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Original Article

Sequential use of capsular typing and whole-genome sequencing-based analysis for transmission of carbapenem-resistant *Acinetobacter baumannii* in a tertiary medical center

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Abstract *Background:* During the COVID-19 pandemic, there has been an increasing trend in healthcare-associated infections (HAIs) caused by carbapenem-resistant *Acinetobacter baumannii* (CRAB), posting a global public health concern. The heightened sensitivity of whole-genome sequencing (WGS) renders it an optimal and potent tool for monitoring outbreaks and tracing the transmission routes of nosocomial pathogens.
Method: We collected CRAB isolates from March 1, 2023, to April 6, 2023 in Chang Gung Memorial Hospital Lin Kou branch, a tertiary medical center in northern Taiwan. Any two or more isolates with the same identifiable capsular K-locus (KL) types were selected, and analyzed via WGS to identify putative transmission clusters, combined with epidemiologic and retrospective analysis on medical records to confirm risk factors and hidden transmission chains.

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Result: A total of 48 non-redundant CRAB isolates were collected, belonging to ST2 of Pasteur MLST scheme and identifiable KL types of KL2, KL3, KL9, KL10, KL22, KL52. Excluding the KL types that was only found in 1 case, KL2 (n = 9, 22.5 %), KL3 (n = 24, 60 %), KL9 (n = 3, 7.5 %), and KL10 (n = 4, 10 %) were selected for further WGS analysis. Four distinct transmission clusters comprised of 2, 3, 10, and 23 cases were identified on a basis of phylogenetic status. 12 probable transmission chains were revealed, and 2 hidden transmission routes can be speculated.
Conclusion: This study referred to some hidden transmission chains that may be missed from traditional surveillance measures. Despite its low prevalence and high cost currently, implementing WGS could be a efficient, prompt, and unequivocal option for future MDRO infection control.

Introduction

Acinetobacter baumannii is an aerobic, Gram-negative bacillus that has been isolated from various environmental sources, including soil, water, animals, and humans, where it acts as normal flora of the human skin and is frequently recovered from the throat and respiratory tract of hospitalized patients.[1] It serves as an opportunistic pathogen, contributing to nosocomial pneumonia and bacteremia.[2] During the Iraq War in 2003, *A. baumannii* bacteremia emerged in military medical facilities, leading to its acquisition of the notoriety term "Iraqibacter".[3]

As *A. baumannii* can survive under harsh conditions and persist on surfaces like doorknobs, tables, and beds, it posts a significant threat in hospitals, especially to immunocompromised and critically ill patients.[1,4] Resistance to carbapenems, commonly used empirical antibiotics for *A. baumannii*, is rising,[5—7] with carbapenem-resistant *Acinetobacter baumannii* (CRAB) linked to higher in-hospital mortality rates compared to carbapenem-susceptible ones.[8,9] The World Health Organization (WHO) has identified antibiotic-resistant *A. baumannii* as a critical priority pathogen, categorizing it among the most serious MDR pathogens in the ESKAPE group called "ESKAPE".[3]

During the COVID-19 pandemic, numerous studies have indicated an increasing trend of healthcare-associated infections (HAIs) caused by CRAB.[6,10,11] Interestingly, while SARS-CoV-2 infection itself has not been considered a risk factor for MDRO isolation,[6,10,12] the prevalence of MDROs has nonetheless risen, underscoring the critical importance of infection control measures.

Whole-genome sequencing (WGS), a cutting-edge and state-of-the-art method, has proven to be invaluable in the practice of infection control.[13,14] In contrast to traditional molecular methods aimed at identifying genetic relationships, WGS can comprehensively assess all strains of microorganisms. Its heightened sensitivity renders it an optimal and potent tool for monitoring outbreaks and tracing the transmission routes of potential nosocomial pathogens.[15,16]

In this study, we analyzed risk factors of CRAB acquisition in a medical center during March 1, 2023, to April 6, 2023. Sequentially utilizing capsular typing and WGS to investigate the potential transmission routes, with the aim of evaluating the application of WGS in infection control efforts.

Material and methods

Study design & population

This study was conducted at Chang Gung Memorial Hospital (CGMH)-Lin Kou branch, a 3700-bed medical center in northern Taiwan, from March 1, 2023, to April 6, 2023. We collected specimens isolated with CRAB, defined by the presence of $\geq$1 positive culture result of specimens from any site of body. Nosocomial CRAB infection was defined as the patient with positive culture of CRAB exhibited signs and symptoms of infection, such as fever, chillness, desaturation, increased need of supplemental oxygen therapy, or any documented symptoms related to the site that isolated with CRAB, more than 48 h after hospital admission. The cases with positive culture but not fulfilled the definition of CRAB infection would be classified as colonization. Patients under 18 years of age or those with incomplete medical records were excluded. This protocol was approved by the institutional review board of CGMH (Approval number: * 202300613B0).

Bacterial isolates and antibiotic susceptibility testing

Identification of *A. baumannii* was conducted using matrix-assisted laser desorption-time of flight mass spectrometry (MALDI-TOF-MS). Susceptibility to all tested antibiotics was determined according to the Clinical and Laboratory Standards Institute (CLSI) interpretive criteria 30th edition for the disc diffusion method. For Colistin, our laboratory referred to CLSI M100 27th edition for disc diffusion. Automatic reading and interpretation of results of disc method was performed with ADAGIO™ system.

