Genomic relatedness and dissemination of *bla*$_{NDM-5}$ amongst *Acinetobacter baumannii* isolated from hospital environments and clinical specimens (#78051)

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Genomic relatedness and dissemination of *bla*$_{NDM-5}$ amongst *Acinetobacter baumannii* isolated from hospital environments and clinical specimens

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

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

Results: A total of 106 *A. baumannii* isolates were obtained from 312 hospital environmental samples. A high prevalence samples with *A. baumannii* colonization was detected from Ambu bag (77.9%) follow by air samples (60.0%). We found 93.4% environmental isolates were multidrug-resistant *A. baumannii* (MDRAB). The most acquired antibiotic resistance genes (ARGs) identified included *bla*$_{OXA-23}$ (80.2%), *bla*$_{NDM}$ (78.30%), and *bla*$_{OXA-58}$ (0.94%). Sixty-one *A. baumannii* isolates were collected from patient specimens in the same ward. Among all *A. baumannii* clinical isolates, MDRAB was found in 81.97% and extremely drug-resistant *A.baumannii* (XDRAB) in 55.74%. The most ARGs identified was *bla*$_{OXA-23}$ (80.33%) followed by *bla*$_{NDM}$(55.74%). Genetic diversity of all isolates using rep-PCR could be divided into 33 genotypes. Genome size of eight *A. baumannii* ranged from 3.78 - 4.01 Mb. We found six of eight isolates to be *bla*$_{NDM-5}$ -harboring *A. baumannii*. Mobile genetic elements (MGEs) such as integron1 (*intI*1) located upstream of *bla*$_{NDM-5}$ was observed. Phylogenomic relationship of core and pan genome as well as the SNP count matrix revealed genetic similarity of *A. baumannii* environmental and clinical isolates 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 guiding infection control of *A. baumannii* infection.

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Abstract

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Introduction

Acinetobacter baumannii has emerged as an important pathogen related to hospital-acquired infections worldwide. This pathogen is the major cause behind ventilator-associated pneumonia (VAP), bacteremia, urinary tract infections, wound infections, and meningitis (*Nutman et al., 2016*). The emergence of antibiotics-resistant *A. baumannii,* especially multidrug resistant *A. baumannii* (MDRAB) and extensively drug-resistant *A. baumannii* (XDRAB), has increased and seriously challenges the treatment of these bacterial infections (*Kyriakidis et al., 2021*). National Antimicrobial Resistance Surveillance Thailand (NARST) reports that the prevalence of carbapenem resistant *Acinetobacter baumannii-~~calcoaceticus~~* complex infection in ICU of 51 hospitals in Thailand was higher than 80% (*NARST, 2021*). The major mechanisms of carbapenem resistance ~~mechanisms~~ among *A. baumannii* ~~was~~ the production of antibiotic-hydrolysing enzymes that belong to Ambler class D, ~~carbapenem-hydrolyzing class D~~ lactamases (CHDLs), and class B metallo-lactamases (MBLs) (*Ibrahim et al., 2021*). Class D carbapenemases are encoded 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 bla_{NDM} gene. This gene was reported in Thailand since 2017 (*Leungtongkam et al., 2018*).Till date, twenty-four 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*). Carbapenem-resistant *A. baumannii* (CRAB) found on the ICU surfaces and genome sequencing 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, which exhibits multidrug resistance phenotype and that it belongs to some widely spread clonal complexes 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 with pathogenicity (*Wright et al., 2016*). Whole-genome sequencing studies comparing distinct clinical isolates and environments 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 environment *A. baumannii* isolates in 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 - April 2021. Naresuan University is 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 Ward (MED-1), Medicine-woman Ward (MED-2), and three intensive care units: ICU Cardio-Vascular-Thoracic surgery (ICU-1), ICU Surgery (ICU-2), and ICU Medicine (ICU-MED). The source of the samples included the staff contact samples, which included stethoscope (n=15), chart (n=15), computer/keyboard (n=15), counter (n=15), medical lab coats (n=15), restroom door handles (n=15), telephone (n= 15), and dressing trolley (n= 15). Patient contact samples were bedrails (n=15), bedsheet (n=15), suction tube (n=15), patient table (n=15), curtain (n=15), humidifiers (n=15), intravenous bottle (IV) stand (n=15), ventilator (n=15), ventilator monitor (n=9), water from ventilator (n=9), suction tube (n=9), and Ambu bag (n=9). Other environmental samples were collected from air (n=15), sink (n=15), and water from sink (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) in 9 cm diameter Petri dishes. Petri dishes were exposed for 24 hrs. The other environmental surfaces were collected using cotton swab soaked in 0.85% normal saline, then placed in the transfer media. The swab samples were enriched in Luria-Bertani Broth (LB) by shaking at 160 rpm and 37°C for 18–24 hours and then cultured in Leed Acinetobacter Media (LAM) ~~Himedia, India~~ at 37°C for 24–48 hours. Cultures with pink colonies were selected for further evaluation using Gram's stain and ~~biochemical testing~~. 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 disk diffusion method using fifteen antibiotics: amikacin (30 µg), cefepime (30 µg), cefotaxime (30 µg), cefoperazone/sulbactam (75 and 30 µg), ceftazidime (30 µg), ceftriaxone (30 µg), ciprofloxacin (5 µg), gentamicin (10 µg), imipenem (10 µg), meropenem (10 µg), piperacillin/tazobactam (100 and 10 µg), tetracycline (30 µg), tigecycline (15 µg), ~~colistin~~ (10µg), 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 (2017)~~ and Piewngam *et al* (2014) (*Piewngam et al., 2014*). All isolates were defined to be NRAB, MDRAB, CRAB, and XDRAB as previously described by Magiorakos et al ~~(2012)~~ (*Magiorakos et al., 2012*).

