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ORIGINAL ARTICLE

Early detection of a possible multidrug-resistant *Acinetobacter baumannii* outbreak in the local hospital setting by using random amplified polymorphism DNA-polymerase chain reaction (RAPD-PCR), oxacillinase gene profiles, and antibiograms

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ABSTRACT

Background and Objectives: Detecting the source of a potential outbreak of multidrug resistant (MDR) *Acinetobacter baumannii* is necessary to be investigated. This study aimed to detect the possibility of *A. baumannii* outbreak in a hospital setting using a combination of random amplified polymorphism DNA- polymerase chain reaction (RAPD-PCR), antibiograms, and the presence of oxacillinase genes.

Materials and Methods: The antibiogram of 31 clinical isolates and six environmental isolates of *A. baumannii* were determined by Vitek® 2 Compact. Oxacillinase genes (OXA-23, -24, -51, and -58) were detected by PCR, and RAPD-PCR was conducted using DAF-4 and ERIC-2 primers. The Similarity Index and dendrogram were generated using GelJ v2.3 software.

Results: The antibiograms showed that all MDR *A. baumannii* isolates has very limited susceptibility to cephalosporins, but mostly susceptible to tigecycline. All isolates were positive for $bla_{\text{OXA-51-like}}$ gene, thirty-two of 37 total isolates (86.5%) were positive for $bla_{\text{OXA-23-like}}$ gene, and none were positive for $bla_{\text{OXA-24-like}}$ and $bla_{\text{OXA-58-like}}$ genes. RAPD-PCR showed that the DAF-4 primer on average had more band visualization and lower Similarity Index's variation compared to the ERIC-2. The discriminatory power of DAF-4 was 0.906. There was a significant correlation between the DAF-4 dendrogram pattern with the antibiogram ($r=0.494$, $p<0.001$) and the presence of $bla_{\text{OXA-23-like}}$ gene ($r=0.634$, $p<0.001$) from all ICU A isolates. Six out of fourteen ICU A isolates belonged to the same cluster with >95% Similarity Index, while one clinical isolate having an identical dendrogram and antibiogram pattern with an environmental isolate within this cluster.

Conclusion: There is a high probability of MDR *A. baumannii* outbreak within ICU A detected by multiple analysis of RAPD-PCR, antibiogram and the $bla_{\text{OXA-23-like}}$ gene profiles. This combinatorial approach is conceivable to mitigate possible outbreak situations of *A. baumannii* in the local hospital without sophisticated microbiology laboratory.

Keywords: *Acinetobacter baumannii*; Antibiogram; Hospital; Outbreak; Oxacillinase; Random amplified polymorphic DNA

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INTRODUCTION

Acinetobacter baumannii is a bacterium classified in the ESKAPE group (*Enterococcus faecium, Staphylococcus aureus, Klebsiella pneumoniae, A. baumannii, Pseudomonas aeruginosa*, and *Enterobacter* species) and is a global threat to human health due to the increasing prevalence of multidrug resistant organisms (1). Based on antimicrobial resistance (AMR) data collected through a case-finding surveillance system, the Global Antimicrobial Surveillance System (GLASS) from the World Health Organization (WHO) identified *Acinetobacter* spp. as one of the high-priority pathogens that causes infection in humans (2). There have been some reports of hospital outbreaks from various locations, and *A. baumannii* has become endemic in some locations (3). Hospitalized and vulnerable patients have a higher risk of *A. baumannii* infections due to this bacteria's ability to persist in low-nutrition environments, especially in the hospital environment (4). Patients with *A. baumannii* infections have high mortality and treatment failure rates (5). The mortality rates of patients with *A. baumannii* infections ranged between 13.6% and 57.6% (6-8). Furthermore, *A. baumannii* has emerged as a significant multidrug resistance (MDR) healthcare-associated infections (HAIs) pathogen worldwide, and carbapenem-resistant *A. baumannii* (CRAB) is a major concern (9).

The percentage of CRAB has been reported to be up to 90% in areas surrounding the Mediterranean, including Southern Europe, the Middle East, and North Africa (10). A multi-center study in China reported approximately 39.8% patients in Intensive care units (ICUs) were infected by multidrug-resistance (MDR) *A. baumannii* (11). The prevalence of MDR *A. baumannii* at King Fahad University hospital is 3.37% with the mortality rate is 40.81% (12). Likewise, the mortality rate is higher in critically ill patients (74%), and ventilator-associated pneumonia (VAP) is the most common source of infections (13, 14). On the other hand, carbapenem is considered an antibiotic therapy for MDR *A. baumannii* infections (15). One of the resistance mechanisms to carbapenem is β-lactamase enzyme production by the bacteria. Oxacillinases (OXA) have been reported in clinical isolates of *A. baumannii* associated with hospital outbreaks. OXA belongs to Ambler class D β-lactamases, which are serine hydrolases that have the ability to hydrolyse oxacillin and carbapenem in *A. baumannii* (16). *A. baumannii* intrinsically has a $bla_{\text{OXA-51-like}}$ and additional groups of OXA-acquired carbapenemases, including $bla_{\text{OXA-23-like}}$, $bla_{\text{OXA-24-like}}$, $bla_{\text{OXA-58-like}}$, and $bla_{\text{OXA-123-like}}$. The presence of these varieties of OXA enzymes could aid in the development of resistance to β-lactam ring-containing antibiotics (16).

Preventing the transmission of resistant organisms, including MDR *A. baumannii*, is a major public health priority (17). Hand hygiene has been the most efficient, simple, and low-cost method to limit both the transmission between patients and between patients and the environment. Nonetheless, this method is frequently found to have variable adherence from 4-100% compliance, and nonadherence is found to be higher in ICUs compared to other settings (18). A study in Brazil investigated the hand hygiene compliance of health care workers using an electronic hand hygiene monitoring system and showed that the hand hygiene compliance of health care workers in ICUs remained relatively low through the end of the study period (19). Despite health care workers' hands being the major vector of cross-transmission of pathogens, *A. baumannii* can be transmitted through the vicinity of affected patients or colonizers, such as bed rails, tables, sinks, doors, feeding tubes, linens fomites, curtains and even medical equipment (20).

A current modality for understanding epidemiological outbreaks and identifying cross-transmission of *A. baumannii* infections in the hospital setting is molecular typing. The function of molecular typing is to investigate the spreading and clonality relationship among bacterial strains and their geographical spread. This is also an important method to measure and identify the source of the original infection in the hospital (21). Some examples of these techniques are pulsed-field gel electrophoresis (PFGE), whole genome sequencing (WGS), and random amplified polymorphism DNA-polymerase chain reaction (RAPD-PCR). PGFE is the gold standard for investigating *A. baumannii* outbreaks that occur in well-defined spatial and temporal span intervals (22). The new high-throughput technique recently used is WGS. Both of these techniques are expensive, have limited accessibility, and need reliable laboratory technicians (22). RAPD-PCR is useful as a rapid, easy and simple methodology, although it has several limitations (22). A combination of RAPD-PCR with an antibiogram and the presence of OXA genes could

be promising to support the specific spatiotemporal result. This study was designed to utilize the combination of RAPD-PCR, antibiograms, and resistance genes as a screening tool for detecting *A. baumannii* outbreaks in local hospital settings with limited microbiology laboratories.