Case-control study and patient data

For each CRAB case, two control cases without culture proof of CRAB, and other nosocomial multidrug-resistant (MDR) strains in previous 3 months, were selected from hospitalized patients matched by onset date and ward, using random sampling. Demographic characteristics and clinical information of the CRAB cases and controls were retrieved from computerized data in the CGMH hospital

information systems (HIS). The data encompassed age, gender, the date of the first positive culture with CRAB acquisition, the ward and date of any transfers, invasive medical procedures, central venous lines/catheters, examinations received prior to CRAB isolation, and prior antibiotic use within 1 month before the acquisition of CRAB.

Capsular locus (KL) typing

As KL2, KL22, KL10, and KL52 were reported as major K-types of CRAB in Taiwan,[17] we further determine the prevalence of the four K-types in our collections. wzy-PCR K-typing was applied for detecting KL2/KL81, KL3/KL22, KL10, and KL52 using specific wzy primers previously described[17] (Supplementary Table S1). If the strains positive for KL2/KL81, cgmA PCR was further performed to differentiate KL2 from KL81 because cgmA was present in KL81 but not in KL2. Similarly, KL3 and KL22 share almost identical wzy, and thus were further distinguished by cgmA PCR (cgmA was present in KL22 but not in KL3).

Whole-genome sequencing and analysis

Representative isolates will undergo sequencing using the Illumina MiSeq platform by 2x150 bp paired-end approach. The quality filtering and adapter trimming of raw reads were performed using BBduk tools. (version 38.79, https://sourceforge.net/projects/bbmap). Subsequently, the draft genomes were assembled into contigs using SPAdes v3.15.1.[18]

Sequence type (ST) identification will be confirmed using the MLST-sequence type tool included in the *A. baumannii* database from the Pasteur Institute, mlst v 2.19.0 (https://github.com/tseemann/mlst). Multilocus sequence typing (MLST) and capsular typing will be determined using Kaptive.[19] A phylogenetic analysis will be conducted using kSNP v3,[20] with the optimum value of K determined to be 19 by kSNP-Kchooser, and a phylogenetic tree was visualized using FigTree v1.4.4.7. We used SNPdragon (https://github.com/FordeGenomics/SNPdragon) to call SNP with default setting. And then it calculates pairwise counts of core SNP differences. We defined a screening threshold of 20 SNPs for pairwise SNP distances, and isolates with SNP distance $\leq$20 SNPs to be in a same cluster.[13]

About analysis of the antibiotic resistance genes (ARGs), the Resistance Gene Identifier (RGI) v6.0.3 and Comprehensive Antibiotic Resistance Database (CARD) database v3.2.9 [21] were used to identify antibiotic resistance genes in draft genomes with default parameters. Additionally, we specifically aligned the genes of interest by using BLAST (v2.13.1+).

The detailed parameter of WGS analyzing methods was listed in Supplementary Table S2.

Transmission analysis

We defined isolates with SNP distance $\leq$20 SNPs as genomically related putative transmission cluster. To evaluate the likelihood of epidemiological relatedness in a cluster, patients were classified as 'probable transmission' if they were at the same ward in the same time period, 'possible transmission' if they were admitted to the same ward but in different time period within 60 days, and 'unlikely transmission' for the rest of the cases. These classifications were modified from the definition published previously.[14,22]

Statistical analysis

The clinical data collected from the CRAB cases and matched control patients were displayed with frequencies (n) and percentages (%) in categorial variables, and in continuous variables we gave mediums and interquartile range (IQR). Dichotomous variables were analyzed using chi-square test or Fisher's exact test, while Student's *t*-test or Kruskal-Wallis test were used for continuous variables. Factor with a P value < 0.1 were further included in the multivariate analyses. All analyses were conducted using IBM SPSS software version 20 (SPSS Inc., Chicago, IL, USA). P values less than 0.05 were considered statistically significant.

Result

KL typing of CRAB

Over the study period, a total of 48 non-redundant *A. baumanni* isolates were collected. By *wzy*-PCR with type-specific *wzy* primers, the distribution of capsular types was as follows: KL2 (n = 9, 18.8 %), KL3 (n = 24, 50.0 %), KL9 (n = 3, 6.3 %), KL10 (n = 4, 8.3 %), KL22 (n = 1, 2.1 %), KL52 (n = 1, 2.1 %), and other (n = 6, 12.5 %). For exploring possible transmission chain, two or more isolates with the same identifiable capsular types were chosen into this study. 40 isolates were selected, and the distribution of KL types was as following: KL2 (n = 9, 22.5 %), KL3 (n = 24, 60 %), KL9 (n = 3, 7.5 %), and KL10 (n = 4, 10 %).

Demographic characteristics and clinical factors of cases and control

The general features of the study population and controls are described in Table 1. In these 40 isolates, there are 14 (30 %) women and 26 (70 %) men, with a median age of 67.75 (IQR = 16.4) years. Isolates were collected from tip of central-venous catheter (KL3: n = 1, 2.5 %), sputum/broncho-alveolar lavage (BAL) (KL2: n = 7, 17.5 %; KL3: n = 18, 45 %; KL9: n = 1, 2.5 %; KL10: n = 3, 7.5 %), urine (KL2: n = 1, 2.5 %; KL3: n = 2, 5 %; KL9: n = 1, 2.5 %; KL10: n = 1, 2.5 %), ascites (KL2: n = 1, 2.5 %), and wound/surgical site (KL3: n = 3, 7.5 %; KL9: n = 1, 2.5 %). 32 cases (80 %) were defined as CRAB infection and the other 8 cases (20 %) were classified as colonization.