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*$_{\text{NDM}}$ were performed using primers (Table S1). Genomic DNA of each isolate was extracted from the overnight cultures using 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 condition described by Leungtongkam ~~(2018),~~ PCR-banding patterns and rep-PCR types were analyzed and interpreted as previously described (*Leungtongkam et al., 2018*).

Whole Genome Sequencing and bioinformatics analysis

The whole ~~genome of eight representative *A.*~~ *baumannii* isolates from 4 wards; four from hospital environments and four from clinical isolates (AE17, AC59, AC30, AC02, AE63, AE28, AE73, AE23, AE106, and AC09). Phylogenetic tree based antibiotic resistance pattern and antibiotic resistance genes of *A. baumannii* isolated from the same ward were performed (Figure S1-S5) and the bacteria presented similar ARG pattern were selected for whole genome sequencing. All strains were cultured onto an LB agar plate and incubated overnight at 37°C. Genomic DNA was extracted using PureDire Genomics DNA Isolation Kit (BIO-HELIX, New Taipei City, Taiwan). The extracted DNA was quantified by nanodrop (company, city, country). 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* isolates have been deposited in NCBI's database under Sequence Read Archive (SRA) with Bioproject PRJNA862456 (https://www.ncbi.nlm.nih.gov/sra/PRJNA862456).

Genome assembly and annotation

Raw sequencing reads were trimmed by using the Trim Galore v0.6.7 with default settings and by using the 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 the Prokka v1.14.6 with default options (*Seemann, 2014*).

Identification of MLST, antimicrobial resistance, and virulence genes

The remaining contigs were subjected to detect drug-resistance and virulence genes by using the Abricate v1.0.1 with default settings (*Seemann, 2016*) against the CARD and VFDB databases (*Alcock et al., 2020; Liu et al., 2022*). MLST types were determined by using the MLST v2.0 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 default parameters to identify pan- and core-genes (*Page et al., 2015*). The resultant

core-genes among eight genomes were concatenated and prior to construction of a pan-genome tree in the CSI phylogeny 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 the FigTree v1.4.4 (https://tree.bio.ed.ac.uk/software/figtree/). The SNP count matrix of all selected genomes was calculated in the snp-dists v0.6.3 with default settings (*Seemann, 2019*).

