Molecular epidemiology and antimicrobial resistance phenotypes of *Acinetobacter baumannii* isolated from patients in three hospitals in southern Vietnam

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**Abstract**

Multidrug resistance in the nosocomial pathogen *Acinetobacter baumannii* limits therapeutic options and impacts on clinical care. Resistance against carbapenems, a group of last-resort antimicrobials for treating multidrug-resistant (MDR) *A. baumannii* infections, is associated with the expression (and over-expression) of carbapenemases encoded by the *bla*<sub>OXA</sub> genes. The aim of this study was to determine the prevalence of antimicrobial-resistant *A. baumannii* associated with infection in three hospitals in southern Vietnam and to characterize the genetic determinants associated with resistance against carbapenems. We recovered a total of 160 *A. baumannii* isolates from clinical samples collected in three hospitals in southern Vietnam from 2012 to 2014. Antimicrobial resistance was common; 119/160 (74%) of isolates were both MDR and extensively drug resistant (XDR). High-level imipenem resistance (>32 μg ml<sup>-1</sup>) was determined for 109/117 (91.6%) of the XDR imipenem-nonsusceptible organisms, of which the majority (86.7%) harboured the *bla*<sub>OXA-51</sub> and *bla*<sub>OXA-23</sub> genes associated with an ISAb1 element. Multiple-locus variable number tandem repeat analysis segregated the 160 *A. baumannii* into 107 different multiple-locus variable number tandem repeat analysis types, which described five major clusters. The biggest cluster was a clonal complex composed mainly of imipenem-resistant organisms that were isolated from all three of the study hospitals. Our study indicates a very high prevalence of MDR/XDR *A. baumannii* causing clinically significant infections in hospitals in southern Vietnam. These organisms commonly harboured the *bla*<sub>OXA-23</sub> gene with ISAb1 and were carbapenem resistant; this resistance phenotype may explain their continued selection and ongoing transmission within the Vietnamese healthcare system.

**INTRODUCTION**

*Acinetobacter baumannii* has emerged globally as one of the leading causes of nosocomial infections. This emergence is partly associated with the remarkable ability of the organism to become resistant to multiple antimicrobials, which leaves limited treatment options [1]. The use of carbapenems as last-resort antimicrobials for the treatment of *A. baumannii* infections has been hindered by the rapid development of resistance against this important group of antimicrobials [2–4]. Carbapenem resistance in *A. baumannii* is associated with a variety of mechanisms, but expression (and over-expression) of carbapenemases plays the most important role [5, 6]. The main carbapenem-hydrolysing class D β-lactamases in *A. baumannii* include the intrinsic *bla*<sub>OXA-51</sub> and the acquired *bla*<sub>OXA-23</sub>, *bla*<sub>OXA-24/40</sub> and *bla*<sub>OXA-58</sub> β-lactamases [7, 8]. Furthermore, the presence of specific insertion elements, such as an ISAb1, upstream of these *bla*<sub>OXA</sub> genes leads to the up-regulation of their expression, resulting in further decreased susceptibility against carbapenems [9, 10].

The clonal spread of multidrug-resistant (MDR) *A. baumannii* within and among hospitals has been recorded internationally [11, 12]. This trend has been observed in Vietnam, and a significant increase in carbapenem-resistant
A. baumannii was observed in a sentinel Vietnamese infectious disease hospital over the last decade, with a rise in the number of MDR A. baumannii isolates harbouring blaOXA-23 and blaOXA-51 [13]. Here we aimed to further consider the emergence of antimicrobial-resistant A. baumannii in Vietnam. Exploiting a collection of A. baumannii clinical isolates from three hospitals in southern Vietnam between 2012 and 2014, we investigated their antimicrobial susceptibility profile, their carbapenem resistance gene complement and their genetic relatedness.

