Research Article

Detection of Antimicrobial Resistance Genes Associated with Carbapenem Resistance from the Whole-Genome Sequence of Acinetobacter baumannii Isolates from Malaysia

Mohan Rao,1 Fairuz A. Rashid,1 Surianti Shukor,1 Rohaidah Hashim,1 and Norazah Ahmad2

1Bacteriology Unit, Infectious Disease Research Centre, Institute for Medical Research, Ministry of Health, Kuala Lumpur, Malaysia
2Infectious Disease Research Centre, National Institute of Health, Ministry of Health, Shah Alam, Malaysia

Correspondence should be addressed to Mohan Rao; mogaeviridae@gmail.com

Received 7 October 2019; Revised 20 December 2019; Accepted 9 January 2020; Published 2 April 2020

Academic Editor: José Ramón Blanco

Copyright © 2020 Mohan Rao et al. This is an open access article distributed under the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

Background. The spread of carbapenem-resistant A. baumannii (CrAb) is gaining worldwide attention. The spread of this pathogen is largely due to its ability to acquire various resistance genes of intrinsic and extrinsic origins that confer unpredictable susceptibility to β-lactams. The aim of this study was to analyze β-lactamase genetic compositions of CrAb in Malaysia. Methods. Whole-genome sequencing (WGS) was carried out on 13 CrAb isolates from clinical samples in Malaysia from 2011 to 2016. Results. Endotracheal aspirate was the dominant clinical sample source (n = 6), and only one isolate was obtained from wound swab. A total of 6 sequence types (STs) of the Oxford scheme were identified, including 4 reported STs and 2 novel STs. Eleven isolates were classified into clonal complex 92 (CC92/ICII), among which ST195 and ST208 were the most prevalent STs. All 13 CrAb isolates harbored multiple β-lactamase genes. blaOXA-23 (n = 13) and blaOXA-66 (n = 11) were the dominant carbapenemase gene families found in these isolates. All isolates harbor blaADC, blaOXA-51-like and blaOXA-23-like genes. blatem (n = 7), blaNDM-1 (n = 3), blaCARB-8 (n = 1), and blaper-3 (n = 1) are amongst other β-lactamase genes found in this study. ISAba1 was found upstream to blaOXA-23 (n = 13), blaOXA-66 (n = 1), and blaADC (n = 11). All blaNDM-1 isolates had ISAba125 (mobile genetic element) upstream to the genes. All isolates were positive for Tn2006/2008 and Tn2009 but were negative for Tn2007. Conclusion. Most of the isolates were grouped under the CC92 clonal complex which belongs to international clonal lineage 2. These findings predict that carriage of carbapenem-resistant genes possibly constitutes the underlying basis of high level of international clone II prevalence. Therefore, molecular surveillance and antimicrobial stewardship are essential in implementing policies to prevent and control the spread of CrAb in hospital settings.

1. Introduction

Acinetobacter baumannii (A. baumannii) is an infectious agent that has been the leading cause of hospital-acquired infections [1]. It is an opportunistic pathogen that poses significant threat to public health and associated with high mortality [2]. A. baumannii nosocomial infection is now common throughout the world [3, 4]. Selection of an appropriate empirical antimicrobial agent is extremely difficult due to its unpredictable antimicrobial resistance genes which are commonly acquired via mobile genetic elements [5].

A. baumannii belongs to a group of clinically important organism, known as ESKAPE. It is predominantly found among health care-associated organisms that have the potential of substantial antibiotic resistance [6]. A. baumannii infection usually involves excretory organ systems that contain high level of fluids. The most common sites of infection are respiratory tract, urinary tract, and peritoneal cavity and highly associated with indwelling devices such as endotracheal tube, urinary catheter, Tenckhoff catheter, and intravascular catheter [7].