Results

A. baumannii strains isolated from hospital environment and clinical isolates

A total of 106 *A. baumannii* isolates were obtained from 312 hospital environmental (HE) samples (33.97%). The isolates associated with patient contact were Ambu bag (77.9%), bedrails (66.7%), suction tube (66.7%), water from ventilator (55.6%), bedsheet (53.3%), patient table (33.3%), humidifiers (33.3%), ventilation (33.3%), curtain (33.3%), and IV stand at 13.3%. The isolates involved staff contact and other environments belonging to air (60.0%), keyboard (53.3%), counter (46.7%), medical lab coats (42.9%), dressing trolley (33.3%), stethoscope (26.7%), chart (26.7%), door handles (6.7%), and telephone (6.7%). However, we did not find *A. baumannii* isolates on sink, water from sink, and ventilator monitor (Table S2). Of the 312 environmental samples collected from each ward, we found high *A. baumannii* contamination from ICU Surgery to be 52.9% (36/~~38~~) followed by medicine-woman ward to be 40.74% (22/54), ICU medicine 38.2% (26/68), medicine-man ward 27.8% (5/54), and ICU cardio-vascular-thoracic surgery 10.3% (7/68) (Table S2). Among 106 *A. baumannii* isolates from hospital environment, a high rate of *A. baumannii* ~~was~~ obtained from ICU Surgery (33.96%) followed by ICU medicine (24.5%) (Table 1). Sixty-one *A. baumannii* isolates were collected from patient specimens in the same ward, which were from ICU medicine (24.59%), medicine-man ward (24.59%), ICU surgery (19.67%), medicine-woman ward (16.40%), and ICU cardio-vascular-thoracic surgery ward (14.75%) (Table 1).

Antibiotic susceptibility patterns of *A. ~~B~~aumannii* 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 *A. Baumannii* clinical isolates were highly resistant to ceftazidime (90.16%) and ceftriaxone (90.16%). All isolates were sensitive to colistin and tigecycline. The prevalence of CRAB from the hospital environments was 100%. MDRAB was found in 93.40%, and XDRAB was found in 44.34%. For *A. baumanniifrom* clinical samples, CRAB was found in 81.97%, MDRAB in 81.97%, XDRAB in 55.74%, and NRAB in 16.39% (Table S3).

Antibiotic resistance genes and rep-PCR typing

The intrinsic bla_{OXA-51} was detected in all *A. baumannii* environmental isolates (ABHE) but 96.7% (59/61) of clinical isolates. Oxacillinase gene, bla_{OXA-23}, was the most detected at 80.20% (85/106) in ABHE and 80.33% (49/61) in clinical isolates. The bla_{OXA-58} gene was detected in one of ABHE (0.94%) and one of clinical isolates (1.64%). The bla_{NDM} gene was detected in 78.3% (83/106) of ABHE and in 55.74% (34/61) of clinical isolates. The bla_{OXA-24} gene was not detected in any of the isolates (Table S3). Rep-PCR typing was performed and fingerprinting represented 33 different DNA patterns consisting of amplicon sizes ranging from 500 to 4,000 bp. The genotype was named T1 to T33. The high prevalence genotype of ABHE was T30 at 21.7% (23/106) followed by T23 at 17% (18/106), and T2 at 15 % (15/106). The high prevalence genotype of AC was T4 at 34.4% (21/61) followed by T23 at 29.5 % (18/61). Heat map representing antibiotic susceptibility patterns, antimicrobial resistance genes, and rep-PCR typing from 5 wards is showed in Figure S1-S5. We found a 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 profile. No association was found between rep-PCR typing of ABHE and *A. baumannii* clinical isolates. Eight isolates 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 isolates and clinical isolates

Eight isolates of *A. baumannii* from clinical and ABHE isolates were analyzed. The four ABHE isolates were AE03 (bedrail), AE17 (patient table), AE106 (Ambu bag), and AE73 (dressing trolley). The four clinical isolates were AC02 (Blood-Hemoculture), AC59 (sputum), AC09 (sputum), and AC23 (right hepatic drain). AC02 and AE03 were obtained from MED-1 ward. AC59 and AE17 were obtained from MED-2 ward. AC09 and AE106 were derived from ICU-1 ward. AC23 and AE73 were derived from ICU-2 ward. The genome characterization of the isolates is summarized in Table 3. 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 size of eight *A. baumannii* strains were ranging from 37.8 to 40.2 Mb and GC contents from 38.8 to 39%.