**METHODS**

**Clinical sample collection, microbial identification and antimicrobial susceptibility testing**

A total of 252 non-duplicate Acinetobacter spp. isolates were cultured from patients admitted to three hospitals in southern Vietnam between 2012 and 2014. The Acinetobacter spp. isolates were further characterized at Oxford University Clinical Research Unit (OUCRU) using classical methods. A total of 252 non-duplicate Acinetobacter spp. isolates from three hospitals in southern Vietnam between 2012 and 2014. The isolates were cultured from patients admitted to three hospitals in southern Vietnam between 2012 and 2014. The Acinetobacter spp. isolates were further characterized at Oxford University Clinical Research Unit (OUCRU) using classical methods.

**Antimicrobial susceptibility testing** was performed by disc diffusion and interpreted according to the Clinical and Laboratory Standards Institute (CLSI) guidelines [17]. The tested antimicrobials varied among hospitals and included amikacin, amoxicillin/sulbactam, ciprofloxacin, ceftazidime, cefpodoxime, cefotaxime, cefoperazone/sulbactam, colistin, cefepime, netilmicin, gentamicin, imipenem, meropenem, levofloxacin, trimethoprim/sulfamethoxazole, ticarcillin/clavulanic acid, piperacillin and piperacillin/tazobactam. Susceptibility against imipenem was measured for all isolates by E-test (bioMérieux) at OUCRU; the CLSI-approved breakpoints for imipenem ≥8 µg ml⁻¹ and ≤2 µg ml⁻¹ were considered as resistant and susceptible, respectively [17].

**Identification of carbapenemase genes and ISAba1**

DNA was extracted from the 160 A. baumannii isolates using the Wizard DNA extraction kit, following the manufacturer’s recommendations (Promega). Extracted DNA from all organisms was diluted to a concentration of 25 ng µl⁻¹ prior to PCR amplification using five specific primer pairs to detect the blaOXA-51, blaOXA-23, blaOXA-24 and blaOXA-58 (Table 1) [18]. PCR amplification was performed in a 25 µl reaction volume containing 5 µl template DNA, 0.2 µM each primer (IDT), 1 x AptaTaq (Roche) master mix and 1 x EvaGreen (Biotium). The PCR amplifications were 40 cycles of 15 s at 95 °C and 60 s at 55 °C. A melting curve analysis was performed under the following conditions: 1 min denaturation at 95 °C, 1 min annealing at 55 °C, 200 cycles of 0.2 °C increments (20 s each) starting at 55 °C. The specific melting temperatures for the blaOXA-23, blaOXA-24, blaOXA-51 and blaOXA-58 PCR amplicons were 82.2±0.5 °C, 78.0±0.5 °C, 78.5±0.5 °C and 80.6±0.5 °C, respectively. The presence of ISAba1 region upstream of blaOXA-23 gene was detected as previously described [19].

**Multiple-locus variable number tandem repeat analysis**

Multiple-locus variable number tandem repeat analysis (MLVA) (as previously described [20] with some modifications [13]) was used to genotype the 160 A. baumannii. Briefly, genomic DNA from each A. baumannii isolate was subjected to three multiplex PCR amplifications in a total volume of 10 µl, which included 2 µl DNA template, 1× buffer enzyme, 1.5 mM MgCl₂, 0.2 mM dNTPs, 0.5 × DoctorBand, 1 U h-taq polymerase (Solgent), and 0.15 µM 3002 F/R, 0.3 µM 1988 F/R and 0.15 µM 3468 F/R for the first PCR amplification, 0.3 µM 3530 F/R, 0.3 µM 2396 F/R and 0.15 µM 845 F/R for the second PCR amplification and 0.3 µM 2240 F/R and 0.4 µM 826 F/R for the third PCR amplification. The PCR programme was as follows: 95 °C denaturation for 15 min followed by 35 cycles of 20 s at 95 °C, 30 s at 50 °C and 120 s at 72 °C and a final cycle of 7 min at 72 °C. The amplicon size and the number of repeat units for a specific amplicon at each locus were determined by capillary electrophoresis fragment analysis and sequenced. The amplicon sequencing was performed by 1-BASE (Malaysia). The resulting data were analysed using BioEdit 7.0.9.0 (http://tandem.bu.edu/cgi-bin/trdb/trdb.exe), which was used to determine the number of repeat units. A minimum-spanning tree displaying the genetic relationships between MLVA types from the whole data set was reconstructed using the Phyloviz program (https://online.phyloviz.net/index). To elucidate clonal relationships, MLVA profiles were analysed using the goeBURST algorithm [21]. Related MLVA types differing in one of eight loci were assigned to a clonal complex (CC).