Carbapenem-resistant A. baumannii (CrAb) was identified as the critical organism based on the global priority
pathogen list proposed by the World Health Organization (WHO). It has been concluded that development of new antimicrobial is the current focus globally [8]. CrAb has become a major concern among healthcare facilities due to its rising prevalence. In countries of the Arab League and Vietnam, prevalence of CrAb has been reported ranging from 50 to 88%, whereas in the United Kingdom, it ranges from 40 to 70% [9, 10]. According to the National Surveillance Antibiotic Resistance database, CrAb prevalence in Malaysia ranges from 50 to 60% and remained static since year 2008 up to 2016 [11]. However, several studies from different hospitals in Malaysia showed CrAb prevalence higher than the national surveillance [12].

Nonjudicious use of antibiotics has led A. baumannii to rapidly acquire antimicrobial resistance genes from the environment. At the same time, selective antimicrobial pressure induces genome rearrangement associated with chromosomally (intrinsic) encoded antimicrobial resistance genes which has resulted in transposition of insertion sequence (IS) as a promoter for various CHDLs [13]. A. baumannii possesses blaOXA-23-like, an intrinsic carbapenem-hydrolysing oxacillinase gene. The expression of this gene may vary with the presence of ISAbd1 as a promoter [14]. It also acquires certain blaOXA and blanon-OXA group genes from plasmids [15]. Predominantly acquired blaOXA-group gene is blaoxa-23-like whereas the most prevalent blanon-OXA group gene is blanDM1 [16].

This study is aimed to analyze the molecular characteristics of 13 A. baumannii isolates obtained from hospitalized patients in Malaysia with underlying carbapenem-resistant phenotype.

2. Methods

Of 1933 A. baumannii isolates collected from various hospitals throughout Malaysia from year 2011 to 2016, we selected 13 carbapenem-resistant A. baumannii (CrAb) isolates that were resistant to carbapenems. No genes (blanDM, blaoxa, blapcr, blavim, and blaim) were found using in-house PCR. These isolates were recovered from patients receiving intensive care and were isolated from respiratory secretion, urine, rectal swabs, and pus. The initial identification test based on biochemical methods was performed using API 20E (bioMérieux, LaPlane, France). Antimicrobial susceptibility was determined by the disc diffusion method for gentamicin, amikacin, ciprofloxacin, cefepime, ceftazidine, aztreonam, imipenem, meropenem, ertapenem, and ampicillin-sulbactam according to Clinical and Laboratory Standards Institute (CLSI) criteria. Minimum inhibitory concentrations (MICs) of imipenem, meropenem, and ertapenem were determined by the E-Test method according to CLSI criteria. CrAb is defined as an A. baumannii isolate that is resistant to meropenem, ertapenem, and imipenem with an MIC value of ≥4 μg/ml via ETest. Table 1 summarizes study isolates, types of specimen, and the genebank identification.

Total DNA of these strains was extracted by using a MasterPure™ DNA Purification Kit (Epicentre, Madison, Wisconsin, USA) and quantified using Qubit 2.0™ Fluorometer (Life Technologies, Carlsbad, CA). DNA libraries were prepared using a Nextera DNA Flex Library Prep Kit (Illumina Inc.), according to the manufacturer’s instructions. Sequence data for all strains were obtained using an Illumina Nextseq platform (Illumina Inc., San Diego, CA, USA). Raw sequence quality trimming was carried out as described by SPAdes version 3.9.1 for de novo assembly and confirmation [17].

Average nucleotide identity (ANI) was calculated by using a gANI tool calculator, ANI calculator software version 1.0. ANI values above 95% between genomes of these isolates denote the same species [18]. Multilocus sequence typing (MLST) analysis was streamlined via the MLST program against PubMLST database via MLST version 2.6 software. Oxford scheme of A. baumannii was used for MLST analysis. The 7 housekeeping genes were gltA, gyrB, gdhB, recA, cpn60, gpi, and rpoD [19]. New alleles and STs were submitted to the curator of the database, and new ST numbers were allotted. Clonal complexes were assigned by eBURST and were defined as single locus and double-locus variants with an outgroup A. baumannii strain ATCC 17978 as a reference (GenBank accession number: CP000521.1) [20]. Antimicrobial resistance genes (AMR) were confirmed by the CARD and resistance gene identifier (Resfinder) via Abricate-Version 0.8 software [21]. kSNP version 3.0 was