ARGs and virulence genes of eight *A. baumannii* isolates showed genetic similarity of *A. baumannii* among hospital environments and clinical isolates (Figure 1AB). We found that thirty-eight antibiotic resistance genes belong to eleven antibiotic classes in all *A. baumannii* strains. Glycylcycline/tetracycline (*ade*R, *ade*S, *ade*A, *ade*B), fluoroquinolone/tetracycline (*ade*F, *ade*G, *ade*H, *ade*L), AdeIJKmultidrug (*ade*I, *ade*J, *ade*K, *ade*N), macrolide (*abe*S, *amv*A), fluoroquinolone (*aba*Q, *abe*M), fosfomycin (*aba*F), and carbapenems (bla_{OXA-23}) were detected. Additionally, tetracycline (*tet*B), glycylcycline/tetracycline (*ade*C), macrolide (*mph*E, *msr*E, aadA5, armA, *aph*(3")-Ib, *aph*(6)-Id), carbapenems (bla_{ADC-73}, bla_{OXA-66},bla_{NDM-5}), sulfonamide (*sul*1), and diaminopyrimidine (*dfr*A17) were detected in AE30, AE106, AE73, AC02, AC09, and AC23 isolates. However, *tet*(39), bla_{ADC-10}, bla_{ADC-6}, and bla_{OXA-91} were detected only in AE17 and AC59. Genetic contexts of bla_{NDM-5} releveled 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* isolates revealed that genes were involved in biofilm formation (*ade*F, *ade*G, *ade*H, *bap*, *csu*A/B, *csu*A, *csu*B, *csu*C, *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,

277 *ent*E), gene regulation (*aba*I, *aba*R, *bfm*R, *bfm*S), serum resistance (*pbp*G), and host
278 cell adherence (*omp*A) (Figure 1B).
279 Phylogenomic relationship of core and pan genome showed in Figure 2AB
280 revealed two major clades. *A. baumannii* strains obtained from ICU-1, ICU-2, and Med-
281 1 wards were in the same clade, while *A. baumannii* strains obtained from Med-2 ward
282 were in different clades. The SNP count matrix of all selected genomes confirmed that
283 the high number of SNPs of AC59 and AE17 derived from Med-2 ward were
284 comparable with other *A. baumannii* strains (Figure 2C).
285

286 ## Discussion

287 *A. baumannii* is an opportunistic pathogen that causes hospital-acquired
288 infections in patients who have high risk factors such as patients in intensive care units
289 (ICUs). This bacterium is extremely capable of surviving, spreading, and developing
290 antibiotic resistance in the hospital ward (*Vázquez-López et al., 2020*). In this study, we
291 investigated three ICUs and two Medicine wards from University hospital using a
292 culture-based technique to identify nosocomial infection-associated bacteria. A total of
293 106 isolates of *A. baumannii* were isolated from 312 samples, which were frequently
294 staff and patient contact. The highest number of staff and patient contact samples that
295 found *A. baumannii* was keyboard (53.3%) and Ambu bag (77.9%). The prevalence of
296 *A. baumannii* in the hospital environment was supported by previous studies
297 Shamsizadeh *et al* (2017) reported that *A. baumannii* was detected in environmental
298 samples with the highest recovery in intensive care units (ICUs) (*Shamsizadeh et al.,*
299 *2017*). This is in agreement with our study that we isolated the highest number of *A.*
300 *baumannii* from two ICUs. Previous study demonstrated that *A. baumannii* was isolated
301 from hospital sinks, bed rails, water systems, and medical equipment, particularly in
302 ICUs and surgical units (*Ibrahim et al., 2021*). We detected a high number of *A.*
303 *baumannii* from bedrails (66.7%). However, we did not obtain *A. baumannii* from
304 hospital sinks and water from sink. Airborne route also plays an important role in
305 transmission of *A. baumannii* infections in hospitals (*Ayoub Moubareck et al., 2020*).
306 Our study confirmed this since a high number of *A. baumannii* was isolated from air

(60.0%). Our study demonstrated that *A. baumannii* may be associated with hospital-acquired outbreaks due to its ability to spread and colonize in hospital environments.