**Multilocus sequence typing**

Multilocus sequence typing (MLST) was used to genotype 23 A. baumannii isolates that were selected for further characterization. The primers for MLST profiling were accessed from the A. baumannii MLST scheme website (http://pubmlst.org/abauumannii/info/primerst_Oxford.shtml) [22]. Extracted genomic DNA (2 µl) was amplified in a PCR containing 0.4 µM each primer (IDT), 1 U h-taq polymerase (Solgent), 200 µM each dNTP, 3 mM MgCl₂ and 1 x PCR buffer in a total volume of 25 µl. The PCR programme was as follows: 95 °C denaturation for 15 min followed by 40
cycles of 30 s at 95 °C, 30 s at 56 °C and 90 s at 72 °C and a
final cycle of 6 min at 72 °C. PCR amplicons were visualized
on 2 % (w/v) agarose gels and sequenced by 1-BASE
(Malaysia). MLST sequences were uploaded to the A. baum-
nannii MLST sequence type database (http://pubmlst.org/
abaumannii/) to determine the allele and sequence type
(ST).

RESULTS

Antimicrobial resistance in A. baumannii

The 160 A. baumannii isolates from three hospitals during the
period of investigation were recovered from sputum
(64/160; 40 %), pus (8/160; 5 %), blood (3/160; 1.9 %) and
fluid aspirates (3/160; 1.9 %); data regarding the origins of
the additional clinical isolates (82/160, 51.3 %), which were
predominantly from hospital C, were not available. The
majority of patients were adults, with a median age of 71
years (age range, 20–101 years). No differences were
observed between the three hospitals for the source of iso-
lates or patient ages. However, there was a significant dis-
pparity in the sex of the patients, with male patients a more
common source of the isolates (100/160; 62.5 %) than
female patients (P=0.013; Chi-squared test).

Antimicrobial susceptibility testing was performed within
the hospital laboratories at the time of isolation; 17 differ-
ing antimicrobials were tested, with only 5 antimicrobials con-
sistently tested between the three sites. The prevalence of
antimicrobial resistance in the A. baumannii isolates was
high, with >70 % of the organisms tested exhibiting resis-
tance against all assayed antimicrobials. The exceptions
were colistin (13/116; 11 %), cefoperazone/sulbactam (53/90;
58.9 %) and ampicillin/sulbactam (70/108; 64.8 %). The
antimicrobial resistance profile to individual antimicrobials
was similar between locations, with the exception of colistin
(1/78, 1.3 % and 12/38, 31.6 % resistance in hospital C and
A, respectively) and ampicillin/sulbactam (38/44, 86.4 %
and 32/64, 50 % resistance in hospital B and C, respectively)
(Table 2).

By definition, MDR isolates are resistant to at least one
agent in three or more antimicrobial families and
extensively drug-resistant (XDR) isolates are nonsusceptible
to at least one agent in all but one or two antimicrobial fam-
ilies [23]. Using these criteria for the 160 A. baumannii
tested, 119 were MDR, including 27/38 (71.1 %) isolates
from hospital A, 38/44 (86.4 %) isolates from hospital B and
54/78 (69.2 %) isolates from hospital C. Following the above
definitions, all MDR strains were similarly XDR, and a com-
parison of MDR/XDR and non-MDR/non-XDR, in terms of
non-susceptibility, revealed some distinct characteristics.
Notably, XDR strains exhibited high-level resistance against
all antimicrobials tested, except for cefoperazone/sulbactam
(50 %) and colistin (8.6 %). However, the antimicrobial
resistance profiles of the non-XDR isolates were different
from all other bacterial isolates, displaying a higher preva-
ience of resistance against third- and fourth-generation
cephalosporins.

