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Investigation of an outbreak of carbapenem resistant *Acinetobacter baumannii* in an intensive care unit during the COVID-19 epidemic

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Abstract

Background During the Coronavirus Disease 2019 (COVID-19) epidemic, the strain on intensive care units (ICUs) has increased, which made them more vulnerable to the threat of multidrug-resistant organism (MDRO).

Aim This study aims to investigate an outbreak of carbapenem resistant *Acinetobacter baumannii* (CRAB) infection in a general adult ICU of a tertiary hospital in China during the COVID-19 epidemic and evaluate the effectiveness of intervention measures.

Methods Demographic and clinical data of 37 patients were collected, and 230 environmental samples were collected. Whole genome sequencing (WGS) analysis was performed on clinical and environmental isolates. An evolutionary tree was constructed based on the WGS data. The infection control team implemented a bundle of MDRO interventions, including a termination of COVID-19 infection control measures and implementation of 'three-step' cleaning and disinfection method.

Findings There were 37 patients found to have CRAB infection or colonization in the ICU from December 2022 to April 2023, of whom 35 were hospital-acquired. 12 CRAB isolates were obtained from the environment and medical equipment. Through WGS analysis, the CRAB strains from the medical environment and bronchoscopes were found to be highly homologous to those from patients' clinical specimens. This demonstrated that the infection outbreak was caused by the lack of MDRO prevention and control measures. Following intervention, the CRAB detection rate gradually declined, with no positive samples for CRAB found in the ICU environment or on bronchoscopes.

Conclusion The infection control measures for COVID-19 conflicted with basic MDRO prevention and control strategies, likely contributing to the outbreak. Therefore, established infection prevention and control measures should be consistently followed, as they represent the most effective approach to preventing MDRO.

Keywords Carbapenem resistant *Acinetobacter baumannii*, Intensive care unit, COVID-19, Outbreaks, Infection control bundle

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Introduction

Acinetobacter baumannii stands out as one of the most common pathogens responsible for health-care infections (HAIs), contributing to a spectrum of illnesses including pneumonia, bacteremia, skin and soft tissue infections, meningistis, urinary tract infections and so on [1–3]. In recent decades, with the use of broad-spectrum antibiotics, the detection rate of CRAB has been increasing year by year, especially in developing countries. Iran, for instance, reported a staggering detection rate of CRAB reaching 85.1% [2], while in China, 71.4% of CRAB contamination was identified in intensive care units (ICUs) [3]. Recognizing its significant threat to public health, the World Health Organization (WHO) has classified CRAB as a critical pathogen on its global priority list of antibiotic-resistant bacteria [2].

Patients in the ICU often require invasive procedures (such as ventilators, urinary catheters, central venous catheters, etc.) and the use of broad-spectrum antibiotics (such as carbapenems, aminoglycosides, etc.), which increases the risk of colonization and infection with multidrug-resistant bacteria, particularly CRAB. On the other hand, CRAB possesses multiple antibiotic resistance mechanisms, enabling it to resist the majority of antibiotics, which complicates infection treatment. Additionally, *A. baumannii* exhibits strong resistance to desiccation and disinfectants, allowing it to survive for extended periods in healthcare environments. These characteristics facilitate its transmission through environmental contamination and enable *A. baumannii* to cause outbreaks [4]. The onset of the Coronavirus Disease 2019 (COVID-19) has further exacerbated the incidence of such strain in ICUs. Studies revealed a notable surge in the infection and colonization rate of CRAB by 1.5-621.6% during the COVID-19 pandemic [5], with numerous reports documenting CRAB outbreaks in ICUs worldwide [6–9]. For instance, in February 2020, an ICU in a New Jersey acute care hospital experienced an outbreak affecting 34 patients [6]. Similarly, in March of the same year, a severe CRAB outbreak occurred simultaneously in five ICUs at a tertiary care teaching hospital in France [7]. Even in regions with historically low CRAB prevalence, such as Switzerland, a tertiary hospital encountered a CRAB infection outbreak in September 2020 [9]. These reports illustrated that during the COVID-19 pandemic, CRAB outbreaks were more likely to occur in ICUs.

On December 8, 2022, the Chinese government announced further optimization of COVID-19 infection prevention and control measures [10]. On December 26, it declared the lifting of Class A infectious disease prevention and control measures for COVID-19 infections [11]. After that, the country experienced its initial wave of COVID-19 infections. During the pandemic, a general adult ICU in a tertiary hospital admitted a total of 39 COVID-19 patients, 19 of whom were detected with CRAB during their hospitalization. One month later, the number of COVID-19 patients in the ICU gradually decreased, but a CRAB infection outbreak occurred. Subsequently, 17 non-COVID-19 patients were detected with CRAB. The hospital infection control team promptly initiated an investigation into the outbreak and implemented a bundle of intervention measures, with particular emphasis on a three-step cleaning and disinfection protocol for bed unit environments. Ultimately, the CRAB outbreak was was successfully controlled. This study aims to delve into the underlying causes of the outbreak and assess the efficacy of the implemented intervention strategies.

Methods

Clinical setting

The hospital is a prominent 710-bed tertiary care university teaching hospital situated in the southeast of China, with a well-equipped general adult ICU with twenty-four beds, comprising eight single rooms, six double rooms, and one four-person room, spanning an approximate total area of 1200 m^2. The hospital infection control team comprises two physicians, two infection control nurses, a microbiologist, one nurses and two physicians from the ICU staff. Since the inception of the ICU, a robust infection prevention and control program (IPC) has been established, accompanied by the continuous implementation of active surveillance of HAIs through the Hospital Infection Case Monitoring System (HICMS).

HICMS was an active surveillance software that captured real-time nosocomial infection data from various information systems such as the Hospital Information System (HIS), Laboratory Information System (LIS), Electronic Medical Record (EMR), Radiology Information System (RIS) and so on. It provided multidimensional alerts to infection control specialists regarding potential infections. The warning indicators included fever, positive microbial cultures, abnormal biochemical markers, use of antimicrobial drugs, undergoing surgery and so on. Once identified as a hospital-acquired infection, the system saved the data and generated statistical reports and trend charts. Additionally, if more than three cases of infection with the same pathogen occurred in a ward or department within a short period, or if the infection rate significantly increased compared to the same period last year, HICMS would issue an alert for a suspected infection outbreak.

Throughout the year 2022, in the ICU, the number of CRAB infection cases was 2, with an infection case rate of 0.33%. The active surveillance of HAIs in the ICU involved systematic monitoring to detect and prevent

infections. It included regular screening, early identification of pathogens, and prompt intervention to reduce transmission and improve patient safety. The regular screening policy entailed collecting lower respiratory tract specimens from patients upon their initial admission to the ICU to screen for common multidrug-resistant organisms. These included methicillin-Resistant *Staphylococcus aureus* (MRSA), carbapenem-resistant *Acinetobacter baumannii* (CRAB) and *Klebsiella pneumoniae* (CRKP), carbapenem-resistant *Pseudomonas aeruginosa* (CRPA), and vancomycin-resistant Enterococci (VRE).

In our hospital, each ward was equipped with a clinical pharmacist who assists the department's physicians in the proper use of antibiotics. The hospital's computer system automatically reviewed every antimicrobial prescription. The use of high-level antibiotics, such as carbapenems, was strictly regulated. Since the inception of this ICU, a program of antimicrobial stewardship had been in place.

