WGS-Based Analysis of Carbapenem-Resistant Acinetobacter baumannii in Vietnam and Molecular Characterization of Antimicrobial Determinants and MLST in Southeast Asia

Gamal Wareth 1,2,3,* Gamal Wareth, Jörg Linde 1, Ngoc H. Nguyen 4,* Tuan N. M. Nguyen 5, Lisa D. Sprague 1, Mathias W. Pletz 2 and Heinrich Neubauer 1

Abstract: Carbapenem-resistant Acinetobacter baumannii (A. baumannii, CRAb) is an emerging global threat for healthcare systems, particularly in Southeast Asia. Next-generation sequencing (NGS) technology was employed to map genes associated with antimicrobial resistance (AMR) and to identify multilocus sequence types (MLST). Eleven strains isolated from humans in Vietnam were sequenced, and their AMR genes and MLST were compared to published genomes of strains originating from Southeast Asia, i.e., Thailand (n = 49), Myanmar (n = 38), Malaysia (n = 11), Singapore (n = 4) and Taiwan (n = 1). Ten out of eleven Vietnamese strains were CRAb and were susceptible only to colistin. All strains harbored ant(3')-Ila, armA, aph(6)-Id and aph(3')-I genes conferring resistance to aminoglycosides, and blaOXA-51 variants and blaADC-25 conferring resistance to ß-lactams. More than half of the strains harbored genes that confer resistance to tetracyclines, sulfonamides and macrolides. The strains showed high diversity, where six were assigned to sequence type (ST)/2, and two were allocated to two new STs (ST/1411-1412). MLST analyses of 108 strains from Southeast Asia identified 19 sequence types (ST), and ST/2 was the most prevalent found in 62 strains. A broad range of AMR genes was identified mediating resistance to ß-lactams, including cephalosporins and carbapenems (e.g., blaOXA-51-like, blaOXA-23, blaADC-25, blaADC-73, blaTEM-1, blaNDM-1), aminoglycosides (e.g., ant(3')-Ia, aph(3')-Ib, aph(6)-Id, armA and aph(3')-Ia), phenicoles (e.g., antBb), tetracyclines (e.g., tet.B and tet.39), sulfonamides (e.g., sul.1 and sul.2), macrolides and lincosamide (e.g., mhp.E, msr.E and abaF). CRAb and core genome MLST (cgMLST) showed an extreme diversity among the strains. Several strains isolated from different countries clustered together by cgMLST; however, different clusters shared the same ST. Developing an action plan on AMR, increasing awareness and prohibiting the selling of antibiotics without prescription must be mandatory for this region. Such efforts are critical for enforcing targeted policies on the rational use of carbapenem compounds and controlling AMR dissemination and emergence in general.

Keywords: Acinetobacter baumannii; carbapenem-resistant; MDR; WGS; Vietnam; Southeast Asia

1. Introduction

Acinetobacter (A.) baumannii is a notorious Gram-negative pathogen associated with a multitude of severe nosocomial infections and high mortalities in intensive care units (ICUs) [1]. The pathogen is known to cause ventilator-associated pneumonia, bloodstream, skin and urinary tract infections and secondary meningitis [2,3]. A. baumannii is intrinsically
resistant to many antibiotics and may acquire resistance via mutational changes in chromosomal structure and through horizontal gene transfer [4]. The spread and dissemination of multidrug-resistant (MDR) and extremely drug-resistant (XDR) *A. baumannii* have become a public health concern in both developing and developed countries. The prevalence of resistance towards last-resort antibiotics such as carbapenems and colistin is increasing globally [4,5]. The treatment of carbapenem-resistant *A. baumannii* (CRAb) is becoming a global challenge. CRAb is emerging worldwide, and the majority of these isolates often show MDR or XDR patterns [6,7]. They are also associated with an increased length of stay at hospital ICUs [8]. CRAb is associated with increased mortalities in patients with bloodstream infections in low- and middle-income countries [9]. It is considered one of the most common pathogens of nosocomial infections in Southeast Asia [10].

Vietnam is one of the Southeast Asian countries with the highest resistance prevalences amongst Gram-negative pathogens in the Asia-Pacific region [11]. It is considered the “hottest” spot of MDR *A. baumannii* in Asia. *A. baumannii* was considered one of the most frequent causes of ventilator-associated respiratory infection [12–14] and secondary meningitis [15] in Vietnamese ICUs. From 2008 to 2011, 101 clinical *A. baumannii* strains were isolated from ICU patients in two medical settings in Vietnam [16]. Between 2012 and 2014, 252 *Acinetobacter* spp. were isolated from patients admitted to three hospitals in southern Vietnam. Among them, 160 were confirmed as *A. baumannii* [17]. Between 2014 and 2015, *A. baumannii* posed serious therapeutic problems in ICU patients at a major tertiary hospital in Ho Chi Minh City [18]. Several separate studies have been carried out on *A. baumannii* in Southeast Asian countries and showed increases in the general distribution of *A. baumannii* in hospitals in Vietnam [12,17–20], Malaysia [21], Thailand [22], the Philippines [23] and Indonesia [24]. However, no comparison of the circulating strains and no multinational transboundary studies have been carried out to investigate the resistance profiles, MLST and the lineage of *A. baumannii* in the Southeast Asia region.

Thus, the current study aimed to analyze the whole-genome sequencing (WGS) data of carbapenem-resistant *A. baumannii* isolates obtained from Vietnamese patients and compare their AMR genes, MLST types and genetic diversity with published genomes of strains from Southeast Asia.

2. Results and Discussion

2.1. Whole-Genome Sequencing Data, MLST and cgMLST Analysis

Genome sequencing of 11 Vietnamese *A. baumannii* isolates yielded an average read length of 262,090 per isolate (range 1,137,862–2,537,360). The isolates’ mean coverage was 105.9-fold (range from 72-fold to 149-fold) (Table S1). To check for possible contamination and accurate species identification, the software Kraken2 was used, which classifies each read (or contig) [25]. At the genus level, the first match for all isolates was always “*Acinetobacter*”, on average, 97.61% of the reads (maximum 99.18%, minimum 89.61%). At the species level, the first match for all 11 isolates was always “*Acinetobacter baumannii*”, on average, 78.24% of the reads. Genome assembly yielded an average genome size of 4,070,357 bp with a minimum of 3,792,190 bp. The GC content was, on average, 38.97%. The mean N50 of the 11 assembled genomes was 88.347 bp (range 41,904–184,445 bp) (Table S2). MLST analyses of eleven Vietnamese *A. baumannii* strains based on the Pasteur scheme allocated nine strains into a distinct ST. Six strains were assigned to ST/2, two were assigned to ST/571 and one was assigned to ST/164. Two isolates were allocated into two new STs (ST/1411-1412) (Table 1).

MLST analysis confirmed the considerable genetic diversity of *A. baumannii* in the investigated strains from Southeast Asia. Six strains were removed as they did not fit our quality criteria. MLST analyses based on the Pasteur scheme identified 19 sequence types (ST) for 107 strains, and a strain from Thailand could not be assigned to a distinct sequence type due to new alleles. ST/2 was the most prevalent sequence type circulating in the Southeast Asian countries. It was found in 62 strains isolated from humans from Vietnam, Myanmar, Thailand, Singapore and Malaysia, followed by ST/164 and ST/16, which were
found in nine and six strains, respectively. A strain obtained from the soil in Malaysia was assigned to ST/46.

**Table 1.** Strain types (ST) of 108 *A. baumannii* strains isolated in Southeast Asia according to the Pasteur scheme.

| MLST | Frequency | Country |
|------|-----------|---------|
| ST/2 | 62        | Vietnam (6), Myanmar (19), Thailand (33), Singapore (3), Malaysia (1) |
| ST/164 | 9  | Vietnam (1), Myanmar (5), Thailand (3) |
| ST/16 | 6         | Myanmar (2), Thailand (4) |
| ST/23 | 4         | Myanmar (3), Thailand (1) |
| ST/25 | 4         | Myanmar (3), Thailand (1) |
| ST/1 | 3         | Myanmar (3) |
| ST/215 | 3  | Thailand (3) |
| ST/571 | 3  | Vietnam (2), Thailand (1) |
| ST/374 | 2  | Malaysia (2) |
| ST/575 | 2  | Malaysia (2) |
| ST/46 | 1         | Malaysia, soil (1) |
| ST/52 | 1         | Thailand (1) |
| ST/109 | 1     | Myanmar (1) |
| ST/129 | 1     | Taiwan (1) * |
| ST/220 | 1     | Malaysia (1) |
| ST/360 | 1     | Malaysia (1) |
| ST/739 | 1     | Malaysia (1) |
| ST/1411 | 1    | Vietnam (1), new ST |
| ST/1412 | 1    | Vietnam (1), new ST |
| ND | 1         | Thailand (1) |
| 20 | 108       | Total |

* Taiwan is part of the area of Greater China and is used as an example of a trading country with Southeast Asian countries.

