrobial agents was determined using a BD Phoenix™ Automated Microbiology System (Becton Dickinson), as described previously [14]. Antimicrobial susceptibility was confirmed by a disk diffusion method, following the guidelines and criteria of the Clinical Laboratory Standards Institute [16]. The definition of multidrug resistant was isolates with resistance 3 classes of following antimicrobial agents: antipseudomonal cephalosporins, antipseudomonal carbapenems, β-lactam–β-lactamase inhibitor combinations, antipseudomonal fluoroquinolones, and aminoglycosides [9, 17].

Molecular fingerprinting by* repetitive extragenic palindromic-based polymerase chain reaction (*REP-PCR)

The isolated *Acinetobacter* species were prepared for extraction of genomic DNA as described

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previously [14]. Consensus primers for the repetitive extragenic palindromic sequences found in many bacterial chromosomes, including those of *Acinetobacter* species, were used in the REP-PCR [18-20]. To prepare bacterial genomic DNA, the bacterial pellets were resuspended in 600 µl of lysis buffer (20 mM Tris-Cl, pH 7.5, 10 mM EDTA, 40 mM NaCl, 0.2% SDS, and 200 µg/ml protease K) and incubated at 50°C for 45 min. DNA was extracted with phenol/chloroform solution (1:1). The paired primers REP-1 (5'-IIIGCGCCGICATCAGGC-3') and REP-2 (5'-ACGTCTTATCAGGCCTAC-3') were used to amplify putative REP-like elements in the bacterial genomic DNA [19]. The procedures for amplification by REP-PCR and interpretation of fingerprint profiles were followed as previously described [19]. A negative control containing all components except the DNA extract, which was replaced with 5 µl of sterile distilled H_2O, was included in each PCR run to rule out reagent contamination. Gel electrophoresis was used to separate the REP-PCR amplification products, which were compared to molecular weight standards. The standard strain of *A. baumannii* (ATCC 19606) was compared with isolated strains from patients and environmental samples. Each isolate was run in duplicate, and fingerprint profiles were interpreted by researchers who were blinded to the clinical data. For detection of genes encoding $bla_{\text{OXA-23-like}}$ and $bla_{\text{OXA-51-like}}$, a multiplex PCR assay was performed as described previously [21].

Genotyping by pulsed-field gel electrophoresis (PFGE)

Bacterial isolates from patients and environment were genotyped using PFGE according to manual protocol (Bio-Rad, Hercules, CA, USA). PFGE analysis was carried out as described previously [22]. The bacterial genomic DNA was prepared and digested with *Apa*I (New England

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BioLabs). The digested DNA fragments were subjected to PFGE, which was conducted with 6V/cm gradient, at 14°C, and with 3–8 s pulse interval for 10.5h followed by 12–20 s pulse interval for 10.5h. The gel was stained and analyzed using BioNumerics software (Applied Maths). Pulsotypes were assigned to same clusters with >80% similarity from the dendrograms.

Statistical analysis

Comparisons between different patient groups were analyzed by X^2 tests for categorical variables or paired *t*-tests for continuous variables. A *P*-value of less than 0.05 was considered statistically significant.

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Results

Molecular characterization of nosocomial outbreak with MDR-Acb

From 1 January 2009 to 31 December 2009, there were 64 isolates of MDR-Acb from 53 patients and 23 environmental surveys in the ICU-A and ICU-B. All 64 isolates were found to be resistant to all beta-lactams (except sulbactam), fluoroquinolones, aminoglycosides (except three isolates sensitive to amikacin), and trimethoprim-sulphamethoxazole. The sites for environmental surveys including ventilator surface, bed rail, bedside curtain, monitors and tables. From the 53 patients, 42 clinical isolates were available for genomic fingerprinting, with 29 from the ICU-A and 13 from the ICU-B. The origin sites of positive cultures in ICU-A and ICU-B were as followed: 4 tips of central vascular catheter, 3 urine, 5 wound pus, 9 sputum, 12 blood and 2 body fluids in ICU-A; 2 tips of central vascular catheter, 2 urine, 2 wound pus, 9 sputum, 2 blood and 1 body fluids in ICU-B. Of the 23 environmental surveys, nine yielded positive cultures (6 from the ICU-A and 3 from the ICU-B) resulting in 22 samples that were suitable for genomic fingerprinting. There was no positive culture from hands of healthcare workers.