MATERIALS AND METHODS

Ethic statement. Approval for the experiment was obtained from the Research Ethics Commission of the Faculty of Medicine, Udayana University, Bali, Indonesia with number 1546/UN14.2.2.VII.14/LT/2021.

Bacterial isolates. Thirty-one *A. baumannii* clinical isolates were used in this study. The isolates were isolated from clinical specimens in the Clinical Microbiology Laboratory, Professor Dr. I.G.N.G. Ngoerah Hospital, Denpasar, Bali, Indonesia, during September-October 2020. There were also six environmental isolates from Intensive Care Unit "A" (ICU A) collected in November 2020 as part of our first-line response to analyse the probability of *A. baumannii* outbreak within ICU A. All clinical and environmental isolates were stored as glycerol stocks in a -80°C freezer that was monitored thoroughly. The isolates were recultured on MacConkey agar. *A. baumannii* ATCC 43498 was used as a control, while *Klebsiella pneumoniae* ATCC 13883, *Streptococcus pyogenes* ATCC 19615, *Pseudomonas aeruginosa* ATCC 9027, *Staphylococcus aureus* ATCC 25293, and *Escherichia coli* ATCC 8739 were used as comparison strains for molecular typing using RAPD-PCR experiments.

Antimicrobial susceptibility test. The antimicrobial susceptibility test was conducted using Vitek® 2 Compact (bioMérieux, Marcy-l'Etoile, France).

Oxacillinase gene detection. Chromosomal DNA from all bacterial isolates was extracted using a commercial Roche MagNA Pure LC™ DNA I kit, according to the manufacturer's instructions (Roche Diagnostics GmbH, Mannheim, Germany). Furthermore, plasmid extraction was performed using QIAprep spin Miniprep (QIAGEN GmbH, Germany) based on the manufacturer's instructions. Polymerase chain reaction (PCR) was conducted to detect $bla_{\text{OXA-23-like}}$, $bla_{\text{OXA-24-like}}$, $bla_{\text{OXA-51-like}}$, and $bla_{\text{OXA-58-like}}$ genes under the following conditions: predenaturation at 94°C for 2 min; 30 cycles of denaturation at 94°C for 25 sec, annealing at 52°C for 40 sec, and extension at 72°C for 50 sec; and final extension at 72°C for 6 min. The forwards and reverse primers for the four oxacillinase genes are described in Table 1 (23, 24). A total volume of 25 µL for each reaction consisted of 12.5 µL Go Taq® 2x Green Master Mix, Promega (Promega Corporation, Madison, USA); 1 µL (10 µM) of forwards and reverse primer, 1 µL (approx. 10-100 ng) DNA template, and 9.5 µL of nuclease-free water. The amplified PCR products were subjected to gel electrophoresis with 1.5% agarose and visualized by Enduro™ GDS (Labnet International Inc).

Random amplified polymorphism DNA polymerase chain reaction (RAPD-PCR). Two different RAPD-PCR primers, a decamer DAF-4 (5'-CGGCAGCGCC-3') and a 22-mer ERIC-2 (5'-AAGTAAGTGACTGGGGTGAGCG-3'), were used in this study (25). The optimum amplification conditions for DAF-4 were predenaturation at 94°C for 2 min; 40 cycles of denaturation at 94°C for 40 sec, annealing at 45°C for 40 sec, and extension at 72°C for 40 sec; and final extension at 72°C for 5 min. A total volume of 50 µL for each reaction consisted of 25 µL of Go Taq® 2x Green Master Mix, Promega (Promega Corporation, Madison, USA); 1 µL (10 µM) of DAF-4 primer, 1 µL (approx. 10-100 ng) DNA template, and 23 µL of nuclease-free water. The optimum amplification conditions for ERIC-2 were predenaturation at 94°C for 3 min; 35 cycles of denaturation at 94°C for 30 sec, annealing at 25°C for 1 min, and extension at 68°C for 3 min; and final extension at 68°C for 10 min. A total volume of 25 µL for each reaction consisted of 12.5 µL of Go Taq® 2× Green Master Mix, Promega (Promega Corporation, Madison, USA); 1 µL (10 µM) of ERIC-2 primer, 1 µL (approx. 10-100 ng) DNA template, and 10.5 µL of nuclease-free water. RAPD-PCR using both primers was performed for chromosomal DNA for all bacterial isolates. In addition, chromosomal DNA of *A. baumannii* ATCC 43498, *Pseudomonas aeruginosa* ATCC 9027, *Escherichia coli* ATCC 8739, *Staphylococcus aureus* ATCC 25923, *Klebsiella pneumoniae* ATCC 13883, and *Streptococcus pyogenes* ATCC 19615 were used for comparison to generate a dendrogram and similarity index. The amplified PCR products were subjected to gel electrophoresis with 1.5% agarose and visualized by Enduro™ GDS (Labnet International

Table 1. Oligonucleotide sequence of primers for $bla_{\text{OXA-23-like}}$, $bla_{\text{OXA-24-like}}$, $bla_{\text{OXA-51-like}}$, and $bla_{\text{OXA-58-like}}$ genes (24).

Gene	Primer	Target amplicon
Oxa-23 forwards	5'-GATCGGATTGGAGAACCAGA-3'	501 bp(*)
Oxa-23 reverse	5'-ATTTCTGACCGCATTTCCAT-3'	
Oxa-24 forwards	5'-GGTTAGTTGGCCCCCTTAAA-3'	246 bp
Oxa-24 reverse	5'-AGTTGAGCGAAAAGGGGATT-3'	
Oxa-51 forwards	5'-TAATGCTTTGATCGGCCTTG-3'	353 bp
Oxa-51 reverse	5'-TGGATTGCACTTCATCTTGG-3'	
Oxa-58 forwards	5'-CGATCAGAATGTTCAAGCGC-3'	599 bp
Oxa-58 reverse	5'-ACGATTCTCCCCTCTGCGC-3'	

(*) bp: base pairs

Inc). All RAPD-PCR and electrophoresis were replicated three times with a minimum interval of one week for each replicate.

Cluster analysis. Cluster analysis was performed using GelJ v.2.3 software. A dendrogram and similarity index were generated by unweighted pair group method with arithmetic mean (UPGMA) analysis with 4% tolerance of the Dice coefficient (26). An arbitrary value of the 95% similarity index was determined as the cluster relatedness threshold to calculate discriminatory power by using Simpson's index of diversity formula as described previously (27).

Statistical analysis. All datasets generated from this study were analysed using Microsoft Excel of Windows 2019, and SPSS v. 28. The Mann–Whitney U test was used to assess the mean difference between the number of antibiotic resistances, the presence of oxacillinase genes, and the DAF-4 similarity index between the same or different dendrogram clusters. Spearman's rank correlation test was used to reveal the correlation between the DAF-4 similarity index antibiogram and the presence of oxacillinase genes.

RESULTS

Sample characteristics. Thirty-one clinical isolates of *A. baumannii* were isolated from patients treated in Intensive Care Units A to D (ICU A to ICU D) and eight common hospital wards (Wards E to L). The majority of clinical isolates were cultured from sputum (67.7%), followed by blood culture (19.4%), and 6.45% coming from both wound swabs and cerebrospinal fluid. Meanwhile, six environmental isolates were cultured from medical devices and one from patient's bed linen within ICU A (Table 2).