Compared to the control group, all CRAB cases in this study exhibited no statistically significant differences in age, gender, and Charlson scores. Notable difference presented in prevalence of lymphoma while comparing all CRAB cases to controls (P = 0.02). Moreover, 7 cases used immunosuppressants in this study (29.2 %), making a significance compared with control group (P = 0.02), and they were exclusively in KL3 group. About antimicrobial agents

Table 1 Demographic characteristics, underlying diseases, sources of infection, clinical characteristics of patients with carbapenem-resistant *Acinetobacter baumannii*.

Variables	All Case n = 40	KL2 n = 9	KL3 n = 24	KL9 n = 3	KL10 n = 4	Control n = 70	*P* for comparing case and control	*P* for comparing KL2/3/9/10
Male, number (%)	26 (65)	6 (66.7)	16 (66.7)	1 (11.1)	3 (75)	44 (62.9)	0.82	0.67
Age (years), median (IQR)	67.8 (16.4)	62.6 (24.4)	67.9 (14.7)	76.7 (52.3)	64.5 (15.3)	69.1 (19.3)	0.82	0.44
Charlson score, median (IQR)	4.5 (5)	5 (4.5)	5 (5)	10 (11)	4 (3)	4.5 (5)	0.99	0.52
Underlying conditions, number (%)								
Diabetes mellitus[a]	18 (45)	3 (33.3)	12 (50)	1 (11.1)	2 (50)	25 (35.7)	0.34	0.87
Liver cirrhosis	0	0	0	0	0	4 (5.7)	0.12	NA
Hypertension	18 (45)	3 (33.3)	12 (50)	1 (11.1)	2 (50)	36 (51.4)	0.52	0.87
Coronary artery disease	3 (7.5)	0	2 (8.3)	0	1 (25)	12 (17.1)	0.16	0.54
Congestive heart failure	3 (7.5)	0	2 (8.3)	0	1 (25)	14 (20)	0.08	0.54
Chronic renal insufficiency	16 (40)	2 (22.2)	11 (45.8)	1 (11.1)	2 (50)	7 (10)	0.36	0.63
COPD, asthma	8 (20)	2 (22.2)	4 (16.7)	2 (22.2)	0	6 (8.6)	0.14	0.22
Autoimmune disease	4 (10)	0	4 (16.7)	0	0	11 (15.7)	0.80	0.80
Tumor with metastases	6 (15)	2 (22.2)	3 (12.5)	1 (11.1)	0	11 (15.7)	0.92	0.55
Leukemia	0	0	0	0	0	0	NA	NA
Lymphoma	3 (7.5)	0	2 (8.3)	1 (11.1)	0	0	0.02	0.26
Solid malignancy	14 (35)	4 (44.4)	6 (25)	1 (11.1)	1 (25)	22 (31.4)	0.70	0.91
Use of immunosuppressant	7 (17.5)	0	7 (29.2)	0	0	3 (4.3)	0.02	0.21
Tested in ICU, number (%)	28 (70)	7 (77.8)	17 (70.8)	2 (66.7)	2 (50.0)	44 (62.9)	0.45	0.79
Antibiotics, number (%)								
Third- and fourth-generation Cephalosporin and piperacillin-tazobactam	24 (60)	5 (55.6)	14 (58.3)	2 (66.7)	3 (75.0)	30 (42.9)	0.08	0.91
Carbapenem	23 (57.5)	3 (33.3)	13 (54.2)	1 (33.3)	2 (50.0)	24 (34.3)	0.02	0.49
Quinolone	11 (27.5)	3 (33.3)	6 (25.0)	0	2 (50.0)	7 (10)	0.02	0.50
Glycopeptides[b], Linezolid	22 (55)	7 (77.8)	10 (41.7)	1 (33.3)	4 (100.0)	20 (28.6)	<0.01	<0.05
Tigecycline, Colistin	14 (35)	3 (3.3)	7 (29.2)	2 (66.8)	2 (50.0)	4 (5.7)	<0.01	0.55
Antifungal agents	11 (27.5)	4 (44.4)	4 (16.7)	1 (33.3)	2 (50.0)	3 (4.3)	0.02	0.29
Catheters and tubing, number (%)								
CVC	32 (80)	6 (66.7)	21 (87.5)	2 (66.7)	3 (75.0)	34 (48.6)	<0.01	0.34
Foley catheter	36 (90)	7 (77.8)	22 (91.7)	3 (100.0)	4 (100.0)	37 (52.9)	<0.01	0.68
NG/ND tube	36 (90)	9 (100.0)	20 (83.3)	3 (100.0)	4 (100.0)	40 (57.1)	<0.01	0.80
Endotracheal tube, tracheostomy	29 (72.5)	8 (88.9)	17 (70.8)	1 (33.3)	3 (75.0)	25 (35.7)	<0.01	0.31
Other[c]	29 (72.5)	7 (77.8)	17 (70.8)	1 (33.3)	4 (100.0)	44 (62.9)	0.30	0.26
Procedure & examinations								
Gastroscopy	8 (20)	4 (44.4)	3 (12.5)	1 (33.3)	0	7 (10)	0.14	0.11
Colonoscopy	0	0	0	0	0	3 (4.3)	0.18	NA
Bronchoscopy	14 (35)	3 (33.3)	9 (37.5)	1 (33.3)	1 (25.0)	7 (10)	<0.01	0.97
Echocardiogram	16 (40)	5 (55.6)	7 (29.2)	1 (33.3)	3 (75.0)	35 (50)	0.31	0.24
Abdominal ultrasound	3 (7.5)	1 (11.1)	2 (8.3)	0	0	11 (15.7)	0.21	1.00
Lung ultrasound	8 (20)	2 (22.2)	4 (16.7)	1 (33.3)	1 (25.0)	13 (18.6)	0.85	0.79
Operation	15 (37.5)	5 (55.6)	7 (29.2)	1 (33.3)	2 (50.0)	35 (50)	0.21	0.52
14 days mortality (%)	3 (7.5)	1 (11.1)	4 (16.7)	1 (33.3)	0	1 (1.4)	0.04	0.63
30 days mortality (%)	5 (12.5)	2 (22.2)	3 (12.5)	0	0	3 (4.3)	0.03	0.63