<table>
<thead>
<tr>
<th>Isolate</th>
<th>Specimen type</th>
<th>NCBI biosample no.</th>
<th>GenBank accession no.</th>
</tr>
</thead>
<tbody>
<tr>
<td>A. baumannii</td>
<td>CRE1071/16</td>
<td>SAMN11513371</td>
<td>SWLT00000000</td>
</tr>
<tr>
<td>A. baumannii</td>
<td>CRE1159/16</td>
<td>SAMN11513372</td>
<td>SWLS00000000</td>
</tr>
<tr>
<td>A. baumannii</td>
<td>CRE157/16</td>
<td>SAMN11513373</td>
<td>SWLR00000000</td>
</tr>
<tr>
<td>A. baumannii</td>
<td>CRE158/16</td>
<td>SAMN11513374</td>
<td>SWLQ00000000</td>
</tr>
<tr>
<td>A. baumannii</td>
<td>CRE245/15</td>
<td>SAMN11513375</td>
<td>SWLP00000000</td>
</tr>
<tr>
<td>A. baumannii</td>
<td>CRE341/15</td>
<td>SAMN11513376</td>
<td>SWLQ00000000</td>
</tr>
<tr>
<td>A. baumannii</td>
<td>CRE400/16</td>
<td>SAMN11513377</td>
<td>SWLN00000000</td>
</tr>
<tr>
<td>A. baumannii</td>
<td>CRE449/14</td>
<td>SAMN11513378</td>
<td>SWLM00000000</td>
</tr>
<tr>
<td>A. baumannii</td>
<td>CRE569/14</td>
<td>SAMN11513379</td>
<td>SWLL00000000</td>
</tr>
<tr>
<td>A. baumannii</td>
<td>CRE645/15</td>
<td>SAMN11513380</td>
<td>SWLK00000000</td>
</tr>
<tr>
<td>A. baumannii</td>
<td>CRE648/15</td>
<td>SAMN11513381</td>
<td>SWLJ00000000</td>
</tr>
<tr>
<td>A. baumannii</td>
<td>CRE85/16</td>
<td>SAMN11513382</td>
<td>SWLJ00000000</td>
</tr>
<tr>
<td>A. baumannii</td>
<td>CRE98/16</td>
<td>SAMN11513383</td>
<td>SWLH00000000</td>
</tr>
</tbody>
</table>
used to identify pan-genome single-nucleotide polymorphism (SNP) [22]. A SNP phylogenetic tree was drawn based on pairwise whole-genome sequence via Type Genome Server using multiple reference strains that belong to the Acinetobacter baumannii complex group. Ugene-PRO and ISfinder applications were used to analyze the presence of mobile genetic elements [23]. The sequence of this whole-genome shotgun project has been deposited in GenBank under Genome submission: SUB5536145 with the Bio-Project ID: PRJNA539835.

3. Results

The genome sequence size of the 13 isolates in this study ranged from 3,832,120 to 4,246,682 base pair (bp), with contigs ranging from 70 to 104, which encodes 3577 to 4003 coding sequences, 50 to 53 tRNA, 3 rRNA, and 1 tmRNA. Six of 13 (46%) isolates were obtained from endotracheal aspirate followed by urine culture (4 (31%)), rectal swab (2 (15.4%)), and wound swab (1 (7.7%)). No isolate of CrAb was cultured from blood samples. Average nucleotide identity (ANI) of all isolates was above 95% which concludes that they belonged to the same species of bacteria. However, 3 paired isolates shared 100% identity of ANI although obtained from different sources and states. Those isolates were CRE400/16-CRE245/15, CRE645/16-CRE648/15, and CRE1071/16-CRE1159/16. Isolates from our study were compared with a genome of reference strain. The SNP-based phylogenetic tree showed that most of the isolate genomes were closely associated with each other and belonged to international clone II.