The 64 MDR-Acb isolates for fingerprinting were distributed among four different PFGE patterns, pulsotypes A to D (Fig. 1). Among these four types, the most frequent isolate was type A, which accounted for 58 isolates (90.6%), with 40 isolates from patient samples and 18 isolates from environmental samples. The other six samples were determined to be type B (one patient isolate from blood), type C (one patient isolate from urine and one environmental isolate), and type D (three environmental isolates). A second molecular approach, REP-PCR, was used to validate the

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bacterial genomic patterns and distribution of the clinical isolates [14]. The data from the REP-PCR analysis confirmed the four distinct genomic profiles and the distribution of the 64 clinical isolates among the four profiles (data not shown).

To further determine whether the 2009 MDR-Acb outbreak strain is the same as the 2006 outbreak strain that occurred in the old building of this Hospital [14], a sample from the 2006 strain was analyzed using PFGE and compared with samples from the 2009 outbreak. As shown in figure 1, the 2006 outbreak strain (pulsotype A, ICU-2006) from the old hospital district was identical to the predominant 2009 outbreak strains (pulsotype A, ICU-A-15 and ICU-B-3) from the new hospital building. The evidence indicates that type A MDR-Acb was the major causative nosocomial outbreak strain in our hospital in 2009. Moreover, the same strain was responsible for the 2006 nosocomial outbreak.

Antimicrobial susceptibilities in MDR-Acb isolates

The $bla_{\text{OXA-23-like}}$ and $bla_{\text{OXA-51-like}}$ genes were detected in clinical MDR-Acb isolated both in 2006 and 2009 outbreaks (Fig. 1). Analysis of antimicrobial susceptibilities revealed that the antibiogram were changed somewhat between the two outbreaks. As shown in figure 2, there was a higher rate of cefepime resistance in 2009 (100%) than in 2006 (77.8%) ($P < 0.05$). It is noted that resistance rates to imipenem were increased from 82.5% in 2006 to 100% in 2009 ($P < 0.05$). Similar results were also found for ampicillin-sulbactam (SAM) and piperacillin/tazobactam (TZP), the resistance rates were slightly increased from 2006 to 2009. However, all the MDR-Acb isolates remained susceptible to colistin (100%). The results indicate that imipenem and cefepime resistance

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are the most significant change in MDR-Acb among the two outbreaks.

Surveillance of MDR-Acb outbreaks in surgical ICUs

The timeline and spatial distribution of patients, and environmental surveys with positive cultures of MDR-Acb in the ICU-A and ICU-B are represented in figure 3. There were 11 clinical isolates (six in the ICU-A and five in the ICU-B) that were not available for genomic fingerprinting. Those isolates are referred to as unavailable MDR-Acb in the figure 3. In the ICU-A, there were only three beds (numbered 12, 13, and 19) without any MDR-Acb–infected patients in 2009 (Fig. 3A). Bed number 5 had the most cases of infection, and this bed also had a positive environmental survey. The peak in the outbreak of type A MDR-Acb infection in the ICU-A occurred between October 2009 and December 2009 (Fig. 3B). In the ICU-B, more than half of the beds had patients who acquired an infection with MDR-Acb (Fig. 3C). The genomic fingerprint for all available MDR-Acb isolates from patient and environmental samples in the ICU-B was confirmed as pulsotype A. The peak of the epidemic in the ICU-B occurred in October 2009 (Fig. 3D). The evidence indicates that pulsotype A MDR-Acb is the major outbreak strain in two surgical ICUs in the newly constructed hospital building.

Discussion

Nosocomial infection with *Acinetobacter* species is associated with a broad range of infections, including pneumonia, septicaemia, urinary tract infections, wound infections, and meningitis [3-5]. Infection with MDR-Acb is thought to increase mortality rates among hospitalized patients [23, 24].