[a] Diabetes mellitus: only included type 1 diabetes mellitus was included in this study.
[b] Glycopeptides: including Vancomycin, Teicoplanin.
[c] Other catheter and tubing: chest pigtail, chest tube, PiCCO, port-A, A-V shunt, Hickman catheter, double-lumen catheter, A-line, vacuum ball, drainage tube, jejunostomy/ileostomy.
Abbreviations: IQR, interquartile range; COPD, chronic obstructive pulmonary disease; ICU, intensive care unit; CVC, central venous catheter; NG/ND tube, nasogastric tube/nasoduodenal tube.

used, case group tended to receive carbapenems ($P = 0.02$), fluroquinolones ($P = 0.02$), glycopeptides and linezoid ($P < 0.01$), tigecyclines and colistin ($P < 0.01$), and antifungal agents ($P = 0.02$). Catheters and tubing were commonly used in case group and showed statistical significance compared to the control group in central venous catheter (CVC) ($P < 0.01$), Foley catheter ($P < 0.01$), nasogastric tube (NG tube) and nasoduodenal tube (ND tube) ($P < 0.01$), and endotracheal tube and tracheostomy ($P < 0.01$). Bronchoscopy was the only exam that was significantly more frequent to be performed on CRAB cases than control group ($P < 0.01$). The all-cause 14-day and 30-day mortality rates after admission were significantly higher in case group.

Among these four groups of different KL types, no significant differences were found in age, sex, underlying diseases including Charlson score, catheter and tubing, procedures and examinations, and all-cause mortality rates at 14 and 30 days. The only borderline statistically significant finding was the use of glycopeptide and linezolid ($P < 0.05$).

Risk factors for CRAB acquisition

Univariate analysis between the 40 patients acquired CRAB and 70 individually matched controls showed significant differences in third- and fourth-generation cephalosporins and piperacillin-tazobactam ($P = 0.09$), carbapenems ($P = 0.02$), fluroquinolone ($P = 0.02$), glycopeptides and linezolid ($P < 0.01$), tigecycline and colistin ($P = 0.03$), antifungal agents ($P = 0.02$), CVC catheter ($P < 0.01$), Foley catheter ($P < 0.01$), NG/ND tubes ($P < 0.01$), endotracheal tube and tracheostomy ($P < 0.01$), and bronchoscopy ($P < 0.01$). On multivariate analysis, third- and fourth-generation cephalosporins and piperacillin-tazobactam ($P = 0.02$), fluroquinolone ($P = 0.03$), glycopeptides and linezolid ($P = 0.02$), Foley catheter ($P = 0.04$), and bronchoscopy ($P = 0.04$) were independently associated with the acquisition of CRAB (Table 2).

Antibiotic susceptibility

All these 40 isolates were non-susceptible to ceftazidime, cefepime, gentamicin, imipenem, meropenem, and piperacillin-tazobactam. Non-susceptible rate of amikacin (97.5 %), cefoperazone-sulbactam (92.9 %), cefepime (97.5 %), ampicillin-sulbactam (92.9 %), and ciprofloxacin (97.5 %) were high. In the regiments suggested for CRAB treatment, the non-susceptible rate to Tigecycline was up to 80 %, and Colistin was 7.5 % with 3 isolates was resistant to it (Table 3).

Whole-genome sequencing and antimicrobial resistance genes

The WGS analysis on the 40 isolates revealed that all of them belonged to sequence type 2 (ST2) of Pasteur MLST

Table 2 Univariate and multivariate logistic regression analyses of the risk factors in patients acquired *A. baumannii*.