Identification and investigation of the outbreak

An infection outbreak is defined by the CDC Field Epidemiology Manual as more cases of disease than expected in a particular area or in a particular population over a specific period of time [12]. Throughout the year of 2022, there were only two cases of CRAB infections in this ICU, which represented an infection rate of 0.33%. Going into January 2023, the detection rate of CRAB in the ICU increased rapidly. By 15 January, 7 CRAB infections had occurred in this ICU in just two weeks, far exceeding its baseline level. Based on the definition, the IPC determined that an infection outbreak occurred in this ICU. At the same time, our surveillance system (HICMS) also warned of a suspected CRAB outbreak in the ICU. The IPC team immediately launched an investigation into the infection outbreak. Comprehensive demographic and clinical data were systematically collected for all CRAB-positive patients, encompassing gender, age, assigned bed, admission diagnosis, time of ICU admission, CRAB detection onset, specimen type, results of drug susceptibility tests, invasive procedures (e.g., surgeries, endotracheal tube placements, central venous catheterizations, urinary catheter insertions, etc.), antimicrobial therapies (including antibiotic choice, dosage, regimen, and administration timing), presence of colonization or infection, and eventual patient outcome (Fig. 1).

The outbreak definition

A case was defined as a patient admitted to the ICU between 15 December 2022 and 31 March 2023 with a diagnosis of hospital-acquired CRAB infection or colonization.

Hospital acquired infection (HAI) was delineated in accordance with the criteria outlined by the Centers for Disease Control and Prevention [13–16]. Episodes of colonization or infection were deemed ICU-acquired if they occurred 48 h or more following ICU admission. Conversely, non-ICU acquired events denoted CRAB isolates detected either upon a patient's ICU entry or within the initial 48 h thereafter. CRAB infection was defined as the identification of CRAB in a non-sterile site (e.g., soft tissue, respiratory samples, etc.) and in a sterile site (e.g.,blood, cerebrospinal fluid, or pleural fluid) accompanied by clinically evident signs of infection (e.g., fever, elevated white blood cell count, heightened inflammatory markers and abnormal imaging). On the other hand, CRAB colonization characterized by the presence of CRAB in a patient´s clinical specimen without concomitant clinical manifestations of disease.

Environmental investigation

CRAB is primarily transmitted through hand contact. Therefore, the sampling sites selected were high-touch environmental surfaces, such as bed rails, buttons, treatment tables and so on. From 15 January 2023 to 30 January 2023, the IPC team conducted sampling of high-touch environmental surfaces within the ICU including patients' bed units (such as bed railings, bedside tables, bedside buttons and end tables) and medical environment (such as treatment tables, nurse mobile stations). On 18 February 2023, surface samples were obtained from three bronchoscopy transfer carts, each comprising three layers, encompassing handrails, frames, and both inner and outer surfaces of the box. The sampling of high-touch surfaces and bronchoscopy transfer carts were performed using TSA contact plate (HuanKai Microbial, Guangdong, China). The sampling method involved pressing two TSA contact plates, each with an area of 25cm^2, onto the surface of the object for 10 s before retrieval. The TSA plates were subsequently incubated at 37 °C for 48 h, and any suspicious colonies were inoculated onto 5% sheep's blood agar and Mac Conkey plates for further microbiological analysis. On 22 February 2023 the IPC team inspected three disinfected bronchoscopes in the ICU. The biopsy channel of bronchoscopy was cultured using the flush-brush-flush method [17]. The endoscope channels were tested by flushing 50 ml of sterile Difo Letheen Broth neutralizing recovery medium (Becton, Dickinson and Company, Sparks, MD) through the biopsy channels. The medium was then filtered through sterile 0.45 μm membrance filters, placed onto nutrient agar in petri plates, incubated at 37 °C for 48 h. Any suspicious colonies were collected for microbiological analysis. We conducted quarterly monitoring of the cleaning and disinfection quality of bronchoscopes, using the methods described above. Up until the outbreak of infection, all test results were qualified. The bronchoscope cleaning and disinfection process included pre-cleaning,

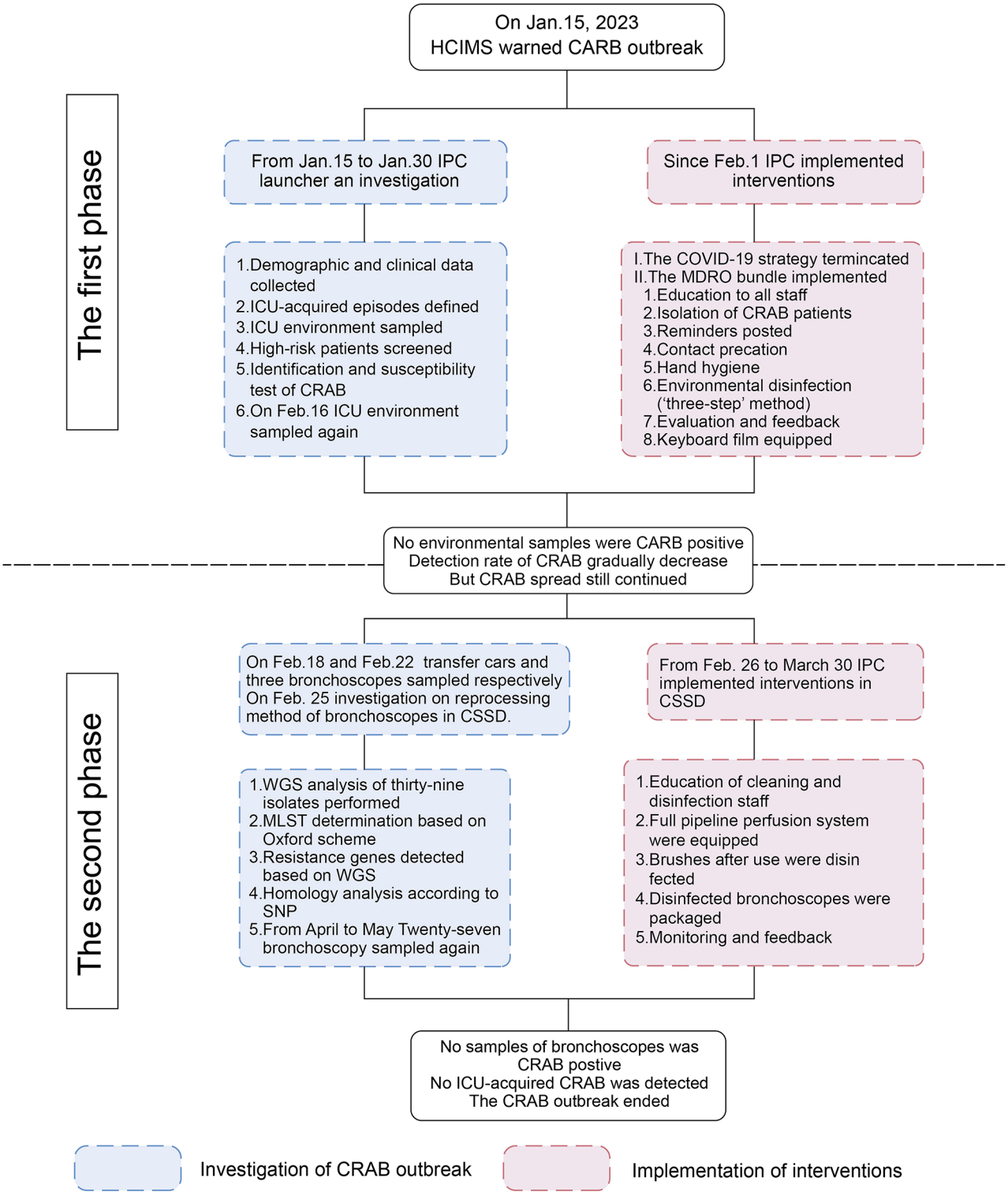

Fig. 1 The flow diagram of the outbreak investigation and implementation of interventions in this study. HICMS: the Hospital Infection Case Monitoring System; CRAB: Carbapenem resistant *Acinetobacter baumannii*; IPC: the infection prevention and control program; ICU: Intensive care unit; MDRO: multidrug-resistant organism; CSSD: the Central Sterile Supply Department; WGS: Whole genome sequencing analysis; MLST: Multi-locus sequence typing; SNP: Single-nucleotide polymorphism

leak testing, manual cleaning, rinsing, high-level disinfection, final rinsing, drying, and proper storage. High-level disinfection was performed by soaking in ortho-phthalaldehyde (OPA) for 5 min.