The MLST analyses showed that more than half of the human strains (62 out of 107) in the current study were assigned to ST/2 (Pasteur). ST/2 belongs to the international clone II and is the most dominant type globally [26]. In previous studies, most of the CRAb isolates were found belong to the ST2 lineage in strains isolated from Thailand [22] and Myanmar [27] and among strains isolated from all five countries that contributed to this study. From 2008 to 2011, 101 clinical *A. baumannii* strains were isolated from patients in two medical settings in Vietnam. Most of the *A. baumannii* isolates obtained from a hospital in Hanoi were ST/91 and ST/231, whereas almost all strains from a hospital in Ho Chi Minh City were ST/136, ST/195 and ST/254 [16]. ST/1, ST/575 and ST/109 were found only in Myanmar, while ST/46, 220, 360, 374 and ST/739 were found only in Malaysia. ST/52 and ST/215 were found only in Thailand, while ST/129 was found only in Taiwan (Table 1). The published knowledge on the general distribution of STs of *A. baumannii* in the population of Southeast Asia is scarce. These findings are, moreover, in agreement with those of Gaiarsa et al., who demonstrated that the Pasteur scheme is more appropriate for epidemiological studies of *A. baumannii* [28] and proposed it to be the scheme of choice in parallel with cgMLST.

In the current study, we applied the cgMLST scheme included in the SeqSphere+ Ridom software tools, using a gene-by-gene approach to compare and describe the ge-
ographical relationship and the lineage among *A. baumannii* strains from Vietnam and Southeast Asia. The Vietnamese strains showed high diversity. Five strains appeared in three different clusters, and six strains were unique singletons. WGS showed a higher discriminatory power than conventional typing methods [6]. It allows a more precise traceback analysis of the strains, particularly when analyzing carbapenem-resistant *A. baumannii* isolates, which threaten the global healthcare system. We observed 14 different clusters (1 to 14) using cgMLST SeqSphere+ analysis. Clusters 1 and 5 contained isolates from Thailand and Myanmar. However, isolates were obtained from different countries and clustered in two distinctive clusters but shared the same Pasteur sequence type (ST/2). Cluster 11 contained one strain from Thailand and one from Vietnam and shared the same Pasteur sequence type (ST/164). Our analyses demonstrate that the strains are highly diverse. However, there was a good correlation between MLST and cgMLST clusters. All strains in the same cluster were assigned to the same ST. The predominant type ST/2 based on the Pasteur scheme could be sub-divided into eleven different clusters and distinct lineages when cgMLST was employed. These results show a superior discriminatory ability of cgMLST compared to the conventional MLST [29].

2.2. Antibiotic Susceptibility Testing (AST) and AMR Determinants of Vietnamese Strains

Antibiotic resistance is a serious problem in the Asia-Pacific region, and *A. baumannii* strains are among the most accounted for Gram-negative bacteria (GNB) causing urinary tract infections in this region [30]. Ten out of eleven *A. baumannii* strains originating from Vietnam were MDR, displayed resistance to fluoroquinolones (ciprofloxacin and levofloxacin), carbapenems (imipenem, meropenem and ertapenem), third-generation cephalosporins (cefotaxime, ceftazidime, ceftazidime/avibactam and cefotolozane/tazobactam), fourth-generation cephalosporins (cefepime), piperacillin, piperacillin/tazobactam, chloramphenicol and fosfomycin and were susceptible only to colistin. Resistance to the tetracycline derivative tigecycline (MIC = 1 µg/mL) and trimethoprim/sulfamethoxazole (MIC > 4/76 µg/mL) was seen in nine strains (Table S3). All MDR strains (*n* = 10) were carbapenem-resistant with MIC values of >8 µg/mL to imipenem (IMP) and >0.5 µg/mL to ertapenem (ERT). The MIC values to meropenem (MER) ranged from 32 to 128 µg/mL, higher than the MIC value (16 µg/mL) reported for the *A. baumannii* DMS06669 strain isolated in a Vietnam hospital [31]. From 2012 to 2014, resistance to cephalosporins, fluoroquinolones and carbapenems was >90% among 904 *A. baumannii* isolates from patients with hospital-acquired or ventilator-associated pneumonia in Vietnam [13], while colistin (MIC90, ≤0.25 mg/L) and tigecycline (MIC90, 4 mg/L) showed appreciable activity against *A. baumannii*. MIC90 for meropenem and imipenem was >32 mg/L in more than 80% of 74 strains isolated from tracheal aspirate specimens taken from patients with suspected ventilator-associated pneumonia from January 2011 to June 2012 [32]. All MIC values of tested antibiotics in the current study are shown in Table 2. One strain showed susceptibility to almost all tested antibiotics. It may have been isolated from a patient from rural areas where the use of antibiotics is seldom because they always depend on medicinal plants in treatment, in contrast to other patients.

The genome of all strains (100%) harbored *ant*(3′)-Ia, which confers resistance to aminoglycosides, while the *armA* gene was found in eight (72.7%) isolates, and *aph*(6)-Ia and *aph*(3′)-Ib were found in seven (63.6%) isolates. All isolates harbored at least one of the *bla*OXA-51 variants and *bla*ADC-25, which confer resistance to β-lactams, while *bla*OXA-23 and *bla*TEM-1 were found in nine (81.8%) and six (54.5%) isolates, respectively. More than half of the Vietnamese strains harbored genes that confer resistance to tetracyclines, sulfonamides and macrolides (Table S4).
Table 2. The MIC (µg/mL) for the 11 sequenced Vietnamese A. baumannii strains as evaluated with MICRONAUT software.

| ID     | CIP | LEV | AMK | COL | CMP | FOS | TGC | T/S | PIP | PIT | CTX | CAZ | CAA | CTA | CEP | IMP | MER | ERT |
|--------|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| 18Y0059| >2  | >2  | >32 | ≤1  | >16 | >64 | >1  | >4/76| >16 | ≤64 | >2  | >128| >16/4| >8/4 | >128| >8  | >64 | >0.5|
| 18Y0060| >2  | >2  | >32 | ≥1  | >16 | >64 | >1  | >4/76| >16 | ≤64 | >2  | >128| >16/4| >8/4 | >128| >8  | >64 | >0.5|
| 18Y0061| >2  | >2  | >32 | ≤1  | >16 | >64 | >1  | 4/76 | >16 | >64 | >2  | >128| >16/4| >8/4 | >64 | >8  | >64 | >0.5|
| 18Y0064| >2  | >2  | >32 | ≤1  | >16 | >64 | >1  | >4/76| >16 | ≤64 | >2  | >128| >16/4| >8/4 | >64 | >8  | >128| >0.5|
| 18Y0065| >2  | >2  | >32 | ≤1  | >16 | >64 | >1  | <2/38| >16 | >64 | >2  | >128| >16/4| >8/4 | >128| >8  | >64 | >0.5|
| 18Y0066| >2  | >2  | >32 | ≤1  | >16 | >64 | >1  | 4/76 | >16 | >64 | >2  | >128| >16/4| >8/4 | >64 | >8  | >128| >0.5|
| 18Y0067| >2  | >2  | >32 | ≤1  | >16 | >64 | >1  | >4/76| >16 | >64 | >2  | >128| >16/4| >8/4 | >64 | >8  | >64 | >0.5|
| 18Y0068| ≥0.25| >0.5| ≤4  | ≤1  | >16 | >64 | ≤0.25| >4/76| ≤8  | ≤4/4 | ≤2  | ≤1 | ≤1/4 | ≤1/4 | ≤1  | ≤1 | ≤1  | ≤125| >0.25|
| 18Y0072| >2  | >2  | >4  | ≤1  | >16 | >64 | ≤0.25| <1/19| >16 | >64 | >2  | >128| >16/4| >8/4 | >128| >8  | >32 | >0.5|
| 18Y0074| >2  | >2  | >32 | ≤1  | >16 | >64 | 0.5 | >4/76| >16 | >64 | >2  | >128| >16/4| >8/4 | >32 | >8  | >64 | >0.5|
| 18Y0075| >2  | >2  | >32 | ≤1  | >16 | >64 | >1  | >4/76| >16 | >64 | >2  | >128| >16/4| >8/4 | >128| >8  | >64 | >0.5|

The prevalence of MDR/CRAb was very high. The results are in agreement with previous reports: most CRAb strains display resistance to at least one compound in three or more antimicrobial categories and are designated as MDR [6,7]. Resistance against imipenem was seen in 91.6% of XDR A. baumannii strains isolated from patients in three hospitals in southern Vietnam between 2012 and 2014 [17]. A strain resistant to all tested classes of antibiotics except ciprofloxacin and colistin was isolated from the sputum of a patient with hospital-acquired pneumonia at the general hospital of Dong Nai [19]. Examination of 79 strains recovered from patients with pneumonia in Thong Nhat Dong Nai General Hospital showed carbapenem resistance and MDR in 80% and 90% of isolates, respectively [33]. This study highlights the very high prevalence of MDR/CRAb in the General Hospital of Phutho, Hanoi. Clinically, A. baumannii has become a notorious hospital-acquired pathogen worldwide [26], and the emergence of MDR/CRAb has serious consequences in the healthcare system in Southeast Asia [10]. The general distribution of A. baumannii is increasing in hospitals in Vietnam [19], Thailand [22,34], the Philippines [23], Malaysia [21], Indonesia [24] and Taiwan [35]. Our results support previous data from Southeast Asian hospitals, where a substantial increase in the MDR/CRAb isolation rate was demonstrated [10]. Identification of the genetic determinants associated with carbapenem resistance in A. baumannii is helping to explain the continuous selection and ongoing transmission within the healthcare system of Vietnam and other Southeast Asian countries.

