Genomic relatedness and dissemination of *bla*$_{\text{NDM-5}}$ among *Acinetobacter baumannii* isolated from hospital environments and clinical specimens in Thailand

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Background:*Acinetobacter baumannii* (*A. baumannii*) is an important cause of nosocomial infection, especially in intensive care units (ICUs). It has the propensity to tolerate various environments and multiple classes of antibiotics. Our study aimed to characterize the comparative genomes of *A. baumannii* from hospital environments and clinical isolates. **Methods:** Clinical and environmental *A. baumannii* isolates were collected from a university hospital. Antibiotic susceptibility testing was performed, antibiotic resistance genes (ARG) were characterized, and repetitive element palindromic-PCR (rep-PCR) typing was performed. Eight representative *A. baumannii* isolated from environmental and clinical samples from the same wards were selected for whole-genome sequencing (WGS) using the Illumina platform. Results: A total of106 *A. baumannii* isolates were obtained from 312 hospital environmental samples. A high percentage of samples with *A. baumannii* colonization was detected from AMBU bags (77.9%), followed by bedrails (66.7 %) and suction tubes (66.7%). We found that 93.4% of the environmental isolates were multidrug-resistant *A. baumannii* (MDRAB), and 44.7% were extremely drug-resistant *A. baumannii* (XDRAB). *bla*$_{\text{OXA-23}}$ *bla*$_{\text{NDM,}}$ and *bla*$_{\text{OXA-58}}$ were present in 80.2%, 78.3%, and 0.9% of all isolates, respectively. Sixty-one *A. baumannii* isolates were collected from patient specimens in the same ward. Among all *A. baumannii* clinical isolates, MDRAB and XDRAB accounted for 82% and 55.7%, respectively. The most dominant ARG identified was *bla*$_{\text{OXA-23}}$ (80.3%), followed by *bla*$_{\text{NDM}}$(55.7%). The genetic diversity of all isolates using rep-PCR could be divided into 33 genotypes. The genome size of eight *A. baumannii* ranged from 3.78-4.01 Mb. We found six of eight strains to be *bla*$_{\text{NDM-5}}$ -harboring *A. baumannii*. Mobile genetic

elements (MGEs), such as integron1 (*intI*1), located upstream of bla_{NDM-5} were observed. The phylogenomic relationship of the core and pan genomes as well as the single nucleotide polymorphism (SNP) count matrix revealed the genetic similarity of *A. baumannii* environmental and clinical strains obtained from the same ward. **Conclusion:** This study confirmed that *A. baumannii* colonized in hospital environments were the main reservoir of nosocomial infection and provides critical information to guide the control of *A. baumannii* infection.

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Abstract

Background: *Acinetobacter baumannii* (*A. baumannii*) is an important cause of nosocomial infection, especially in intensive care units (ICUs). It has the propensity to tolerate various environments and multiple classes of antibiotics. Our study aimed to characterize the comparative genomes of *A. baumannii* from hospital environments and clinical isolates.

Methods: Clinical and environmental *A. baumannii* isolates were collected from a university hospital. Antibiotic susceptibility testing was performed, antibiotic resistance genes (ARG) were characterized, and repetitive element palindromic-PCR (rep-PCR) typing was performed. Eight representative *A. baumannii* isolated from environmental and clinical samples from the same wards were selected for whole-genome sequencing (WGS) using the Illumina platform.

Results: A total of 106 *A. baumannii* isolates were obtained from 312 hospital environmental samples. A high percentage of samples with *A. baumannii* colonization was detected from AMBU bags (77.9%), followed by bedrails (66.7%) and suction tubes (66.7%). We found that 93.4% of the environmental isolates were multidrug-resistant *A. baumannii* (MDRAB), and 44.7% were extremely drug-resistant *A. baumannii* (XDRAB). $bla_{\text{OXA-23}}$ $bla_{\text{NDM,}}$ and $bla_{\text{OXA-58}}$ were present in 80.2%, 78.3%, and 0.9% of all isolates, respectively. Sixty-one *A. baumannii* isolates were collected from patient specimens in the same ward. Among all *A. baumannii* clinical isolates, MDRAB and XDRAB accounted for 82% and 55.7%, respectively. The most dominant ARG identified was $bla_{\text{OXA-23}}$ (80.3%), followed by bla_{NDM} (55.7%). The genetic diversity of all isolates using rep-PCR could be divided into 33 genotypes. The genome size of eight *A. baumannii* ranged from 3.78-4.01 Mb. We found six of eight strains to be $bla_{\text{NDM-5}}$ -harboring *A. baumannii*. Mobile genetic elements (MGEs), such as integron1 (*intI*1), located upstream of $bla_{\text{NDM-5}}$ were observed. The phylogenomic relationship of the core and pan genomes as well as the single nucleotide polymorphism (SNP) count matrix revealed the genetic similarity of *A. baumannii* environmental and clinical strains obtained from the same ward.

Conclusion: This study confirmed that *A. baumannii* colonized in hospital environments were the main reservoir of nosocomial infection and provides critical information to guide the control of *A. baumannii* infection.

Introduction

Acinetobacter baumannii has emerged as an important pathogen related to hospital-acquired infections worldwide. This pathogen is the major cause of ventilator-associated pneumonia (VAP), bacteremia, urinary tract infections, wound infections, and meningitis (*Nutman et al., 2016*). The emergence of antibiotic-resistant *A. baumannii,* especially MDRAB and XDRAB, has increased and seriously challenged the treatment of these bacterial infections (*Kyriakidis et al., 2021*). National Antimicrobial Resistance Surveillance Thailand (NARST) reported that the prevalence of carbapenem-resistant *Acinetobacter baumannii* complex infection in the ICUs of 51 hospitals in Thailand was higher than 80% (*NARST, 2021*). The major mechanism of carbapenem resistance among *A. baumannii* is the production of antibiotic-hydrolyzing enzymes that belong to Ambler Class D β-lactamases (CHDLs) and class B metallo-lactamases (MBLs) (*Ibrahim et al., 2021*). Class D carbapenemases encode acquired $bla_{\text{OXA-23}}$, $bla_{\text{OXA-24}}$, and $bla_{\text{OXA-58}}$. These genes have been reported in many countries all over Asia, including China, Korea, Thailand, Vietnam, and Malaysia (*Hsu et al., 2017*). Major MBLs in *A. baumannii* are encoded by the bla_{NDM} gene and has been reported in Thailand since 2017 (*Leungtongkam et al., 2018*). To date, twenty-four New Delhi metallo-beta-lactamase (NDM) variants have been identified in more than 60 bacterial species, including *Acinetobacter* spp., and several variants have the ability to enhance carbapenemase activity (*Wu et al., 2019*).