Variables	Cases (n = 40)	Controls (n = 70)	Univariate analysis		Multivariate analysis	
			Odds ratio (95%CI)	*P* value	Odds ratio (95%CI)	*P* value
Demographic characteristics						
Male (%)	26 (65)	44 (62.9)	1.1 (0.5–2.5)	0.82	–	
Age, median (IQR)	67.75 (16.4)	69.1 (19.3)	1.0 (0.9–1.0)	0.68	–	
Underlying diseases						
COPD, asthma (%)	8 (20)	6 (8.6)	2.3 (0.7–6.8)	0.15	–	
Charlson score, median (IQR)	4.5 (5)	4.5 (5)	1.0 (0.9–1.2)	0.87	–	
Tested in ICU, number (%)	28 (70)	44 (62.9)	1.4 (0.6–3.2)	0.45	–	
Antibiotics, number (%)						
Third- and fourth generation Cephalosporin and/or piperacillin-tazobactam	24 (60)	30 (42.9)	2.0 (0.9–4.4)	0.09	4.9 (1.3–17.8)	0.02
Carbapenem	23 (57.5)	24 (34.3)	2.6 (1.2–5.8)	0.02	2.0 (0.6–7.0)	0.29
Fluroquinolone	11 (27.5)	7 (10)	3.4 (1.2–9.7)	0.02	5.4 (1.2–25.4)	0.03
[a]Glycopeptide, Linezolid	22 (55)	20 (28.6)	3.1 (1.4–6.9)	<0.01	4.1 (1.3–13.0)	0.02
Tigecycline, Colistin	14 (35)	4 (5.7)	8.9 (2.7–29.5)	0.03	4.6 (0.9–22.5)	0.06
Antifungal agents	11 (27.5)	3 (4.3)	3.4 (1.2–9.7)	0.02	0.6 (0.1–2.5)	0.48
Catheters and procedures, number (%)						
CVC	32 (80)	34 (48.6)	4.2 (1.7–10.5)	<0.01	1.4 (0.4–5.4)	0.58
Foley catheter	36 (90)	37 (52.9)	8.0 (2.6–25.0)	<0.01	5.2 (1.1–24.9)	0.04
NG, ND tube	36 (90)	40 (57.1)	6.8 (2.2–21.0)	<0.01	1.8 (0.4–9.1)	0.47
Endotracheal tube, tracheostomy	29 (72.5)	25 (35.7)	4.7 (2.0–11.1)	<0.01	1.0 (0.3–3.7)	0.95
Exams, number (%)						
Gastroscopy	8 (20)	7 (10)	2.3 (0.7–6.8)	0.15	–	
Bronchoscopy	14 (35)	7 (10)	4.8 (1.8–13.4)	<0.01	4.9 (1.1–21.7)	0.04

[a] Glycopeptides: including Vancomycin, Teicoplanin.

Abbreviations: CI, confidence interval; COPD, chronic obstructive pulmonary disease; ICU, intensive care unit; CVC, central venous catheter; NG/ND tube, nasogastric tube/nasoduodenal tube.

Table 3 In vitro antimicrobial susceptibility in various capsular types of CRAB.

	KL2 (n = 9)			KL3 (n = 24)			KL9 (n = 3)			KL10 (n = 4)		
Antimicrobial resistance	S (%)	I (%)	R (%)	S (%)	I (%)	R (%)	S (%)	I (%)	R (%)	S (%)	I (%)	R (%)
Amikacin	1 (11.1)	0	8 (88.8)	0	0	24 (100)	0	0	3 (100)	0	0	4 (100)
Ceftazidime	0	0	9 (100)	0	0	24 (100)	0	0	3 (100)	0	0	4 (100)
Ciprofloxacin	0	0	9 (100)	1 (4.2)	0	23 (95.8)	0	0	3 (100)	0	0	4 (100)
Colistin	9 (100)	0	0	21 (87.5)	0	3 (12.5)	3 (100)	0	0	4 (100)	0	0
Cefoperazone-sulbactam	1 (11.1)	4 (44.4)	4 (44.4)	1 (4.2)	1 (4.2)	22 (91.7)	1 (33.3)	2 (66.7)	0	0	0	4 (100)
Cefepime	0	0	9 (100)	0	0	24 (100)	0	1 (33.3)	2 (66.7)	0	0	4 (100)
Gentamicin	0	0	9 (100)	0	0	24 (100)	0	0	3 (100)	0	0	4 (100)
Imipenem	0	0	9 (100)	0	0	24 (100)	0	0	3 (100)	0	0	4 (100)
Meropenem	0	0	9 (100)	0	0	24 (100)	0	0	3 (100)	0	0	4 (100)
Ampicillin-sulbactam	1 (11.1)	2 (22.2)	6 (66.6)	0	2 (8.3)	22 (91.7)	0	2 (66.7)	1 (33.3)	0	0	4 (100)
Tigecycline	3 (33.3)	3 (33.3)	3 (33.3)	3 (12.5)	21 (87.5)	0	1 (33.3)	2 (66.7)	0	1 (25)	3 (75)	0
Piperacillin-tazobactam	0	0	9 (100)	0	0	24 (100)	0	0	3 (100)	0	0	4 (100)

scheme. Carbapenem resistance genes among these 40 CRAB strains included carbapenemase genes, like *blaOXA-66* (n = 40, 100 %), and *blaOXA-23* (n = 25, 62.5 %), and resistance-nodulation-division (RND) carbapenem multidrug efflux pump genes *adeI* (n = 40, 100 %) and *adeK* (n = 40, 100 %). Other genes represented antimicrobial resistance included aminoglycoside (*APH(3′)-Ia*: n = 16, 40 %; *armA*: n = 37, 92.5 %), tetracyclines (*adeA*: n = 37, 92.5 %; *adeC*: n = 31, 92.5 %; *adeL*: n = 39, 97.5 %; *adeG*: n = 38, 95 %), cephalosporin (*ADC-30*: n = 37, 92.5 %; *TEM-1*: n = 19, 47.5 %), erythromycin (*msrE*: n = 39, 97.5 %), and sulfonamide (*sul1*: n = 39, 97.5 %; *sul2*: n = 12, 30 %).