2.3. Predicted Phenotype and AMR Determinants of A. baumannii from Southeast Asia

To compare the findings of AMR determinants in Vietnam to neighboring countries, we downloaded and analyzed A. baumannii sequence data from Southeast Asia. The frequency and percentage of resistance genes conferring specific antibiotic resistance were identified in 108 A. baumannii whole genomes. Our WGS approach identified different AMR genes, among which at least 47 genes confer resistance to β-lactams, 18 to aminoglycosides, 8 to phenicolics, 4 to tetracyclines, 3 to sulfonamides and 3 to macrolides and lincosamide (Table S4).

2.3.1. Resistance to β-Lactams

In total, forty-seven AMR genes mediating resistance to β-lactams, including cephalosporins and carbapenemases, were identified. The Ambler class D β-lactamases were present in all strains. The variants of the intrinsic blaOXA-51-like carbapenemase gene were found in 103 (95.5%) strains; blaOXA-66 was the most frequent and was found in 68 (61%) isolates, followed by blaOXA-91, blaOXA-402 and blaOXA-64. Five isolates (4.5%) were devoid of blaOXA-51-like. The blaOXA-23 variant was found in 90 (83%) isolates, and blaOXA-58 was found in 13 (12%) isolates. In terms of Ambler class A β-lactamases, blaTEM-1 was...
found in 55 (51%) isolates, followed by \( \text{bla}_{\text{PER}} \) in 8 (7.5%) isolates, \( \text{bla}_{\text{CARB}}-16 \) in 5 isolates, \( \text{bla}_{\text{VEB}}-21 \) in 4 isolates and \( \text{bla}_{\text{SHV}}-5 \) in 1 strain. Regarding Ambler class B \( \beta \)-lactamases, \( \text{bla}_{\text{NDM}}-1 \) was found in nine (8%) isolates, and \( \text{bla}_{\text{IMP}}-14 \) was found in two strains. Sixteen \textit{Acinetobacter}-derived cephalosporinase \( \text{bla}_{\text{ADC}} \) variants of the Ambler class C \( \beta \)-lactamases were identified. The \( \text{bla}_{\text{ADC}}-25 \) variant was the most frequent variant and was detected in all isolates (100%), followed by \( \text{bla}_{\text{ADC}}-73 \) in 50 (46%) isolates, \( \text{bla}_{\text{ADC}}-52 \) in 8 isolates, \( \text{bla}_{\text{ADC}}-30 \) and \( \text{bla}_{\text{ADC}}-26 \) in 7 isolates and \( \text{bla}_{\text{ADC}}-76 \) in 6 isolates (Table S4).

Four Ambler classes of \( \beta \)-lactamases (i.e., classes A, B, C and D) were identified in the current study. Various resistance genes conferring resistance to carbapenems and cephalosporins were found in \textit{A. baumannii} isolated from Southeast Asia. The \( \text{bla}_{\text{OXA}}-23 \) and \( \text{bla}_{\text{OXA}}-51 \)-like variants were among the most frequent AMR genes identified. Both are currently spreading on plasmids and associated with resistance to all \( \beta \)-lactam compounds, including carbapenems [21,36]. The ADC \( \beta \)-lactamases are cephalosporinases with extended-spectrum resistance to cephalosporins. All strains harbored \( \text{bla}_{\text{ADC}}-25 \), and approximately half of the strains (46%) harbored \( \text{bla}_{\text{ADC}}-73 \), which are considered significant determinants responsible for cephalosporin resistance in \textit{A. baumannii} [37]. More than half of the strains (51%) harbored \( \text{bla}_{\text{TEM}}-1 \). It encodes a class A \( \beta \)-lactamase and has been found in CRAB strains from different regions worldwide [38–40].

### 2.3.2. Resistance to Aminoglycosides

In the 108 analyzed strains, 18 AMR genes conferring resistance to aminoglycosides were identified. Aminoglycoside-modifying enzymes (AMEs), including acetyltransferases (AAcTs), methyltransferase (\textit{armA}), phosphotransferases (\textit{APHs}) and nucleotidyltransferases (\textit{ANTs}), were identified. The new subclass of intrinsic aminoglycoside nucleotidyltransferase, \textit{ANT}(3")-IIa, was widely distributed and was found in almost all strains (99%), followed by the intrinsic aminoglycoside O-phosphotransferase \textit{aph}(6)-IId and \textit{aph}(3")-Ib found in 83 (77%) strains. To date, over a hundred AMEs have been described, and AACs represent the largest group of AMEs [41]. In the current study, AAC genes were detected in 12% of the isolates, while \textit{ANT} and \textit{APH} genes were distributed in almost all isolates (Table 3). Moreover, the intrinsic aminoglycoside methyltransferase (MET) \textit{armA} was found in 73 (76.5%) isolates. \textit{ArmA} is the most important class of plasmid-mediated MET enzymes that confer resistance to gentamicin in \textit{Enterobacteriaceae} [42]. Aminoglycosides are broad-spectrum antibiotics used against a wide range of infections caused by Gram-negative bacteria in clinical settings. However, their efficacy has been reduced by resistance development [7,43]. This finding highlights the diversity of aminoglycoside-resistant \textit{A. baumannii} strains mediated by \textit{ANTs}, \textit{APHs}, \textit{armA} and AAcTs of AMEs in Southeast Asian countries.

### 2.3.3. Resistance to Phenicoles, Tetracyclines, Macrolides, Sulphonamides and Rifamycin

At least eight genes encoding resistance to phenicoles (\textit{cmlA}, \textit{catB8}, \textit{cmlA1}, \textit{floR}, \textit{cmlA5}, \textit{catB3}, \textit{catA1} and \textit{cmlA6}) were identified. \textit{CatB8} was the most frequent and found in 14 (13%) isolates, followed by \textit{cmlA} in 6 isolates and \textit{floR} in 4 isolates. Most \textit{A. baumannii} isolates are intrinsically resistant to chloramphenicol [44]. However, genes encoding resistance to phenicoles were found only in 13% of the isolates. Four genes encoding resistance to tetracycline (\textit{tetB}, \textit{tet39}, \textit{tetA}, \textit{tetM}) were identified. \textit{TetB} was identified in 79 (73%) isolates, and \textit{tet39} was identified in 14 (14%) isolates. \textit{TetB} is a tetracycline efflux protein that confers resistance to tetracycline but not to tigecycline [45]. In the current survey, it was found in the majority of strains. Two genes encoding macrolide resistance were identified. \textit{Mph.E} and \textit{msr.E} were identified in 77% and 79% of the isolates, respectively. Three genes encoding resistance to sulphonamides (\textit{sul1}, \textit{sul2} and \textit{sul3}) were identified. \textit{Sul2} and \textit{sul1} variants were found in 56% and 29% of the strains, respectively, while \textit{sul3} was found in one isolate. Both \textit{sul2} and \textit{sul1} are mediated by transposons and plasmids [46]. The presence of one or both genes in \textit{A. baumannii} isolates might confer resistance to trimethoprim/sulphamethoxazole. The \textit{arr-2} gene confers resistance to rifampicin was found in 14 (13%) strains (Table 3). Several resistance mechanisms for different antibiotic
classes exist in \textit{A. baumannii} \cite{47} and were found in the current study. Genes encoding resistance to macrolides, tetracyclines, sulfonamides and phenicoles were seen in 77\%, 73\%, 66\% and 13\% of the isolates, respectively. The circulation of genes at such high frequency in Southeast Asia is alarming and highlights the urgent need to take effective control measures.

Table 3. AMR genes detected in 108 whole-genome sequences of \textit{A. baumannii} originating from Southeast Asia.