Screening and microbiological method

All patients expected to remain in the ICU for more than 24 h underwent screening for CRAB carriage. CRAB screening was a temporary outbreak measure rather than a routine practice. This involved collecting respiratory tract samples upon admission and weekly thereafter, including sputum, lavage fluid and tracheal swabs. Respiratory tract samples collected from non-ventilated patients and ventilated patients. Clinical and environmental samples were cultured on 5% sheep's blood agar and Mac Conkey plates (bioMerieux, France) and then incubated at 37 °C in 5% CO_2 for 24 h. Phenotypic identification of *A. baumannii* was accomplished through biochemical reactions, including oxidase, catalase, sugar fermentation, motility, citrate utilization, and the ability to grow at 41 °C and 44 °C. Suspicious colonies underwent testing using the Vitek 2 automated system with GN cards (bioMérieux, France). Antibiotic susceptibility testing was conducted via Vitek 2 automated system with AST-N335 cards (bioMérieux, France), with MIC determination and interpretation results aligned with those of the the Clinical and Laboratory Standards Institute [18]. Imipenem MICs were determined using the E-test (AB Biodisk, Solna, Sweden). Molecular confirmation was achieved through PCR to detect the intrinsic OXA-51 resistance gene.

Prevention and control strategies before intervention

Since the Chinese government adjusted the COVID-19 prevention and control strategy on 8 December 2022, the SARS-CoV-2 virus rapidly disseminated across China. Subsequently, beginning on 20 December 2022 the ICU commenced admitting COVID-19 patients. In an effort to mitigate COVID-19 transmission within hospitals, the IPC mandated that staff wear specific personal protective equipment (PPE) before entering the ICU patient area, including N95 masks, medical caps, isolation gowns, gloves and face masks. Furthermore, it was required to remove PPE upon exiting the patient area. However, when caring for patients infected or colonized with MDROs, staff were not instructed to replace PPE or utilize an additional isolation gown on top of the original one. This prevention and control strategy remained in effect until 31 January 2023.

Implementation of intervention

Starting from February 2023, the COVID-19 prevention and control measures in the ICU were discontinued. Medical staff did not need to wear any other PPE except for N95 masks when entering patient area. Instead, a bundle of MDRO infection control and prevention measures was implemented. (1) Education: The IPC provided MDRO training to all ICU staff, including physicians, nurses, cleaning personnel, and nursing workers. (2) Isolation: Patients with CRAB were promptly isolated in single rooms. When single rooms were insufficient, CRAB patients were cohorted to a four-person room at the end of the ward (beds 21 to 24), maintaining a certain distance from non-CRAB patients. Patients originating from long-term care facilities or who had been hospitalized in the ICU within the past three months would be isolated until CRAB screenings returned negative results. (3) Reminders: The "CRAB" logo was displayed at the head of the patient's bed, and a blue line symbolizing contact isolation was marked on the patient's wrist strap. (4) Contact precaution: HCWs worn medical gloves, isolation gowns and N95 masks before entering MDRO patients' room. Upon leaving the room, all PPE would be removed. (5) Hand hygiene: HCWs strictly adhered to the WHO's five moments for hand hygiene. (6) Environmental disinfection: The three-step method was employed for cleaning and disinfecting the bed unit environment. Non-disposable towels were used for the method, and the disinfectant was a chlorine-based solution with a concentration of 500 mg/ml. Disposable disinfectant wipes were used to wipe the surfaces of medical equipment. The primary component of the disinfectant wipes was a compound double-chain quaternary ammonium salt, with a concentration of 1.85 g/L. All disinfection procedures were to be carried out once every 8 h. Upon patient discharged, terminal disinfection of the bed unit was performed, encompassing air disinfection via hydrogen peroxide aerosols. Post-terminal disinfection, the bed unit environment underwent CRAB sampling to verify disinfection effectiveness. (7) Evaluation and Feedback: The IPC team monitored HCWs' hand hygiene compliance daily and assessed cleaned surfaces using fluorescein spray with UV torches. HCWs' hand hygiene compliance was assessed using the World Health Organization (WHO) hand hygiene observation form, which included compliance at five key moments: before touching a patient, before clean/aseptic procedures, after touching a patient, after exposure to body fluids, and after touching a patient's surroundings. Cleaning quality assess: On the first day, we used a fluorescent marker to label all surfaces of the patient's bed unit that require cleaning (these marks were only visible under ultraviolet light). On the second day, these fluorescent marks were inspected using an ultraviolet light. If all the marks were completely removed, it indicated good cleaning quality. If the marks remained, it suggested lapses in the cleaning process. Surveillance results were provided as feedback on a weekly basis. (8) Keyboard film: Since May 2022,

all computers in patient areas of the ICU were equipped with keyboard films, which were replaced twice daily (distinguished by color). Procedures for cleaning, disinfecting, and drying the keyboard film were diligently implemented.

The "three-step" clean and disinfection

All objects within the bed unit were meticulously classified and labeled with different colors (Fig. 2), each corresponding to a specific cleaning and disinfection protocol. The objects were categorized into three zones: (1) Clean zones: Marked in blue, these areas, including medication preparation vehicle, bed end table, equipment cabinet, and mobile nurse station, were cleaned using blue towels and were primarily where nurses operated. (2) Semi-Contaminated Zones: Designated in yellow, these encompassed the immediate surroundings of the patient, such as bed railings, bedside tables, and bed head panels. Yellow towels were utilized for cleaning these areas. (3) Contaminated Zones: Identified by red labels, these zones included the patient's black and yellow trash cans. Brown towels were employed for cleaning and disinfecting these areas. The disinfection procedure followed a sequential approach, beginning with the clean zones, then progressing to the semi-contaminated zones, and finally addressing the contaminated zones. The disinfectant is a chlorine-based solution with a concentration of 500 mg/mL. The cleaners were required to change the towels and perform hand hygiene between different bed units to prevent cross-contamination.

Whole genome sequencing

To delve deeper into the outbreak investigation, whole genome sequencing (WGS) analysis was conducted on 39 CRAB isolates, comprising 28 isolates from the patients' clinical samples and 11 isolates from the environment and medical equipment. Because microbiological laboratories did not store specimens and strains from discharged patients in the past, 7 CRAB isolates from patients were not obtained. On the other hand, one CRAB strain from a bronchoscope transport case was not included in this WGS analysis. The research team considered it more meaningful to analyze the isolate from the inner lumen of the bronchoscope and, in an effort to conserve funds, chose not to include this strain. DNA quantification was performed using the Qubit Equalbit 1X dsDNA HS Assay Kit and Qubit fluorometers (Invitrogen). WGS was performed utilizing Illumina NovaSeq platform alongside the TIANSeq Fast DNA Library Kit (Illumina) (TianGen Biotech, Beijing, China). Sequence reads were assembled using SPAdes v3.15.5 [19], while genome annotation was achieved using the NCBI Prokaryotic Genome Annotation Pipeline (PGAP). The identified open reading frames (ORFs) were uploaded to the ResFinder v3.0 web server for identifying resistance genes and the MLST web server for the Pasteur and OXford scheme MLST analysis (www.genomicepidemiology.org) [20]. Draft genome assemblies of *A. baumannii* strains reported in this study have been deposited at NCBI under the BioProject accession number PRJNA1097824.

