ลักษณะระดับโมเลกุลของเชื้อ *Acinetobacter baumannii* ดื้อยาคาร์บาพีเนมที่แยกได้จากโรงพยาบาลเอกชนแห่งหนึ่งในกรุงเทพมหานคร

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บทคัดย่อ

Acinetobacter baumannii **เป็นแบคทีเรียแกรมลบรูปร่างท่อนสั้น ไม่หมักย่อยน้ำตาลพบได้ทั่วไปทั้งในน้ำและดิน จัดเป็นแบคทีเรียก่อโรคหนึ่งในกลุ่ม ESKAPE มีศักยภาพในการดื้อยาต้านจุลชีพ ในการจัดลำดับแบคทีเรียดื้อยาปฏิชีวนะขององค์การอนามัยโลกเพื่อการใช้ยาอย่างมีประสิทธิภาพ พบว่า** ***A. baumannii*** **ดื้อยาคาร์บาพีเนม (carbapenem-resistant** ***A. baumannii*****, CRAB) เป็นเชื้อก่อโรคที่มีความสำคัญในระดับวิกฤต การศึกษาแบบย้อนหลังนี้มีวัตถุประสงค์เพื่อศึกษาลักษณะระดับโมเลกุลของเชื้อ CRAB ที่แยกได้จากโรงพยาบาลเอกชนแห่งหนึ่งในกรุงเทพมหานคร ระหว่างปี พ.ศ. 2564-2565 พบ** ***A. baumannii*** **จำนวน 244 ไอโซเลท จากสิ่งส่งตรวจของผู้ป่วย 166 คน เป็น CRAB จำนวน 171 ไอโซเลท (ร้อยละ 70.1) พบอัตราการดื้อยากลุ่มคาร์บาพีเนมของเชื้อ** ***A. baumannii*** **เพิ่มขึ้นจากร้อยละ 64.4 ในปี 2563 เป็นร้อยละ 73.8 ในปี 2564 ลดลงเป็นร้อยละ 66.4 ในปี 2565 และร้อยละ 61.5 ในปี 2566 การบริโภคยากลุ่มคาร์บาพีเนม (defined daily doses (DDD) ต่อ 1,000 วันนอนที่ศึกษาต่อปี) สูงถึงร้อยละ 63.71 ในปี 2564 ลดลงเป็นร้อยละ 37.41 ในปี 2566 จากแนวโน้มในช่วงปี 2563-2566 พบความสอดคล้องกันระหว่างการบริโภคยาคาร์บาพีเนมและอัตราการดื้อยา กลุ่มคาร์บาพีเนมของเชื้อ** ***A. baumannii*** **มีรูปแบบความไวต่อยาต้านจุลชีพจำนวน 19 รูปแบบ คัดเลือกเชื้อ CRAB จำนวน 30 ไอโซเลท มาตรวจหายีนดื้อยาคาร์บาพีเนมและยีน integron-integrase ด้วยปฏิกิริยาลูกโซ่โพลีเมอเรส พบยีนดื้อยา** ***bla***$_{\text{OXA-23-like}}$ **28 ไอโซเลท (ร้อยละ 93.3) ยีน** ***bla***$_{\text{OXA-58-like}}$ **3 ไอโซเลท (ร้อยละ 10.0) และ** ***intI1*** **28 ไอโซเลท (ร้อยละ 93.3) โดยสรุปจากการศึกษาพบร้อยละ 2.3 ของ CRAB ไอโซเลทเป็นแบคทีเรียที่ดื้อยาทุกกลุ่ม และการพบยีนดื้อยา** ***bla***$_{\text{OXA-23-like}}$**,** ***bla***$_{\text{OXA-58-like}}$ **และอินทิกรอนชนิดที่ 1 แสดงถึงเชื้อที่ดื้อยาสูงและสามารถถ่ายทอดยีนดื้อยาไปสู่รุ่นลูกและแบคทีเรียอื่น ๆ ได้ การค้นพบนี้เน้นย้ำความสำคัญของการเฝ้าระวังและการใช้ยาปฏิชีวนะที่มีประสิทธิภาพ เป็นมาตรการที่เข้มงวดในการป้องกันและควบคุมการแพร่กระจายของ CRAB ในสถานพยาบาล**

คำสำคัญ: *A. baumannii* *bla*$_{\text{OXA-23-like}}$ *bla*$_{\text{OXA-58-like}}$ อินทิกรอน การดื้อยา

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Molecular Characterization of Carbapenem Resistant *Acinetobacter baumannii* Isolated from a Private Hospital in Bangkok