The 119 XDR organisms included 117 (98.3 %) imipenem-
susceptible organisms (Table 3). The two imipenem-
susceptible isolates were alternatively resistant to merope-
nem. High-level imipenem resistance (>32 µg ml
−1) was
determined for 109/117 (91.6 %) of the XDR imipenem-
nonsusceptible organisms. For the 41 non-XDR isolates, 26
(63.4 %) were susceptible and 15 (36.6 %) were resistant to
imipenem (Table 3). The proportion of imipenem-nonsus-
ceptible isolates from hospitals A, B and C were 79.5 %
(62/78), 84.2 % (32/38) and 86.4 % (38/44), respectively; these
proportions were not significantly different (P=0.6; Chi-
squared test).

Genetic determinants associated with carbapenem
resistance in A. baumannii

We next investigated the presence of blaOXA-51, blaOXA-23,
blaOXA-24, blaOXA-58, and ISAba1 in the 160 A. baumannii
isolates. The resulting combinations of carbapenem resis-
tance genes are shown in Table 4. The proportion of isolates
harbouring blaOXA-51, with or without the blaOXA-23 and
blaOXA-58 genes and ISAba1 displayed no significant differ-
ence between the three hospitals (P=0.629, P=0.617 and
P=0.416, respectively). No isolates generated a PCR ampli-
con for the blaOXA-24 gene. The majority of isolates (128/
160; 80 %) carried the blaOXA-51 and the blaOXA-23 gene
accompanied by an upstream ISAba1; 61/78 (78.2 %), 30/38

Table 1. Primers used for PCR amplification of the blaOXA genes

| Primers  | Sequence (5′→3′) | Length (bp) | Temp. (°C) | Product (bp) |
|----------|-----------------|-------------|------------|--------------|
| OXA-23-F | CACTAGGAGAAGCCATGAAGC | 21 | 55.0 | 114 |
| OXA-23-R | CAGCCATTACGAAAACAAATAGC | 22 | 55.0 | 114 |
| OXA-24-F | GCTAAATGCTTTATATCGGGCTAG | 24 | 55.0 | 141 |
| OXA-24-R | ACTGGAACCTGCTGAATAGC | 20 | 55.0 | 141 |
| OXA-51-F | GAAGTAAGCGTGTGGTTGATAG | 22 | 55.0 | 148 |
| OXA-51-R | GCCCTCTGCTAGGAGTATATT | 20 | 55.0 | 148 |
| OXA-58-F | ATATTTAAGTGGAAGTGAAGGCC | 23 | 55.0 | 110 |
| OXA-58-R | CGTGCAATTCCTGATACTACAGG | 23 | 55.0 | 110 |
| ISAba1-F | CAGAATGCAGCAGGTGTT | 17 | 56.0 | 520 |
| ISAba1-R | CGACGAAATACTGACAC | 18 | 56.0 | 520 |
(78.9%) and 37/44 (84.1%) of organisms carrying these genes originated in hospitals A, B and C, respectively.

We found that the majority of isolates (26/27; 96.3%) harbouring the \textit{bla}\textsubscript{OXA-51} gene only were susceptible to imipenem. All isolates (130/160, 81.3%) that carried a \textit{bla}\textsubscript{OXA-51} and \textit{bla}\textsubscript{OXA-23} (with or without IS\textsubscript{Aba1}) exhibited high-level resistance against imipenem (Table 4). However, the imipenem susceptibility of the three isolates harbouring the combination of \textit{bla}\textsubscript{OXA-51} and \textit{bla}\textsubscript{OXA-58} genes was inconclusive, with two susceptible and one nonsusceptible organism.

There was no significant difference in the prevalence of imipenem resistance associated with the differing \textit{bla}\textsubscript{OXA} gene combinations between the three hospitals (Table 4).