A. baumannii has the ability to survive on hospital surfaces and equipment for long periods. Hospital surface contamination of *A. baumannii* is closely correlated with the transmission of the bacteria to patients, causing episodes of bacteremia and/or sepsis (*Markogiannakis et al., 2008*). Genome sequencing of carbapenem-resistant *A. baumannii* (CRAB) found on ICU surfaces revealed that the CRAB isolates from ICU environment were linked with those of clinical origin (*Yasir et al., 2022*). *A. baumannii* isolates were recovered from surrounding ICU bed surfaces, and these isolates

exhibited a multidrug resistance phenotype and belonged to some widely spread clonal complexes (CCs) of clinical *A. baumannii* isolates (*Rocha et al., 2018*).

Comparative genomics research can help assess the bacterial evolution, resistance mechanisms, and pathogenicity of bacterial pathogens at the genome-wide level; it is also useful in the ensuing study of virulence factors involved in pathogenicity (*Wright et al., 2016*). Whole-genome sequencing studies comparing distinct clinical and environmental isolates have improved our understanding of the evolution of *A. baumannii*. In this study, we aimed to investigate the resistance rates and epidemiological characteristics of clinical and environmental *A. baumannii* isolates. Then, we determined the draft genome sequence of eight clinical and eight environmental *A. baumannii* strains from the same wards to perform comparative genomic analysis.

Materials & Methods

Samples

Clinical and environmental *A. baumannii* isolates were collected from Naresuan University Hospital between December 2020 and April 2021. Naresuan University is a level III hospital with 400 beds located in the lower northern region of Thailand. Hospital environment and clinical isolates were collected from five wards, which were two medical wards, Medicine-man (MED-1) and Medicine-woman (MED-2), and three intensive care units, the ICU Cardio-Vascular-Thoracic Surgery (ICU-1), ICU Surgery (ICU-2), and ICU Medicine (ICU-MED). The sources of the samples included staff contact samples, which included samples collected from stethoscopes (n=15), charts (n=15), computers/keyboards (n=15), nurse station counters (n=15), medical lab coats (n=15), restroom door handles (n=15), telephones (n= 15), and dressing trolleys (n= 15). Patient contact samples were collected from bedrails (n=15), bedsheets (n=15), suction tubes (n=15), patient tables (n=15), curtains (n=15), humidifiers (n=15), intravenous (IV) stands (n=15), ventilators (n=15), ventilator monitors (n=9), water from ventilators (n=9), suction tubes (n=9), and AMBU bags (n=9). Other environmental samples were collected from the air (n=15), sinks (n=15), and water from sinks (n=15).

The protocol was approved by the Naresuan University Institutional Biosafety Committee, and the project number was NUIBC MI62-09-42.

Isolation and identification of *A. baumannii* from hospital environments

The air samples were collected using Leeds Acinetobacter Medium (LAM) (Hi-media, India) in 9 cm diameter Petri dishes. Petri dishes were exposed for 24 hrs. The other samples from environmental surfaces were collected using cotton swabs soaked in 0.85% normal saline and then placed in transfer media. The swab samples were enriched in Luria-Bertani broth (LB) (Hi-media, India) by shaking at 160 rpm at 37°C for 24 hours and then cultured in Leed Acinetobacter Media (LAM) at 37°C for 24 hours. Cultures with pink colonies were selected for further evaluation using Gram's stain and biochemical tests (catalase, oxidase, TSI, citrate). Molecular identification of the bacterial isolates was confirmed by polymerase chain reaction (PCR) using 16S rRNA, *rpo*B, and $bla_{\text{OXA-51}}$ primers (Table S1).

Determination of antibiotic susceptibility

Antibiotic susceptibility testing was performed according to the disk diffusion method using 12 antibiotics: piperacillin/tazobactam (100 and 10 µg), ceftazidime (30 µg), cefepime (30 µg), cefotaxime (30 µg), ceftriaxone (30 µg), imipenem (10 µg), meropenem (10 µg), gentamicin (10 µg), amikacin (30 µg), tetracycline (30 µg), ciprofloxacin (5 µg), and trimethoprim/sulfamethoxazole (1.25 and 23.75 µg). The plates were incubated at 37 °C for 24 hours. The zones of inhibition determined whether the microorganism was susceptible, intermediately resistant, or resistant to each antibiotic according to the Clinical and Laboratory Standards Institute (CLSI) guidelines (2022). All isolates were defined as non-drug-resistant *A. baumannii* (NRAB), MDRAB, CRAB, and XDRAB as previously described by Magiorakos *et al*.

PCR amplification of antibiotic resistance genes and rep-PCR typing

As mentioned earlier, PCR assays to detect $bla_{\text{OXA-23}}$, $bla_{\text{OXA-24}}$, $bla_{\text{OXA-58}}$, and bla_{NDM} were performed using the primers shown in Table S1. The genomic DNA of each isolate was extracted from the overnight cultures using a PureDirex Genomics DNA Isolation Kit (BIO-HELIX, New Taipei City, Taiwan). Rep-PCR was performed by using genomic DNA as a template for PCR amplification with the ERIC-2 primer (Table S1) with the

conditions described by Leungtongkam *et al*. PCR-banding patterns and rep-PCR types were analyzed and interpreted as previously described (*Leungtongkam et al., 2018*).