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Abstract

Acinetobacter baumannii **is a non-fermentative Gram-negative coccobacillus commonly found in water and soil, categorized as the ESKAPE pathogens with high antimicrobial resistance. Carbapenem-resistant *A. baumannii* (CRAB) is a critical-priority pathogen on the WHO list. This retrospective study investigated the molecular characterization of CRAB isolates from a private hospital in Bangkok during 2021-2022. A total of 244 *A. baumannii* isolates were collected from clinical specimens of 166 patients, of which 171 (70.1%) were CRAB. CRAB isolation rates increased from 64.4% in 2020 to 73.8% in 2021, followed by decreases to 66.4% in 2022 and 61.5% in 2023. Carbapenem consumption (defined daily doses (DDD) per 1,000 patient-days) peaked at 63.71 in 2021 before declining to 37.41 in 2023. Trends from 2020-2023 revealed a consistency between carbapenem use and CRAB isolation rates. Nineteen distinct antimicrobial susceptibility patterns were identified. Thirty CRAB isolates were determined for carbapenem-resistant genes and integron-integrase gene using polymerase chain reaction (PCR). Out of 30 tested isolates, the $bla_{\text{OXA-23-like}}$ gene was detected in 28 isolates (93.3%), the $bla_{\text{OXA-58-like}}$ in 3 isolates (10.0%), and *intI1* in 28 isolates (93.3%). In conclusion, 2.3% of the CRAB isolates displayed pan-drug resistance. The presence of $bla_{\text{OXA-23-like}}$, $bla_{\text{OXA-58-like}}$, and Class 1 integron genes indicates their high resistance and potential for both vertical and horizontal gene transfer to other bacteria. These findings emphasize the importance of routine surveillance and a robust antibiotic stewardship program to enforce stringent measures for preventing and controlling the spread of CRAB in healthcare settings.**

Keywords: *A. baumannii*, $bla_{\text{OXA-23-like}}$, $bla_{\text{OXA-58-like}}$, integron, resistance

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Introduction

The high prevalence of multidrug-resistant (MDR) gram-negative bacteria, particularly *Acinetobacter baumannii*, has been reported, with carbapenem resistant strains emerging in Southeast Asian countries e.g. Thailand, Laos, Vietnam, Malaysia, Brunei, Singapore, Philippines, and Indonesia. *A. baumannii* is a non-fermentative Gram-negative coccobacillus commonly found in water and soil; and is among the ESKAPE pathogens: *Enterococcus faecium*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *A. baumannii*, *Pseudomonas aeruginosa*, and *Enterobacter* species. Carbapenem-resistant *A. baumannii* (CRAB) has been the most common pathogen causing hospital-acquired infections (HAIs) during these few decades, particularly ventilator-associated pneumonia and bloodstream infections. The spread of CRAB has been recognized as a significant public health concern in both developed and developing countries.[1-2] The mortality rate associated with *A. baumannii* infections varies widely across studies and clinical settings. Reported rates range from 26.0% to 55.7%, depending on the context and resistance patterns.[3] In an ICU setting, one study documented a mortality rate of 21.6%.[4]

Acinetobacter species are capable of accumulating multiple antibiotic resistance genes, leading to the development of multidrug-resistant or extensively drug-resistant strains[5] which frequently express resistance mechanisms in nosocomial strains of *Acinetobacter* including enzymatic drug inactivation. Carbapenem is a highly effective β-lactam antimicrobial agent and can be used to treat many types of infections caused by 3rd and 4th generation cephalosporins resistant bacteria. However, an increase in carbapenem-resistant Enterobacterales (CRE) has been reported. There are several mechanisms for carbapenem resistance, especially carbapenemase production in drug-resistant bacteria. The carbapenemase enzyme can break down carbapenems, e.g. imipenem, meropenem, ertapenem, as well as other β-lactam types such as penicillins and cephalosporins (ceftazidime, cefotaxime, ceftriaxone). Large varieties of carbapenemases belonging to three molecular classes of β-lactamases have been identified in Enterobacterales: the Ambler class A, class B, and class D β-lactamases.[6]

The carbapenem usage, even in the short term, or certain antibiotic usage in the long term, increased the risk of CRAB infection. The National Antimicrobial Resistance Surveillance Center, Thailand (NARST) reported that the prevalence of carbapenem-resistant *Acinetobacter* spp. in Thailand increased from 14.4% in 2000 to 74.7% in 2021.[7] The use of carbapenems should be considered to control CRAB. Antimicrobial stewardship programs that restrict excessive antibiotic usage and introduce alternative antibiotic regimens

have also been revealed to be an effective means of controlling MDR *A. baumannii* outbreaks.[8] *A. baumannii* infections are widely distributed in all regions of Thailand.[9] *A. baumannii* accounts for most human infections, and is among species collectively called as "*Acinetobacter baumannii* complex or ACB complex" including *A. calcoaceticus* and *A. pittii*.[10]