Antibiogram and oxacillinase gene detection. The antimicrobial susceptibility characteristics of all *A. baumannii* clinical isolates were assessed as part of routine microbiology testing in our hospital. As shown in Fig. 1, most clinical and environmental isolates were susceptible to three antibiotics, including tigecycline, sulfamethoxazole-trimethoprim, and amikacin, with susceptibilities of 97.3%, 75.7%, and 70.3%, respectively. Only seven out of the 37 total isolates (18.9%) were susceptible to meropenem. All isolates showed total resistance to cefoperazone, cefixime, cefuroxime, and cefazolin. The presence of oxacillinase genes in *A. baumannii* isolates was detected in this study. All isolates harboured the $bla_{\text{OXA-51-like}}$ gene in the chromosome and plasmid. A total of 29 of 37 *A. baumannii* isolates (78.4%) were positive for $bla_{\text{OXA-23-like}}$ gene both in chromosomes and plasmids, while three isolates (8.1%) were found to have the $bla_{\text{OXA-23-like}}$ gene in their chromosomes or plasmid. Only five isolates (13.5%) were negative for $bla_{\text{OXA-23-like}}$ gene both in chromosomes and plasmids. No $bla_{\text{OXA-24-like}}$ and $bla_{\text{OXA-58-like}}$ genes were found in all isolates' chromosomes or plasmids. Based on Spearman's rank correlation test, there was a strong correlation between the antibiogram pattern and the presence of $bla_{\text{OXA-23-like}}$ genes ($r=0.696$, $p<0.001$). Mean difference analysis showed that the total number of antibiotic resistant which harbored $bla_{\text{OXA-23-like}}$ was higher compared to isolates without $bla_{\text{OXA-23-like}}$ in chromosome, plasmid, or both (Mann–Whitney U test, $U=161.5$, $p<0.001$). These statistical analyses suggest the presence of $bla_{\text{OXA-23-like}}$ gene significantly determine the antibiotic resistance phenotype of *A.*

Table 2. Descriptive data from 31 clinical isolates and six environmental isolates of MDR *A. baumannii*.

Strain ID	Ward	Specimen's origin	Antibiogram profile	Total of antibiotic resistant	Oxa-23	
					Chromosome	Plasmid
D136	ICU A	Blood	sam-tzp-cz-cxm-cfm-cfp-caz-cro-fep-mem-cip-sxt	12	+	+
Envi A	ICU A	Suction drainage 01	sam-tzp-cz-cxm-cfm-cfp-caz-cro-fep-mem-gm-cip	12	+	+
Envi B	ICU A	Suction drainage 02	sam-tzp-cz-cxm-cfm-cfp-caz-cro-fep-mem-gm-cip	12	+	+
Envi C	ICU A	Suction machine 03	cz-cxm-cfm-cfp	4	(-)	(-)
Envi D	ICU A	Suction pipe 01	sam-tzp-cz-cxm-cfm-cfp-caz-cro-fep-mem-gm-cip	12	+	+
Envi E	ICU A	Suction pipe 02	sam-tzp-cz-cxm-cfm-cfp-caz-cro-fep-mem-gm-cip	12	+	+
ICU/Envi F	ICU A	Bed 01 Linen	sam-tzp-cz-cxm-cfm-cfp-caz-cro-fep-mem-an-gm-cip	13	+	+
L51	ICU A	Cerebrospinal fluid	sam-tzp-cz-cxm-cfm-cfp-caz-cro-fep-mem-gm-cip	12	+	+
Sp 14	ICU A	Sputum	sam-tzp-cz-cxm-cfm-cfp-caz-cro-fep-mem-gm-cip	12	+	+
Sp 23II	ICU A	Sputum	sam-tzp-cz-rox-cxm-cfp-caz-cro-fep-mem-an-gm-cip-tgc	14	+	+
Sp 32	ICU A	Sputum	sam-tzp-cz-cxm-cfm-cfp-caz-cro-fep-mem-gm-cip	12	+	+
SP 44	ICU A	Sputum	sam-tzp-cz-cxm-cfm-cfp-caz-cro-fep-mem-an-gm-cip	13	+	+
Sp 58	ICU A	Sputum	sam-tzp-cz-cxm-cfm-cfp-caz-cro-fep-mem-gm-cip	12	+	+
Sp 79	ICU A	Sputum	cz-cxm-cfm-cfp	4	(-)	(-)
D206	ICU B	Blood	tzp-cz-cxm-cfm-cfp-caz-cro-fep-gm-cip	10	(-)	(-)
D47	ICU B	Blood	sam-tzp-cz-cxm-cfm-cfp-caz-cro-fep-mem-an-gm-cip-sxt	14	+	+
Sp 70II	ICU C	Sputum	sam-tzp-cz-cxm-cfm-cfp-caz-cro-fep-mem-gm-cip	12	+	(-)
Sp 75I	ICU D	Sputum	cz-cxm-cfm-cfp	4	+	+
D176	Ward E	Blood	sam-tzp-cz-cxm-cfm-cfp-caz-cro-fep-mem-an-gm-cip-sxt	14	+	+
Sp 52	Ward E	Sputum	sam-tzp-cz-cxm-cfm-cfp-caz-cro-fep-mem-gm-cip	12	+	+
Sp 70	Ward E	Sputum	sam-tzp-cz-cxm-cfm-cfp-caz-cro-fep-mem-gm-cip	12	+	+
Sp 77	Ward E	Sputum	sam-tzp-cz-cxm-cfm-cfp-caz-cro-fep-mem-gm-cip	12	+	+
Sp 85	Ward E	Sputum	cz-cxm-cfm-cfp	4	(-)	(-)
D78	Ward F	Blood	sam-tzp-cz-cxm-cfm-cfp-caz-cro-fep-mem-gm-cip	12	+	+
Sp 38II	Ward F	Sputum	sam-tzp-cz-cxm-cfm-cfp-caz-cro-fep-mem-gm-cip	12	+	+
Sp 39	Ward F	Sputum	sam-tzp-cz-cxm-cfm-cfp-caz-cro-fep-mem-gm-cip	12	+	+
Sp 43I	Ward F	Sputum	cz-cxm-cfm-cfp	4	(-)	(-)
Sp 73I	Ward F	Sputum	sam-tzp-cz-cxm-cfm-cfp-caz-cro-fep-mem-an-gm-cip	13	+	+
Sp 75	Ward F	Sputum	sam-tzp-cz-cxm-cfm-cfp-caz-cro-fep-mem-gm-cip	12	+	+
Sp 43	Ward G	Sputum	sam-tzp-cz-cxm-cfm-cfp-caz-cro-fep-mem-cip	11	+	(-)
Sp 89	Ward G	Sputum	sam-tzp-cz-cxm-cfm-cfp-caz-cro-fep-mem-gm-cip-sxt	13	+	+
PS 24	Ward H	Wound swab	sam-tzp-cz-cxm-cfm-cfp-caz-cro-fep-mem-an-gm-cip-sxt	14	+	+
Sp 55	Ward H	Sputum	sam-tzp-cz-cxm-cfm-cfp-caz-cro-fep-mem-an-gm-cip-sxt	14	+	+
D209	Ward I	Blood	tzp-cz-cxm-cfm-cfp-caz-cro-fep-gm-cip	10	(-)	+
L38	Ward I	Cerebrospinal fluid	sam-tzp-cz-cxm-cfm-cfp-caz-cro-fep-mem-an-gm-cip-sxt	14	+	+
PS 23	Ward J	Wound swab	sam-tzp-cz-cxm-cfm-cfp-caz-cro-fep-mem-an-gm-cip-sxt	14	+	+
Sp 18	Ward K	Sputum	sam-tzp-cz-cxm-cfm-cfp-caz-cro-fep-mem-an-gm-cip-sxt	14	+	+