Whole-genome sequencing and bioinformatics analysis

Eight representative *A. baumannii* strains from four wards, four from hospital environments (AE17, AE30, AE73, AE106) and four from clinical isolates (AC02, AC09, AC23, and AC59) were analyzed. We selected two *A. baumannii* strains from each ward that were isolated from the same time frame and showed similar antibiotic susceptibility profiles and ARG patterns. All strains were cultured onto an LB agar plate and incubated overnight at 37°C. Genomic DNA was extracted using a PureDire Genomics DNA Isolation Kit (BIO-HELIX, New Taipei City, Taiwan). The extracted DNA was quantified by a nanodrop (Hercuvan, Cambridge, UK). The purified genomic DNA was used to construct libraries followed by sequencing with the Illumina HiSeq 2500-PE125 platform at Macrogen, Korea. The nucleotide sequences of the eight *A. baumannii* strains have been deposited in NCBI's database under Sequence Read Archive (SRA) with Bioproject PRJNA862456 (https://www.ncbi.nlm.nih.gov/sra/PRJNA862456). The genome of *A. baumannii* ATCC17978 (CP000521) was used as a reference strain for comparison with the eight *A. baumannii* strains.

Genome assembly and annotation

Raw sequencing reads were trimmed by using Trim Galore v0.6.7 with default settings and by using Unicycler v0.4.8 with default parameters prior to assembly (*Krueger et al., 2012; Wick et al., 2017*). The assembled contigs that were larger than 300 bp in length were selected and subjected to further bioinformatic analysis. The remaining contigs were annotated by using Prokka v1.14.6 with default options (*Seemann, 2014*).

Identification of MLST, antimicrobial resistance, and virulence genes

The remaining contigs were subjected to detection of drug-resistance and virulence genes by using Abricate v1.0.1 with default settings (*Seemann, 2016*) against the comprehensive antibiotic resistance database (CARD) and virulence factor database (VFDB) (*Alcock et al., 2020; Liu et al., 2022*). Multilocus sequence typing (MLST) was performed by using MLST v2.0, which is accessible from the Center for Genomic

Epidemiology (www.genomicepidemiology.org). The gene arrangement analysis of bla_{NDM-5} was performed using Easyfig version 2.1 (*Sullivan et al., 2011*).

Phylogenomic relationships

The selected genomes of eight *A. baumannii* were subjected to Roary v3.13.0 with the default parameters to identify pan- and core genes (*Page et al., 2015*). The resultant core genes among the eight genomes were concatenated prior to the construction of a pangenome tree in the CSI phylogeny, which is accessible from the Center for Genomic Epidemiology (www.genomicepidemiology.org) (*Kaas et al., 2014*). A core-genome tree was constructed based on the presence/absence of identified core-genes and visualized in FigTree v1.4.4 (https://tree.bio.ed.ac.uk/software/figtree/). The SNP count matrix of all selected genomes was calculated in snp-dists v0.6.3 with default settings (*Seemann, 2019*).

Statistical analyses

Statistical analyses were performed using Stata (Stata 12.0 Corporation). The comparisons of the proportions of antibiotic resistance between *A. baumannii* obtained from the two different origins were analyzed by using the Z-test. The comparisons of antibiotic resistance among *A. baumannii* collected from the five hospital wards were analyzed by using the chi-square test. P values <0.05 were considered to be a statistically significant difference.

Results

***A. baumannii* strains isolated from the hospital environment and clinical isolates**

A total of 106 *A. baumannii* isolates were obtained from 312 hospital environmental samples (33.97%). The isolates associated with patient contact from AMBU bags, bedrails, suction tubes, water from ventilators, bedsheets, patient tables, humidifiers, ventilators, curtains, and IV stands were found in 77.9%, 66.7%, 66.7%, 55.6%, 53.3%, 33.3%, 33.3%, 33.3%, 33.3%, and 13.3% of the samples, respectively. The isolates associated with staff contact and other environments from the air, keyboards, counters, medical lab coats, dressing trolleys, stethoscopes, charts, restroom door handles, and telephones were found in 60.0%, 53.3%, 46.7%, 42.9%, 33.3%, 26.7%, 26.7%, 6.7%, 6.7%, and 6.7% of the samples, respectively (Table S2).

However, we did not find *A. baumannii* isolates on sinks, water from sinks, or ventilator monitors (Table S2). Of the 312 environmental samples collected from each ward, we found the highest *A. baumannii* contamination in the samples obtained from ICU Surgery, with a rate of 52.9% (36/38), followed by those obtained from the Medicine-woman (40.7%; 22/54), ICU Medicine (38.2%; 26/68), Medicine-man (27.8%; 5/54), and ICU Cardiovascular-Thoracic Surgery (10.3%; 7/68) wards (Table S2).

During the investigation of the prevalence of *A. baumannii* isolates from the hospital environments of various wards, we found the highest rate of *A. baumannii* in the ICU Surgery ward (33.9%), followed by the ICU Medicine (24.5%), Medicine-woman (20.8%), Medicine-man (14.2%), and ICU Cardio-Vascular-Thoracic surgery (6.6%) wards (Table 1). *A. baumannii* isolates were found in the patient specimens collected from the ICU Medicine (24.6%), Medicine-man (24.6%), ICU Surgery (19.7%), Medicine-woman (16.4%), and ICU Cardio-Vascular-Thoracic surgery (14.8%) wards (Table 1).

Antibiotic susceptibility patterns of *A. baumannii* isolates

All *A. baumannii* isolates were subjected to antimicrobial susceptibility testing, and the results are shown in Table 2. *A. baumannii* isolates from hospital environments were highly resistant to meropenem (100%), cefotaxime (100%), ceftazidime (100%), and ceftriaxone (100%), while the *A. baumannii* clinical isolates were highly resistant to ceftazidime (90.2%) and ceftriaxone (90.2%). NRAB was detected in only 16.39% of *A. baumannii* clinical isolates. A high prevalence of MDRAB and CRAB was detected in *A. baumannii* isolated from hospital environment (ABHE) (93.4% and 100%) and clinical isolates (82.0% and 92.0%) with p value < 0.05, as shown in Table 3. The prevalence of XDRAB in *A. baumannii* isolates from hospital environments and clinical isolates was 44.7% and 55.7%, respectively. (Table 3). Among the five wards, a high prevalence of XDRAB was detected in *A. baumannii* isolates from ICU Surgery (Table 4).