The major issue of CRAB is an emergent problem in Thailand. The extensive use of carbapenems causes more widespread drug-resistant bacteria. An academic medical center in Thailand reported the statistically significant negative correlation between carbapenem consumption and the susceptibility rate of *A. baumannii* to imipenem and meropenem in the data from the non-ICU wards.[11] Similarly, significant positive correlation between carbapenem consumption and the imipenem resistance rate of *A. baumannii* was exhibited in China ($p < 0.01$).[12]

Genetic characterization of CRAB is important in several aspects, such as understanding resistance mechanisms, tracking clonal lineages, infection control, and global surveillance. Molecular methods are important tools for epidemiological studies of outbreaks and hospital-associated infections, such as identification of pathogen reservoirs and mode of transmission, distinguishing outbreaks from endemic strains. Although PCR is susceptible to contamination from extraneous DNA, the advantage of PCR allows for the efficient and rapid detection of resistant genes with high sensitivity and specificity, enabling accurate identification, necessitating complementary diagnostic methods. Carbapenemase genes commonly used for investigation are $bla_{\text{OXA-23-like}}$, $bla_{\text{OXA-58-like}}$, bla_{IMP}, bla_{VIM}, bla_{NDM}, and bla_{KPC}. The molecular epidemiology of *A. baumannii* has been widely studied in outbreaks of infections in many countries worldwide. There were sporadic reports that focused on a few hospitals in the region for genetic relatedness of MDR *A. baumannii* isolates in Thailand.[13] The aims of this study were to determine the molecular characters and resistance patterns in CRAB at a private tertiary-care hospital in Bangkok.

Materials and Methods

At a private tertiary-care hospital in Bangkok, Thailand, the trend between carbapenem consumption and the isolation rate of CRAB was determined during 2019-2023. CRAB isolated from patients were obtained from the stock culture for molecular work during 2021-2022.

1. Bacterial identification and antimicrobial susceptibility test of *A. baumannii*

For ethical approval, this study was reviewed and approved by the Ethical Review Committee for Human Research, Faculty of Public Health, Mahidol University, to consider to be compiled with a research

with exemption category, protocol number MUPH 171/2022. The clinical specimens were collected at the routine request of the physician. Bacterial identification and antimicrobial susceptibility testing were performed with the Microscan Walkaway automated system (Siemens, Sacramento, USA), according to the manufacturer's instructions. Antimicrobial susceptibility test was performed by the microdilution method according to Clinical and Laboratory Standards Institute (CLSI) guidelines. Minimum inhibitory concentration (MIC) results of antimicrobial agents were classified as resistant, intermediate, or susceptible based on the CLSI, 2022, document M100-Ed32. The American Type Culture Collection (ATCC) strains used for validation tests were *Escherichia coli* ATCC 25922 and *Pseudomonas aeruginosa* ATCC 27853. Antibiotic tested for aminoglycoside (gentamicin, tobramycin, and amikacin), carbapenem (meropenem, and imipenem), cephalosporin (cefotaxime, cefepime, and ceftazidime), β-lactamase inhibitor (piperacillin-tazobactam), quinolone (ciprofloxacin, and levofloxacin), tetracycline, folate pathway (trimethoprim-sulfamethoxazole), and polymyxin (colistin). *A. baumannii* that were resistant to imipenem or meropenem were considered as CRAB. Isolation rates of CRAB were calculated from all *A. baumannii* isolated during the same period. CRAB isolated from patients admitted at a private tertiary-care hospital in Bangkok, Thailand, during 2021-2022 were obtained from the stock culture which was stored in 20% glycerol at -80°C for molecular work.

2. Extraction and purification of genomic DNA

Genomic DNA from CRAB colonies grown on MacConkey agar overnight at 35°C was extracted and purified by QIAamp DNA mini kit according to the manufacturer's recommendations (QIAGEN, Germany). The purified DNA from each selected isolate was evaluated for both quality and quantity using a nanodrop spectrophotometer by measuring absorbance at 260-280 nm and stored at -20°C until used for further experiments.