List of abbreviations for antibiotic class: Cefazolin (cz); Cefuroxime (cxm); Cefixime (cfm); Cefoperazone (cfp); Cefepime (fep); Ceftazidime (caz); Ceftriaxone (cro); Ciprofloxacin (cip); Meropenem (mem); Gentamicin (gm); Ampicillin/Sulbactam (sam); Amikacin (an); Tigecycline (tgc); Trimethoprim/Sulfamethoxazole (sxt); Piperacillin/Tazobactam (tzp)

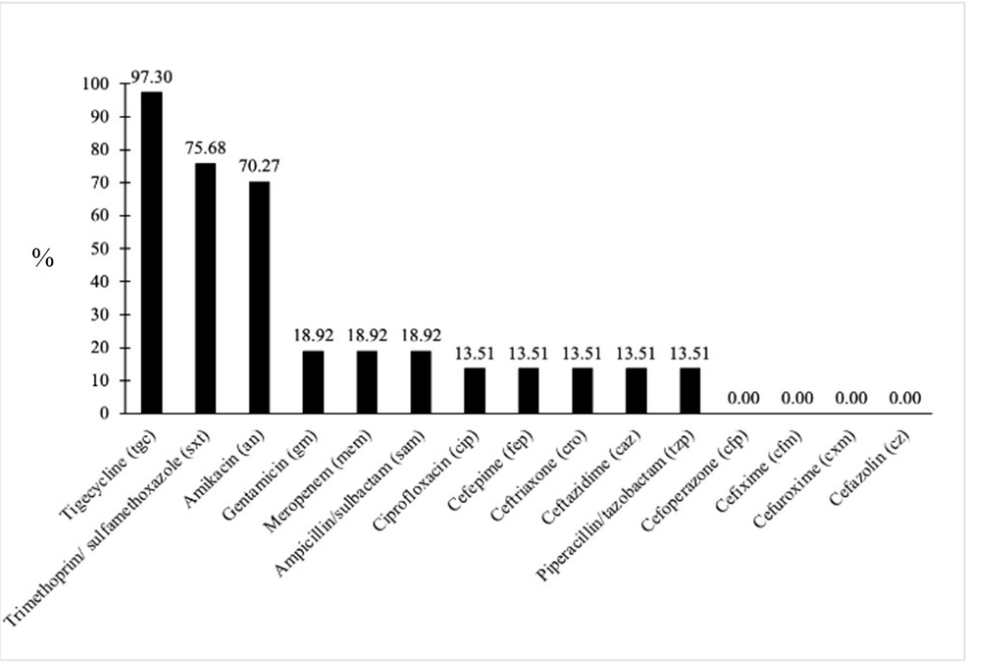


Fig. 1. Antibiotic susceptibility results from 31 clinical isolates of MDR *A. baumannii*. Antibiotic susceptibility was determined automatically by Vitek® 2 Compact.

baumannii isolates in Professor Dr. I.G.N.G. Ngoerah Hospital.

Random amplified polymorphism DNA polymerase chain reaction (RAPD-PCR). Cluster relatedness pattern. The RAPD-PCR procedure conducted in this study by using the DAF-4 primer had, on average, 11 successive band visualizations compared to 7 bands from the ERIC-2 primer. To analyse the intra-laboratory reproducibility of the DAF-4 and ERIC-2 primers, we generated a similarity index and dendrogram from three replication (triplicate) experiments. The similarity index variations of DAF-4 and ERIC-2 from triplicate experiments were 12% (range: 0-46%) and 24% (range: 0-75%), respectively. To better visualize these variations, we ran electrophoresis of each of the five isolates from the first and second replication experiments side-by-side in the same agarose gel, as shown in Figs. 2A and 2B.

To increase the validation of the similarity index and dendrogram pattern, we included five other bacterial species, i.e., *Pseudomonas aeruginosa* ATCC 9027, *Escherichia coli* ATCC 8739, *Staphylococcus aureus* ATCC 25923, *Klebsiella pneumoniae* ATCC 13883, and *Streptococcus pyogenes* ATCC 19615 alongside *A. baumannii* ATCC 43498. One result of the DAF-4 primer from the first replication experiment successfully distinguished *S. pyogenes* ATCC 19615, *P. aeruginosa* ATCC 9027, *S. aureus* ATCC 25923, and *E. coli* ATCC 8739 with maximum 74.3% of similarity index from the rest of the *A. baumannii* isolates (Fig. 3). The discriminatory power of DAF-4 by using an arbitrary value of 95% similarity was 0.906, which categorized into 25 clusters. Higher discriminatory power suggested more accuracy in clustering the isolates. Although this study did not intend to cluster

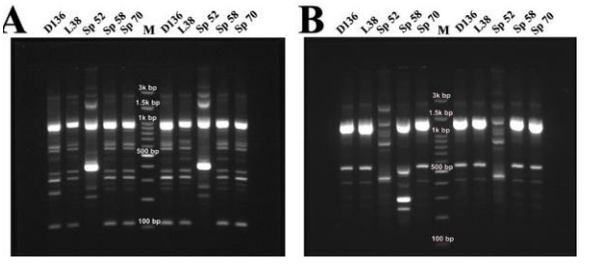


Fig. 2. Result of RAPD-PCR and dendrogram analysis from clinical isolates of MDR *A. baumannii*. Intralaboratory reproducibility from RAPD-PCR experiment using two primers: DAF-4 (Fig. 2A) and ERIC-2 (Fig. 2B). Fig. 2A-2B: Side-by-side electrophoresis of five isolates from the 1st replication (left side of marker) and 2nd replication (right side of marker). Sample 1= isolate D136; sample 2 = isolate L38; sample 3 = isolate Sp 52; sample 4 = Sp 58; sample 5 = isolate Sp 70; M= marker.