Antibiotic resistance genes and rep-PCR typing

16S rRNA and *rpo*B genes were detected in all *A. baumannii* isolates. The intrinsic $bla_{\text{OXA-51}}$ gene was detected in all ABHE and 96.7% (59/61) of clinical isolates. The oxacillinase gene, $bla_{\text{OXA-23}}$was the most frequently detected gene at 80.20%

(85/106) in ABHE and 80.33% (49/61) in clinical isolates (Table 3). The *bla*$_{OXA-58}$ gene was detected in one ABHE (0.94%) and one clinical isolate (1.64%). The *bla*$_{NDM}$ gene was detected in 78.3% (83/106) of ABHE (p value < 0.05) compared to 55.74% (34/61) of clinical isolates. The *bla*$_{OXA-24}$ gene was not detected in any of the isolates. Among the five wards, a high prevalence of *bla*$_{OXA-23}$ was detected in ICU Cardio-Vascular-Thoracic Surgery, and a high prevalence of *bla*$_{NDM}$ was detected in ICU Surgery (p value < 0.05) (Table 4).

Rep-PCR typing was performed, and fingerprinting represented 33 different DNA patterns consisting of amplicon sizes ranging from 500 to 4,000 bp. The genotypes were named T1 to T33. The major genotype of ABHE was T30 at 21.7% (23/106), followed by T23 at 17% (18/106) and T2 at 15% (15/106). The major genotype of the *A. baumannii* clinical isolates was T4 at 34.4% (21/61), followed by T23 at 29.5 % (18/61). Heatmaps representing the antibiotic susceptibility patterns, antimicrobial resistance genes, and rep-PCR typing from the five wards is shown in Figures S1-S5. We found genetic similarity between ABHE and *A. baumannii* clinical isolates in each ward with antibiotic susceptibility patterns and antimicrobial resistance genes since most *A. baumannii* strains in the same ward showed similar profiles. No association was found between rep-PCR typing of ABHE and *A. baumannii* clinical isolates (Figures S1-S5). Eight strains of *A. baumannii* with similar profiles from four wards were selected for genome sequencing.

Comparative genomic and phylogenomic analysis of *A. baumannii* from hospital environmental and clinical isolates

Eight strains of *A. baumannii* from clinical and environmental isolates were analyzed and compared with the genome of *A. baumannii* ATCC17978. The four ABHE were AE17 (patient table), AE30 (bedrail), AE73 (dressing trolley), and AE106 (AMBU bag). The four clinical isolates were AC02 (blood hemoculture), AC09 (sputum), AC23 (sputum), and AC59 (right hepatic drain). AC02 and AE03 were obtained from the Medicine-man ward. AC59 and AE17 were obtained from the Medicine-woman ward. AC09 and AE106 were derived from the ICU Cardio-Vascular-Thoracic Surgery ward. AC23 and AE73 were derived from the ICU Surgery ward. The genome characterization

of the isolates is summarized in Table 5. The genome analysis revealed that AC02, AE30, AC09, AE106, AC23 and AE73 belong to ST2 based on the Pasteur MLST scheme. However, AC59 and AE17 belong to ST164. The predicted genome sizes of the eight *A. baumannii* strains ranged from 3.78 to 4.01 Mb compared to the genome of ATCC17978, which was 3.97 Mb.

ARGs and virulence genes of eight *A. baumannii* strains showed genetic similarity among *A. baumannii* hospital environments and clinical isolates but were slightly different from the genome of ATCC17978 (Figure 1AB). The ARGs detected in all eight *A. baumannii* strains as well as ATCC 17978 encoded macrolide resistance genes (*amvA*) and a number of genes encoding efflux pumps involved in resistance in glycylcycline/tetracycline (*adeR, adeS, adeA, adeB*), fluoroquinolone/tetracycline (*adeF, adeG, adeH, adeL*), fluoroquinolone (*abaQ, abeM*), fosfomycin (*abaF*), and multidrug resistance (*adeI, adeJ, adeK, adeN, abeS*). We identified 23 ARGs present in only some *A. baumannii* strains, which encoded the efflux pump (*adeC*) and genes involved in resistance to tetracycline (*tet(39*), *tetB*), cephalosporins (bla_{ADC-10}, bla_{ADC-6}, bla_{ADC-73}, bla_{ADC-79}, bla_{TEM-1}, bla_{TEM-12}), carbapenems (bla_{OXA-23}, bla_{OXA-66}, bla_{OXA-91}, $bla_{OXA-259}$), macrolide (*mphE, msrE*), aminoglycoside (*aadA5, armA, aph(3'')-Ib, aph(6)-Id*), sulfonamide (*sul*1, *sul*2), and integron-encoded dihydrofolate reductase (*dfrA17*).

A class B β-lactamase gene, bla_{NDM-5}, that hydrolyzes virtually all β-lactam antibiotics, including carbapenems, was detected in six strains except ATCC17978, AE17 and AC59 (Figure 1AB). Genetic contexts of bla_{NDM-5} revealed mobile genetic elements (MGEs), such as integron1 (*intI1*), IS91 family transposase, and transposase (*ISAba125*), along with other AGRs, *ant(3")-Ia*, *qacEΔ1,* and *sul1,* located upstream and downstream of bla_{NDM-5} (Figure 1C).

Analysis of the virulence genes of eight *A. baumannii* strains and ATCC17978 revealed that the genes were involved in biofilm formation (*adeF, adeG, adeH, bap, csuA/B, csuA, csuB, csuC*, *csu*D, *csu*E, *pga*A, *pga*B, *pga*C, *pga*D), enzyme phospholipase (*plc*C, *plc*D), immune evasion (*lps*B, *lpx*A, *lpx*B, *lpx*D, *lpx*L, *lpx*M), iron uptake (*bar*A, *bar*B, *bas*A, *bas*B, *bas*C, *bas*D, *bas*F, *bas*G, *bas*I, *bas*J, *bau*A, *bau*B, *bau*C, *bau*D, *bau*E, *bau*F, *ent*E), gene regulation (*aba*I, *aba*R, *bfm*R, *bfm*S), serum resistance (*pbp*G), and host cell adherence (*omp*A) (Figure 1B). The genes involved in

capsule polysaccharide synthesis (*weoB*) and the gene encoding glycosyltransferase in lipopolysaccharide (LPS) biosynthesis (*lpsB*) were detected in only one strain, ATCC 17978 and AC09 (Figure 1B).