| Antibiotic Class | AMR Resistance Genes | Mechanism | Predicted Phenotype | Origin of Strains |
|------------------|-----------------------|-----------|---------------------|-------------------|
| **Aminoglycosides** |                       |           |                     |                   |
| **Gene Family**   | **Frequency (%)**     | **Mechanism** |                     |                   |
| \textit{ant(3\textsuperscript{*})-Ia} | 107 (99\%) | NUT: Nucleotidyltransferase | Streptomycin, spectinomycin | Viet, Myan, Thai, Sing, Mala, Tiaw |
| \textit{aph(3\textsuperscript{*})-Ib} | 83 (77\%) | PHT: Phosphotransferase | Streptomycin | Viet, Myan, Thai, Sing, Mala, Tiaw |
| \textit{aph(6)-Id} | 83 (77\%) | PHT: Phosphotransferase | Streptomycin | Viet, Myan, Thai, Sing, Mala, Tiaw |
| \textit{armA\textsubscript{1}} | 73 (63.5\%) | MET: Methyltransferase | Gentamicin | Viet, Myan, Thai, Sing, Mala, Tiaw |
| \textit{aph(3\textsuperscript{*})-Ia} | 53 (49\%) | PHT: Phosphotransferase | Kanamycin | Viet, Myan, Thai, Sing, Mala, Tiaw |
| \textit{aadA1} | 22 (20\%) | NUT: Nucleotidyltransferase | Streptomycin | Viet, Myan, Thai, Sing, Tiaw |
| \textit{aph(3\textsuperscript{*})-Via} | 15 (14\%) | PHT: Phosphotransferase | Amikacin, kanamycin | Viet, Myan, Thai |
| \textit{ant(2\textsuperscript{*})-Ia} | 14 (13\%) | NUT: Nucleotidyltransferase | Gentamicin, kanamycin | Viet, Myan, Thai |
| \textit{aac(6\textsuperscript{*})-Ib} | 12 (11\%) | ACT: Acetyltransferase | Gentamicin | Viet, Myan, Thai, Tiaw |
| \textit{aac(3)-Ild} | 11 (10\%) | ACT: Acetyltransferase | Gentamicin | Myan, Thai |
| **\textbeta-lactams** |                       |           |                     |                   |
| **Gene Family**   | **Frequency (%)**     | **Mechanism** |                     |                   |
| \textit{blaOXA-51-like} | 103 (95.5\%) | Ambler class D \beta-lactamases | \beta-lactam (carbapenem) | Viet, Myan, Thai, Sing, Mala, Tiaw |
| \textit{blaOXA-66} | 68 (61\%) | blaOXA-51 variant | \beta-lactam (carbapenem) | Viet, Myan, Thai, Sing, Mala, Tiaw |
| \textit{blaOXA-91} | 10 (9\%) | blaOXA-51 variant | \beta-lactam (carbapenem) | Viet, Myan, Thai |
| \textit{blaOXA-23} | 90 (83\%) | Ambler class D \beta-lactamases | \beta-lactam (carbapenem) | Viet, Myan, Thai, Sing, Mala, Tiaw |
| \textit{blaOXA-58} | 13 (12\%) | Ambler class D \beta-lactamases | \beta-lactam (carbapenem) | Viet, Myan, Thai, Mala |
| \textit{blaTEM-1} | 55 (51\%) | Ambler class A \beta-lactamases | \beta-lactam | Viet, Myan, Thai, Sing, Mala |
| \textit{blaADC-25} | 108 (100\%) | Ambler class C \beta-lactamases | \beta-lactam (cephalosporin) | Viet, Myan, Thai, Sing, Mala, Tiaw |
| \textit{blaADC-73} | 50 (46\%) | Ambler class C \beta-lactamases | \beta-lactam (cephalosporin) | Viet, Myan, Thai |
| \textit{blaNDM-1} | 9 (8.5\%) | Ambler class B \beta-lactamases | \beta-lactam (carbapenem) | Viet, Myan, Thai |
| **Phenicoles** |                       |           |                     |                   |
| \textit{catB8} | 14 (13\%) | Enzymes inactivation | Chloramphenicol | Viet, Myan, Thai, Tiaw |
| **Macrolide** |                       |           |                     |                   |
| \textit{mph.E} | 83 (77\%) | Enzymes inactivation | Macrolide | Viet, Myan, Thai, Sing, Mala, Tiaw |
| \textit{msr.E} | 85 (79\%) | Antibiotic efflux | Macrolide | Viet, Myan, Thai, Sing, Mala, Tiaw |
| **Sulfonamides** |                       |           |                     |                   |
| \textit{sl1} | 31 (29\%) | Antibiotic target replacement | Sulfonamide | Viet, Myan, Thai, Sing, Mala, Tiaw |
| \textit{sl2} | 71 (66\%) | Antibiotic target replacement | Sulfonamide | Viet, Myan, Thai, Sing, Mala, Tiaw |
| **Tetracyclines** |                       |           |                     |                   |
| \textit{tet.39} | 15 (14\%) | Antibiotic efflux | Tetracycline | Viet, Myan, Thai |
| \textit{tet.B} | 79 (73\%) | Antibiotic efflux | Tetracycline | Viet, Myan, Thai, Sing, Mala, Tiaw |
| **Rifamycin** |                       |           |                     |                   |
| \textit{arr-2} | 14 (13\%) | Rifamycin | Myan, Thai |                 |

Viet (Vietnam), Myan (Myanmar), Thai (Thailand), Sing (Singapore), Mala (Malaysia), Tiaw (Taiwan).
2.3.4. Antibiotic Efflux Pumps

Four categories of efflux pumps were found in *A. baumannii* isolates of Southeast Asian origin, including the resistance-nodulation-division (RND) superfamily, the major facilitator superfamily (MFS), the multidrug and toxic compound extrusion (MATE) family and the small multidrug resistance (SMR) family transporters. Among these different pumps, the MFS transporter (*amvA*), RND (*adeFGH, adeIJK and adeL*), SMR (*abeS*) and MATE (*abeM*) were most frequent and found in almost all isolates (99–100%). RND efflux pump-coding genes (*adeN, adeR, adeS* and *adeAB*) were found in 92.2%–94.5% of the strains (Table S4). Resistance mediated by antibiotic efflux pump-encoding genes is well documented in *A. baumannii* [7,20]. Efflux pumps play significant roles in developing AMR in *A. baumannii* [48]. The chromosomally encoded tripartite efflux pump *adeABC* is a worldwide-distributed RND superfamily efflux pump in *A. baumannii* and was found in approximately 80% of the clinical strains. The overexpression of *adeABC* efflux pumps is mainly associated with reduced susceptibility to tigecycline [49], fluoroquinolones, tetracycline, chloramphenicol and erythromycin, confers resistance to aminoglycosides [50,51] and may contribute resistance to carbapenems [39]. The circulation of numerous efflux pumps with high frequency suggests a significant rise in *A. baumannii* antibiotic resistance in Southeast Asia.

Acinetobacter baumannii AbaF was found in 104 (96%) strains isolated from all contributing countries. *AbaF* is a major facilitator superfamily (MFS) antibiotic efflux pump interfering with protein synthesis. Its expression in *E. coli* increases resistance to fosfomycin. The high frequency of resistance to fosfomycin was seen in the tested strains and in a previous study on *A. baumannii* [7]. It has been reported that the *abaF* gene is involved in fosfomycin resistance in *A. baumannii* and plays a role in biofilm formation and virulence mechanisms [52].

2.4. Acquired Resistance in *A. baumannii* of Southeast Asian Origin

Examination of all strains in the current study, either sequenced strains from Vietnam or downloaded genomes from PlasmidFinder [53] and Platon [54] as two different tools to investigate the potential presence of plasmids, failed to detect plasmids or plasmid replicons in all strains, except for one strain from Myanmar which harbored only two replicons (Col.MG828._1 and Col8282_1). This information is included in Table S5. Moreover, the comprehensive ResFinder server [55] was used to investigate the potential acquired AMR genes in the *A. baumannii* strains. ResFinder can identify acquired genes and chromosomal mutations mediating AMR in a total or partial DNA sequence. Several genes encoding resistance to aminoglycosides, β-lactams, tetracycline, sulfonamides and macrolides were found. The *Acinetobacter* -derived cephalosporinase *blaADC.25* conferring resistance to cephalosporin was identified in all isolates (100%), and the two genes conferring resistance to carbapenems, *blaOXA-23* and *blaOXA-51-like* (*blaOXA-66* variant), were detected in 83% and 61% of the strains, respectively. Moreover, different variants of *blaTEM, blaCARB, blaIMP, blaVEB, blaper, aph.6.Id, aph.3.1a, aph.3.1b, tet, sul* and *armA* were identified using the ResFinder database. The presence of various plasmids in the genome of *A. baumannii* [56] and its ability to acquire foreign DNA [57,58] enhance the acquisition of AMR genes. Several reports suggested that mobile genetic elements play significant roles in the horizontal transfer of AMR genes in *A. baumannii*, particularly genes that confer resistance to aminoglycosides, chloramphenicol and tetracycline [59–61]. Identification of a wide variety of AMR genes by ResFinder in the current study highlights the role of horizontal gene transfer in the development of resistance in *A. baumannii* in Southeast Asia.

3. Materials and Methods

3.1. Identification of Bacterial Isolates and Antibiotics Susceptibility Testing (AST)

In total, eleven non-repetitive *A. baumannii* strains isolated from Vietnamese patients at the General Hospital of Phutho, Hanoi, were received by the Institute of Bacterial Infections and Zoonoses (IBIZ, Jena) for species confirmation and typing. The strains were isolated from blood, sputum, CSF and abscess samples of patients admitted to
the hospital in 2017. The agreement for receiving the genetic samples according to the Nagoya Protocol was obtained from the Natural Resources and Environment Minister. No additional ethical approval was required. All strains were identified at species level using a combination of matrix-assisted laser desorption/ionization mass spectrometry (MALDI-TOF MS) with a log value of >2.300 and the intrinsic \textit{bla}$_{\text{OXA-51-like}}$-PCR [62]. The minimum inhibitory concentration (MIC) was determined by the broth microdilution method using an automated MICRONAUT-S system (Micronaut, MERLIN Diagnostics GmbH, Bornheim-Hersel Germany) according to the manufacturer’s instructions. The results were evaluated as susceptible, intermediate and resistant automatically with the built-in MICRONAUTS software. The MIC values for a panel of the 18 antibiotics were interpreted according to the Clinical and Laboratory Standards Institute (CLSI) breakpoint guidelines available for \textit{A. baumannii} as previously described [7].