The construction of the phylogenetic tree involved six main steps: First, genome sequence of *Acinetobacter pittii*

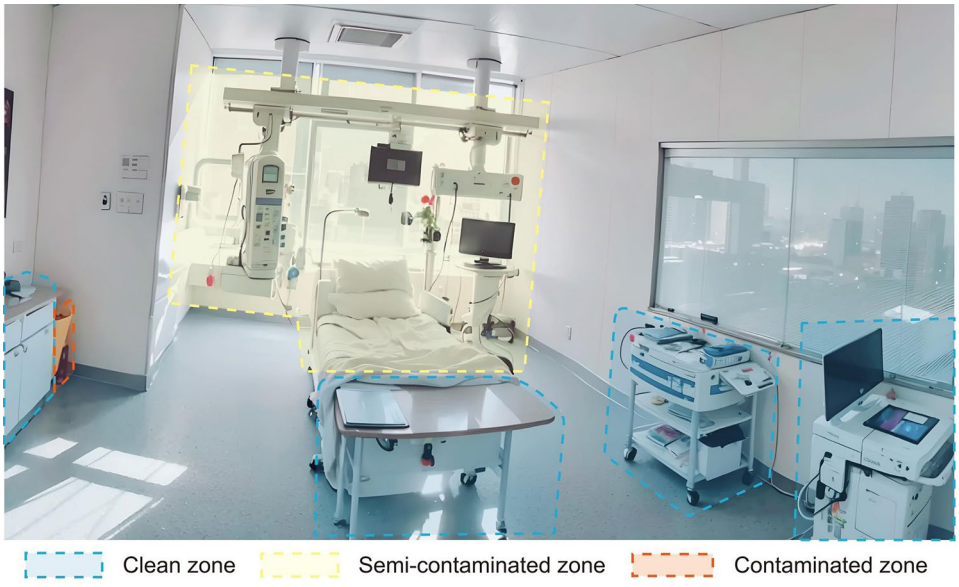

Fig. 2 The "three-step" clean and disinfection of patient unit (1) The clean zone was marked in blue, including a medication preparation vehicle, a bed end table, an equipment cabinet, and a mobile nurse station. (2) The semi-contaminated zone was marked in yellow, including bed railings, bedside tables, and bed head panels. (3) The contaminated zone was marked in red, including the patient's black trash can and yellow trash can. The procedure of cleaning and disinfection was first to the clean zone, then to the semi-contaminated zone, and finally to the contaminated zone

CIP 70.29, a closely related reference strain, was retrieved from the NCBI GenBank database. Second, Roary version 3.13.0 [21, 22] was used to analyze the pan-genome of 38 strains (37 *A. baumannii* and 1 *A. pittii*), generating a core gene alignment file containing 1,637 conserved genes. Third, SNP-sites version 2.5.1 [23] was employed to extract single nucleotide polymorphism (SNP) sequences from the core gene alignment. Fourth, jModelTest 2 version 2.1.10 [24] was utilized to determine the optimal nucleotide substitution model, identified as the General Time Reversible (GTR) model. Fifth, RAxML-NG version 1.2.1 [25] was used to build a maximum likelihood phylogenetic tree under the GTR substitution model, with 1,000 bootstrap replicates. Sixth, the resulting tree was imported into iTOL version 6.8.5 [26] for refinement. Branches with bootstrap support values < 50 were collapsed, and the tree was rerooted using the *A. pittii* CIP 70.29 branch. The final tree (in Newick format) was exported, and heatmaps were generated using iTOL.

Statistical analysis

Descriptive statistics were were employed to characterize the demographic and clinical characteristics of the patients, encompassing measures such as mean and standard deviation for continuous variables, and median and interquartile range (IQR) for non-normally distributed continuous variables. Categorical variables were summarized using proportions. All statistical analyses were conducted using the Statistical Package for the Social Sciences for Windows (version 26; SPSS, Chicago, IL) software.

Results

Demographic and clinical characteristics of CRAB patients

From 1 December 2022 to 30 April 2023, a total of 37 patients with CRAB infection or colonization were identified (Supplementary Table S1). Among them, 19 patients' SARS-CoV-2 nucleic acid tests were positive. There were 14 ICU-acquired CRAB infections, 20 ICU-acquired colonization and 3 non ICU-acquired colonization and infections. The ICU-acquired CRAB infections included 7 ventilator-associated pneumonias (VAPs), 4 pneumonias, 1 bacteremia, 1 systemic infection, and 1 central line-associated bloodstream infection (CLABSI). Hospital-acquired CRAB pneumonia developed in 33.3% of 18 patients with pre-existing disease on admission. In terms of device usage, there were 37 (100.0%) urinary catheterizations, 13 (81.1%) mechanical ventilations, and 35 (94.6%) central venous catheters. During this period, 9 patients succumbed to their conditions during hospitalization, resulting in a mortality rate of 24.3% (Table 1). Among the nine deceased patients, three were infected with CRAB. The mortality rate of CRAB was 8.1%.

Outbreak description

Since the announcement by the Chinese government on 8 December 2022 to further optimization of COVID-19 infection prevention and control measures. The ICU experienced a surge in COVID-19 patients, with the average daily number of hospitalized patients increasing from 15 to 24. To curb the spread of COVID-19, the ICU implemented a series of prevention and control measures. However, the contact isolation for MDRO patients was discontinued, ultimately leading to the widespread of CRAB within the ICU. The weekly number of CRAB detection increased from 0 to 7. The HICMS warned of a suspected CRAB outbreak in the ICU on 15 January 2023. The IPC team immediately launched an investigation. The timeline of events is as follows:

(1) Initial investigation (15 Jan.2022-30 Jan.2023): The IPC team collected clinical data from CRAB-positive patients and sampled the ICU environment and medical equipment. A total of 173 environmental samples were collected, from which 10 CRAB strains were isolated. WGS analysis confirmed that the CRAB strains from the environment were highly homologous to those isolated from patient clinical specimens. This confirmed the IPC team's hypothesis that the CRAB outbreak was closely related to the discontinuation of MDRO prevention and control measures. Healthcare workers did not change gloves and gowns after contact with CRAB patients or their environment. Contaminated protective equipment led to the transmission of CRAB when caring for the next patient.

(2) Implementation of MORO bundle (on 1 Feb.2023): A bundle of MRO intervention measures was implemented in the ICU, including contact precaution, hand hygiene, environmental disinfection, education of staff and so on. After the intervention, the IPC team conducted another round of environmental sampling on 17 February. A total of 173 samples were collected, and no CRAB was detected. At this time, the CRAB detection rate also significantly decreased, dropping from a peak of 7 cases per week to 3 cases. However, the detection rate had not yet fallen to zero, indicating that transmission was still ongoing. To identify potential sources of transmission, the IPC team again sampled medical equipment that could potentially cause cross-contamination. Ultimately, a CRAB strain was isolated in a bronchoscope transport car. This suggested that the outbreak might be related to contaminated bronchoscopes.