The phylogenomic relationship of the core and pan genomes of eight *A. baumannii* and ATCC17978 strains shown in Figure 2AB revealed three major clades. The *A. baumannii* strains obtained from the ICU-1, ICU-2, and Med-1 wards were in the same clade, while the *A. baumannii* strains obtained from the Med-2 ward were in different clades. The genome of ATCC17978 showed different clades from all eight *A. baumannii* strains. The SNP count matrix of all selected genomes confirmed that the high number of SNPs of AC59 and AE17 derived from the Med-2 ward were comparable with other *A. baumannii* strains (Figure 2C).

Discussion

A. baumannii is an opportunistic pathogen that causes hospital-acquired infections in patients who have high risk factors, such as patients in intensive care units (ICUs). This bacterium is extremely capable of surviving, spreading, and developing antibiotic resistance in hospital wards (*Vázquez-López et al., 2020*). In this study, we investigated *A. baumannii* from three ICUs and two medicine wards from a university hospital to identify nosocomial infection-associated bacteria. A total of 106 isolates of *A. baumannii* were isolated from 312 environmental samples, which were frequently in contact with staff and patients. The highest numbers of staff and patient contact samples with *A. baumannii* colonization were from AMBU bags (77.9%) and keyboards (53.3%). Shamsizadeh *et al.* (2017) reported that *A. baumannii* was detected in environmental samples with the highest recovery in intensive care units (ICUs). This is in agreement with our study in which we isolated the highest number of *A. baumannii* from two ICUs. A previous study demonstrated that *A. baumannii* was isolated from hospital sinks, bed rails, water systems, and medical equipment, particularly in ICUs and surgical units (*Ibrahim et al., 2021*). We detected a high number of *A. baumannii* from AMBU bags (77.9%), followed by bedrails (66.7%) and suction tubes (66.7%). However, we did not obtain *A. baumannii* from hospital sinks or water from sinks. In addition, a previous study reported that the airborne route also plays an important role

in the transmission of *A. baumannii* infections in hospitals (*Ayoub Moubareck et al., 2020*). Our study confirmed that a high number of *A. baumannii* was isolated from air (60.0%). *A. baumannii* was associated with hospital-acquired outbreaks due to its ability to spread in the air environment and colonize hospital utensils.

MDRAB and CRAB were described as major resistant strains that caused hospital outbreaks in Thailand (*Leungtongkam et al., 2018*; *Chukamnerd et al., 2022*). High prevalence rates of both MDRAB and CRAB were found in this study. We found that the resistance rate of *A. baumannii* isolated from hospital environments was higher than that isolated from clinical samples. In addition, all *A. baumannii* isolates isolated from hospital environments were resistant to meropenem (100%), cefotaxime (100%), ceftazidime (100%), and ceftriaxone (100%), and all isolates were CRAB. The results were in contrast with a Chinese study showing that *A. baumannii* isolated from the hospital environment was more susceptible to most antimicrobial agents (*Ying et al., 2015*).

Our data showed that *A. baumannii* isolated from hospital environments and clinically isolated from the same ward possessed similar antibiotic susceptibility profiles, and ARG patterns represented the outbreak clone in each ward (Figure S1–S5). Among all isolates, the results showed that $bla_{\text{OXA-23}}$ was the most frequent carbapenemase gene detected. This result suggests that $bla_{\text{OXA-23}}$ was the major cause of carbapenem resistance in *A. baumannii* isolates from hospital environments and clinical samples in our hospitals. This result was supported by Leungtongkam *et al.* (2018), who detected $bla_{\text{OXA-23}}$ in all *A. baumannii* isolates from four tertiary hospitals in Thailand. Jain et al. (2019) reported that $bla_{\text{NDM-1}}$ was the most frequent gene detected in *A. baumannii* isolated in both clinical and environmental samples from India (*Jain et al., 2019*). Interestingly, we found a high prevalence of bla_{NDM} among both the hospital environment and clinical sample isolates. Compared to a previous report from Thailand, a low rate of bla_{NDM} was detected in *A. baumannii* isolates from hospitals in northern and southern Thailand (*Leungtongkam et al., 2018*; *Chukamnerd et al., 2022*).

Genomic analysis of eight representative MDRAB strains found that the major ST type (AC02, AE30, AC09, AE106, AC23, and AE73) was ST2. It has been reported that MDRAB sequence type ST2 was the most prevalent in Thailand. The AC59 and AE17

strains were designated ST164, which was also reported in Thailand (*Khuntayaporn et al., 2021*). NDM-producing organisms have become endemic in the Indian subcontinent, and numerous epidemics have been recorded worldwide. Genomic analysis found that the AC02, AE30, AC09, AE106, AC23, and AE73 strains possess an NDM-5 metallo-β-lactamase gene. This is the first report regarding the detection of an NDM-5-producing *A. baumannii* from hospital environments and clinical samples in Thailand. The emergence of the bla_{NDM-5} gene was mostly identified in *Escherichia coli*. To date, only one report by Khalid *et al.* (2020) identified *A. baumannii* harboring bla_{NDM-5} from the neonatal intensive care unit (NICU) of an Indian Hospital, but it was not present in environmental isolates (*Hamidian et al., 2019*). Our PCR study identified the bla_{NDM} gene but could not specifically identify the NDM variant. The outbreak clone harboring bla_{NDM-5} was revealed using WGS. Mobile genetic elements such as insertion sequences, transposons, and integrons can mobilize $bla N_{DM-5}$ (*Wu et al., 2019*). Our WGS analysis revealed *intI1* located upstream of bla_{NDM-5} (Figure 1C). A previous report on *E. coli* detected bla_{NDM-5} to be located in a complex of class 1 integrons together with *aadA2, aac(3)-IIa, mph(A), sul1, tet(A), and dfrA12* (*Alba et al., 2021*). In this study, we found *ant(3")-Ia*, *qacEΔ1,* and *sul1.*

WGS of eight strains revealed a high number of ARGs in accordance with previous reports in Thailand (*Kongthai et al., 2021; Wareth et al, 2021*; *Chukamnerd et al, 2022*). Among the eight strains, the antibiotic resistance gene patterns of *A. baumannii* differed among wards but were similar in the same ward. A high number of acquired ARGs was detected. Horizontal gene transfer among *A. baumannii* and other bacterial species colonizing the hospital environment may play an important role in the movement of these acquired ARGs. Interestingly, we found that the virulence gene patterns of *A. baumannii* strains from four wards were quite similar (Figure 1B). These findings indicated that all *A. baumannii* strains from the four wards were derived from the same ancestor and employed the same pathogenic mechanisms to cause disease. The phylogenomic relationship of the core and pan genomes as well as the SNP count matrix revealed the genetic similarity of *A. baumannii* strains obtained from the same ward. This is in agreement with a previous study by Yasir et al. (2022), in which genome

sequencing revealed that *A. baumannii* isolated from hospital environments was linked with those of clinical origin (*Yasir et al., 2022*).