3. Polymerase chain reaction-based detection

The extracted DNA was used as a template for PCR reactions. PCR amplification was performed by using specific primers for carbapenem resistance genes: $bla_{\text{OXA-23-like}}$-F, 5' GATCGGATTGGAGAACCAGA 3'; $bla_{\text{OXA-23-like}}$-R, 5' ATTTCTGACCGCATTTCCAT 3'; $bla_{\text{OXA-58-like}}$-F, 5' AAGTATTGGGGCTTGTGCTG 3'; $bla_{\text{OXA-58-like}}$-R, 5' CCCCTCTGCGCTCTACATAC 3'; and PCR condition as previously described.[14] The specific primer for integron-integrase gene is the *intI1*-F, 5' CAGTGGACATAAGCCTGTTC 3'; *intI1*-R, 5' CCCGAGGCATAGACTGTA 3', and PCR condition as previously described.[15] The amplicon sizes of each gene were as follows: 501 bp for $bla_{\text{OXA-23-like}}$; 599 bp for $bla_{\text{OXA-58-like}}$; and

160 bp for *intI1*. The amplified DNA was electrophoresed in 1.5% agarose gel stained with RedSafe and the gel was visualized under the UV transilluminator for photo documentation. The molecular weight of the amplified product was determined using a molecular weight marker. DNA sequencing of each PCR product was performed. The results of DNA sequencing data were analyzed using BLAST on the website www.ncbi.nlm.nih.gov.

4. Data analysis

Descriptive analysis of patient characteristics, resistance patterns, isolation rate, carbapenem resistant genes, and Class 1 integrons were presented in percentage. Carbapenem consumption data during 2019-2023 were collected from the pharmacy dispensing database. WHO ATC classification system and defined daily dose (DDD) were used for this study. Carbapenem was defined as an antibacterial for group J01 of the WHO ATC classification system. The utilization of antibiotics was converted to DDD according to the Guidelines for ATC classification and DDD assignment 2021. DDD per 1,000 patient-days was calculated for carbapenem consumption.[16] The trend between carbapenem consumption and the isolation rate of CRAB was determined over the five-year period. Differences in CRAB isolation rates between years were assessed using the Chi-square test, with statistical significance set at $p < 0.05$. Where applicable, post-hoc pairwise comparisons were performed using Fisher's exact test for multiple comparisons. All statistical analyses were conducted using R software (version 4.4.1).

Results

1. The trend between CRAB isolation rate and carbapenem consumption

The isolation rate of CRAB was 71.0% in 2019, followed by a decrease to 64.4% in 2020. The isolation rate peaked at 73.8% in 2021, the highest in five years, before declining to 66.4% in 2022 and further to 61.5% in 2023. However, no statistically significant difference in CRAB isolation rates was observed across the five-year period ($p = 0.351$). The trend of CRAB isolation rates and carbapenem usage during 2019 and 2023 is shown in Fig. 1. The Carbapenem resistance rate of *A. baumannii* and carbapenem consumption are in the same trend during 2020-2023. A similar trend was observed in carbapenem consumption (DDD per 1,000 patient-days), which increased from 53.05 in 2020 to 63.71 in 2021 and decreased to 52.83 and 37.41 in 2022 and 2023, respectively. Between 2020 and 2023, carbapenem consumption and CRAB isolation rates exhibited parallel trends.

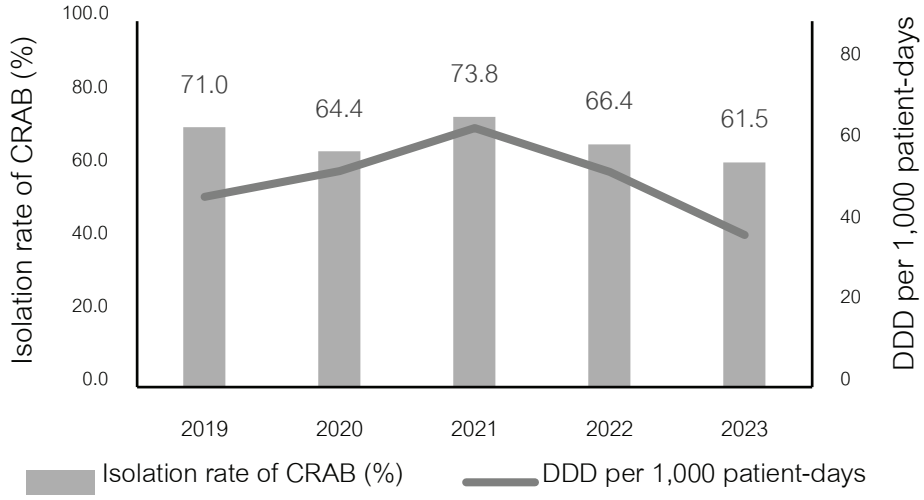


Fig. 1 Trend of carbapenem usage and isolation rate of CRAB during 2019-2023.