A high prevalence rate of multidrug resistant *A. baumannii* was found in this study. The resistant rate of *A. baumannii* isolated from hospital environments was higher than that isolated from clinical samples. Our results also showed that a high percentage of *A. baumannii* isolated from hospital environments were resistant to meropenem, cefotaxime, ceftazidime, and ceftriaxone, and all isolates were CRAB. Our results were higher than that reported in another Chinese study where resistance rates approached 10% against many antibiotics among carbapenem resistant ~~Acinetobacter~~ isolates (*Ying et al., 2015*). However, 93.40% of *A. baumannii* isolated hospital environments and 81.97% of *A. baumannii* isolated clinical isolates were multidrug resistant. Multidrug resistant *A. baumannii*, carbapenem resistant in particular, has a propensity to cause infections (*Ibrahim et al., 2021*).

Our data showed that *A. baumannii* isolated from hospital environments and clinically isolated from the same ward possess similar antibiotic resistance patterns and ARGs pattern represent the outbreak clone in each wards (Figure S1–S5). Among all isolates, the results showed that $bla_{\text{OXA-23}}$ was the most frequent gene detected. This result suggests that the $bla_{\text{OXA-23}}$ was the main cause of the resistance of *A. baumannii* isolates from hospital environments and clinical samples in our hospitals. This result was supported by Kongthai et al (2021) who revealed that the $bla_{\text{OXA-23}}$ was detected in all *A. baumannii* isolated from four tertiary hospitals in Thailand (*Kongthai et al., 2021*). Jain et al (2019) reported that NDM-1 was the most frequent gene detected in *A. baumannii* isolated among both the clinical and environments (*Jain et al., 2019*). Interestingly, we found high prevalence of bla_{NDM} among both the hospital environment and clinical sample isolates. As compared to the previous report from Thailand, 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 sequencing type ST2 was determined to be the most prevalent in Thailand. AC59 and AE17 strains were designated to be ST164, which was also reported in

Thailand (*Khuntayaporn et al., 2021*). NDM-producing organisms have become endemic in the Indian subcontinent and numerous transfers have been recorded worldwide. Genomic analysis found that AC02, AE30, AC09, AE106, AC23, and AE73 isolates 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 bla_{NDM-5} gene was mostly identified in ~~Enterobacteriaceae~~, especially in *Escherichai coli*. To date only one report by Khalid *et al.* (2020) identified *A. baumannii* harbored bla_{NDM-5} from neonatal intensive care unit (NICU) of an Indian Hospital, but it is not present in environmental isolates (*Hamidian et al., 2019*). Our PCR study can identify the bla_{NDM} gene but cannot specifically identify the NDM variant. The outbreak clone harbored bla_{NDM-5} was revealed using WGS.

Mobile genetic elements such as insertion sequences, transposons, and integrons can mobilize the *bla*N_{DM-5} (*Wu et al., 2019*). Our WGS analysis observed *intI1* located upstream of bla_{NDM-5} (Figure1C). Previous report in *E.coli* detected bla_{NDM-5} to be located in complex of class 1 integron together with *aadA2, aac(3)-IIa, mph(A), sul1, tet(A), and dfrA12* (*Alba et al., 2021*). Compared to this study, we found *ant(3")-Ia*, *qacEΔ1,* and *sul1.*