Conclusions

In conclusion, in this study, we presented a whole-genome analysis of eight *A. baumannii* strains from hospital environments and clinical samples. Our data revealed the epidemiological characteristics of similar antibiotic susceptibility profiles, antibiotic resistance genes, virulence genes, clonal complexes, core genomes, pan genomes, and single nucleotide polymorphisms among clinical and environmental *A. baumannii* isolates from the same ward.

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Abbreviations

ARG: antibiotic resistance gene

ABHE: *A. baumannii* isolated from hospital environment

CARD: comprehensive antibiotic resistance database

CRAB: carbapenem-resistant *A. baumannii*

MDRAB: multidrug-resistant *A. baumannii*

MLST: multilocus sequence typing

NDM: New Delhi metallo-beta-lactamase

NRAB: non drug- resistant *A. baumannii*

SNP: single nucleotide polymorphism

VFDB: virulence factor database

XDRAB: extremely drug-resistant *A. baumannii*

WGS: whole-genome sequencing

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Figure 1

Detections of antibiotic resistance, virulence genes, and genetic contexts of *A. baumannii* harboring bla_{NDM-5} among 8 representative *A. baumannii* strains and ATCC 17978.

(A) The pattern of acquired resistance genes, (B) virulence factor-associated genes in the *A. baumannii* genomes, and (C) genetic contexts and comparison of the gene arrangement of six *A. baumannii* isolates harboring $bla_{NDM-5.}$ The arrows indicate genes located upstream and downstream of bla_{NDM-5}, including Integron1 (*intI*1), BsuBI-PstI family restriction endonuclease (Bsu-PstI), aminoglycoside 3''-nucleotidyltransferase (*ant(3")-Ia*), quaternary ammonium compound efflux (*qacEΔ1*), sulfonamide resistance (*sul1*), IS91 family transposase, cytochrome c-type biogenesis protein (*DsbD*), N-(5'-phosphoribosyl) anthranilate isomerase (*trpF*), bleomycin resistance protein (ble_{MBL}), New Delhi metallo-beta-lactamase 5 (bla_{NDM-5}), and transposase (IS*Aba125*).

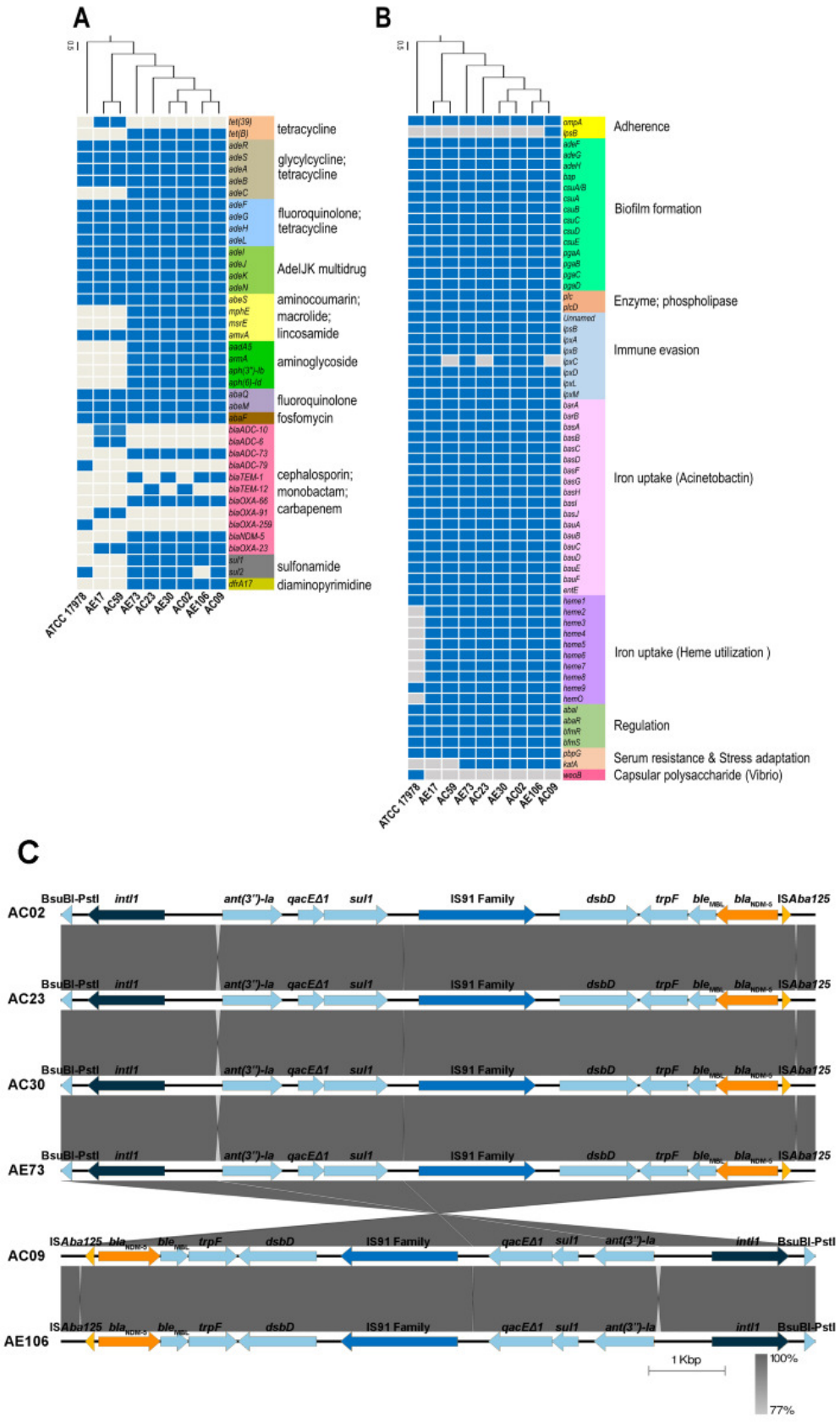
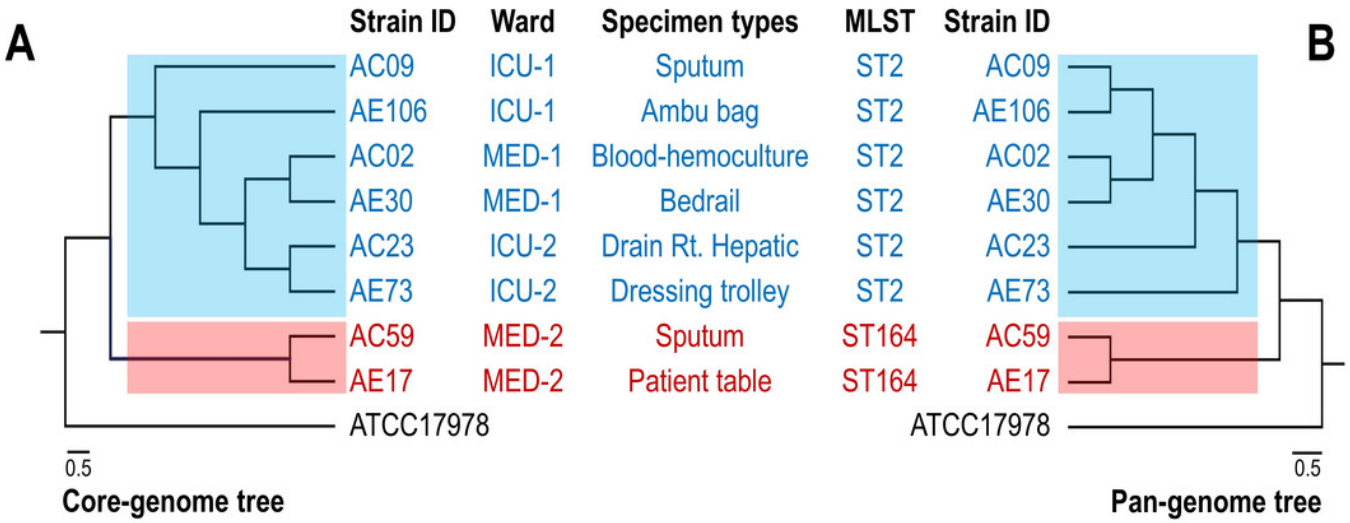