3.2. Whole-Genome Sequencing and Collection of Sequence Data from Southeast Asia

DNA extraction was performed using the High-Pure template preparation kit (Roche Applied Sciences, Mannheim, Germany) according to the manufacturer’s instructions. The library preparation and paired-end sequencing on an Illumina MiSeq sequencer were performed for the 11 Vietnamese \textit{A. baumannii} strains as previously described [7]. Briefly, the Nextera XT DNA Library Prep Kit (Illumina, Inc., San Diego, CA, USA) was utilized to prepare the sequencing library, followed by paired-end sequencing on an Illumina MiSeq sequencer (Illumina, San Diego, CA, USA).

Raw sequencing data were downloaded from NCBI’s Sequence Read Archive (https://www.ncbi.nlm.nih.gov/sra/?term, accessed on 1 November 2020) according to the following criteria: Search for species [\textit{Acinetobacter baumannii}] with the geo_loc_name_country_continent [Asia] provided 1025 genomes. The results were filtered, and the search items following the Library Layout [paired], Library Source [genomic] and Platform [Illumina] were eligible for inclusion. Raw sequence data of all \textit{A. baumannii} strains belonging to the search criteria from Southeast Asian countries were included in the study. Data of 103 \textit{A. baumannii} strains were extracted. Of these, 49 \textit{A. baumannii} genomes were from Thailand (BioProjects: PRJNA623108, PRJNA627433, PRJNA647677 and PRJNA389557), 38 were from Myanmar (BioProject: PRJDB8528), 11 were from Malaysian BioProjects (PRJNA565663 and PRJNA185400), four were from a Singapore BioProject (PRJNA627433), and one was from a Taiwanese BioProject (PRJNA627433). No genomes were found for Vietnam, Indonesia, the Philippines, Cambodia, Laos, Brunei and Timor-Leste (Table 4).

| No. | Country       | Geographical Location | No. of Strains | No. of Strains Analyzed | Source of Sequence |
|-----|---------------|-----------------------|----------------|-------------------------|--------------------|
| 1   | Thailand      | Southeast Asia        | 49            | 49                      | NCBI               |
| 2   | Myanmar       | Southeast Asia        | 38            | 37                      | NCBI               |
| 3   | Malaysia      | Southeast Asia        | 11            | 7                       | NCBI               |
| 4   | Vietnam       | Southeast Asia        | 11            | 11                      | IBIZ/FLI           |
| 5   | Singapore     | Southeast Asia        | 4             | 3                       | NCBI               |
| 6   | Indonesia     | Southeast Asia        | 0             | 0                       | -                  |
| 7   | Philippines   | Southeast Asia        | 0             | 0                       | -                  |
| 8   | Cambodia      | Southeast Asia        | 0             | 0                       | -                  |
| 9   | Laos          | Southeast Asia        | 0             | 0                       | -                  |
| 10  | Brunei        | Southeast Asia        | 0             | 0                       | -                  |
| 11  | Timor-Leste   | Southeast Asia        | 0             | 0                       | -                  |
| 12  | Taiwan        | East Asia/Trade country| 1             | 1                       | NCBI               |
|     | Total         |                       | 114           | 108                     |                    |
3.3. Bioinformatic Data Analysis

Data analysis of sequences from eleven samples sequenced within this study and downloaded sequences was performed with the pipeline WGSBAC (v2.0.0) [7,63,64]. In short, raw sequencing data quality was controlled by WGSBAC with FastQC (v. 0.11.5) [65], and coverage was determined. Shovill (v. 1.0.4), based on SPAdes (v3.14.0) [25], was used for assembly. Assembly quality was checked with QUAST (v. 5.0.2) [66]. Kraken 2 (v. 2.0.7 beta) [67], in combination with the database MiniKraken (v2), was used to classify reads and assemblies and to check for contamination. In silico determination of classical multilocus sequence typing (MLST) was performed based on the assembled genomes using mlst software (v. 2.16.1) according to the Acinetobacter baumannii#2 scheme published by Diancourt and coworkers and referred to as the Pasteur scheme [68]. For antimicrobial resistance profiling and determination of AMR genes, the databases from NCBI AMR Finder Plus [69], ResFinder [55] and CARD [70] were used. Core-Genome MLST (cgMLST) was performed by employing the software Ridom SeqSphere+, version 5.1.0 (Ridom GmbH, Münster, Germany) [71]. PlasmidFinder [53] and Platon [54] were used to investigate the potential presence of plasmids and plasmid replicons.

4. Conclusions and the Way Forward

Southeast Asia encompasses eleven countries with a wide diversity in history, culture and religion: Brunei, Myanmar (Burma), Cambodia, Timor-Leste, Indonesia, Laos, Malaysia, the Philippines, Singapore, Thailand and Vietnam. According to the Southeast Asia Infectious Disease Clinical Research Network, a previous multinational, multicenter cross-sectional study showed that Acinetobacter was among the causative pathogens of sepsis in South Asian countries [72] and that CRAb was the most common nosocomial pathogen associated with infection in ICUs in this region [10]. In silico analysis of 108 whole genomes of A. baumannii strains isolated from Southeast Asia successfully assigned 107 strains into distinct STs using the Pasteur scheme. MLST analysis confirmed the considerable diversity of A. baumannii, and ST/2 was the most prevalent sequence type. The strains harbored a wide variety of AMR genes mediating resistance mostly to β-lactams (including cephalosporins and carbapenems), aminoglycosides, phenicols, tetracyclines, sulfonamides and macrolides. However, the strain resistance phenotype is unknown except for Vietnamese strains. Several antibiotic resistance mechanisms for various antibiotic classes were observed, including β-lactamases, aminoglycoside-modifying enzymes, permeability defects, alteration and replacement of antibiotic target sites, enzymatic inactivation, multidrug efflux pumps and acquisition of AMR genes. A. baumannii combines clonal spread with high genetic flexibility. The clonal spread of ST/2 is obvious, but there is a lot of evolutionary dynamic outside ST/2 and, most concerning, also within ST2. Aminoglycosides mediating resistance genes ant(3′)-Ia, aph(6)-Id, armA and aph(3′)-Ib, and blaOXA-64, blaOXA-23, blaTEM-1 and blaADC-73 genes mediating resistance to β-lactams, as well as tetB mediating resistance to tetracyclines, are the highest variable genes within ST/2. These might be genes playing a role in improving the adaptation to the environment. Therefore, both decreased antibiotic consumption and infection prevention and control (IPC) (mainly tackling the clinical component of the spread) are required to counteract this pathogen’s spread.

In general, the prevalence of MDR increases due to limited infection control measures and the lack of antimicrobial stewardship teams in healthcare settings. Moreover, antibiotics are sold without a prescription in rural and urban pharmacies in many developing countries, including Southeast Asian countries [73]. Notably, awareness of antibiotic resistance is missing, particularly in rural areas [73]. Uncontrolled travel throughout this region and transboundary trade of animals and foodstuffs have contributed to increasing AMR prevalence. Resistance to carbapenems is increasingly being reported in Southeast Asia health facilities where the antibiotic is not routinely used and is emerging despite its restricted uses due to high costs. A way forward for this region is to design multinational collaborative efforts geared towards investigating the molecular epidemiology of...
CRAB and its burden on healthcare systems and understanding the underlying genetic mechanisms associated with resistance to carbapenems. In 2013, the Vietnamese Ministry of Health was the first ministry in the WHO Western Pacific region to develop a national action plan on AMR. The antimicrobial resistance reference laboratory and surveillance program were initiated in Vietnam in 2017 [74]. However, within a region characterized by open borders, an environment with high prevalences of AMR organisms and patient self-treatment, a multinational transboundary surveillance program is urgently needed [75]. Multinational unified antimicrobial stewardship and broad-scale collaboration would ultimately enable the persons in charge to identify existing hotspots, possible reservoirs and possible practices, cultures and attitudes that may predispose different communities to CRAB infections. Control of selling antibiotics without prescription and increasing awareness are required. Implementing the Vietnamese way, i.e., developing a national action plan on AMR and establishing an antimicrobial resistance reference laboratory, is a supreme priority. Such efforts would be critical in creating targeted policies on carbapenem compounds’ rational use and controlling AMR’s dissemination and emergence in general. This will be significant because carbapenems are expected to become cheaper and readily available in the future in most countries.

Supplementary Materials: The following are available online at https://www.mdpi.com/article/10.3390/antibiotics10050563/s1. Table S1: The average read length and the coverage of 108 A. baumannii isolates from Southeast Asia. Table S2: The data of genome assembly of 11 A. baumannii isolates from Vietnam. Table S3: The results of antibiotic susceptibility testing of Vietnamese A. baumannii isolates. Table S4: Metadata, MLST, AMR genes and the predicted antibiotic classes of 108 A. baumannii isolates from Southeast Asia. Table S5: The results of PlasmidFinder for all A. baumannii strains provide some information about the presence of replicons.

Author Contributions: G.W., H.N., L.D.S. and M.W.P. conceptualization, designed research, analyzed data and wrote the paper; J.L. downloaded sequences and performed the bioinformatic analysis; N.H.N. and T.N.M.N. collected the samples and made a preliminary identification of the Vietnamese strains; G.W. performed the work and wrote the first draft. All authors read and approved the final manuscript.

Funding: This research received no external funding.