2. Types of specimens and antimicrobial susceptibility of CRAB isolates

Of the 244 *A. baumannii* isolates during 2021-2022, 171 (70.%1) that were resistant to imipenem or meropenem by microdilution method were identified as CRAB. Male patients accounted for a higher proportion of CRAB isolates (62.6%) compared to female patients (37.4%). The median age of patients with CRAB infection was 64 years (range: 27-102 years), with a majority (58.9%) being over 60 years old. Sputum was the most common specimen source, contributing to 94 isolates (55.0%). The distribution of clinical isolates according to specimen types and sources is detailed in Table 1. CRAB were resistant to gentamicin (158/171; 92.4%), tobramycin (153/171; 89.5%), amikacin (146/171; 85.4%), meropenem (170/171; 99.4%), imipenem (168/171; 98.2%), cefotaxime (166/171; 97.1%), cefepime (166/171; 97.1%), ceftazidime (165/171; 96.5%), piperacillin-tazobactam (166/171; 97.1%), ciprofloxacin (166/171; 97.1%), levofloxacin (165/171; 96.5%), tetracycline (161/171; 94.1%), trimethoprim-sulfamethoxazole (158/171; 92.4%), and colistin (4/171; 2.3%), respectively.

Table 1 Clinical characteristics of patients, specimens, and antimicrobial resistance by broth microdilution method (n = 171).

Characteristics	Number (%) of CRAB isolates	Antimicrobial agent	Resistant No (%)
Gender		Gentamicin	158 (92.4)
Female	40 (37.4)	Tobramycin	153 (89.5)
Male	67 (62.6)	Amikacin	146 (85.4)
Ages (years)		Meropenem	170 (99.4)
< 15	0 (0.0)	Imipenem	168 (98.2)
15 - 60	44 (41.1)	Cefotaxime	166 (97.1)
> 60	63 (58.9)	Cefepime	166 (97.1)
Specimen type		Ceftazidime	165 (96.5)
Sputum	94 (55.0)	Piperacillin-tazobactam	166 (97.1)
Pus	11 (6.4)	Ciprofloxacin	166 (97.1)
Tissue	17 (9.9)	Levofloxacin	165 (96.5)
Blood	21 (12.3)	Tetracycline	161 (94.1)
Body fluid	11 (6.4)	Trimethoprim-sulfamethoxazole	158 (92.4)
Urine	17 (9.9)	Colistin	4 (2.3)

3. Resistance patterns of 171 CRAB isolates

The nineteen different resistance patterns of 171 CRAB isolates according to the CLSI standard for broth microdilution method were revealed in Table 2 based on their resistance from the most to the least resistance patterns. Thirty isolates of CRAB were selected for the detection of carbapenemase genes. Twenty-seven isolates were obtained from the group with the most resistance *i.e.*, resistance pattern 2 (resistant to all 13 tested antibiotics except colistin) and one isolate each from pattern 1 (resistant to all tested antibiotics), pattern 13 (resistant to 8 tested antibiotics, intermediate to 2 tested antibiotics, and susceptible to 4 tested antibiotics), and pattern 18 (susceptible to 8 tested antibiotics, intermediate to 4 tested antibiotics but resistant to only 2 tested antibiotics).

Table 2 The 19 resistance patterns of CRAB isolates from patients (n = 171).

Pattern	Profile	Isolates	%
1	R14	4	2.3
2	R13/I1(CL)	136	79.5
3	R12/I2(TE, CL)	1	0.6
4	R12/I1(CL)/S1(AK)	7	4.1
5	R12/I1(CL)/S1(T/S)	1	0.6
6	R12/I1(CL)/S1(P/T)	1	0.6
7	R11/I1(CL)/S2(TO, AK)	5	2.9
8	R10/I2(TE, CL)/S2(CAZ, T/S)	1	0.6
9	R10/I1(CL)/S3(IMP, P/T, T/S)	1	0.6
10	R10/I1(CL)/S3(LVX, TE, T/S)	1	0.6
11	R10/I1(CL)/S3(GM, TO, AK)	4	2.3
12	R9/I1(CL)/S4(GM, TO, AK, T/S)	2	1.2
13	R8/I2(TE, CL)/S4(GM, TO, AK, T/S)	1	0.6
14	R7/I2(TE, CL)/S5(GM, TO, AK, CAZ, T/S)	1	0.6
15	S9/I2(CFT, CL)/R3(IMP, CAZ, P/T)	1	0.6
16	S9/I3(CFT, TE, CL)/R2(MER, P/T)	1	0.6
17	S8/I5(IMP, CFT, P/T, TE, CL)/R1(MER)	1	0.6
18	S8/I4(TO, CFT, TE, CL)/R2(IMP, MER)	1	0.6
19	S11/I1(CL)/R2(IMP, MER)	1	0.6