(3) Bronchoscopy investigation and intervention (22 Feb. 2023–30 March 2023):

Table 1 Demographic and clinical characteristics of CRAB positive patients from Dec. 1, 2022 to Apr. 30, 2023

Variable	No. of patients (%) *n* = 37
Age (years), mean ± SD	70.0 ± 13.3
Male %	27 (73.0)
SARS-CoV-2 nucleic acid test positive	19 (51.4)
Diagnoses upon admission	
Pneumonia	18 (48.7)
Cerebral hemorrhage	6 (16.2)
Esophageal malignancy	3 (8.1)
Sepsis	3 (8.1)
Chronic renal failure	1 (2.7)
Hydrocephalus	1 (2.7)
Mediastinal infection	1 (2.7)
Myasthenia gravis	1 (2.7)
Pulmonary fungal infection	1 (2.7)
Urinary tract infection	1 (2.7)
Site of isolatetes (*n* = 57)	
Sputum	32 (56.1)
BALF	16 (28.1)
Blood	2 (3.5)
Catheter head	1 (1.8)
Lung tissue	1 (1.8)
Secretion	1 (1.8)
Ascites	1 (1.8)
Hydrothorax	1 (1.8)
Drainage	1 (1.8)
Urine	1 (1.8)
Device	
Urinary catheter	37 (100.0)
Central venous catheter	35 (94.6)
Bronchoscopy	35 (94.6)
Endotracheal tube	30 (81.1)
Surgery	14 (37.8)
CRRT	12 (32.4)
Pleural drainage	6 (16.2)
Received bronchoscopy before first detecting CRAB	18 (48.7)
Total hospitalization (days) M(P_{25}, P_{75})	25.0(18.5, 43.0)
Total ICU stay (days) M(P25, P75)	20.0(16.0, 32.5)
Pattern of CRAB acquisition	
ICU-acquired colonization	20 (54.1)
Non ICU-acquired colonization	1 (2.7)
ICU-acquired infection	14(37.8)
Non ICU-acquired infection	2(5.4)
Types of ICU-acquired infections (*n* = 14)	
VAP	7 (50.0)
HAP	4 (28.6)
CLABSI	1 (7.1)
Bacteremia	1 (7.1)
Systemic infection	1 (7.1)

Table 1 (continued)

Variable	No. of patients (%) *n* = 37
Outcome	
In-hospital mortality	9 (24.3)

CRAB: Carbapenem-resistant Acinetobacter baumannii

BALF: Bronchoalveolar lavage fluid

CRRT: Continuous renal replacement therapy

VAP: Ventilator associated pneumnia

HAP: Hospital acquired pneumonia

CLABSI: Central line associated-bloodstream infection

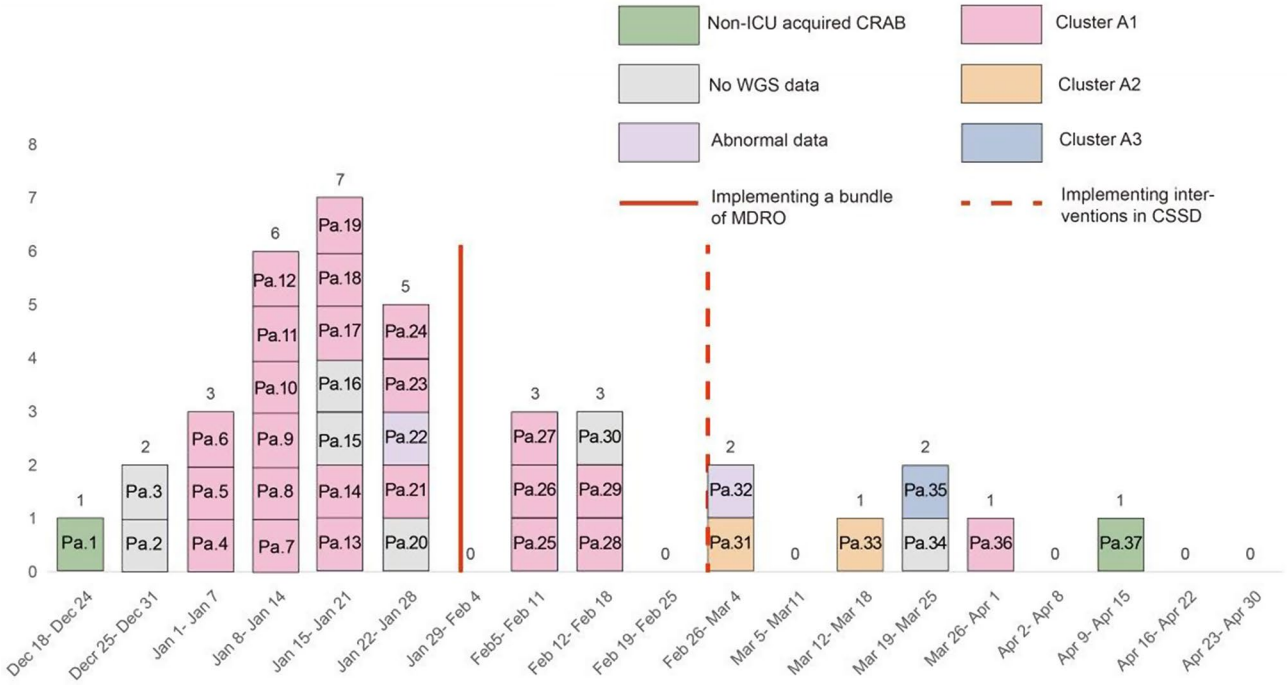

Fig. 3 Timeline of 37 CRAB patients detected in the ICU from December 2022 to April 2023. CRAB: Carbapenem-resistant *Acinetobacter baumannii*; ICU: intensive care unit; MDRO: multidrug-resistant organism; CSSD: the Central Sterile Supply Department

On February 22, all bronchoscopes in the ICU (a total of 3) were tested. As a result, one CRAB strain was isolated from the lumen of one bronchoscope. WGS analysis confirmed that it was highly homologous to the strains isolated from patients. The IPC team immediately launched an investigation into the Central Sterile Supply Department (CSSD) responsible for bronchoscope reprocessing. Investigation revealed deficiencies in the cleaning and disinfection process of bronchoscopes, including incomplete manual perfusion, lack of high-level disinfection for cleaning brushes, and improper storage. Interventions were implemented at CSSD from 26 February to 30 March, including process redevelopment, staff education and training, equipment upgrades, and packaging improvements. Subsequent random inspections revealed no CRAB-positive bronchoscopes, and no ICU-acquired CRAB cases were detected within the following four weeks (Fig. 3).

Detection of CRAB in ICU environment

During the investigation and intervention phases, the IPC team collected a total of 230 environmental samples from various locations within the ICU (Table 2). Sample types included bedside buttons, bedside tables, bedside armrests, treatment tables, nurses' mobile cars, bronchoscopy, bronchoscope transfer carts and bronchoscope leak detectors. Among these samples: eight CRAB isolates were recovered from the bed unit environment, two isolates were obtained from the medical environment, and two isolates were associated with the bronchoscope and its transfer cart. Notably, one CRAB was isolated from the bed end armrest after the terminal disinfection of the bed unit on 6 February 2023. Subsequently, terminal disinfection was repeated in the bed unit, and no further CRAB was detected. Following the implementation of the 'three-step' method and the utilization of fluorescein spray to monitor cleaning effectiveness, no

Table 2 Detection of CRAB in the ICU patient environment

Item	Number of samples (*n*)	Number of CRAB (*n*)	Detection rate (%)
Bedside left button	24	4	16.7
Bedside right button	24	1	4.2
Bed end table	24	1	4.2
Bedside table	24	1	4.2
Left armrest by the bed	10	0	0.0
Right armrest by the bed	10	0	0.0
Bedside armrest	9	0	0.0
Bed end armrest	9	1	11.1
Treatment table	24	2	8.3
Nurse mobile car	15	0	0.0
Bronchoscopy	3	1	33.3
Bronchoscopy transfer cart	51	1	2.0
Bronchoscopy leak detector	3	0	0.0

CRAB: Carbapenem-resistant *Acinetobacter baumannii*;ICU: intensive care unit

CRAB-positive samples were detected in the entire ICU. Similarly, post-intervention inspections in the Central Sterile Supply Department (CSSD) revealed no further detection of CRAB from disinfected bronchoscopes. The intervention measures effectively controlled the spread of CRAB in the ICU.