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Previous studies reported that in ICUs, outbreaks of infection with MDR-Acb often result from cross-transmission among patients [11, 13, 25]. Additionally, patients in ICUs who received invasive procedures (central vascular catheterization, Foley, catheterization, mechanical ventilation, nasogastric tube, or haemodialysis) showed a significantly increased risk of infection with MDR-Acb [12, 14]. Molecular epidemiology is often used to discriminate strains of MDR-Acb during nosocomial outbreaks [14, 18, 26]. In the current study, the outbreak investigation took place in the clinical setting of two surgical ICUs (ICU-A and ICU-B) in a new building at our hospital. The new building provides critical care for patients who may have been transferred from emergency departments at our hospital or others. It is important to note that all of the enrolled patients in this study received critical care with invasive procedures and were hospitalized in ICUs; thus, these patients were likely at a higher risk for acquiring MDR-Acb [27].

Our data indicated that the outbreak MDR-Acb isolates contained at least 2 genes in class D carbapenemas, $bla_{\text{OXA-23-like}}$ and $bla_{\text{OXA-51-like}}$, which contributed to imipenem resistance. This result was similar to a previous report that both $bla_{\text{OXA-23-like}}$ and $bla_{\text{OXA-51-like}}$ genes were detected in carbapenem-resistant *A. baumannii* isolates in central Taiwan [28]. Noticeably, the capapenem and cefepime resistance rates were increased significantly from 82.5% and 77.8% in 2006 to both 100% in 2009, respectively. The deteriorating antimicrobial susceptibilities may result from both the dissemination of the major outbreak strain and the persistent selective pressure of clinical prescribed antibiotics in our hospital.

In this study, we used molecular approaches to determine that type A (referred to as type 1 in

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the 2006 outbreak [14]) MDR-Acb is a predominant clonal strain in our hospital regardless of the district. Several reports have revealed that major outbreaks of *Acinetobacter* species clones often involve the same genotype in clinical and environmental samples in ICUs [11, 12]. These findings indicate that the predominant clonal strains might have higher tolerance and virulence, lead to resistance to environmental cleaning or infection control interventions, and lead to more severe infections and poor patient outcomes, as mentioned by other study [11, 25]. The recurrence of major outbreaks of MDR-Acb clones in our hospital therefore needs to be surveyed continuously and brought to the attention of healthcare workers more frequently.

Patients and healthcare workers were not exposed to the newly constructed building before it began operating in October 2008. Additionally, the new hospital district is located more than 500 meters from the old hospital district. After opening the new building, most patients, healthcare workers, and old medical equipment (including beds, monitors, ventilators, *etc.*) were transferred from the old district to the newly constructed building. There was no share of healthcare workers or equipments between two ICUs except ventilator rarely. This may be the main transmission route by which the same MDR-Acb strain emerged in the new district, despite attempts to disinfect the old medical equipment and the admission of new patients from emergencies or other hospitals. Our findings are similar to the circumstances surrounding outbreaks of MDR-Acb infection in the United States military healthcare system [29]. Additionally, several studies have documented the spread of Acb through different modes, including contamination of medical equipment, computer devices, and the surrounding environment, or transmission by healthcare workers and during

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medical procedures [11-13].

Comprehensive and multifaceted infection control interventions are necessary to effectively reduce nosocomial outbreaks of Acb infection [11-15, 30]. During the outbreak in our hospital in 2006, a series of sustained infection control programs was enforced to stop the emergence of new cases [14]. The recurrence of the second outbreak of MDR-Acb infection might be attributed to patient carry-over of this virulent bacterium or inadequately disinfected medical equipment during the transfer from the old district building to the new one. Decreased attention of medical staff to the ongoing infection control program may have occurred in the new environment, which was not associated with the previous outbreak of nosocomial infections, and this might have accelerated the outbreak in the new building. In May 2009 in the ICU-A and in October 2009 in the ICU-B, enhanced infection control procedures (other than standard universal precautions) were implemented and included repeated environmental surveys and disinfections until no further positive cultures were found and the decreased use of unnecessary antibiotics; however the result was a decrease in case numbers, not eradication of the infection. Furthermore, the number of infected ICU-A patients rose by the end of 2009, resulting in prolonged enforcement of the infection control interventions described above, combined with repeated education and monitoring of medical staff.