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: All study data are included in the article and supporting information. The data have also been submitted to the European Nucleotide Archive (ENA). The project accession number is PRJEB43552.

Acknowledgments: We thank Gernot Schmooock, Johannes Solle, Claudia Grosser and Birgit Schikowski for excellent technical assistance.

Conflicts of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

References
1. Kurihara, M.N.L.; Sales, R.O.; Silva, K.E.D.; Maciel, W.G.; Simionatto, S. Multidrug-resistant Acinetobacter baumannii outbreaks: A global problem in healthcare settings. Rev. Soc. Bras. Med. Trop. 2020, 53, e20200248. [CrossRef]
2. Tian, H.; Chen, L.; Wu, X.; Li, F.; Ma, Y.; Cai, Y.; Song, S. Infectious complications in severe acute pancreatitis: Pathogens, drug resistance, and status of nosocomial infection in a university-affiliated teaching hospital. Dig. Dis. Sci. 2019. [CrossRef] [PubMed]
3. Metan, G.; Zarakolu, P.; Otlu, B.; Tekin, I.; Aytac, H.; Bolek, E.C.; Metin, B.C.; Arsava, E.M.; Unal, S. Emergence of colistin and carbapenem-resistant Acinetobacter calcoaceticus-Acinetobacter baumannii (CCR-Acb) complex in a neurological intensive care unit followed by successful control of the outbreak. J. Infect. Public Health 2019. [CrossRef]
4. Pormohammad, A.; Mehdinejadiani, K.; Gholizadeh, P.; Mohtavinejad, N.; Dadashi, M.; Karimaei, S.; Safari, H.; Azimi, T. Global prevalence of colistin resistance in clinical isolates of Acinetobacter baumannii: A systematic review and meta-analysis. Microb. Pathog. 2019. [CrossRef] [PubMed]
5. Theriault, N.; Tillotson, G.; Sandrock, C.E. Global travel and Gram-negative bacterial resistance; implications on clinical management. *Expert Rev. Anti-Infect. Ther.* 2020. [CrossRef]

6. Nodari, C.S.; Cayô, R.; Strelng, A.P.; Lei, F.; Wille, J.; Almeida, M.S.; de Paula, A.I.; Pignatari, A.C.C.; Seifert, H.; Higgins, P.G.; et al. Genomic analysis of carbapenem-resistant *Acinetobacter baumannii* isolates belonging to major endemic clones in South America. *Front. Microbiol.* 2020, 11, 584603. [CrossRef]

7. Wareth, G.; Linde, J.; Hammer, P.; Nguyen, N.H.; Nguyen, T.N.M.; Splettstoesser, W.D.; Makarewicz, O.; Neubauer, H.; Sprague, L.D.; Pletz, M.W. Phenotypic and WGS-derived antimicrobial resistance profiles of clinical and non-clinical *Acinetobacter baumannii* isolates from Germany and Vietnam. *Int. J. Antimicrob. Agents* 2020, 56, 106127. [CrossRef] [PubMed]

8. Niu, T.; Xiao, T.; Guo, L.; Yu, W.; Chen, Y.; Zheng, B.; Huang, C.; Yu, X.; Xiao, Y. Retrospective comparative analysis of risk factors and outcomes in patients with carbapenem-resistant *Acinetobacter baumannii* bloodstream infections: Cefoperazone-sulbactam associated with resistance and tigecycline increased the mortality. *Infect. Drug Resist.* 2018, 11, 2021–2030. [CrossRef]

9. Stewartson, A.J.; Marimuthu, K.; Sengupta, S.; Allignol, A.; El-Bouseary, M.; Carvalho, M.J.; Hassan, B.; Delgado-Ramirez, M.A.; Arora, A.; Bagga, R.; et al. Effect of carbapenem resistance on outcomes of bloodstream infection caused by *Enterobacteriaceae* in low-income and middle-income countries (PANORAMA): A multinational prospective cohort study. *Lancet. Infect. Dis.* 2019, 19, 601–610. [CrossRef]

10. Suwantarat, N.; Carroll, K.C. Epidemiology and molecular characterization of multidrug-resistant Gram-negative bacteria in Southeast Asia. *Antimicrob. Resist. Infect. Control* 2016, 5, 15. [CrossRef] [PubMed]

11. Kiratisin, P.; Chongthaleong, A.; Tan, T.Y.; Lagamayo, E.; Roberts, S.; Garcia, J.; Davies, T. Comparative in vitro activity of carbapenems against major Gram-negative pathogens: Results of Asia-Pacific surveillance from the COMPACT II study. *Int. J. Antimicrob. Agents* 2012, 39, 311–316. [CrossRef]

12. Phu, V.D.; Nadjm, B.; Duy, N.H.A.; Co, D.X.; Mai, N.T.H.; Trinh, D.T.; Campbell, J.; Khiem, D.P.; Quang, T.N.; Loan, H.T.; et al. Ventilator-associated respiratory infection in a resource-restricted setting: Impact and etiology. *J. Intensive Care* 2017, 5, 69. [CrossRef]

13. Biedenbach, D.J.; Giao, P.T.; Hung Van, P.; Su Minh Tuyet, N.; Thi Thanh Tuan, N.; Bu Thanh Tuan, N.; Badal, R.E. Antimicrobial-resistant *Pseudomonas aeruginosa* and *Acinetobacter baumannii*: From patients with hospital-acquired or ventilator-associated pneumonia in Vietnam. *Clin. Ther.* 2016, 38, 2098–2105. [CrossRef]

14. Nhu, N.T.K.; Lan, N.P.H.; Campbell, J.L.; Parry, C.M.; Thompson, C.; Tuyen, H.T.; Hoang, N.V.M.; Tam, P.T.T.; Le, V.M.; Ngà, T.V.T.; et al. Emergence of carbapenem-resistant *Acinetobacter baumannii* as the major cause of ventilator-associated pneumonia in intensive care unit patients at an infectious disease hospital in southern Vietnam. *J. Med. Microbiol.* 2014, 63, 1386–1394. [CrossRef] [PubMed]

15. Trung, N.T.; Van Son, T.; Quyen, D.T.; Anh, D.T.; Sang, V.V.; Lam, N.X.; Manh, N.D.; Duong, V.P.; Cuong, B.T.; Tuyen, Q.D.; et al. Significance of nucleic acid testing in diagnosis and treatment of post-neurosurgical meningitis caused by multidrug-resistant *Acinetobacter baumannii*: A case report. *J. Med. Case Rep.* 2016, 10, 313. [CrossRef]

16. Tada, T.; Miyoshi-Akiyama, T.; Kato, Y.; Ohmagari, N.; Takeshita, N.; Hung, N.V.; Phuong, D.M.; Thu, T.A.; Binh, N.G.; Anh, N.Q.; et al. Emergence of 16S rRNA methylase-producing *Acinetobacter baumannii* and *Pseudomonas aeruginosa* isolates in hospitals in Vietnam. *BMJ Infect. Dis.* 2013, 13, 251. [CrossRef] [PubMed]

17. Tran Anh, N.; Nga, T.V.T.; Tuan, H.M.; Tran, N.S.; Y, D.M.; Vinh Chau, N.V.; Baker, S.; Duong, H.H.T. Molecular epidemiology and antimicrobial resistance phenotypes of *Acinetobacter baumannii* isolated from patients in three hospitals in southern Vietnam. *J. Med. Microbiol.* 2017, 66, 46–53. [CrossRef]

18. Tran, G.M.; Ho-Le, T.P.; Ha, D.T.; Tran-Nguyen, C.H.; Nguyen, T.S.M.; Pham, T.T.N.; Nguyen, T.A.; Nguyen, D.A.; Hoang, H.Q.; Tran, N.V.; et al. Patterns of antimicrobial resistance in intensive care unit patients: A study in Vietnam. *BMJ Infect. Dis.* 2017, 17, 429. [CrossRef]

19. Si-Tuan, N.; Ngo, H.M.; Nhat, L.D.; Nguyen, C.; Pham, H.Q.; Hung, N.T. Genomic features, whole-genome phylogenetic and comparative genomic analysis of extreme-drug-resistant ventilator-associated pneumonia *Acinetobacter baumannii* strain in a Vietnam hospital. *Infect. Genet. Evol.* 2020, 80, 101478. [CrossRef]

20. Leus, I.V.; Weeks, J.W.; Bonífay, V.; Smith, L.; Richardson, S.; Zgurskaya, H.I. Substrate specificities and efflux efficiencies of RND efflux pumps of *Acinetobacter baumannii*. *J. Bacteriol.* 2018, 200. [CrossRef]

21. Rao, M.; Rashid, F.A.; Shukor, S.; Hashim, R.; Ahmad, N. Detection of antimicrobial resistance genes associated with carbapenem resistance from the whole-genome sequence of *Acinetobacter baumannii* isolates from Malaysia. *Can. J. Infect. Dis. Med. Microbiol.* 2020, 2020, 5021064. [CrossRef]

22. Thirapanmethee, K.; Srisiri, A.N.T.; Houngsaitong, J.; Montakantikul, P.; Khuntayaporn, P.; Chomnawang, M. OXA-Type β-Lactamase genes among carbapenem-resistant *Acinetobacter baumannii* clinical isolates in Thailand. *Antibiotics* 2020, 9, 864. [CrossRef] [PubMed]