S, susceptible; I, intermediate; R, resistant; AK, amikacin; CPE, cefepime; CFT, cefotaxime; CAZ, ceftazidime; CP, ciprofloxacin; CL, colistin; GM, gentamicin; IMP, imipenem; LVX, levofloxacin; MER, meropenem; P/T, piperacillin-tazobactam; TE, tetracycline; TO, tobramycin; T/S, trimethoprim-sulfamethoxazole

4. Detection of carbapenem resistance genes and class 1 integron-integrase gene

Carbapenemase genes were found in 28 (93.3%) of 30 CRAB isolates: 28 isolates with $bla_{\text{OXA-23-like}}$, and 3 isolates with $bla_{\text{OXA-58-like}}$, Class 1 integron-integrase gene was found in 28 studied CRAB isolates (93.3%) (Fig. 2 and Table 3). From the comparison of resistance patterns with gene patterns in 30 CRAB isolates shown in Table 3, five gene patterns among 27 CRAB isolates that had resistance pattern 2 were determined. Gene pattern 3 of CRAB which carried $bla_{\text{OXA-23-like}}$ and *intI1* but negative for $bla_{\text{OXA-58-like}}$ was the most common (23 isolates) gene pattern among the resistance pattern 2, followed by gene pattern 1 which carried all 3 genes (2 isolates). However, not only in resistance pattern 2 but also gene pattern 3 was revealed in resistance pattern 13. The data indicated that among all CRAB isolates, the $bla_{\text{OXA-23-like}}$ gene was the most prevalent. Notably, among CRAB isolates (n = 30) obtained from various sources, the presence of at least one carbapenemase-related gene or integron integrase gene was observed in 100.0% (20/20) of sputum samples from intubated patients, as well as in 100.0% of isolates from blood (3/3), tissue (3/3), urine (2/2), pus (1/1), and body fluid (1/1) (Table 4).

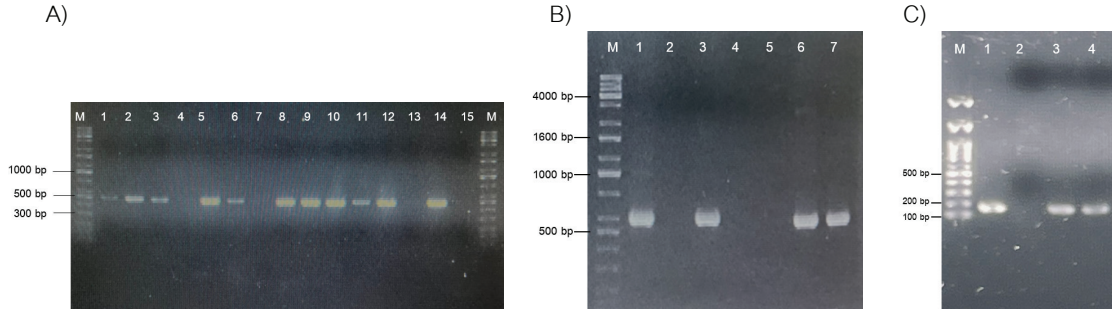


Fig. 2 Gel electrophoresis of PCR products of carbapenem resistant genes and integron integrase gene in CRAB isolated from patients. Lane M are 100 bp standard size marker. Lane 1 are positive control. A) $bla_{\text{OXA-23-like}}$ (501 bp); lane 2-14 are amplicons of CRAB isolates; lane 15 is negative control. B) $bla_{\text{OXA-58-like}}$ (599 bp); lane 2 is negative control; lane 3-7 are amplicons of CRAB isolates. C) *intI1* (160 bp); lane 2 is negative control; and lane 3-4 are amplicons of CRAB isolates.

Table 3 Resistance patterns and carbapenem resistance gene, and integron-integrase gene patterns of 30 CRAB isolates.

Resistance patterns	No. of isolates	Gene patterns	No. of isolates (n = 30)	*bla*$_{\text{OXA-23-like}}$	*bla*$_{\text{OXA-58-like}}$	*intI1*
1	1	5	1	+	-	-
2	27	1	2	+	+	+
		2	1	+	+	-
		3	23	+	-	+
		4	1	-	-	+
13	1	3	1	+	-	+
18	1	4	1	-	-	+

Table 4 Distribution of carbapenem resistance genes, and integron-integrase gene in CRAB isolates by specimen.