CRAB clustering and probable transmission chain

The maximum likelihood (ML) phylogenetic tree based on core genome alignment is presented in Fig. 1. As we defined previously the SNP distance ≤20 SNPs, 4 putative transmission clusters in our CRAB cases were revealed and named as cluster A to D. The clusters were mostly congruent to KL profiles, except 2 cases in cluster B and 1 in cluster C (n = 3, 92.5 %). For each cluster that includes more than 2 cases, further analysis on epidemiological data were applied (Fig. 2). The isolates were denoted according to their sequential analysis numbers such as MQ230510-001, and we simplified them as S01 in our article.

In cluster A, 2 cases were defined to unlikely transmission by the definition aforementioned (Fig. 2A). S22 had bronchoscopy prior to S27, and after S27 received bronchoscopy, CRAB was isolated from sputum specimen of him after 4 days, and this could be the hidden transmission chain. In cluster B, 4 probable transmission chain can be found (Fig. 2B). 3 cases were unlikely transmission and no related procedure or exam with other cases in cluster C before their acquisition of CRAB. In cluster C (Fig. 2C), 5 probable transmission chain was revealed. Case S01 was transferred from 13G ward to 10B ward right after CRAB was isolated, becoming the beginning of outbreak at 10B. Furthermore, case S01, S05, and S09 were defined as unlikely transmission, but they all received echocardiogram within 30 days before isolation of CRAB, and this could be a hidden transmission chain. Case S13 and S34 were unlikely transmission cases because they didn't have any connection identified with other case in this cluster. Lastly, the cluster D included 3 cases (Fig. 2 D), and 2 probable transmission chain at 2E and 2F ward. Detailed transmission chain is presented in Supplementary Table S3.

Discussion

This study documents the risk factors, spread, and transmission of CRAB within a medical center encompassing approximately 3700 beds over a concise timeframe. We conducted an analysis of strains potentially linked to the outbreak, utilizing capsular typing to delineating putative transmission clusters. The following WGS facilitated a comprehensive evaluation of antimicrobial resistance characteristics and pairwise SNP distance for a more detailed maximum likelihood (ML) analysis. Combined with epidemiologic analysis, we identify the overlapping admission, locations, procedures, and exams, enabling the

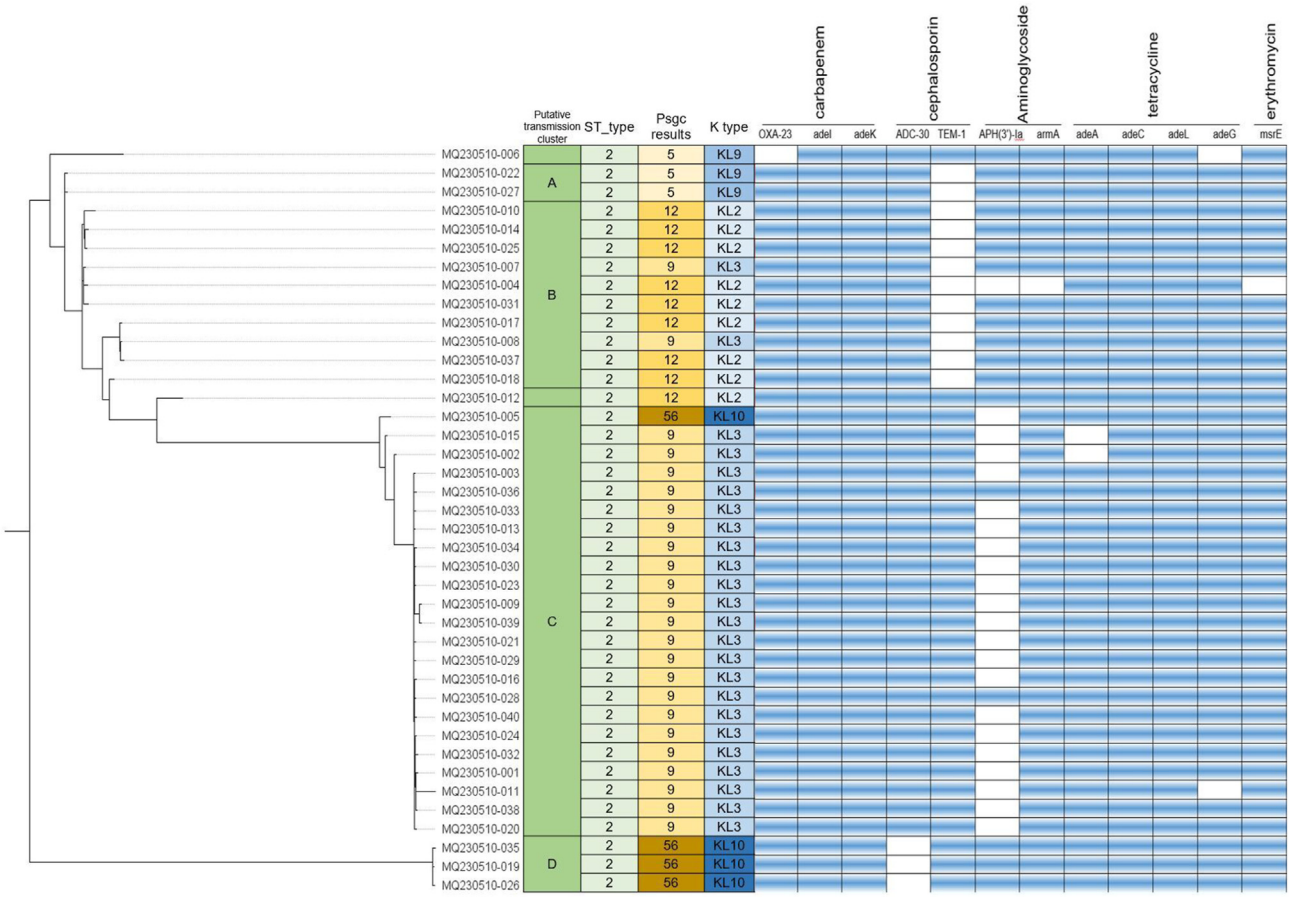

Fig. 1. The maximum likelihood phylogenetic tree and comparison of CRAB cases.