A
B
C
tetracycline
glycylcycline; tetracycline
fluoroquinolone; tetracycline
AdeIJK multidrug
aminocoumarin; macrolide; lincosamide
aminoglycoside
fluoroquinolone
fosfomycin
cephalosporin; monobactam; carbapenem
sulfonamide
diaminopyrimidine
Adherence
Biofilm formation
Enzyme; phospholipase
Immune evasion
Iron uptake (Acinetobactin)
Iron uptake (Heme utilization)
Regulation
Serum resistance & Stress adaptation
Capsular polysaccharide (Vibrio)
ATCC 17978
AE17
AC59
AE73
AC23
AE30
AC02
AE106
AC09
BsuBI-PstI
intI1
ant(3'')-Ia
qacEΔ1
sul1
IS91 Family
dsbD
trpF
ISAba125
1 Kbp
100%
77%

Figure 2

Phylogenomic relationship among selected representative isolates of *Acinetobacter baumannii* obtained from different wards.

(A) A phylogeny reconstructed from 2,928 concatenated core genes of all analyzed genomes presented with metadata. (B) Hierarchical tree based on the presence/absence of patterns of 4,778 pangenome genes of 8 representative isolates and ATCC 17978. (C) SNP matrix-based heatmap illustrating the number of single nucleotide polymorphisms in the whole genome between the eight strains studied.



C

	AC02	AE30	AC23	AE73	AE106	AC09	AC59	AE17
AC02	0							
AE30	283	0						
AC23	402	317	0					
AE73	446	247	155	0				
AE106	651	604	393	450	0			
AC09	1220	1061	1088	1004	1043	0		
AC59	43731	43843	43739	43826	43789	43858	0	
AE17	43564	43664	43598	43669	43694	43787	547	0

Table 1(on next page)

A. baumannii isolated from hospital environments and clinical samples from various hospital wards.

Table 1: *A. baumannii* isolated from hospital environments and clinical samples from various hospital wards.

Ward		Positive environment		Positive Clinical	
		n	%	n	%
MED-1	Medicine-man ward	15	14.2%	15	24.6%
MED-2	Medicine-woman ward	22	20.8%	10	16.4%
ICU-MED	ICU Medicine	26	24.5%	15	24.6%
ICU-1	ICU Cardio-Vascular-Thoracic Surgery	7	6.6%	9	14.8%
ICU-2	ICU Surgery	36	33.9%	12	19.6%
Total		106	100.00%	61	100.00%

Table 2(on next page)

Frequency of resistance to antimicrobial agents among *A. baumannii* isolates from hospital environments and clinical samples.

Table 2: Frequency of resistance to antimicrobial agents among *A. baumannii* isolates from hospital environments and clinical samples.