Antimicrobial susceptibility testing

Among the 37 CRAB patients, a total of 57 strains were isolated. It was worth noting that strains from the same site in the same patient were considered the same strain, while strains from different sites were defined as separate strains. The distribution of these strains across sample types was as follows: 56.1% were isolated from sputum; 28.1% were isolated from bronchoalveolar lavage. All isolated strains exhibited resistance to carbapenems, quinolones, penicillins plus β-lactamase inhibitors, and third-generation cephalosporins. However, they demonstrated sensitivity to tigecycline (80.7%) and colistin (100.0%) (Supplementary Table S6.). In summary, the strains isolated from the outbreak were resistant to most antibiotics except polymyxin and tigecycline.

Outbreak profiling by WGS analysis

The results analyzed by WGS and bioinformatic analysis on thirty-seven CRAB isolates were shown in Fig. 3. Two isolates' data were abnormal, which were not included. The reason for the abnormal data might be that the DNA of these two strains degraded during transportation, resulting in incomplete data acquisition.

(1) MLST analysis: According to the Pasteur scheme, all thirty-seven isolates belonged to sequence type (ST) 2, which is part of the international clone II lineage. Whereas, analysis using the Oxford scheme revealed the presence of four distinct clones: ST1968 ($n = 14$), ST1806 ($n = 2$), the singleton ST3243, and a Novel sequence type ($n = 20$).

(2) Phylogenetic tree: The phylogenetic tree showed that the 37 strains could be divided into three clusters: A1, A2, and A3. Cluster A1 included 34 strains, cluster A2 included Pa33 and Pa31, and cluster A3 consisted solely of Pa35. To further elucidated the relatedness of isolates involved in this outbreak, we calculated the number of SNP differences between each pair of strains and visualized them in a heatmap (Supplementary Fig. 1). The heatmap clearly revealed results consistent with the phylogenetic tree, showing that the 37 strains can be divided into three clusters: A1, A2, and A3. On the other hand, we conducted a more detailed analysis of the differences among the strains in cluster A1. Based on the characteristics of SNPs, the smaller the number of SNP differences between strains, the more similar they are. Firstly, the pairwise differences between Pa4, Pa6, Pa11, Pa13, Pa19, Pa26, Pa28, Pa29, and E15 were less than 40 SNPs, so they could be identified as the same clonal strain. This demonstrated that a single clonal strain persisted in the ICU, spreading between patients and the environment, as well as between patients, for over a month. Secondly, the genetic distances between E10, E13, E14, E16, and E2 were also less than 40 SNPs apart from each other, hence they could be regarded as the same clonal strain. These strains were isolated from the environments of beds 4, 10, 9, 18, and 17, respectively (Fig. 5). This confirmed cross-contamination of the same clonal within the ICU environment. Thirdly, E1 isolated from a bronchoscope on 22 February, differed from Pa7 (isolated on 8 January) and Pa27 (isolated on 10 February) by 23 and 39 SNPs, respectively. This provided evidence that the bronchoscope was involved in the transmission of CRAB during this outbreak. In summary, we concluded that the transmission of CRAB during this outbreak primarily occurred through contaminated environments and contaminated bronchoscopes.

(3) Drug resistance genes: drug resistance genes revealed the presence of $bla_{\mathrm{OXA-23}}$ and $bla_{\mathrm{OXA-66}}$, which encode Class D beta-lactamases responsible for carbapenem resistance. These genes were carried by all isolates. Additionally, all isolates carried *aph(3)-Ia*, *adcC*, and *abaF* genes, associated with resistance to aminoglycosides, efflux pumps, and fosfomycin, respectively. Most isolates also harbored resistance genes to macrolides, streptogramins, and sulfonamides, contributing to their broad antibiotic resistance profile.

Effects of implementing interventions

The intervention measures yielded significant improvements in various aspects of infection control within the ICU: (1) Reduction in CRAB Detection Rate: The detection rate of CRAB in the ICU environment decreased from 5.8 to 0.0% after intervention (Supplementary Table S2). (2) Enhanced Cleaning and Disinfection of Bronchoscopes: The qualified rate of cleaning and disinfection of bronchoscopes increased from 66.7 to 100.0% following intervention (Supplementary Table S3). (3) Improved Hand Hygiene Compliance: HCWs' Hand hygiene compliance increased from 84.4 to 91.3% after intervention (Supplementary Table S4). (4) Enhanced Cleaning Quality of Bed Units: The IPC team utilized fluorescein spray to assess the cleaning quality of bed units. Following intervention, the clearance rate of fluorescent markers increased significantly from 89.6 to 100.0% (Supplementary Table S5). These results demonstrated the effectiveness of the intervention measures in mitigating environmental contamination, improving cleaning and disinfection practices, enhancing hand hygiene compliance, and ensuring overall cleanliness within the ICU environment. The improvements in these aspects ultimately brought an end to the CRAB outbreak.

Discussion

The CRAB outbreak in the ICU during December 2022 to April 2023 could be divided into two phases. The first phase extended from late December 2022 to mid-February 2023. During this period, adjustments to China's COVID-19 containment strategies led to a surge in ICU admissions of COVID-19 patients. To mitigate viral transmission, the ICU adopted specialized infection control protocols; however, this inadvertently resulted in the discontinuation of contact precautions for MDRO patients. This resulted in an outbreak of CRAB in the ICU. The second phase spanned from mid-February 2023 to the end of March 2023. During this phase, the IPC team implemented a bundle of MDRO infection control and prevention measures in the ICU. While the detection rate of CRAB significantly decreased, it did not drop to zero. Investigations revealed that disinfection failures in bronchoscopes might have been another contributing factor to the outbreak. The IPC team promptly initiated an investigation into the reprocessing of bronchoscopes and implemented targeted interventions. Following these actions, no new cases of CRAB were detected in the ICU. We will elaborate on the factors contributing to the spread of CRAB during each phase and evaluate the effectiveness of the corresponding intervention measures.

During the first phase, there were four factors contributed to this outbreak. (1) Critical condition of COVID-19 patients increased susceptibility to MDRO infection. (2) The severe shortage of human resources and increased workload likely contributed to inadvertent lapses in infection control measures by HCWs. The patient-to-nurse ratio increased from 0.9 to 1.2 in the ICU during this period. (3) The ICU implemented inappropriate control measures, requiring HCWs to wear all PPE before entering the patient area, while not requiring them to change these items. (4) The ICU terminated the previous MDRO contact precautions. This allowed healthcare personnel to care for different patients without needing to change gloves or perform hand hygiene. CRAB was primarily transmitted through contact, and the hands of HCWs were one of the main routes of CRAB transmission. When HCWs care for CRAB-positive patients, their gloves became contaminated with CRAB. Failure to change gloves or perform hand hygiene after patient care led to the spread of CRAB within the ICU.

Many articles also reported similar findings. Thoma et al.. conducted a review of 17 articles concerning MDRO outbreaks during the COVID-19 pandemic. The results showed that most outbreaks were due to inadequate PPE or hand hygiene adherence, PPE shortage, and high antibiotic use [9]. Shinohara et al. reported that to minimize healthcare personnel exposure to COVID-19 patients, they started to conduct medical preparation outside patient rooms before entering [8]. Furthermore, the frequency of certain traditional prevention and control measures has been decreased, such as patient bathing with chlorhexidine gluconate [6], thus, a 43% reduction in CRAB screening tests in the ICU [9]. In summary, many experts believed that some of the preventive and control measures taken during the COVID-19 pandemic instead facilitated the spread of MDRO. Therefore, they recommend promptly reverting to traditional prevention and control strategies [6–9].