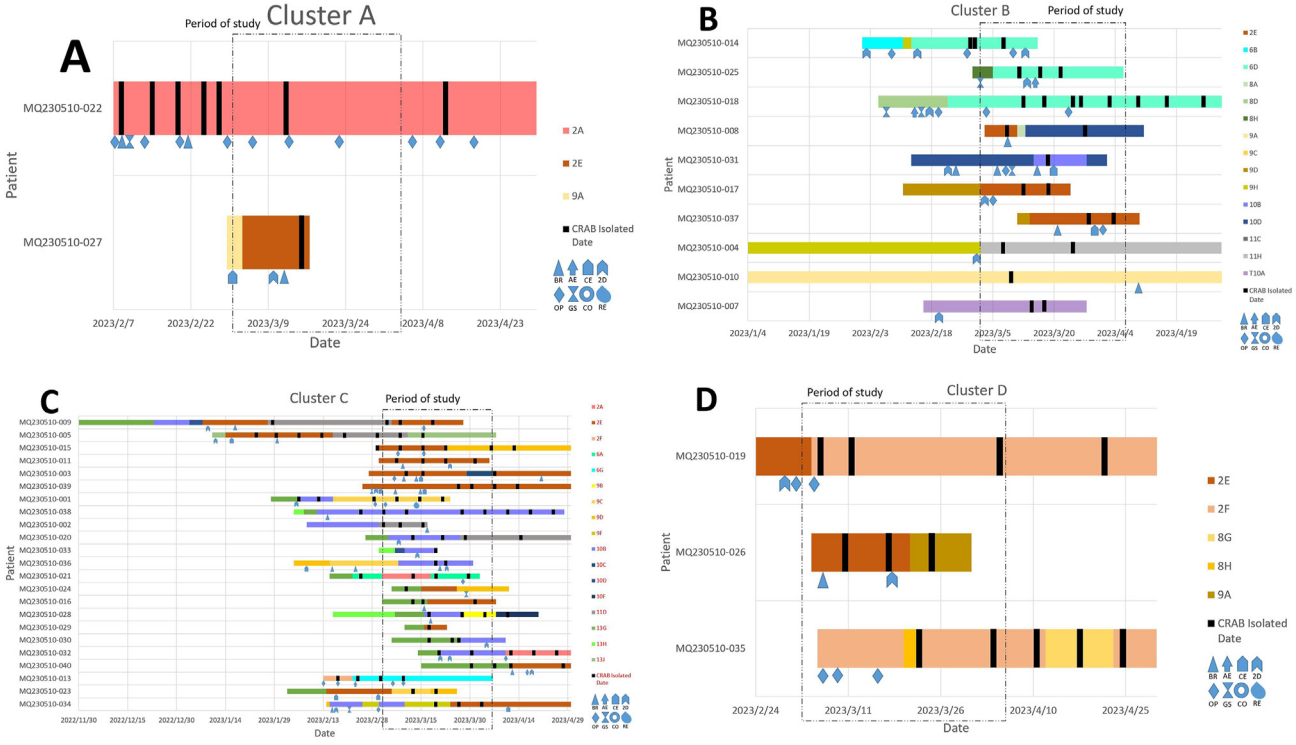

Fig. 2. ABCD. Staying and transferring of CRAB cases between wards

Abbreviations: BR, bronchoscopy; GS: gastroscopy; CO: colonoscopy; AE, abdominal echography; CE, chest echography; 2D: echocardiogram; RE: renal sonography; OP: operation.

identification of hidden transmission chains and their circulation dynamics.

Traditional HAI surveillance is a manual, time-consuming process based on spatial and temporal connections, prone to under- and over-reporting.[23] Seifi's study reported its low sensitivity (27.5 %) and positive predictive value (69 %).[24] Colonization cases, though not included, are crucial in nosocomial pathogen transmission. Effective infection control requires isolating infected cases and monitoring colonization. This study included colonization cases and used WGS to identify clusters and hidden transmission chains early, enabling interventions like environmental sanitation, improved handwashing, and staff hygiene education.

The bacterial envelope of *A. baumannii* plays a crucial role in disease pathogenesis, acting as a barrier to antibiotics and interacting with the host immune system through its unique carbohydrate structures.[25,26] The capsule's hydrophilicity and negative charge hinder phagocyte interaction, reducing complement deposition and phagocytic killing[25] Different capsular types are linked to carbapenem resistance and mortality.[17,27] Our previous study found KL2/KL10/KL22/KL52 as the predominant types in nosocomial CRAB bacteremia, with appropriate antimicrobial therapy within 24 h significantly reducing mortality.[17,27] Capsular typing in this study revealed clustering within the same types, underscoring its utility in identifying transmission chains and improving prognosis.