23. Velasco, J.M.; Valderama, M.T.; Margulieux, K.; Diones, P.C.; Peacock, T.; Navarro, F.C.; Liao, C.; Chua, D.; Macareo, L.; Crawford, J.; et al. Comparison of carbapenem-resistant microbial pathogens in combat and non-combat wounds of military and civilian patients seen at a tertiary military hospital, Philippines (2013–2017). *Mil. Med.* 2020, 185, e197–e202. [CrossRef]

24. Karuniawati, A.; Saharman, Y.R.; Lestari, D.C. Detection of carbapenemase encoding genes in *Enterobacteriaceae, Pseudomonas aeruginosa*, and *Acinetobacter baumannii* isolated from patients at Intensive Care Unit Cipto Mangunkusumo Hospital in 2011. *Acta Med. Indones.* 2013, 45, 101–106.
25. Bankevich, A.; Nurk, S.; Antipov, D.; Gurevich, A.A.; Dvorkin, M.; Kulikov, A.S.; Lesin, V.M.; Nikolenko, S.I.; Pham, S.; Prijibelski, A.D.; et al. SPAdes: A new genome assembly algorithm and its applications to single-cell sequencing. *J. Comput. Biol.* 2012, 19, 455–477. [CrossRef]

26. Hamidian, M.; Nigro, S.J. Emergence, molecular mechanisms and global spread of carbapenem-resistant *Acinetobacter baumannii*. *Microb. Genom.* 2019, 5. [CrossRef]

27. Tada, T.; Uchida, H.; Hishinuma, T.; Watanabe, S.; Tohya, M.; Kuwahara-Arai, K.; Mya, S.; Zan, K.N.; Kirikae, T.; Tin, H.H. Molecular epidemiology of multidrug-resistant *Acinetobacter baumannii* isolates from hospitals in Myanmar. *J. Glob. Antimicrob. Resist.* 2020, 22, 122–125. [CrossRef] [PubMed]

28. Gaiarsa, S.; Batisti Biffignandi, G.; Esposito, E.P.; Castelli, M.; Jolley, K.A.; Brisse, S.; Sasaera, D.; Zarrilli, R. Comparative analysis of the two *Acinetobacter baumannii* multilocus sequence typing (MLST) schemes. *Front. Microbiol.* 2019, 10, 930. [CrossRef] [PubMed]

29. Hua, X.; Zhang, L.; He, J.; Leptihn, S.; Yu, Y. Population biology and epidemiological studies of *Acinetobacter baumannii* in the era of whole-genome sequencing: Is the oxford scheme still appropriate? *Front. Microbiol.* 2020, 11, 775. [CrossRef] [PubMed]

30. Jean, S.S.; Coombs, G.; Ling, T.; Balaji, V.; Rodrigues, C.; Mikamo, H.; Kim, M.J.; Rajasekaram, D.G.; Mendoza, M.; Tan, T.Y.; et al. Epidemiology and antimicrobial susceptibility profiles of pathogens causing urinary tract infections in the Asia-Pacific region: Results from the Study for Monitoring Antimicrobial Resistance Trends (SMART), 2010–2013. *Int. J. Antimicrob. Agents* 2016, 47, 328–334. [CrossRef]

31. Si-Tuan, N.; Ngoc, H.M.; Hang, P.T.T.; Nguyen, C.; Van, P.H.; Huong, N.T. New eight genes identified at the clinical multidrug-resistant *Acinetobacter baumannii* DMS06669 strain in a Vietnam hospital. *Ann. Clin. Microbiol. Antimicrob.* 2017, 16, 74. [CrossRef]

32. Le Minh, V.; Nhu, N.T.K.; Phat, V.V.; Thompson, C.; Lan, N.P.H.; Nga, T.V.T.; Tam, P.T.T.; Tuyen, H.T.; Nhau, T.D.H.; Van Hao, N.; et al. In vitro activity of colistin in antimicrobial combination against carbapenem-resistant *Acinetobacter baumannii* isolated from patients with ventilator-associated pneumonia in Vietnam. *J. Med. Microbiol.* 2015, 64, 1162–1169. [CrossRef] [PubMed]

33. Jeong, S.; Coombs, G.; Ling, T.; Balaji, V.; Rodrigues, C.; Mikamo, H.; Kim, M.J.; Rajasekaram, D.G.; Mendoza, M.; Tan, T.Y.; et al. Epidemiology and antimicrobial susceptibility profiles of pathogens causing urinary tract infections in the Southern Vietnam. *Antibiotics* 2019, 8, 148. [CrossRef] [PubMed]

34. Leungtongkam, U.; Thummeepak, R.; Wongprachan, S.; Thongsuk, P.; Kitti, T.; Ketwong, K.; Runcharoen, C.; Chantratita, N.; Sitthisak, S. Dissemination of bla(OXA-23), bla(OXA-24), bla(OXA-58), and bla(NDM-1) Genes of *Acinetobacter baumannii* isolates from four tertiary hospitals in Thailand. *Microb. Drug Resist.* 2018, 24, 55–62. [CrossRef] [PubMed]

35. Chen, T.L.; Lee, Y.T.; Kuo, S.C.; Hsueh, P.R.; Chang, F.Y.; Siu, L.K.; Fung, C.P. Emergence and distribution of plasmids bearing the bla(OXA-51-like) gene with an upstream ISAba1 in carbapenem-resistant *Acinetobacter baumannii* isolates in Taiwan. *Antimicrob. Agents Chemother.* 2010, 54, 4575–4581. [CrossRef]

36. Khurshid, M.; Rasool, M.H.; Ashfaq, U.A.; Aslam, B.; Waseem, M.; Wang, M. Dissemination of bla(OXA-23)-harbouring carbapenem-resistant *Acinetobacter baumannii* clones in Pakistan. *J. Glob. Antimicrob. Resist.* 2020, 21, 357–362. [CrossRef]

37. Lee, S.Y.; Oh, M.H.; Yun, S.H.; Choi, C.W.; Park, E.C.; Song, H.S.; Lee, H.; Yi, Y.S.; Shin, J.; Chung, C.; et al. Genomic characterization of extensively drug-resistant *Acinetobacter baumannii* strain, KAB03 belonging to ST451 from Korea. *Infect. Genet. Evol.* 2018, 65, 150–158. [CrossRef]

38. Vranić-Ladavac, M.; Bedenić, B.; Minandri, F.; Ištok, M.; Frančula-Zaninović, S.; Ladavac, R.; Visca, P. Carbapenem resistance and acquired class D beta-lactamases in *Acinetobacter baumannii* from Croatia 2009–2010. *Eur. J. Clin. Microbiol. Infect. Dis.* Off. Publ. Eur. Soc. Clin. Microbiol. 2014, 33, 471–478. [CrossRef]

39. Zhu, L.J.; Pan, Y.; Gao, C.Y.; Hou, P.F. Distribution of carbapenemases and efflux pump in carbapenem-resistance *Acinetobacter baumannii*. *Ann. Clin. Lab. Sci.* 2020, 50, 241–246. [CrossRef]

40. Krizova, L.; Poiriel, L.; Nordmann, P.; Nemeč, A. TEM-1 β-lactamase as a source of resistance to sulbactam in clinical strains of *Acinetobacter baumannii*. *J. Antimicrob. Chemother.* 2013, 68, 2786–2791. [CrossRef] [PubMed]

41. Kim, S.Y.; Park, Y.J.; Yu, J.K.; Kim, Y.S. Aminoglycoside susceptibility profiles of *Enterobacter cloacae* isolates harboring the aac(6')-Ib gene. *Korean J. Lab. Med.* 2011, 31, 279–281. [CrossRef] [PubMed]

42. Fritsche, T.R.; Castanheira, M.; Miller, G.H.; Jones, R.N.; Armstrong, E.S. Detection of methyltransferases conferring high-level resistance to aminoglycosides in *Enterobacteriaceae* from North America, Europe, and Latin America. *Antimicrob. Agents Chemother.* 2008, 52, 1843–1845. [CrossRef]

43. Mortazavi, S.M.; Farshadzadeh, Z.; Janabadi, S.; Musavi, M.; Shahi, F.; Moradi, M.; Khoshnood, S. Evaluating the frequency of carbapenem and aminoglycoside resistance genes among clinical isolates of *Acinetobacter baumannii* from Ahvaz, south-west Iran. *New Microbes New Infect.* 2020, 38, 100779. [CrossRef] [PubMed]

44. Roca, I.; Martí, S.; Espinal, P.; Martínez, P.; Gibert, I.; Vila, J. CraA, a major facilitator superfamily efflux pump associated with chloramphenicol resistance in *Acinetobacter baumannii*. *Antimicrob. Agents Chemother.* 2009, 53, 4013–4014. [CrossRef] [PubMed]

45. Roberts, M.C. Update on acquired tetracycline resistance genes. *FEMS Microbiol. Lett.* 2005, 245, 195–203. [CrossRef] [PubMed]

46. Sköld, O. Resistance to trimethoprim and sulfonamides. *Vet. Res.* 2001, 32, 261–273. [CrossRef]

47. Gordon, N.C.; Fareham, D.W. Multidrug-resistant *Acinetobacter baumannii*: Mechanisms of virulence and resistance. *Int. J. Antimicrob. Agents* 2010, 35, 219–226. [CrossRef]