Consequently, our IPC team discontinued the infection control measures for COVID-19 on February 1, 2023, and bolstered contact precautions for CRAB patients. A bundle of MDRO strategies has been implemented, with particular emphasis on the three-step cleaning and disinfection method. This intervention played an crucial role in halting the spread of CRAB in the environment. Firstly, it prevented cleaning personnel from using a single towel to wipe all items in the patient's bed unit. Secondly, the rigorous cleaning sequence compelled cleaners to prioritize disinfecting the clean zone, which included area designated for medications and infusion preparation. Contamination in these areas could lead to severe consequences. Finally, the cleaning and disinfection process may be more practical and cost-effective for low- and middle-income countries. In many high-income countries, the practice of using reusable towels has been phased out in favor of disposable disinfectant wipes. The nurses are primarily responsible for disinfecting the bed

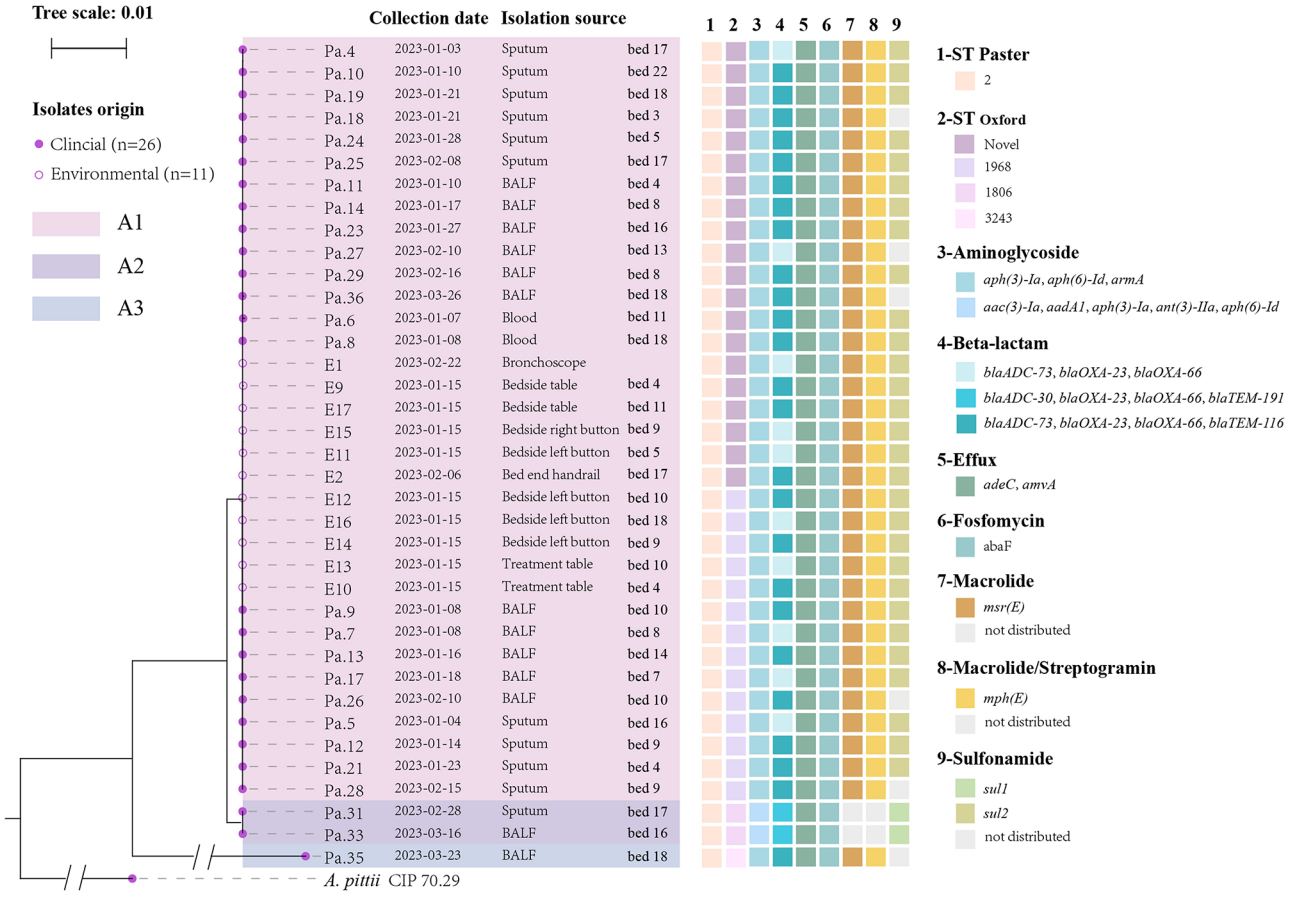

Fig. 4 An evolutionary tree of 37 CRAB isolates based on WGS (26 isolates from the patients and 11isolates from the ICU environment). The genome sequence of *Acinetobacter pittii* CIP 70.29 obtained from the NCBI was served as a reference. There were three distinct clusters could be identified, namely A1, A2, and A3. Patient and environment sequences were named Pa.xx and Exx, respectively. WGS: Whole genome sequencing analysis; CRAB: Carbapenem resistant *Acinetobacter baumannii*; ICU: Intensive care unit; BALF: Bronchoalveolar lavage fluid

unit. However, in developing countries, limited medical resources often cannot cover the expense of high-cost disinfectant wipes. The task of disinfecting bed units is often carried out by individuals lacking a medical background, including those with lower levels of education and income. Under these circumstances, the ICU must establish a specialized disinfection process for bed units to achieve thorough cleaning outcomes while making efficient use of limited resources. Fortunately, the 'three-step' method efficiently eradicated *A. baumannii* from the environment during this outbreak, with the cooperation of other prevention and control measures, the transmission of CRAB was effectively halted. So, this method could be more cost-effective and therefore more suitable for low- and middle-income countries.

On the other hand, we have adopted special approaches to improve the education of the cleaning staff. The approaches included the following three components: offline lectures, on-site drills, and random inspections. Firstly, all cleaning staff attended a course covering the three-step cleaning and disinfection method, hand hygiene, and MDRO prevention and control measures. This enhanced the cleaning staff's awareness of hospital infection prevention and control. Subsequently, on-site drills were conducted in the ICU to ensure that each member thoroughly masters the three-step method. Finally, the hospital infection specialists randomly observed and recorded the operations of the cleaning staff weekly, and the results were regularly fed back to the cleaning supervisor. This achieved continuous quality improvement.

During the second phase, the IPC found that disinfection failures in bronchoscopes might have been another contributing factor to the outbreak. There were many reports detailing MDRO outbreaks attributed to inadequate cleaning and disinfection of endoscopes. One of the most notable incidents was the ERCP postoperative infection that occurred in the United States between 2012 and 2016 [27, 28]. The outbreak was attributed to the ineffective disinfection of the duodenoscope used ERCP procedures. Similarly, the bronchoscopy in the ICU also failed to undergo proper disinfection. The

reason for this failure included the absence of adequate cleaning and disinfection facilities, violations of standard operating procedures by disinfection personnel, and risks associated with the storage method of the bronchoscopy. This serves as a reminder to hospital infection control personnel to remain vigilant regarding the quality of endoscopy cleaning and disinfection, as it directly impacts patient safety.