MLST analysis with the Oxford scheme in this study revealed a total of 4 defined STs and 2 novel STs (Figure 1). The 2 novel STs of year 2014-2015 were submitted and were assigned as ST1947 and ST1948. ST195, accounting for the largest proportion (5/13, 38%), was the major clonal type followed by ST208 (3/13, 23%), ST938 (2/13, 15.4), ST1418 (1/13, 7.7%), ST1947 (1/13, 7.7%), and ST1948 (1/13, 7.7%). Additionally, ST195, ST208, ST938, and ST1948 were double-locus variants of gyrB, gbdB, and gpi genes interchangeably. eBURST analysis showed that these 3 defined STs along with one novel ST clustered in the same CCs (CC92), which was also referred to as global clonal 2 (GC2)/international clonal II (ICII).

All isolates harbored intrinsic \( \text{bla}_{\text{OXA-51-like}} \) class D carbapenemases. \( \text{bla}_{\text{OXA-66}} \) was the most prevalent (11 (85%)), followed by \( \text{bla}_{\text{OXA-64}} \) and \( \text{bla}_{\text{OXA-91}} \), 1 (8%) of each, respectively. An extrinsic \( \text{bla}_{\text{OXA-3}} \)-type carbapenemase gene found was \( \text{bla}_{\text{OXA-23}} \) (100%), while no isolates contained \( \text{bla}_{\text{OXA-24-like}} \) or \( \text{bla}_{\text{OXA-58-like}} \) gene. Interestingly, all isolates...
Table 2: Resistance genes present in carbapenem-resistant *A. baumannii* from year 2011–2016.

<table>
<thead>
<tr>
<th>Strain ID</th>
<th>State</th>
<th>Source</th>
<th>Oxford MLST</th>
<th>Clonal assigned</th>
<th>blaTEM</th>
<th>blaPER</th>
<th>blaOXA-23</th>
<th>blaOXA-66</th>
<th>blaOXA-91</th>
<th>blaOXA-64</th>
<th>blaNDM</th>
<th>blaADC</th>
<th>blaCARB</th>
<th>ISAbA1-ADC</th>
<th>ISAbA1-OXA23-like</th>
<th>ISAbA1-OXA51-like</th>
<th>ISAbA125-NDM</th>
<th>Transposons</th>
</tr>
</thead>
<tbody>
<tr>
<td>REL07/16</td>
<td>Kelantan</td>
<td>Pus</td>
<td>ST195</td>
<td>CC92/IC2</td>
<td>−</td>
<td>−</td>
<td>+</td>
<td>+</td>
<td>−</td>
<td>+</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
</tr>
<tr>
<td>CRE115/16</td>
<td>Selangor</td>
<td>Urine</td>
<td>ST195</td>
<td>CC92/IC2</td>
<td>−</td>
<td>−</td>
<td>+</td>
<td>+</td>
<td>−</td>
<td>−</td>
<td>+</td>
<td>−</td>
<td>−</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>−</td>
<td>−</td>
</tr>
<tr>
<td>CRE157/16</td>
<td>Perak</td>
<td>ETT</td>
<td>ST195</td>
<td>CC92/IC2</td>
<td>−</td>
<td>−</td>
<td>+</td>
<td>+</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>+</td>
<td>−</td>
<td>−</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>CRE158/16</td>
<td>Perak</td>
<td>ETT</td>
<td>ST195</td>
<td>CC92/IC2</td>
<td>−</td>
<td>−</td>
<td>+</td>
<td>+</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>−</td>
<td>+</td>
</tr>
<tr>
<td>CRE245/15</td>
<td>Kuala Lumpur</td>
<td>Rectal Swab</td>
<td>ST938</td>
<td>CC92/IC2</td>
<td>−</td>
<td>−</td>
<td>+</td>
<td>+</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>+</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>+</td>
</tr>
<tr>
<td>CRE341/15</td>
<td>Kuala Lumpur</td>
<td>Urine</td>
<td>ST1947</td>
<td>CC229</td>
<td>−</td>
<td>+</td>
<td>+</td>
<td>−</td>
<td>+</td>
<td>+</td>
<td>−</td>
<td>−</td>
<td>+</td>
<td>−</td>
<td>+</td>
<td>+</td>
<td>−</td>
<td>+</td>
</tr>
<tr>
<td>CRE400/16</td>
<td>Perak</td>
<td>ETT</td>
<td>ST938</td>
<td>CC92/IC2</td>
<td>+</td>
<td>−</td>
<td>+</td>
<td>+</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>−</td>
<td>+</td>
</tr>
<tr>
<td>CRE449/14</td>
<td>Pahang</td>
<td>ETT</td>
<td>ST1948</td>
<td>CC92/IC2</td>
<td>−</td>
<td>+</td>
<td>+</td>
<td>−</td>
<td>−</td>
<td>+</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>−</td>
<td>+</td>
</tr>
<tr>
<td>CRE596/14</td>
<td>Kuala Lumpur</td>
<td>Rectal Swab</td>
<td>ST208</td>
<td>CC92/IC2</td>
<td>+</td>
<td>−</td>
<td>+</td>
<td>+</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>−</td>
<td>+</td>
</tr>
<tr>
<td>CRE645/15</td>
<td>Kuala Lumpur</td>
<td>Rectal Swab</td>
<td>ST208</td>
<td>CC92/IC2</td>
<td>+</td>
<td>−</td>
<td>+</td>
<td>+</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>−</td>
<td>+</td>
</tr>
<tr>
<td>CRE648/15</td>
<td>Kuala Lumpur</td>
<td>Rectal Swab</td>
<td>ST208</td>
<td>CC92/IC2</td>
<td>+</td>
<td>−</td>
<td>+</td>
<td>+</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>−</td>
<td>+</td>
</tr>
<tr>
<td>CRE85/16</td>
<td>Pahang</td>
<td>Urine</td>
<td>ST418</td>
<td>CC224</td>
<td>−</td>
<td>+</td>
<td>−</td>
<td>+</td>
<td>−</td>
<td>+</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>−</td>
<td>−</td>
</tr>
<tr>
<td>CRE98/14</td>
<td>Sarawak</td>
<td>ETT</td>
<td>ST935</td>
<td>CC92/IC2</td>
<td>−</td>
<td>−</td>
<td>+</td>
<td>+</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>−</td>
<td>−</td>
</tr>
</tbody>
</table>