**Genetic relatedness of Vietnamese \textit{A. baumannii} isolates**

We assessed the genetic relationship of the 160 \textit{A. baumannii} isolates by MLVA and identified 107 unique MLVA profiles, which assembled into five major groups in the resulting minimum-spanning tree (Fig. 1a). The largest group consisted of 28, 23 and 47 closely related isolates from hospitals A, B and C, respectively. All isolates, except one, from this group were imipenem resistant (Fig. 1b). The four less common groups included isolates from all three hospitals; these groups contained organisms that were both imipenem resistant and imipenem susceptible. A population snapshot of the 107 MLVA profiles generated in goeBURST revealed the presence of 32 singletons and 8 CCs that we named (a) to (h) (Fig. 1c, d). Within the largest CC(d), isolates from the three hospitals formed three well-delineated sub-clusters (Fig. 1a). CC(a) contained four isolates from hospital B, which shared the same uncommon antimicrobial resistance profile: amikacin susceptible, imipenem and gentamicin resistant (Fig. 1a). CC(c) and CC(d) contained a mixed group of MLVA types, each of which was assigned to isolates from different hospitals (Fig. 1b, c).

### Table 2. Antimicrobial resistance profiles of 160 \textit{A. baumannii} isolates from three Vietnamese hospitals

| Antimicrobial agent | Hospital A (n=38) | Hospital B (n=44) | Hospital C (n=78) |
|---------------------|------------------|------------------|------------------|
| CPD                 | NT               | NT               | 100% (53/53)     |
| CTX                 | NT               | NT               | 98.0% (50/51)    |
| CRO                 | 100% (38/38)     | 100% (43/43)     | 98.3% (59/60)    |
| CAZ                 | 92.1% (35/38)    | 90.0% (36/40)    | 98.0% (50/51)    |
| TCC                 | 84.2% (32/38)    | NT               | 92.0% (46/50)    |
| PIP                 | NT               | 88.6% (39/44)    | NT               |
| IMP                 | NT               | 86.4% (38/44)    | 86.9% (53/61)    |
| FEP                 | NT               | 87.8% (36/41)    | 84.8% (56/66)    |
| MEM                 | 86.8% (33/38)    | 86.4% (38/44)    | 83.3% (64/78)    |
| TZP                 | 86.8% (33/38)    | 88.6% (39/44)    | 81.3% (61/75)    |
| SAM                 | NT               | 86.4% (38/44)    | 50.0% (32/64)    |
| CES                 | 65.8% (25/38)    | NT               | 53.8% (28/52)    |
| GM                  | NT               | 84.1% (37/44)    | 78.0% (39/50)    |
| AMK                 | NT               | NT               | 77.6% (59/76)    |
| AK                  | 71.1% (27/38)    | 77.3% (34/44)    | NT               |
| NEL                 | 73.7% (28/38)    | NT               | 70.1% (47/67)    |
| CIP                 | 84.2% (32/38)    | 88.6% (39/44)    | 85.5% (59/69)    |
| LEV                 | 84.2% (32/38)    | 90.0% (36/40)    | NT               |
| CO                  | 31.6% (12/38)    | NT               | 1.3% (1/78)      |
| SXT                 | NT               | 77.3% (34/44)    | 76.2% (48/63)    |

CPD, cefpodoxime; CTX, cefotaxime; CRO, ceftriaxone; CAZ, ceftazidime; TCC, ticarcillin/clavulanic acid; PIP, piperacillin; IMP, imipenem; FEP, cefepime; MEM, meropenem; TZP, piperacillin/tazobactam; SAM, ampicillin/sulbactam; CES, cefoperazon/sulbactam; GM, gentamicin; AMK, amikacin; AK, amikacin; NEL, neltimicin; CIP, ciprofloxacin; LEV, levofloxacin; CO, colistin; SXT, trimethoprim/sulfamethoxazole; NT, not tested.
We next selected 23 isolates for MLST analysis to better understand the diversity of the organisms causing infections in these hospitals. These 23 organisms included isolates belonging to the same or different clusters from the same hospital and isolates from different hospitals but belonging to the same cluster. Among the 23 isolates, we identified 16 STs; 4 isolates were non-typeable due to the lack of amplification of gpi and rpoD. Isolates YD046, YD047, YD131 and DN008 were confirmed as new STs (Table 5). We lastly overlaid the MLVA and MLST data, aiming to identify concordance between typing systems. All isolates belonging to ST136 (YD031, YD072, YD085, YD086, YD110, YD112) fell into CC(d) (Fig. 1c) and largely possessed the same MLVA loci, with the exception of some minor differences (Table 5). CC(d) also included the two ST493 isolates (DN009, DN017) (Fig. 1c). The two ST805 isolates (YD126, DN043) and the two non-typeable strains (TN100, DN012), which shared the same MLST profile for six genes, belonged to CC (c) (Table 5).