WGS of eight isolates revealed a high number of ARGs in accordance with previous reports of *A. baumannii* WGS isolated in Thailand (*Kongthai et al., 2021; Chukamnerd et al, 2022*; *Wareth et al, 2021*). Among eight isolates, antibiotic resistance gene patterns of *A. baumannii* were different; however, gene patterns from the same ward were similar. Compared to the virulence genes, the patterns were not considerably different. These findings indicated that horizontal gene transfer between *A. baumannii* from environment and clinical isolates is important for the movement of ARGs among *A. baumannii* strains. Phylogenomic relationship of core and pan genome as well as the SNP count matrix revealed 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 the *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* isolated from hospital environment and clinical samples. Our data revealed the epidemiological characteristics of similar antibiotic resistance patterns, antibiotic resistance genes, virulence genes, clonal complex, core genes, pan genes, and single nucleotide polymorphism among clinical and environmental *A. baumannii* isolated from the same ward.

Acknowledgements

The authors would like to thank the staffs of Naresuan university hospitals for collecting the bacterial isolates.

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Figure 1

Detections of antibiotic resistance, virulence genes, and genetic contexts *A. baumannii* harbored blaNDM-5 among 8 representative isolates of *A. baumannii*.

(A) The pattern of acquired resistance genes, (B) virulence factors associated genes in the*A. baumannii*genomes, and (C) Genetic contexts and comparison of the gene arrangement of six *A. baumannii* harbored $bla_{\text{NDM-5.}}$ Arrow indicate gene located upstream and downstream of $bla_{\text{NDM-5}}$that were 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 ($bla_{\text{NDM-5}}$), and Transposase(IS*Aba125*).