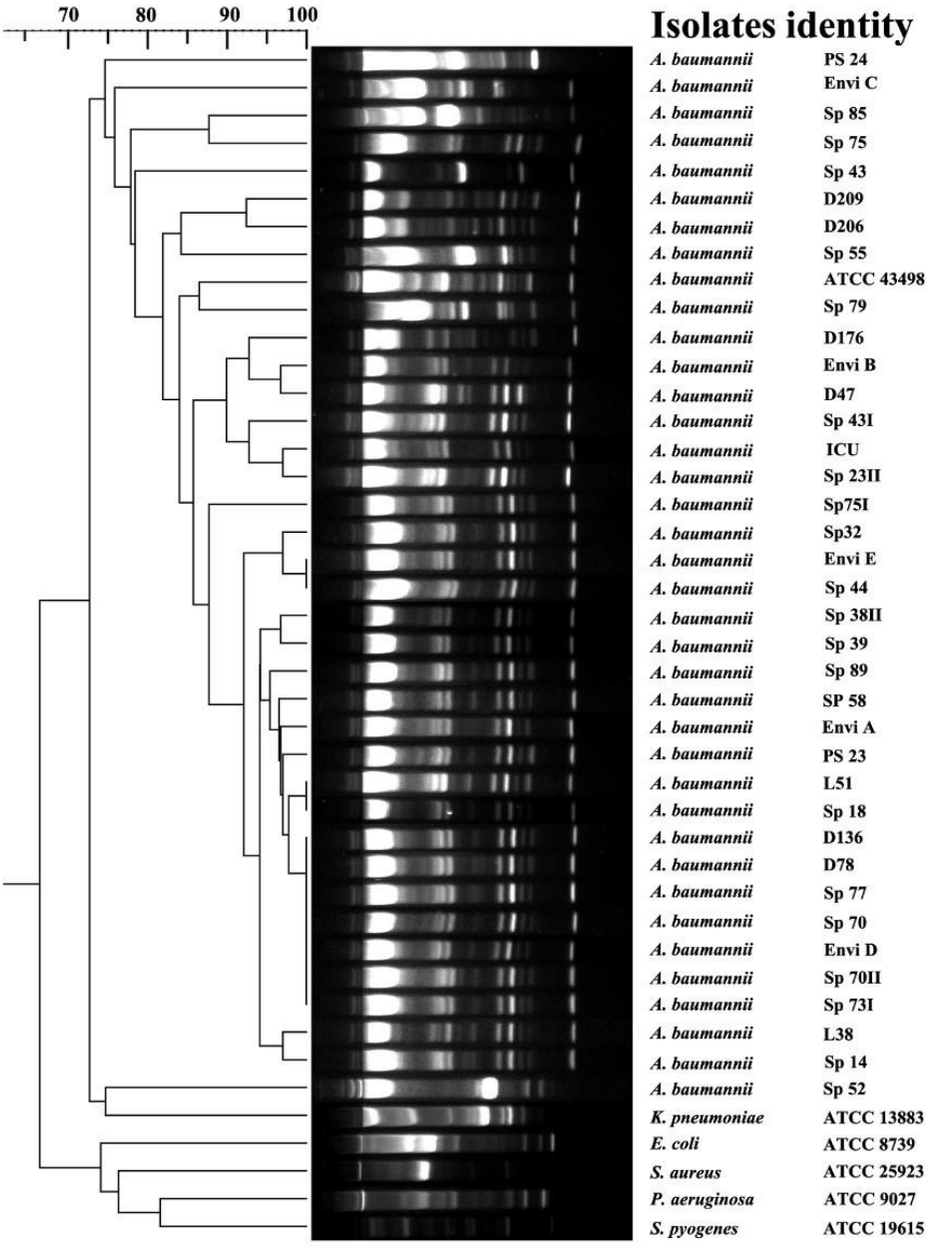


Fig. 3. Dendrogram pattern from the 1st replication experiment including all 37 isolates of MDR *A. baumannii* and six bacterial ATCC controls. This figure was an edited version to make the similarity index scale [upper right] and isolates identity more visible

all of the *A. baumannii* isolates since they originated from various ICUs and hospital wards within Professor Dr. I.G.N.G. Ngoerah General Hospital, but the higher yield of successive band visualization, lower intra-laboratory variation, and high discriminatory power suggested that RAPD-PCR with DAF-4 primer could serve as feasible clustering analysis for *A. baumannii* isolates, especially in the hospital setting with limited microbiology laboratory equipment.

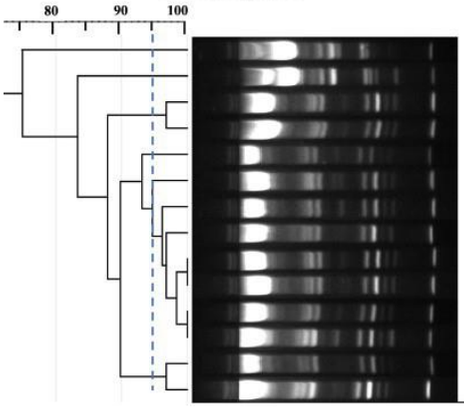
Probability of multidrug resistant (MDR) *Acinetobacter baumannii* outbreak in Intensive Care Unit A (ICU A). Since there were increasing cases of *A. baumannii* in clinical isolates of ICU A between September and October 2020, we utilized RAPD-PCR analysis to determine the genetic relatedness of these *A. baumannii* isolates. This genetic mapping was intended to be used as a surveillance model to assess the probability of a nosocomial outbreak of *A. baumannii*.

In addition to eight clinical isolates of *A. baumannii* that originated from patients, we also conducted an environmental swab within the ICU which generated six successful isolates as follow: Envi A, B, and C originated from three unrelated suction machines; Envi D and Envi E were isolated from two different suction pipes, and ICU was derived from the patient's bed linen (Table 2).

The dendrogram analysis of fourteen *A. baumannii* isolates in ICU A shown in (Fig. 4) with 95% similarity index as the cut-off value (dashed blue line) was categorized into seven clusters. Spearman's rank correlation analysis showed a significant positive correlation between the DAF-4 similarity index and either the antibiogram pattern ($r=0.494$, $p<0.001$) or the presence of the $bla_{\text{OXA-23-like}}$ gene ($r=0.634$, $p<0.001$). Correlation analysis clearly showed that the Envi C and Sp79 isolates, which were cephalosporin-only resistant *A. baumannii* and negative for $bla_{\text{OXA-23-like}}$ gene, had the lowest similarity with the rest of the isolates which were positive for the $bla_{\text{OXA-23-like}}$ gene. Furthermore, six isolates were classified into cluster VI, consisting of three clinical samples and three environmental samples. All isolates within cluster VI harboured $bla_{\text{OXA-23-like}}$ with identical antibiogram results, except for D136, which was resistant to sulfamethoxazole-trimethoprim but susceptible to gentamicin. These data might suggest that one isolate of *A. baumannii* was the original outbreak source for the rest of the isolates belonging to cluster VI. The probable isolate was L51 since it was detected earlier than Sp58 and D136. The L51 isolate had an identical dendrogram, antibiogram, and oxacillinase gene status to Envi D. Detection of environmental isolates of MDR A. baumannii with a high DAF-4 similarity index, identical antibiogram and $bla_{\text{OXA-23-like}}$ gene status to the clinical isolates in cluster VI indicated persistent bacterial presence in medical devices in addition to possible health care workers' role as transmission carriers.

DISCUSSION

Acinetobacter baumannii has been related to HAIs and has become a major hospital pathogen due to multidrug-resistant (MDR) strains (4). A broad spectrum of infectious diseases caused by MDR *A. baumannii* infection are closely associated with device-related infections and high mortality rates, especially in ICUs (28). Approximately 10.6% of patients die as a result of infections caused by MDR *A. baumannii* (29). According to our research, tigecycline had the highest susceptibility percentage with 97.3% susceptibility rate. A high sensitivity percentage of tigecycline was also reported at hospitals in Iran and Turkey with 77.6% and 78.3% respectively (30, 31). On the other hand, a multicenter cross-sectional study in Indonesia showed a contradictory result with a low