In conclusion, our results suggest that the same MDR-Acb strain spread from the old district to a new district building, most likely through patients, healthcare workers, or medical equipment. The present study also indicates that infected patients and the environment are important reservoirs for

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MDR-Acb. Prolonged, aggressive, comprehensive infection control interventions, combined with repeated education and surveillance of healthcare providers, are critical for getting an outbreak of MDR-Acb nosocomial infection under control.

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Author Disclosure Statement

No conflicting financial interests exist.

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Figure legends

FIG. 1. Representative PFGE patterns of MDR-Acb isolates in ICUs at the old district (ICU-2006) and the new building (ICU-A and ICU-B). The dendrogram was produced by BioNumerics software, showing distance calculated by the Dice similarity index of *Apa*I-digested DNA fragments. The degree of similarity is shown in the scale. Similarities >80% assign the same cluster of strains. The isolated locations are shown at the right side of the figure.

FIG. 2. Antimicrobial resistance rates of MDR-Acb isolated in 2006 and 2009. SAM, Ampicillin-sulbactam; TZP, Piperacillin/Tazobactam; SXT, Trimethoprim-sulfamethoxazole. An asterisk indicates $P < 0.05$.

FIG. 3. The spatial (A and C) and timeline (B and D) distribution of patients and environmental surveys with positive cultures of MDR-Acb in the ICU-A (A and B) or ICU-B (C and D). The samples were not available for typing from patients or environments is referred to as unavailable isolates.

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FIG. 1

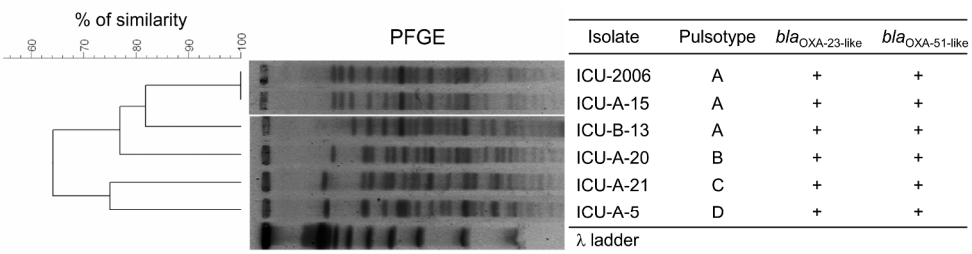

FIG. 1. Representative PFGE patterns of MDR-Acb isolates in ICUs at the old district (ICU-2006) and the new building (ICU-A and ICU-B). The dendrogram was produced by BioNumerics software, showing distance calculated by the Dice similarity index of ApaI-digested DNA fragments. The degree of similarity is shown in the scale. Similarities >80% assign the same cluster of strains. The isolated locations are shown at the right side of the figure.