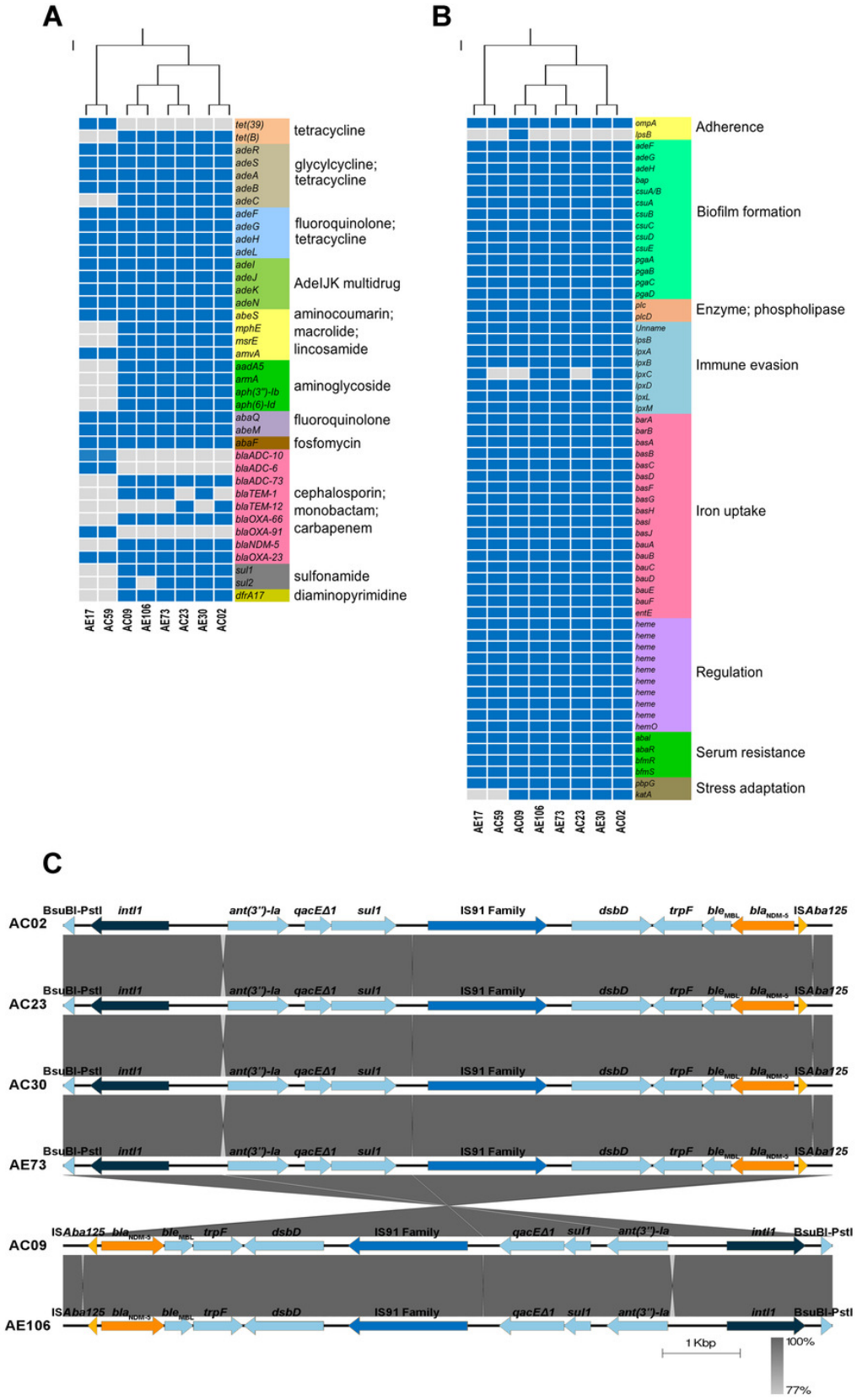
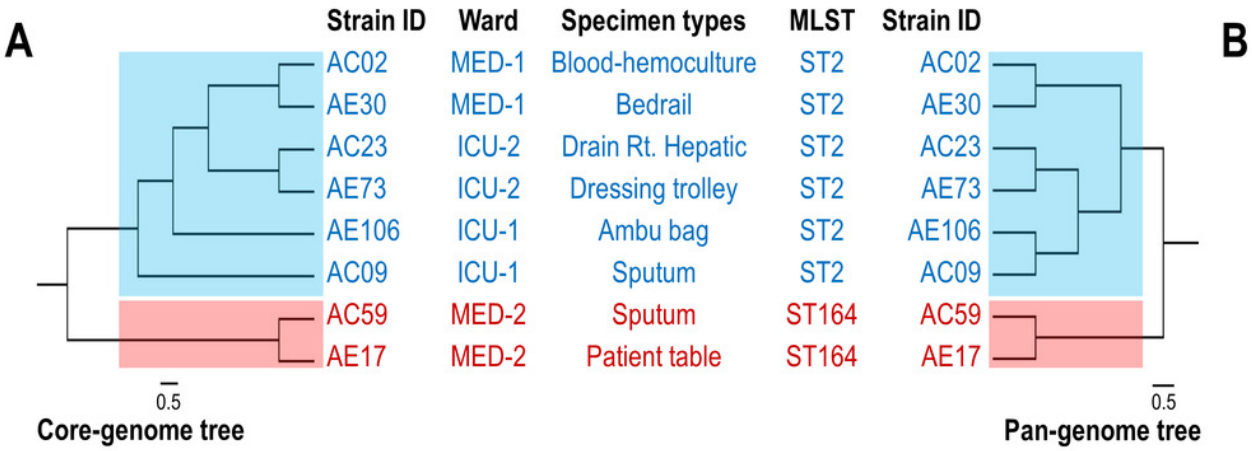