Previous studies have identified several risk factors for CRAB infection, such as mechanical ventilation, broad-spectrum antibiotic use, bronchoscopy, indwelling catheters, and shared rooms.[28—30] Our study found significant associations with piperacillin-tazobactam, third- and fourth-generation cephalosporins, glycopeptides, linezolid, and fluoroquinolones, with tigecycline and colistin showing borderline significance. These findings align with Vasudevan et al.'s report of vancomycin and linezolid being linked to nosocomial resistant Gram-negative bacilli infections, including CRAB.[31] Meric et al. demonstrated a relationship between exposure to third generation cephalosporins and drug resistance,[32] and similarly, β-lactams and carbapenems was identified as an independent risk factor for acquiring CRAB by Falagas et al.[33] The potential mechanism can be antibiotic selection pressure leading to carbapenem resistance and multidrug-resistant strains, though it is controversial.[34]

Moreover, these board-spectrum antibiotics are often prescribed for resistant nosocomial pathogens, implying patients with underlying conditions are more susceptible to CRAB acquisition. To mitigate this risk, it is crucial to monitor and review the use of broad-spectrum antibiotics, ceasing unnecessary empirical antibiotics, and practicing reasonable de-escalation are crucial strategies.

Our study also identified the use of Foley catheters and bronchoscopies as significant factors in the acquisition of CRAB, likely due to contaminated instruments and insufficient infection control protocols. Prolonged indwelling of Foley catheters is a well-established risk factor, emphasizing the need for rigorous disinfection procedures. The literature consistently underscores the critical role of thorough disinfection in preventing nosocomial pathogen transmission, particularly in the context of bronchoscope usage.,[29,35] noting delayed bronchoscope cleaning can result in biofilm formation.[36] Also, skipped disinfection to the whole equipment of bronchoscope and control panel could be a route of transmission. Preventive measures include timely, extended, and manual cleaning, visual confirmation, proper drying, and storage. Few studies report bronchoscope-related infections, highlighting a lack of surveillance.[36,37] Our findings advocate for the utilization of whole-genome sequencing (WGS) to uncover hidden transmission pathways and enable timely, targeted interventions.

Put an eye on the isolates of urine specimens, all of them were acquired from patients having Foley catheter, which made a direct influence on acquisition of CRAB in urinary tract (Table 4). Although, most of the cases were not documented with clear sign or symptom of infection of urinary source. It is reported that fewer than 2 % of patients developed an invasive infection with Acinetobacter colonization in the urine.[38]

Despite the advantages of whole-genome sequencing (WGS), its high cost and complex data analysis are significant limitations. However, studies, such as Forde's, show significant medical cost savings and reduced mortality, emphasizing benefits of WGS.[13] To optimize WGS efficiency for timely infection control, establishing a dedicated WGS lab, refining workflows, and enhancing specimen transport and data retrieval are crucial strategies. This approach ensures rapid identification of transmission chains and effective infection control interventions.

Our study has some limitations. First, our sample size was small and limited to a short time period. Second, there was no environmental screening data and no implementation of corresponding infection control intervention.

In conclusion, this study demonstrates the sequential use of capsular typing and WGS for acquisition and transmission of CRAB in a medical center. This approach reveals potential clusters and hidden transmission chains that may not be detected by traditional surveillance methods, and

Table 4 Source of bacterium culture.

	KL2 (n = 9)	KL3 (n = 24)	KL9 (n = 3)	KL10 (n = 4)	Total (n = 40)
CVC tip	0 (0 %)	1 (2.5 %)	0 (0 %)	0 (0 %)	1 (2.5 %)
Sputum/BAL	7 (17.5 %)	18 (45 %)	1 (2.5 %)	3 (7.5 %)	29 (72.5 %)
Urine	1 (2.5 %)	2 (5 %)	1 (2.5 %)	1 (2.5 %)	5 (12.5 %)
Ascites	1 (2.5 %)	0 (0 %)	0 (0 %)	0 (0 %)	1 (2.5 %)
Wound/surgical site	3 (7.5 %)	0 (0 %)	1 (2.5 %)	0 (0 %)	4 (10 %)

Abbreviations: CVC, central venous catheter; BAL, broncho-alveolar lavage.

provides an efficient, prompt, and unequivocal option for further development of MDRO infection control. While the expenses of maintaining this service may be substantial, they are offset by the potential savings for the healthcare system overall and the heightened prevention of infections acquired during healthcare in susceptible patients.

CRediT authorship contribution statement

Yi-An Way: Writing — review & editing, Writing — original draft, Visualization, Project administration, Investigation, Data curation. **Chong-Wei Huang:** Writing — review & editing, Validation, Project administration, Investigation, Data curation, Conceptualization. **Wei-Chao Liao:** Visualization, Validation, Software, Investigation, Formal analysis. **Shiao-Wen Li:** Funding acquisition, Formal analysis. **Ruei-Lin Chiang:** Visualization, Software, Investigation, Formal analysis. **En-Wei Hsing:** Investigation, Data curation. **Yi-Jiun Pan:** Software, Investigation, Formal analysis, Data curation. **Shian-Sen Shie:** Supervision, Methodology, Conceptualization. **Yu-Chia Hsieh:** Writing — review & editing, Supervision, Resources, Methodology, Investigation, Conceptualization.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jmii.2024.08.014.