Isolates	Sputum (n = 20)	Blood (n = 3)	Tissue (n = 3)	Urine (n = 2)	Pus (n = 1)	Body fluid (n = 1)
Non-gene carriers	0	0	0	0	0	0
At least one-gene carriers	20 (100.0%)	3 (100.0%)	3 (100.0%)	2 (100.0%)	1 (100.0%)	1 (100.0%)

5. DNA Sequencing

Nine amplicons of *bla*$_{\text{OXA-23-like}}$ (5 amplicons), *bla*$_{\text{OXA-58-like}}$ (3 amplicons), and *intI1* (1 amplicon) of 5 CRAB isolates were submitted for DNA sequencing. Then the DNA sequences were compared with nucleotide sequences in the database using the Basic Local Alignment Search Tool or BLAST (www.ncbi.nlm.nih.gov/BLAST). The results confirmed that 5 amplicons were *bla*$_{\text{OXA-23-like}}$ with 98.47-99.35% identity. Three amplicons of *bla*$_{\text{OXA-58-like}}$ were matched with *bla*$_{\text{OXA-58-like}}$ with 99.11-99.47% identity. An amplicon of *intI1* was matched with Class 1 integron-integrase (*intI1*) with 100% identity.

Discussion

The trend between carbapenem consumption and isolation rates of CRAB was observed in our study. From 2019 to 2023, carbapenem consumption was determined from 46.79 DDD per 1,000 patient-days in 2019 to 53.05, 63.71, 52.83, and 37.41 DDD per 1,000 patient-days in 2020-2023. The CRAB isolation rate was 71.0% in 2019 and decreased to 64.4% in 2020, rapidly increasing to 73.8% in 2021. During 2022 and 2023, the isolation rate of CRAB continuously decreased to 66.4% and 61.5%, respectively. While statistical correlation analysis was not feasible due to the use of annually aggregated carbapenem consumption data, our findings demonstrated parallel trends between carbapenem usage and CRAB isolation rates from 2019 to 2023, consistent with the study by Zeng *et al.* (2019) who reported the utilization of carbapenems revealed a positive significant correlation with the imipenem resistance rate of *A. baumannii* ($p < 0.01$) from 2014 to 2018.[12]

The trend between antibiotic consumption and the rising rates of antibiotic-resistant microorganisms is commonly observed following a prolonged period of increased antibiotic use.[17] Notably, an increase in antibiotic consumption precedes a subsequent rise in the prevalence of antibiotic-resistant microorganisms, though these events may not simultaneously occur, supported by evidence reported among hospitalized patients and is further influenced by broader environmental factors, such as the dissemination of resistance genes within the environment.[18] In addition, the development of multidrug-resistant organisms (MDROs) is influenced by numerous factors, including inappropriate prescribing practices.[19] In this study, a direct relationship between specific factors was not observed. This lack of observation could be attributed to the potential effects of other variables, including the co-prescription of antimicrobial agents, the use of carbapenems for infections caused by different bacteria, and the presence of poly-microbial infections. These additional variables may have a substantial impact on the prevalence rate of CRAB in our study.

Most patients in this study were elderly, which patients median age was 64 and the age range of 27-102 years, often presenting with risk factors such as weakened immunity, comorbidities, and prolonged hospital stays. This investigation confirmed the results of several other studies that CRAB is mostly isolated from clinical specimens of sputum, followed by blood, tissue, or urine.[20-21] According to the CLSI standard, 4 CRAB isolates (2.3%) in this study were resistant and 167 isolates (97.7%) were intermediate to colistin. Apart from colistin, amikacin was the second agent mostly active against the CRAB isolates with 14.6% susceptibility which is consistent with 25.3% susceptibility in University Hospital in Khon Kaen, Thailand.[22]

This emphasizes the critical role of CRAB in respiratory infections, particularly among hospitalized patients, addressing the need for regular monitoring and surveillance in clinical settings, especially within intensive care units (ICUs).