Cluster	Sample ID	Antibiotic resistance pattern	Oxa-23 Chr	Oxa-23 Pl	Date collection
I	Envi C	cz-cxm-cfm-cfp	(-)	(-)	Nov 8
II	Sp 79	cz-cxm-cfm-cfp	(-)	(-)	Sep 28
III	Sp 32	sam-tzp-cz-cxm-cfm-cfp-caz-cro-fep-mem-gm-cip	+	+	Sep 14
III	SP 44	sam-tzp-cz-cxm-cfm-cfp-caz-cro-fep-mem-an-gm-cip	+	+	Sep 18
IV	Envi B	sam-tzp-cz-cxm-cfm-cfp-caz-cro-fep-mem-gm-cip	+	+	Nov 8
V	Sp 14	sam-tzp-cz-cxm-cfm-cfp-caz-cro-fep-mem-gm-cip	+	+	Sep 7
VI	Sp 58	sam-tzp-cz-cxm-cfm-cfp-caz-cro-fep-mem-gm-cip	+	+	Oct 15
VI	Envi A	sam-tzp-cz-cxm-cfm-cfp-caz-cro-fep-mem-gm-cip	+	+	Nov 8
VI	D136	sam-tzp-cz-cxm-cfm-cfp-caz-cro-fep-mem-cip-sxt	+	+	Oct 19
VI	Envi E	sam-tzp-cz-cxm-cfm-cfp-caz-cro-fep-mem-gm-cip	+	+	Sep 8
VI	Envi D	sam-tzp-cz-cxm-cfm-cfp-caz-cro-fep-mem-gm-cip	+	+	Nov 8
VI	L51	sam-tzp-cz-cxm-cfm-cfp-caz-cro-fep-mem-gm-cip	+	+	Sep 22
VII	ICU	sam-tzp-cz-cxm-cfm-cfp-caz-cro-fep-mem-an-gm-cip	+	+	Nov 8
VII	Sp 23II	sam-tzp-cz-cxm-cfm-cfp-caz-cro-fep-mem-an-gm-cip-tgc	+	+	Oct 6

Fig. 4. The combined approach was used to assess the probability of MDR *A. baumannii* outbreaks within ICU A. Eight clinical isolates and 6 environmental isolates were subjected to RAPD-PCR (DAF-4 primer) for clustering analysis, routine antibiotic susceptibility testing, and PCR for oxacillinase gene detection. There were seven clusters generated from the dendrogram pattern by using a 95% similarity index as the cut-off point (blue-dashed line). Six isolates were grouped together in Cluster VI, in which clinical isolate L51 shared a 100% similarity index with environmental isolate Envi D and clinical isolate D136 also shared a 100% similarity index with environmental isolate Envi E. (*Chr: chromosome; Pl: plasmid.*)

percentage of tigecycline susceptibility (45.45%) in carbapenem-nonsusceptible *A. baumannii* isolates. This may be due to the wide use of tigecycline in several locations in Indonesia involved in that study (32). The sensitivity percentage of sulfamethoxazole/trimethoprim and amikacin were 75.7%, and 70.3% respectively. A study from a tertiary public hospital in Padang, Indonesia found that the three most sensitive antibiotics to *A. baumannii* were amikacin (74.9%), sulfamethoxazole-trimethoprim (67.1%), and meropenem (62.7%) (33). Our study found that only 22% of isolates (7/32) were susceptible to meropenem. A high percentage of CRAB was found in South Korea, Jordan, and Palestine, with resistance percentages between 87.0% and 97.2% (10). The resistance of carbapenems also contributes to other beta-lactam drugs ineffective (16). Cefoperazone, cefixime, cefuroxime, and cefazolin showed total resistance to *A. baumannii*. A study in China also found total resistance to cefazolin and 71.8% to cefuroxime (34). High resistance percentages to cefoperazone (61%), cefixime (99%), and cefuroxime (96%) were also reported from 250 bacterial isolates in Egypt (35). A high resistance percentage in the cephalosporin class may occur due to *A. baumannii* having an AmpC cephalosporinase-type enzyme that is chromosomally encoded by all *A. baumannii* strains (36). *A. baumannii* commonly does not induce the expression of AmpC-type enzymes. The overexpression is mostly mediated by inserting insertion sequence *A. baumannii* 1 (ISAba1) before AmpC genes and will enhance *A. baumannii*'s resistance to extended-spectrum cephalosporins (36). In addition, the insertion of ISAba1 upstream of the $bla_{\text{OXA-51-like}}$ gene is associated with the overexpression of $bla_{\text{OXA-51-like}}$ enzymes that correlate with cephalosporin resistance (37).

All isolates in this study haboured the $bla_{\text{OXA-51-like}}$ gene. This results also similar in several studies (38, 39). The $bla_{\text{OXA-51-like}}$ gene is utilized as a reliable marker for identification of species *A. baumannii* due to this gene chromosomally located in *A. baumannii* (36). Our study found that 86.5% of clinical isolates were positive for the $bla_{\text{OXA-23-like}}$ gene. In concordance with this result, similar conditions were also observed in a study conducted by Hera et al. that found that 82.4% of isolates were positive for $bla_{\text{OXA-23-like}}$ (38). In addition, 83.6% of *A. baumannii* blood isolates were positive for $bla_{\text{OXA-23-like}}$ (32). In this study, a strong correlation between the antibiogram pattern and the presence of the oxacillinase gene $bla_{\text{OXA-23-like}}$ was found. A previous study also showed a significant association between the $bla_{\text{OXA-23-like}}$ gene and the susceptibility pattern to some antibiotics (32). Furthermore, it was found that most isolates harbouring $bla_{\text{OXA-23-like}}$ (88%) were resistant to meropenem (carbapenem), which is in line with several studies that found $bla_{\text{OXA-23-like}}$ was associated with CRAB (39, 40). The main mechanism of resistance to carbapenem is the production of carbapenemase, which is dominantly encoded by $bla_{\text{OXA-23-like}}$ (36). The insertion sequence ISAba1 in the $bla_{\text{OXA-23-like}}$ promotor is associated with the overexpression of *A. baumannii* carbapenemase (36).

In this study, from the RAPD-PCR experiment, we found that DAF-4 generated more visible bands than ERIC-2. In addition, the discriminatory power of the DAF-4 primer in this study (0.906) was similar to the discriminatory power of pulse-field gel electrophoresis/PFGE (0.892) but far lower than that of whole-genome sequencing/WGS (0.997) from a study conducted by Fitzpatrick et al. in the United States (2017) (41). However, unlike PFGE or WGS, the intra- and interlaboratory reproducibility of RAPD-PCR should be taken cautiously. Based on triplicate RAPD-PCR experiments, the variation of DAF-4 and ERIC-2 primers in this study were 12% and 24%, respectively. Meanwhile, the interlaboratory reproducibility of RAPD-PCR is the main limitation of this typing method because the PCR machine, reagents, and protocol could not be standardized among different laboratories. Despite its limitation of reproducibility, RAPD-PCR holds the greatest advantages as an early surveillance system in local hospital settings (42). The RAPD fingerprinting technique is the cheapest and simplest procedure compared to PFGE or WGS. The cumulative cost of RAPD-PCR per sample is less than US $20, while PFGE and WGS cost approximately US $150 and US $100-300, respectively (43). Lessons learned from the COVID-19 pandemic have provided significant insight into WGS utilization to detect variations in pathogen genomes on a single nucleotide scale. A continuous decrease in the cost of genome sequencing could accelerate WGS utilization as primary molecular typing to assess the probability of bacterial outbreaks in hospital settings. However, the limited financial power to set and maintain advanced laboratories and the need for highly trained bioinformatician staff hinder the widespread use of WGS, es-

pecially in hospitals across low- and middle-income countries.