The CRAB strains isolated during this outbreak were resistant to all antibiotics except tigecycline and colistin. Prior to the CRAB outbreak, the average usage rate of carbapenem antibiotics in this ICU from January to November 2022 was 10.71% (number of patients using this class of antibiotics / total number of inpatients × 100%). After the CRAB outbreak, the average usage rate of carbapenems increased to 20.97% in December 2022 and further rose to 25.40% in January 2023. Following the implementation of a bundle of intervention measures in the ICU, the detection rate of CRAB gradually decreased, and the usage rate of carbapenems also declined. By February 2023, the average usage rate had dropped to 11.76%, to 9.18% in March, and further decreased to 5.21% in April. This further underscored the importance of antimicrobial stewardship in curbing CRAB outbreaks. Stewardship programs enforce evidence-based prescribing practices, curbing unnecessary broad-spectrum antibiotic exposure that drives resistance selection. By promoting targeted therapy guided by susceptibility testing and advocating for antibiotic de-escalation, these programs reduce selective pressure, thereby limiting CRAB dissemination. Additionally, stewardship fosters interdisciplinary collaboration to optimize treatment efficacy while minimizing collateral damage to microbial ecology. Integrating stewardship with infection prevention measures creates a synergistic defense, essential for controlling outbreaks and preserving therapeutic options in healthcare settings.

This study has several limitations. Among the thirty-five patients who acquired CRAB in the ICU, isolates from seven patients were not collected. This was due to two reason. Firstly, when HICMS alerted of a potential outbreak on 15 January 2023, patients with CRAB in December 2022 had already been discharged, thus their isolates could not be collected. Secondly, some patients had short stays in the ICU, and by the time laboratory reports confirmed CRAB positive, they had already been

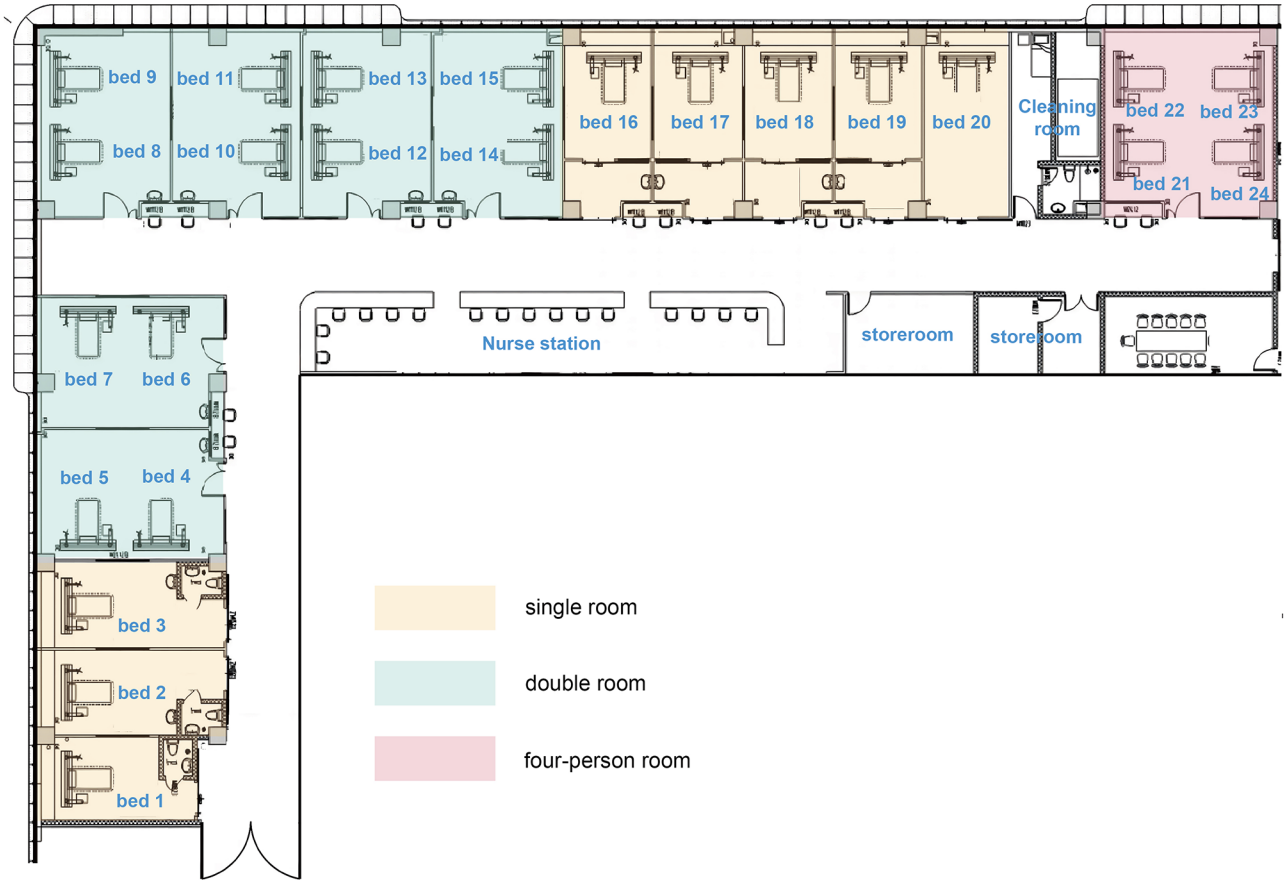

Fig. 5 The architectural layout of the ICU. There were eight single rooms corresponding to beds 1, 2, 3, 16, 17, 18, 19, and 20. Six double rooms spanned from bed 4 to bed 14. One four-person room included beds 21 to 24

discharged, resulting in a delay in collecting their isolates. Our microbiology laboratory did not store specimens or strains from discharged patients in the past. We have raised this issue with the laboratory. Storing target MDROs under surveillance is essential for aiding future outbreak investigations, enabling better analysis, tracking, and response to emerging multidrug-resistant organism threats. Furthermore, this study only selected the respiratory tract as the screening site for CRAB, omitting other sites such as the armpit, groin, skin, and rectum as in other studies. Since there is no consensus on the optimal screening site for CRAB. This screening method might overlook some patients with colonization, but it did not impact the evaluation of the effectiveness of prevention and control measures. Throughout the entire outbreak period, the consistent use of the same screening method, coupled with the implementation of interventions, led to a gradual decline in the detection rate of CRAB, eventually reaching zero for four consecutive weeks. This clearly demonstrated the effectiveness of these measures in containing the spread of CRAB.

Conclusion

Through investigation, this study concluded that the primary reasons for the outbreak were the absence of contact precautions for MDRO patients and the failure of bronchoscope disinfection. Following the implementation of interventions, the detection rate of CRAB significantly decreased in the ICU, indicating the effectiveness of the strategies. Among these interventions, the "three-step" method successfully halted the spread of CRAB in the ICU. This method was environmentally friendly, cost-effective, and suitable for low- and middle-income countries. Regardless of the circumstances, it is crucial to adhere to established prevention and control principles, as they form the cornerstone of our endeavors to safeguard the health and safety of patients.

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s13756-025-01547-0.

Supplementary Material 1

Supplementary Material 2

Supplementary Material 3

Supplementary Material 4

Supplementary Material 5

Supplementary Material 6

Supplementary Material 7

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Author contributions

YK and TL conceived and designed the experiments and wrote the main manuscript text. YK, TL, YZ and HW collected the samples and information.YK and TL performed the experiments in the laboratory.YZ and HW analyzed the study samples and interpretation of the data. YK prepared Figs. 1, 2, 3 and 4; Tables 1, 2 and 3, and wrote the first draft of the manuscript. HL made critical revisions and approval final version. All authors agreed with manuscript results and conclusions, and reviewed and approved of the final manuscrip.

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Data availability

Draft genome assemblies of A. baumannii strains reported in this study have been deposited at NCBI under the BioProject accession number PRJNA1097824.

Declarations

Ethical approval

The study was conducted in accordance with the guidelines of the Helsinki declaration. Outbreak investigations and reporting were performed within the routine processes of the institutional infection control department. This article is a retrospective analysis of an infection outbreak, utilizing anonymized aggregate data and has received an exemption approval from the IRB. Therefore, obtaining informed consent from patients is not required.

Competing interests

The authors declare no competing interests.

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