+ indicates the presence of the resistant gene. MLST sequence type (ST) along with clonal complex is included in the table. *bla*OXA, *bla*NDM, *bla*ADC, *bla*TEM, and *bla*PER are the β-lactamase genes identified. IS: mobile genetic element-insertion sequence; Tn: transposon; ETT: endotracheal tube.
harbored more than one oxacillinase gene. Acinetobacter-derived single-variant cephalosporinase \textit{bla} \textsubscript{ADC} gene was present in all our isolates. A total of 8 (62.5%) isolates harbored \textit{class A} \textit{β}-lactamase gene \textit{bla} \textsubscript{TEM}, 1 isolate carried \textit{bla} \textsubscript{CARB}, and 1 isolate carried \textit{bla} \textsubscript{PER} gene. In addition, 3 (23.1%) isolates carried \textit{class B metallo-β-lactamase (MBL) gene} \textit{bla} \textsubscript{NDM}. Additionally, all isolates were negative for other MBL genes which included \textit{bla} \textsubscript{IMP}, \textit{bla} \textsubscript{VIM}, \textit{bla} \textsubscript{GIM}, and \textit{bla} \textsubscript{SPM}. Table 2 summarizes all the AMR genes detected in this study.

As described in [24], we found that 1 (7.7%) isolate harbors class 1 integron. The presence of mobile genetic element provides strong evidence for the horizontal dissemination of antibiotic resistance genes. At the same time, all \textit{bla} \textsubscript{OXA-23-like} genes were carried by \textit{Tn2006} and \textit{Tn2008} in our study. In addition, we detect insertion sequence (IS) elements in the promoter regions of several AMR genes [25]. All isolates with \textit{bla} \textsubscript{NDM} gene were found to harbor IS\textit{Aba125} upstream to the corresponding gene \textit{bla} \textsubscript{NDM}. IS\textit{Aba1} was found upstream to \textit{bla} \textsubscript{OXA-23} \textsubscript{(n=13)}, \textit{bla} \textsubscript{ADC} \textsubscript{(n=11)}, \textit{bla} \textsubscript{OXA-66} \textsubscript{(n=1)}, and \textit{bla} \textsubscript{CARB} \textsubscript{(n=1)}. IS\textit{91} was found upstream to \textit{bla} \textsubscript{PER} \textsubscript{(n=1)}.