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FIG. 2

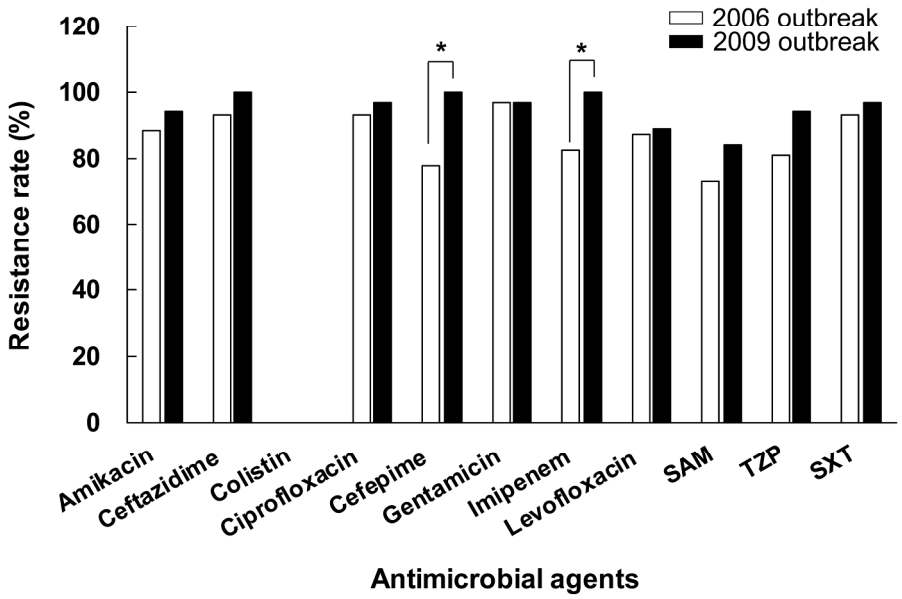

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FIG. 3

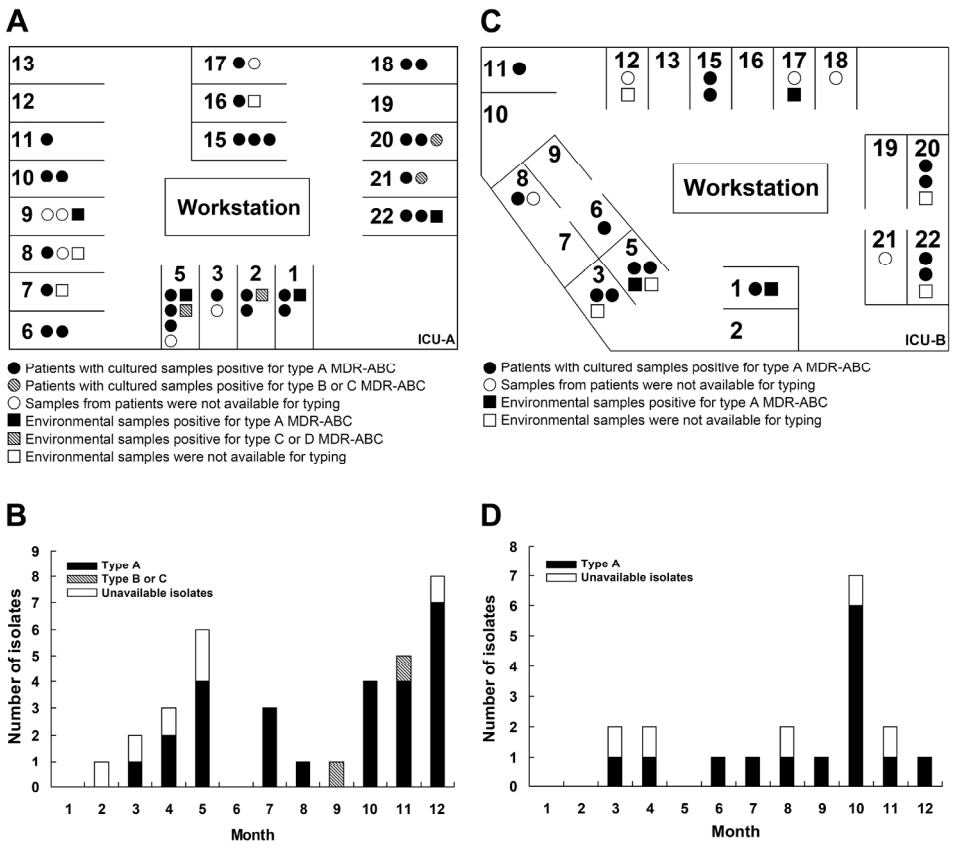

FIG. 3. The spatial (A and C) and timeline (B and D) distribution of patients and environmental surveys with positive cultures of MDR-Acb in the ICU-A (A and B) or ICU-B (C and D). The samples were not available for typing from patients or environments is referred to as unavailable isolates.