Antimicrobial Group	Antibiotics	Resistance	
		hospital Environment	Clinical
β-Lactam combinations	Piperacillin/Tazobactam	80.2%	81.9%
Cephems	Ceftazidime	100.0%	90.2%
	Cefepime	99.1%	85.3%
	Cefotaxime	100.0%	88.3%
	Ceftriaxone	100.0%	90.2%
Carbapenems	Imipenem	77.4%	55.7%
	Meropenem	100.0%	83.6%
Aminoglycosides	Gentamicin	77.4%	70.5%
	Amikacin	62.3%	67.2%
Tetracyclines	Tetracycline	74.5%	73.8%
Fluoroquinolones	Ciprofloxacin	79.2%	83.6%
Folate pathway inhibitors	Trimethoprim/Sulfamethoxazole	88.7%	81.9%

Table 3(on next page)

The statistical analysis for comparing the proportions of antibiotic resistance between *A. baumannii* obtained from two different origins

* Comparison of percentages between two groups by Z-test ** ND; Not determined statistical analysis Note: A p value < 0.05 reflected statistically significant findings . CRAB: carbapenem-resistant *A. baumannii*; MDRAB: multidrug-resistant *A. baumannii*; XDRAB: extremely drug-resistant *A. baumannii*

Table 3. The statistical analysis for comparing the proportions of antibiotic resistance between *A. baumannii* obtained from two different origins

Characteristics	Clinical origin (n=61 isolates)	Environmental origin (n=106 isolates)	*p value (95% CI)
Prevalence of MDRAB	50/61 (82.0%)	99/106 (93.4%)	0.021 (22.2% to 0.7%)
Prevalence of CRAB	50/61 (92.0%)	106/106 (100%)	<0.001 (83.8% to 27.7%)
Prevalence of XDRAB	34/61 (55.7%)	47/106 (44.7%)	0.116 (27.0% to 4.2%)
Prevalence of bla_{OXA-23} positive isolates	49/61 (80.3%)	85/106 (80.2%)	0.983 (12.4% to -12.7%)
Prevalence of bla_{OXA-58} positive isolates	1/61 (1.6%)	1/106 (0.9%)	** ND
Prevalence of bla_{NDM} positive isolates	34/61 (55.7%)	83/106 (78.3%)	0.002 (37.3% to 7.8%)

* Comparison of percentages between two groups by Z-test

** ND; Not determined statistical analysis

Note: A p value < 0.05 reflected statistically significant findings.

CRAB: carbapenem-resistant *A. baumannii*; MDRAB: multidrug-resistant *A. baumannii*; XDRAB: extremely drug-resistant *A. baumannii*

Table 4(on next page)

Proportion comparisons of antibiotic resistance among *A. baumannii* collected from five hospital wards

* overall p value calculated to compare percentages among multiple groups by Chi-square test ** ND; Not determined statistical analysis Note: Bold values denote the highest proportions with statistical significance at the p value < 0.05 level. MED-1 (Medicine-man ward), MED-2 (Medicine-woman ward), ICU-MED (ICU Medicine), ICU-1 (ICU Cardio-Vascular-Thoracic Surgery), ICU-2 (ICU Surgery) CRAB: carbapenem-resistant *A. baumannii*; MDRAB: multidrug-resistant *A. baumannii*; XDRAB: extremely drug-resistant *A. baumannii*

Table 4. Proportion comparisons of antibiotic resistance among *A. baumannii* collected from five hospital wards

Hospital wards/Characteristics	MED-1	MED-2	ICU-MED	ICU-1	ICU-2	*p value
Percentage of MDRAB	29/30 (96.7%)	26/32 (81.3%)	37/41 (90.2%)	15/16 (93.3%)	42/48 (87.5%)	0.386
Percentage of CRAB	29/30 (96.7%)	31/32 (96.9%)	39/41 (95.1%)	15/16 (93.8%)	42/48 (87.5%)	0.490
Percentage of XDRAB	13/30 (43.3%)	11/32 (34.4%)	13/41 (31.7%)	8/16 (50%)	**36/48 (75%)**	<0.001
Percentage of bla_{OXA-23} positive isolates	27/30 (90%)	14/32 (43.8%)	36/41 (87.8%)	**15/16 (93.8%)**	42/48 (87.5%)	<0.001
Percentage of bla_{OXA-58} positive isolates	1/30 (3.3%)	0/32 (0%)	0/41 (0%)	0/16 (0%)	1/48 (2.1%)	** ND
Percentage of bla_{NDM} positive isolates	14/30 (46.7%)	29/32 (90.6%)	22/41 (53.7%)	8/16 (50%)	**44/48 (91.7%)**	<0.001

* overall p value calculated to compare percentages among multiple groups by Chi-square test

** ND; Not determined statistical analysis

Note: Bold values denote the highest proportions with statistical significance at the p value < 0.05 level.

MED-1 (Medicine-man ward), MED-2 (Medicine-woman ward), ICU-MED (ICU Medicine), ICU-1 (ICU Cardio-Vascular-Thoracic Surgery), ICU-2 (ICU Surgery)

CRAB: carbapenem-resistant *A. baumannii*; MDRAB: multidrug-resistant *A. baumannii*; XDRAB: extremely drug-resistant *A. baumannii*

Table 5(on next page)

Medical and general genome features of 8 representatives *A. baumannii* isolated from various hospital wards.

MED-1 (Medicine-man ward), MED-2 (Medicine-woman ward), ICU-1 (ICU Cardio-Vascular-Thoracic Surgery), ICU-2 (ICU Surgery), MDRAB: multidrug-resistant *A. baumannii*, XDRAB: extremely drug-resistant *A. baumannii*

Table 5: Medical and general genome features of 8 representatives *A. baumannii* isolated from various hospital wards.

Strain ID/ Characteristics	AC02	AE30	AC59	AE17	AC09	AE106	AC23	AE73
Ward	MED-1	MED-1	MED-2	MED-2	ICU-1	ICU-1	ICU-2	ICU-2
Specimen types	Blood-hemoculture	Bedrail	Sputum	Patient table	Sputum	AMBU bag	Right Hepatic Drain	Dressing trolley
Antibiotic Resistance	XDRAB	XDRAB	MDRAB	MDRAB	XDRAB	XDRAB	MDRAB	MDRAB
MLST	ST2	ST2	ST164	ST164	ST2	ST2	ST2	ST2
Genome size (bp)	4,016,797	3,966,329	3,958,580	3,786,785	3,934,990	3,949,273	3,925,340	3,955,274
% GC	38.90	38.99	38.87	38.88	38.98	39.00	38.98	38.99
No. of contigs	86	71	96	63	68	76	72	81
Largest contig	340426	292477	481102	306399	303352	292477	360663	292477

MED-1 (Medicine-man ward), MED-2 (Medicine-woman ward), ICU-1 (ICU Cardio-Vascular-Thoracic Surgery), ICU-2 (ICU Surgery), MDRAB: multidrug-resistant *A. baumannii*, XDRAB: extremely drug-resistant *A. baumannii*