4. Discussion

\textit{A. baumannii} of nosocomial origin has been the leading cause of hospital-acquired infections [1]. The nature of this bacterium is that it can be found in the environment, intrinsically carrying the antibiotic resistance gene and posing a significant threat to public health due to its unpredictable antibiotic susceptibility [2, 4, 5]. This study was aimed to determine the genetic mechanisms conferring carbapenem resistance in our local strains.

Most of the clinical isolates in this study obtained from respiratory secretion (tracheal aspirate, sputum, and bronchial alveolar lavage) were similar to the Malaysian local surveillance study. Up to year 2017, nosocomial \textit{A. baumannii} was commonly isolated from respiratory secretion, followed by blood isolation [11, 26]. Likewise, many studies nationwide shared similar findings. \textit{A. baumannii} preferably colonizes or infects the respiratory tract. Such infection commonly occurs in debilitated patients especially in the ICU. Patients of mechanical ventilation and lengthy hospital stay are at risk of \textit{A. baumannii} infection [27, 28].
ST195 was the frequent sequence types observed in our study. At the same time, as expected, most of these CrAb isolates belong to the CC92/IC2 clonal lineage. The predominance of CC92/IC2 in the present study was similar to reports produced in other neighbouring Asian countries such as China and Thailand and consistent with local studies and reports [29–31]. We also found novel strains of different clonal lineage emerging. Based on our study, these new strains have emerged in 2014 and 2015. In [19, 28, 32], the authors have revealed that emergence of newer strains is caused by inappropriate antibiotic usage. It is crucial to study about epidemiology of sequence types as there is a high chance of emergence of newer strains due to inappropriate antibiotic usage.

The accurate identification of A. baumannii is only possible via molecular methods. Molecular characterization of _bla_ _OXA-51-like_ genes detection is carried out along with RNA polymerase β-subunit gene (_rpoB_) and DNA gyrase B gene (_gyrB_) for A. _baumannii_ species identification [35]. All the isolates involved in this study were positive for _bla_ _OXA-51-like_ _rpoB_, and _gyrB_ genes. This finding resonates along with SNP phylogeny and ANI on species-level identification in this study.

A. _baumannii_ is known for its enzymatic degradation mechanism by β-lactamases [36]. The most common carbapenem resistance mechanism found in our study was the existence of various β-lactamases and mobile genetic elements. _bla_OXA-23-like_ and _bla_OXA-51-like_ were the most prevalent, accounting for 100% carbapenem resistance amongst studied isolates. _bla_OXA-51-like_ gene was detected in all the isolates due to its chromosomal-borne nature, naturally occurring in oxacillinase gene [37].

Meanwhile, _bla_OXA-23-like_ gene can be either plasmid or chromosome-borne, resulting in increased rates of carbapenem resistance in healthcare settings due to its mobility in facilitating horizontal genetic transfer. The acquisition of _bla_OXA-23-like_ gene is a major public health concern for its horizontal dissemination and rapid spread [38].

No isolates harbored _bla_OXA-24-like_, _bla_OXA-48-like_ and _bla_OXA-58-like_ genes. Although these genes are disseminated in Europe and Middle East, they remained rare in our local findings [39, 40]. A variant of _bla_OXA-51-like_ found in this study, namely, _bla_OXA-66_ is commonly found in China [41]. At the same time, the present study observed that both _bla_OXA-23-like_ and _bla_OXA-51-like_ genes are found in all the isolates. These findings were similar to [42] as it common to find _A. baumannii_ isolates harbor _bla_OXA-23-like_ and _bla_OXA-51-like_ in the Asian continent, whereas _bla_OXA-51-like_ and _bla_OXA-58-like_ in the western hemisphere.