A
tet(39)
tet(B)
tetracycline
adeR
adeS
adeA
adeB
adeC
glycylcycline; tetracycline
adeF
adeG
adeH
adeL
fluoroquinolone; tetracycline
adeI
adeJ
adeK
adeN
AdeIJK multidrug
abeS
mphE
msrE
amvA
aminocoumarin; macrolide; lincosamide
aadA5
armA
aph(3'')-Ib
aph(6)-Id
aminoglycoside
abaQ
abeM
fluoroquinolone
abaF
fosfomycin
blaADC-10
blaADC-6
blaADC-73
blaTEM-1
blaTEM-12
blaOXA-66
blaOXA-91
blaNDM-5
blaOXA-23
cephalosporin; monobactam; carbapenem
sul1
sul2
sulfonamide
dfrA17
diaminopyrimidine
AE17 AC59 AC09 AE106 AE73 AC23 AE30 AC02
B
ompA
Adherence
ipsB
adeF
adeG
adeH
bap
csuA/B
csuA
csuB
csuC
csuD
csuE
pgaA
pgaB
pgaC
pgaD
Biofilm formation
plc
plcD
Enzyme; phospholipase
Unname
ipsB
lpxA
lpxB
lpxC
lpxD
lpxL
lpxM
Immune evasion
barA
barB
basA
basB
basC
basD
basF
basG
basH
basI
basJ
bauA
bauB
bauC
bauD
bauE
bauF
entE
Iron uptake
heme
heme
heme
heme
heme
heme
heme
heme
heme
hemO
Regulation
abaI
abaR
bfmR
bfmS
Serum resistance
pbpG
katA
Stress adaptation
AE17 AC59 AC09 AE106 AE73 AC23 AE30 AC02
C
AC02
AC23
AC30
AE73
AC09
AE106
BsuBI-PstI
intI1
ant(3'')-Ia
qacEΔ1
sul1
IS91 Family
dsbD
trpF
ble_MBL
bla_NDM-5
ISAba125
1 Kbp
100%
77%

Figure 2

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

(A) A phylogeny reconstructed from 2,928 concatenated core-genes of all analyzed genomes presented with metadata. Hierarchical tree based on the presence/absence of patterns of 4,778 pan-genome genes of 8 representative isolates. (C) SNPs matrix-based heatmap illustrating the number of single nucleotide polymorphism in whole-genome between the isolates studied.



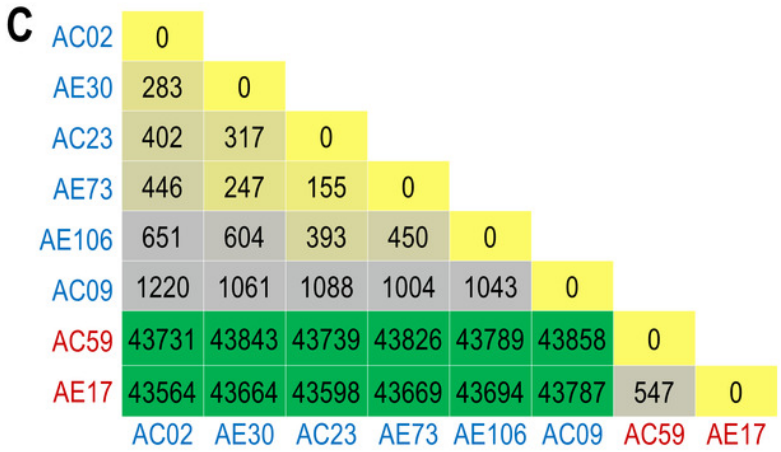


Table 1(on next page)

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	14.15%	15	24.59%
MED-2	Medicine-woman Ward	22	20.76%	10	16.40%
ICU-MED	ICU Medicine	26	24.53%	15	24.59%
ICU-1	ICU Cardio-Vascular-Thoracic surgery	7	6.60%	9	14.75%
ICU-2	ICU Surgery	36	33.96%	12	19.67%
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.

Antibiotics	Resistant	
	Environment	Clinical
Ciprofloxacin	79.24%	83.61%
Gentamicin	77.36%	70.49%
Imipenem	77.36%	55.74%
Meropenem	100.00%	83.61%
Trimethoprim/Sulphamethoxazole	88.68%	81.97%
Amikacin	62.26%	67.21%
Cefotaxime	100.00%	88.52%
Ceftazidime	100.00%	90.16%
Ceftriaxone	100.00%	90.16%
Cefepime	99.06%	85.25%
Tetracycline	74.53%	73.77%
Sulbactam/Cefoperazone	60.38%	54.10%
Piperacillin/Tazobactam	80.19%	81.97%
Colistin	0.00%	0.00%
Tigecycline	0.00%	0.00%

Table 3(on next page)

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

Table 3: Medical and general genome features of 8 representatives 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
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