We intend to use RAPD-PCR using the DAF-4 primer as a molecular typing method in addition to an antibiogram and the presence of oxacillinase genes to assess the probability of MDR *A. baumannii* outbreak within ICU A. As the essential initial procedure, we collected environmental isolates from the entire area surrounding ICU A, including the medical devices. Collecting environmental isolates is crucial since widely reported *A. baumannii* can survive more than 20 days in the environment and medical devices (44, 45). The environmental isolates might serve as reservoirs for ongoing outbreaks in hospital settings. We found six environmental isolates of *A. baumannii* with MDR characteristics and harbouring the $bla_{\text{OXA-23-like}}$ gene, except for Envi C, which showed a cephalosporin-only resistance pattern and was negative for the $bla_{\text{OXA-23-like}}$ gene. By using the 95% similarity index, three environmental isolates (Envi A, D, and E) occupied the same cluster with three clinical isolates (L51, Sp58, and D136), suggesting that one isolate might serve as a source of outbreak that spilled to medical devices and directly infected other patients or indirectly via health worker carryover. Furthermore, Spearman's rank correlation test found that the similarity index (which is reflected in the dendrogram pattern) was significantly correlated with the antibiogram pattern ($r=0.494$, $p<0.001$) or the presence of the $bla_{\text{OXA-23-like}}$ gene ($r=0.634$, $p<0.001$) from clinical and environmental isolates within ICU A. The statistical analysis added more weight and enhanced our reliance on DAF-4 dendrogram analysis. All of the findings reported in this current study and special case analysis from ICU A serve as input for further evaluation of infection and prevention control practices, including standard and transmission precaution protocols, adherence to HAIs bundle practices, medical devices and environmental disinfection protocols in our hospital. The main limitation is small sample size presented in this study. and no confirmatory study with the gold standard methods such as PFGE or WGS. In the beginning of our study, we consider to expand the time frame of this study for 6 months to increase the total samples. But since we deal with the possibility of MDR *A. baumannii*'s outbreak within ICU A, therefore we opt to minimize the time frame only for 2 months in order to analysis the source of outbreak and prevent further spreading.

CONCLUSION

There is a high probability of MDR *A. baumannii* outbreak within ICU A detected by multiple analysis of RAPD-PCR, antibiogram and the $bla_{\text{OXA-23-like}}$ gene profiles. The utilization of routine antibiotic susceptibility tests in combination with oxacillinase gene profiles and RAPD-PCR typing methods is a cost-effective early surveillance system to assess the probability of MDR *A. baumannii* outbreaks in local hospital settings, especially in the hospital with limited microbiology laboratory. This approach could also be expanded to other multidrug-resistant bacteria, such as methicillin-resistant *Staphylococcus aureus* (MRSA) or carbapenem-resistant *Enterobacteriaceae* (CRE), with modification of the specific resistance gene profile and another suitable RAPD-PCR primer for the respective bacteria.

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REFERENCES

1. Kyriakidis I, Vasileiou E, Pana ZD, Tragiannidis A. *Acinetobacter baumannii* antibiotic resistance mechanisms. *Pathogens* 2021; 10: 373.
2. World Health Organization (2020). Global Antimicrobial Resistance Surveillance System (GLASS) Report. Geneva: World Health Organization (WHO).
3. Vashist J, Tiwari V, Das R, Kapil A, Rajeswari MR. Analysis of penicillin-binding proteins (PBPs) in carbapenem resistant *Acinetobacter baumannii. Indian J Med Res* 2011; 133: 332-338.
4. Ibrahim S, Al-Saryi N, Al-Kadmy IMS, Aziz SN. Multidrug-resistant *Acinetobacter baumannii* as an emerging concern in hospitals. *Mol Biol Rep* 2021; 48: 6987-

6998.
5. Alrahmany D, Omar AF, Harb G, El Nekidy WS, Ghazi IM. *Acinetobacter baumannii* infections in Hospitalized patients, treatment outcomes. *Antibiotics (Basel)* 2021; 10: 630.
6. John AO, Paul H, Vijayakumar S, Anandan S, Sudarsan T, Abraham OC, et al. Mortality from acinetobacter infections as compared to other infections among critically ill patients in South India: A prospective cohort study. *Indian J Med Microbiol* 2020; 38: 24-31.
7. Cornejo-Juárez P, Cevallos MA, Castro-Jaimes S, Castillo-Ramìrez S, Velázquez-Acosta C, Martìnez-Oliva D, et al. High mortality in an outbreak of multidrug resistant *Acinetobacter baumannii* infection introduced to an oncological hospital by a patient transferred from a general hospital. *PLoS One* 2020; 15 (7): e0234684.
8. Appaneal HJ, Lopes VV, LaPlante KL, Caffrey AR. Treatment, clinical outcomes, and predictors of mortality among a national cohort of hospitalized patients with Stenotrophomonas maltophilia infection. *Public Health* 2023; 214: 73-80.
9. Yadav SK, Bhujel R, Hamal P, Mishra SK, Sharma S, Sherchand JB. Burden of multidrug-resistant *Acinetobacter baumannii* infection in hospitalized patients in a tertiary care hospital of Nepal. *Infect Drug Resist* 2020; 13: 725-732.
10. Ma C, McClean S. Mapping global prevalence of *Acinetobacter baumannii* and recent vaccine development to tackle it. *Vaccines (Basel)* 2021; 9: 570.
11. Huang H, Chen B, Liu G, Ran J, Lian X, Huang X, et al. A multi-center study on the risk factors of infection caused by multi-drug resistant *Acinetobacter baumannii*. *BMC Infect Dis* 2018; 18: 11.
12. Alotaibi T, Abuhaimed A, Alshahrani M, Albdelhady A, Almubarak Y, Almasari O. Prevalence of multidrug-resistant *Acinetobacter baumannii* in a critical care setting: A tertiary teaching hospital experience. *SAGE Open Med* 2021; 9: 20503121211001144.
13. Hafiz TA, Alghamdi SS, Mubaraki MA, Alghamdi SSM, Alothaybi A, Aldawood E, et al. A two-year retrospective study of multidrug-resistant *Acinetobacter baumannii* respiratory infections in critically Ill patients: Clinical and microbiological findings. *J Infect Public Health* 2023; 16: 313-319.
14. Čiginskienė A, Dambrauskienė A, Rello J, Adukauskienė D. Ventilator-associated pneumonia due to drug-resistant *Acinetobacter baumannii:* Risk factors and mortality relation with resistance profiles, and independent predictors of in-hospital mortality. *Medicina (Kaunas)* 2019; 55: 49.
15. McKay SL, Vlachos N, Daniels JB, Albrecht VS, Stevens VA, Rasheed JK, et al. Molecular epidemiology of carbapenem-resistant *Acinetobacter baumannii* in the United States, 2013-2017. *Microb Drug Resist* 2022; 28: 645-653.
16. El-Kazzaz W, Metwally L, Yahia R, Al-Harbi N, El-Taher A, Hetta HF. Antibiotics antibiogram, prevalence of OXA carbapenemase encoding genes, and RAPD-Genotyping of multidrug-resistant *Acinetobacter baumannii* incriminated in hidden community-acquired infections. *Antibiotics (Basel)* 2020; 9: 603.
17. Bulens SN, Yi SH, Walters MS, Jacob JT, Bower C, Reno J, et al. Carbapenem-nonsusceptible *Acinetobacter baumannii*, 8 US metropolitan areas, 2012-2015. *Emerg Infect Dis* 2018; 24: 727-734.
18. Weinberg SE, Villedieu A, Bagdasarian N, Karah N, Teare L, Elamin WF. Control and management of multidrug resistant *Acinetobacter baumannii:* A review of the evidence and proposal of novel approaches. *Infect Prev Pract* 2020; 2: 100077.
19. Casaroto E, Generoso JR, Tofaneto BM, Bariani LM, Auler MA, Xavier N, et al. Hand hygiene performance in an intensive care unit before and during the COVID-19 pandemic. *Am J Infect Control* 2022; 50: 585-587.
20. Asif M, Alvi IA, Rehman SU. Insight into *Acinetobacter baumannii:* Pathogenesis, global resistance, mechanisms of resistance, treatment options, and alternative modalities. *Infect Drug Resist* 2018; 11: 1249-1260.
21. Kamolvit W, Sidjabat HE, Paterson DL. Molecular Epidemiology and mechanisms of carbapenem resistance of *Acinetobacter* spp. In Asia and Oceania. *Microb Drug Resist* 2015; 21: 424-434.
22. Rafei R, Kempf M, Eveillard M, Dabboussi F, Hamze M, Joly-Guillou M-L. Current molecular methods in epidemiological typing of *Acinetobacter baumannii*. *Future Microbiol* 2014; 9: 1179-1194.
23. Poirel L, Marqué S, Héritier C, Segonds C, Chabanon G, Nordmann P. OXA-58, a novel class D β-Lactamase involved in resistance to carbapenems in *Acinetobacter baumannii*. *Antimicrob Agents Chemother* 2005; 49: 202-208.
24. Woodford N, Ellington MJ, Coelho JM, Turton JF, Ward ME, Brown S, et al. Multiplex PCR for genes encoding prevalent OXA carbapenemases in *Acinetobacter* spp. *Int J Antimicrob Agents* 2006; 27: 351-353.
25. D'Arezzo S, Capone A, Petrosillo N, Visca P; GRAB; Ballardini M, et al. Epidemic multidrug-resistant *Acinetobacter baumannii* related to European clonal types I and II in Rome (Italy). C*lin Microbiol Infect* 2009; 15: 347-357.
26. Heras J, Domínguez C, Mata E, Pascual V, Lozano C, Torres C, et al. GelJ -- a tool for analyzing DNA fingerprint gel images. *BMC Bioinformatics* 2015; 16: 270.
27. De Wolf H, Blust R, Backeljau T. The use of RAPD in ecotoxicology. *Mutat Res* 2004; 566: 249-262.
28. Gómez RF, Castillo A, Chávez-Vivas M. Characteri-