48. Coyne, S.; Courvalin, P.; Perichon, B. Efflux-mediated antibiotic resistance in *Acinetobacter* spp. *Antimicrob. Agents Chemother.* 2011, 55, 947–953. [CrossRef]
49. Lee, Y.T.; Chen, H.Y.; Yang, Y.S.; Chou, Y.C.; Chang, T.Y.; Hsu, W.J.; Lin, I.C.; Sun, J.R. AdeABC Efflux pump controlled by adeRS two-component system conferring resistance to tigecycline, omadacycline and eravacycline in clinical carbapenem-resistant Acinetobacter nosocomialis. Front. Microbiol. 2020, 11, 584789. [CrossRef]

50. Abbott, I.; Cerqueira, G.M.; Bhuiyan, S.; Peleg, A.Y. Carbapenem resistance in Acinetobacter baumannii. Laboratory challenges, mechanistic insights and therapeutic strategies. Expert Rev. Anti-Infect. Ther. 2013, 11, 395–409. [CrossRef]

51. Ranjarb, R.; Zayeri, S.; Afshar, D. High frequency of adeA, adeB and adeC genes among Acinetobacter baumannii isolates. Iran. J. Public Health 2020, 49, 1539–1545. [CrossRef]

52. Sharma, A.; Sharma, R.; Bhattacharyya, T.; Bhandu, T.; Pathania, R. Fosfomycin resistance in Acinetobacter baumannii is mediated by efflux through a major facilitator superfamily (MFS) transporter-AbaF. J. Antimicrob. Chemother. 2017, 72, 68–74. [CrossRef]

53. Carattoli, A.; Zankari, E.; García-Fernández, A.; Voldby Larsen, M.; Lund, O.; Villa, L.; Møller Aarestrup, F.; Hasman, H. In silico detection and typing of plasmids using PlasmidFinder and plasmid multilocus sequence typing. Antimicrob. Agents Chemother. 2014, 58, 3895–3903. [CrossRef]

54. Schwengers, O.; Barth, P.; Falgenhauer, L.; Hain, T.; Chakraborty, T.; Goessmann, A. Platon: Identification and characterization of bacterial plasmid contigs in short-read draft assemblies exploiting protein sequence-based replicon distribution scores. Microb. Genom. 2020, 6. [CrossRef]

55. Zankari, E.; Hasman, H.; Cosentino, S.; Vestergaard, M.; Rasmussen, S.; Lund, O.; Aarestrup, F.M.; Larsen, M.V. Identification of acquired antimicrobial resistance genes. J. Antimicrob. Chemother. 2012, 67, 2640–2644. [CrossRef] [PubMed]

56. Salgado-Camargo, A.D.; Castro-Jaimes, S.; Gutierrez-Rios, R.M.; Lozano, L.F.; Altamirano-Pacheco, L.; Silva-Sanchez, J.; Pérez-Oseguera, A.; Volkow, P.; Castillo-Ramirez, S.; Cevallos, M.A. Structure and evolution of Acinetobacter baumannii plasmids. Front. Microbiol. 2020, 11, 1283. [CrossRef]

57. Krizova, L.; Dijkstra, L.; Nemec, A. Diversity and evolution of AbaR genomice resistance islands in Acinetobacter baumannii strains of European clone I. Antimicrob. Agents Chemother. 2011, 55, 3201–3206. [CrossRef] [PubMed]

58. Lin, M.F.; Lan, C.Y. Antimicrobial resistance in Acinetobacter baumannii: From bench to bedside. World J. Clin. Cases 2014, 2, 787–814. [CrossRef] [PubMed]

59. Lin, M.F.; Chang, K.C.; Yang, C.Y.; Yang, C.M.; Xiao, C.C.; Kuo, H.Y.; Liou, M.L. Role of integrons in antimicrobial susceptibility patterns of Acinetobacter baumannii. Jpn. J. Infect. Dis. 2010, 63, 440–443. [PubMed]

60. Huang, L.Y.; Chen, T.L.; Lu, P.L.; Tsai, C.A.; Cho, W.L.; Chang, F.Y.; Fung, C.P.; Siu, L.K. Dissemination of multidrug-resistant, class 1 integron-carrying Acinetobacter baumannii isolates in Taiwan. Clin. Microbiol. Infect. Off. Publ. Eur. Soc. Clin. Microbiol. Infect. Dis. 2008, 14, 1010–1019. [CrossRef]

61. Rabea, R.A.; Zaki, M.E.S.; Fahmy, E.M.; Fathelbab, A. Molecular study of nodulation division genes and integron genes in Acinetobacter baumannii. Clin. Lab. 2020, 66. [CrossRef]

62. Turton, J.F.; Woodford, N.; Glover, J.; Yarde, S.; Kaufmann, M.E.; Pitt, T.L. Identification of Acinetobacter baumannii by detection of the blaOXA-51-like carbapenemase gene intrinsic to this species. J. Clin. Microbiol. 2006, 44, 2974–2976. [CrossRef] [PubMed]

63. Linde, J.; Homeier-Bachmann, T.; Dangel, A.; Riehm, J.M.; Sundell, D.; Öhrman, C.; Forsman, M.; Tomaso, H. Genotyping of Francisella tularensis subsp. holarctica from hares in Germany. Microorganismos 2020, 8, 1932. [CrossRef]

64. García-Soto, S.; Abdell-Gil, M.Y.; Tomaso, H.; Linde, J.; Methner, U. Emergence of multidrug-resistant Salmonella enterica Subspecies enterica serovar infantis of multilocus sequence type 2283 in German broiler farms. Front. Microbiol. 2020, 11, 1741. [CrossRef] [PubMed]

65. Andrews, S. FastQC: A Quality Control Tool for High Throughput Sequence Data. v. 0.11.5. Available online: https://www.bioinformatics.babraham.ac.uk/projects/fastqc/ (accessed on 1 August 2020).

66. Gurevich, A.; Saveliev, V.; Vyahhi, N.; Tesler, G. QUAST: Quality assessment tool for genome assemblies. Bioinformatics 2013, 29, 1072–1075. [CrossRef] [PubMed]

67. Wood, D.E.; Lu, J.; Langmead, B. Improved metagenomic analysis with Kraken 2. Genome. Biol. 2019, 20, 257. [CrossRef]

68. Diancourt, L.; Passet, V.; Nemec, A.; Dijkstra, L.; Brisse, S. The population structure of Acinetobacter baumannii: Expanding multiresistant clones from an ancestral susceptible genetic pool. PLoS ONE 2010, 5, e10034. [CrossRef] [PubMed]

69. Feldgarden, M.; Brover, V.; Haft, D.H.; Prasad, A.B.; Slotta, D.J.; Tolstoy, I.; Tyson, G.H.; Zhao, S.; Hsu, C.H.; McDermott, P.F.; et al. Validating the AMRFinder tool and resistance gene database by using antimicrobial resistance genotype-phenotype correlations in a collection of isolates. Antimicrob. Agents Chemother. 2019, 63. [CrossRef]

70. Jia, B.; Raphenya, A.R.; Alcock, B.; Waglechner, N.; Guo, P.; Tsang, K.K.; Lago, B.A.; Dave, B.M.; Pereira, S.; Sharma, A.N.; et al. CARD 2017: Expansion and model-centric curation of the comprehensive antibiotic resistance database. Nucleic Acids Res. 2017, 45, D566–D573. [CrossRef]

71. Higgins, P.G.; Prior, K.; Harmsen, D.; Seifert, H. Development and evaluation of a core genome multilocus typing scheme for whole-genome sequence-based typing of Acinetobacter baumannii. PLoS ONE 2017, 12, e0179228. [CrossRef]

72. Network, S.A.I.D.C.R. Causes and outcomes of sepsis in Southeast Asia: A multinational, multicentre cross-sectional study. Lancet. Glob. Health 2017, 5, e157–e167. [CrossRef]

73. Nga do, T.T.; Chuc, N.T.; Hoa, N.P.; Hoa, N.Q.; Nguyen, N.T.; Loan, H.T.; Toan, T.K.; Phuc, H.D.; Horby, P.; Van Yen, N.; et al. Antibiotic sales in rural and urban pharmacies in northern Vietnam: An observational study. BMC Pharmacol. Toxicol. 2014, 15, 6. [CrossRef] [PubMed]
74. Kinh, N.V.; Wertheim, H.F.L.; Thwaites, G.E.; Khue, L.N.; Thai, C.H.; Khoa, N.T.; Thi Bich Ha, N.; Trung, N.V.; Crook, D.; van Doorn, H.R. Developing an antimicrobial resistance reference laboratory and surveillance programme in Vietnam. *Lancet Glob. Health* 2017, 5, e1186–e1187. [CrossRef]

75. Li, R.; van Doorn, H.R.; Wertheim, H.F.L.; Khue, L.N.; Ha, N.T.B.; Dat, V.Q.; Hanh, C.T.; Nga, D.T.T.; Trang, N.N.M.; Nadjm, B.; et al. Combating antimicrobial resistance: Quality standards for prescribing for respiratory infections in Vietnam. *Lancet Glob. Health* 2016, 4, e789. [CrossRef]