MBL carrying _A. baumannii_ isolates are rare nationwide. _bla_NDM_ carrying _A. baumannii_ is not commonly found in our area of study. We have not encountered _bla_NDM_ during previous years or among many local studies [38, 39]. However, to our surprise, a small number (3/13, 24%) of _A. baumannii_ isolates collected during 2014–2016 were positive for _bla_NDM_, indicating recent emergence. Worthy of mentioning is the fact that the isolates carrying _bla_NDM_ gene did not belong to the same clone lineage [43]. This will be the first reporting on _bla_NDM_ gene harboring _A. baumannii_ isolates from clinical samples in Malaysia.

Similar to the _bla_OXA-51-like_ oxacillinase gene, _bla_ADC_ is also a chromosomally encoded acinetobacter-derived
cephalosporinase gene found among all the A. baumannii isolates in this study. This finding indicates intrinsic-species specific gene [44]. blaTEM genes were also found in 3/4 of our isolates. In [45], the authors had demonstrated that carbapenem resistance among CrAb is due to the coexistence of blaOXA-23 and blaTEM. Similar output was observed in this study; however, isolates without blaTEM also exhibit the resistant phenotypes. To a moderate level, blaCARB and blaPER were also detected. No prevalence of colistin-resistant genes was found among these isolates. However, there are A. baumannii isolates found in many case reports from neighbouring countries such as Taiwan, India, and China that are resistant and becoming resistant [46].

CRE341/15 isolate harbors integrase class I indicating sporadic clones. Isolates carrying mobile elements such as integron-encoded integrase gene flanking resistance genes are capable of acquiring and transferring virulence genes via recombination [47]. Transposon played a major role in dissemination of resistant genes. In this study, we observed the presence of transposon Tn2006/2008 in all the isolates carrying blaOXA-23 gene. This finding suggests that blaOXA-23 dissemination might be due to transposition of transposon [48]. In [14], the authors demonstrated that insertion of IS elements upstream to the resistant genes changes the expression level leading to the increased antimicrobial resistance phenotype. Every isolates in this study was found to have ISAba1 upstream to blaOXA,23, blaOXA-66, blaADC,7, and blaCARB. The ISAba125 was also found in the promoter region of all blanDM positive isolates. These findings suggest that the isolates may have other additional mechanisms resistance against carbapenem [49–51].

In summary, we demonstrated the possible clones of A. baumannii resistant to carbapenem and the prevalence of antibiotic resistance genes associated with mobile genetic elements. These findings provide epidemiological data of prevalent local STs as they are getting more diverse and resistant to multiple antibiotics. The presence of insertion sequence may reflect that these organisms readily take up external DNA. These findings are worrisome for its capability of outbreaks and horizontal resistance gene transmission. Molecular surveillance and antimicrobial stewardship are essential in implementing policies to prevent and control the spread of CrAb in hospital settings.

Data Availability

Genome database of this study is available in National Center of Biotechnology Information, NCBI.

Additional Points

Highlights. (i) The spread of carbapenem-resistant Acinetobacter baumannii is gaining global attention. (ii) It is an opportunistic pathogen that poses a significant threat to public health and is associated with high mortality. (iii) Selection of an appropriate empirical antimicrobial agent is extremely difficult due to its unpredictable susceptibility patterns. (iv) The association of a mobile genetic element with the resistant gene is worrisome and presents as an emerging threat to our healthcare settings.

Ethical Approval

This study obtained approval from the National Health Institute Human Research Ethics Committee.

Conflicts of Interest

All authors declare that they have no conflicts of interest.

Acknowledgments

This study was supported by a grant from Ministry of Health, Malaysia (NMRR 18-203-40147). The authors are grateful to the Institute for Medical Research, Kuala Lumpur, and the hospitals in Malaysia for their support and facilities. The authors would like to thank the Director General of Health Malaysia for allowing us to publish our findings.

References


[45] L. Han, J. Lei, J. Xu, and S. Han, “Bla OXA-23-like and bla TEM rather than bla OXA-51-like contributed to a high level of carbapenem resistance in *Acinetobacter baumannii* strains from a teaching hospital in Xi’an, China,” *Medicine*, vol. 96, no. 48, p. e8965, 2017.