zation of multidrug-resistant *Acinetobacter* ssp. strains isolated from medical intensive care units in Cali - Colombia. *Colomb Med (Cali)* 2017; 48: 183-190.

29. Zhou H, Yao Y, Zhu B, Ren D, Yang Q, Fu Y, et al. Risk factors for acquisition and mortality of multidrug-resistant *Acinetobacter baumannii* bacteremia: A retrospective study from a Chinese hospital. *Medicine (Baltimore)* 2019; 98(13): e14937.
30. Alavi-Moghaddam M, Dolati M, Javadi A, Saki BG, Karami-Zarandi M, Khoshnood S. Molecular detection of oxacillinase genes and typing of clinical isolates of *Acinetobacter baumannii* in Tehran, Iran. *J Acute Dis* 2020; 9: 33-39.
31. Şimşek M, Demir C. Determination of colistin and tigecycline resistance profile of *Acinetobacter Baumannii* strains from different clinical samples in a territory hospital in Turkey. *J Health Sci Med Res* 2020; 38: 81-91.
32. Anggraini D, Santosaningsih D, Saharman YR, Endraswari PD, Cahyarini C, Saptawati L, et al. Distribution of carbapenemase genes among carbapenem-non-susceptible *Acinetobacter baumanii* blood isolates in Indonesia: A Multicenter Study. *Antibiotics (Basel)* 2022; 11: 366.
33. Ezeddin MO, Nasrul E, Alia E. Prevalensi dan Pola sensitivitas antibiotik *Acinetobacter baumannii* di RSUP. Dr. M. *Djamil Padang. Maj Kedokt Andalas* 2022; 45: 10-16.
34. Feng D-Y, Zhou J-X, Li X, Wu W-B, Zhou Y-Q, Zhang T-T. Differentiation between *Acinetobacter Baumannii* colonization and infection and the clinical outcome prediction by infection in lower respiratory tract. *Infect Drug Resist* 2022; 15: 5401-5409.
35. Abo safe F, Salah N, Rashed M, Saad S. Susceptibility of aminoglycoside resistant *Acinetobacter baumannii* to antibiotic combinations. *Egypt J Microbiol* 2019; 54: 137-147.
36. Ayoub Moubareck C, Hammoudi Halat D. Insights into *Acinetobacter baumannii*: a review of microbiological, virulence, and resistance traits in a threatening nosocomial pathogen. *Antibiotics (Basel)* 2020; 9: 119.
37. Kurihara MNL, Sales RO, Silva KED, Maciel WG, Simionatto S. Multidrug-resistant *Acinetobacter baumannii* outbreaks: a global problem in healthcare settings. *Rev Soc Bras Med Trop* 2020; 53: e20200248.
38. Nirwati H, Hakim MS, Darma S, Mustafa M, Nuryastuti T. Detection of blaoxa genes and identification of biofilm-producing capacity of *Acinetobacter baumannii* in a tertiary teaching hospital, Klaten, Indonesia. *Med J Malaysia* 2018; 73: 291-296.
39. Anggraini D, Kemal RA, Hadi U, Kuntaman K. The susceptibility pattern and distribution of $bla_{\text{OXA-23}}$ genes of clinical isolate *Acinetobacter baumannii* in a tertiary hospital, Indonesia. *J Infect Dev Ctries* 2022; 16: 821-826.
40. Handal R, Qunibi L, Sahouri I, Juhari M, Dawodi R, Marzouqa H, et al. Characterization of carbapenem-resistant *Acinetobacter baumannii* strains isolated from hospitalized patients in palestine. *Int J Microbiol* 2017: 2017: 8012104.
41. Fitzpatrick MA, Ozer EA, Hauser AR. Utility of whole-Genome sequencing in characterizing *Acinetobacter* epidemiology and analyzing hospital outbreaks. *J Clin Microbiol* 2016; 54: 593-612.
42. van Belkum A, Tassios PT, Dijkshoorn L, Haeggman S, Cookson B, Fry NK, et al. Guidelines for the validation and application of typing methods for use in bacterial epidemiology. *Clin Microbiol Infect* 2007; 13 Suppl 3: 1-46.
43. Sherry NL, Porter JL, Seemann T, Watkins A, Stinear TP, Howden BP. Outbreak investigation using high-throughput genome sequencing within a diagnostic microbiology laboratory. *J Clin Microbiol* 2013; 51: 1396-1401.
44. Towner KJ. *Acinetobacter*: an old friend, but a new enemy. *J Hosp Infect* 2009; 73: 355-363.
45. Obeidat N, Jawdat F, Al-Bakri AG, Shehabi AA. Major biologic characteristics of *Acinetobacter baumannii* isolates from hospital environmental and patients' respiratory tract sources. *Am J Infect Control* 2014; 42: 401-414.