Our findings revealed a related pattern between the most common resistance phenotype (resistance pattern 2) and the dominant resistance genotype (gene pattern 3), highlighting how the high prevalence of $bla_{\text{OXA-23-like}}$ gene likely contributes to the observed carbapenem resistance profiles in our setting. In the present study, 28 (93.3%) of the 30 CRAB isolates were detected for $bla_{\text{OXA-23-like}}$ which was consistent with the reports in Thailand by Boonsilp *et al.* (2024) and Thet *et al.* (2020) that $bla_{\text{OXA-23-like}}$ gene was revealed for 96.7% and 90.0% in CRAB clinical isolates from university hospitals in Bangkok[23] and Khon Kaen,[20] respectively. In agreement with other reports, $bla_{\text{OXA-23-like}}$ was the major resistance determinant of 77.3% among the CRAB clinical isolates from Gorgan, Northern Iran,[24] while studies in Korea and China reported even higher frequencies of 98.7%[25] and 99.5%,[26] respectively. These results further highlight the widespread distribution of the $bla_{\text{OXA-23-like}}$ gene in CRAB isolates across different regions.

In this study, the $bla_{\text{OXA-58-like}}$ gene was detected in three (10.0%) of 30 CRAB isolates corresponding to the *A. baumannii* isolates for 3.2% from a teaching hospital in Taiwan.[27] Obvious that 30% demonstrated positivity to the $bla_{\text{OXA-58-like}}$ gene from clinical isolates in Iraq.[28] It is also commonly found in healthcare facilities worldwide. Likewise, $bla_{\text{OXA-58-like}}$ gene was also detected in CRAB isolates for only 2.2% in the Asia-Pacific region (Australia, China, Hong Kong, Japan, Malaysia, Philippines, South Korea, Taiwan, and Thailand), and 1.4% in Thailand.[29, 22] Some *A. baumannii* isolates were resistant to carbapenems but tests negative for the $bla_{\text{oxa-23-like}}$ and $bla_{\text{oxa-58-like}}$ genes, it may be due to the presence of other resistance genes or alternative resistance mechanisms.

The *intI1* gene is strongly associated with antibiotic-resistance gene dissemination and linked to outbreaks involving MDR pathogens like *A. baumannii*. Epidemic *A. baumannii* strains revealed significantly higher integron carriage than non-epidemic strains, with Class 1 integrons detected in 58.0% of *A. baumannii* isolates.[15] The examination of CRAB isolates revealed the presence of Class 1 integron (*intI1*) in this study in 28 (93.3%) of 30 isolates harbored Class 1 integron, which is consistent with previous research. The highest prevalence Class 1 integrons (99.7%) in *A. baumannii* isolated from various sources such as foods, environments, humans, and animals were reported in Korea.[30] Anane *et al.* reported a prevalence of 80.0% in *A. baumannii* clinical

isolates in South Africa,[31] while Hashemi *et al.* identified Class 1 integron in 60% of *A. baumannii* isolates from burn patients in North Iran.[32] This revealed the cumulative evidence of the widespread occurrence of Class 1 integron in clinical settings, both regionally and internationally. The *intI1* gene facilitates horizontal gene transfer of antibiotic-resistance genes via gene cassettes, contributing to MDR in *A. baumannii*.[33] Understanding the prevalence of Class 1 integron in clinical isolates is important for delineating the genetic mechanisms underlying antibiotic resistance and horizontal gene transfer, thereby informing strategies in healthcare settings for antimicrobial stewardship and infection control measures.

For some limitations to this study, the various resistance patterns such as resistance pattern 19, which was the least resistance pattern, should be selected for genetic characterization to identify the genes and understand the mechanisms. Not only $bla_{\text{OXA-23-like}}$ and $bla_{\text{OXA-58-like}}$ genes, but also bla_{NDM}, which is a metallo β-lactamase gene involved in high-level drug resistance, and bla_{IMP}, bla_{VIM}, and bla_{KPC} genes, which are other carbapenemase genes found in CRAB, should be determined to clarify the resistance mechanisms which are critical for controlling resistance spread and developing targeted treatments.

Conclusion

Analysis of trends during 2020 and 2023, revealed that both carbapenem resistance rates in *A. baumannii* and carbapenem consumption followed similar patterns, suggesting a consistency between carbapenem use and the rise in CRAB infection during this period. In conclusion during 2021-2022, 2.3% of the CRAB isolates displayed pandrug resistance. The Class 1 integron predominantly conferred resistance to antimicrobials which enhances their potential for both vertical and horizontal gene transfer to other bacteria. These findings highlight the importance of routine surveillance and a strong antibiotic stewardship program to enforce stringent measures for preventing and controlling the spread of CRAB in healthcare settings. Future studies should use time-series analyses with finer temporal resolution and multivariate models to clarify the causal link between carbapenem use and resistance in CRAB. Additionally, expanded phenotypic-genotypic analysis using whole genome sequencing may reveal novel mechanisms and improve understanding of resistance dynamics.

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