**DISCUSSION**

Here we aimed to further understand the scope and the genetic basis for carbapenem resistance in *A. baumannii* circulating in Vietnam. Our findings were largely consistent with previously conducted studies on hospital isolates of *A. baumannii*, and our patients had a similar age range and distribution between sexes to previous works [24, 25].

### Table 4. blaOXA gene combinations in 160 Vietnamese isolates of *A. baumannii*

| blaOXA/ISAba1 gene combinations | Imipenem MIC (µg ml⁻¹) | Hospital A n (%) | Hospital B n (%) | Hospital C n (%) | Total n (%) | P value (Fisher’s exact test) |
|---------------------------------|------------------------|------------------|------------------|------------------|------------|-----------------------------|
| **blaOXA-51 only**              | <8                     | 14/78 (17.9)     | 6/38 (15.8)      | 6/44 (13.6)      | 26/160 (16.2) | 0.66                        |
|                                 | ≥8                     | 1/78 (1.3)       | 0                | 0                | 1/160 (0.6)  |                             |
| **blaOXA-51+blaOXA-23**         | <8                     | 0                | 0                | 0                | 0          |                             |
|                                 | ≥8                     | 2/38 (5.3)       | 0                | 0                | 2/160 (1.3)  |                             |
| **blaOXA-51+blaOXA-58**         | <8                     | 0                | 0                | 0                | 0          |                             |
|                                 | ≥8                     | 1/44 (2.3)       | 1/160 (0.6)      |                 |             |                             |
| **blaOXA-51+blaOXA-23+ISAba1**  | <8                     | 0                | 0                | 0                | 0          |                             |
|                                 | ≥8                     | 61/78 (78.2)     | 30/38 (78.9)     | 37/44 (84.1)     | 128/160 (80.0) | 0.57                        |

**Fig. 1.** Population structure of 160 *A. baumannii* isolates by MLVA. Minimum-spanning trees were created using goeBURST algorithm and visualized in Phyloviz. Each MLVA type is represented by a dot with a size proportional to the number of isolates particular to this MLVA type. Green lines link all nodes at the absolute distance of 1 MLVA variant; grey lines show connected pairs of MLVA types. (a) Colours assigned by hospital: hospital A, orange; hospital B, blue; hospital C, green. (b) Colours are assigned by susceptibility against imipenem: red, resistant; pink, susceptible. (c) Colours assigned by hospital as above but stratified by CCs. (d) Colours are assigned by susceptibility against imipenem as above but stratified by CCs.
However, we did detect several interesting insights into antimicrobial-resistant *A. baumannii*. Specifically, we found that, in comparison to XDR strains, non-XDR organisms exhibited a low rate of resistance against aminoglycosides but a high degree of resistance against third- and fourth-generation cephalosporins. The resistance rates associated with the antimicrobials profiled in this study support previous data from Vietnamese hospitals, which have shown a substantial increase in the rate of isolation of MDR Gram-negative organisms [26].

A survey of colistin usage in 2012 found that a common practice in Vietnam was to provide a loading dose; this may encourage the development of resistance against this last-line drug [27]. The prevalence of resistance to colistin observed in our study (31.6%), especially for hospital A, supports this notion, whereas previous data from Vietnam in 2009 found that all screened *A. baumannii* were colistin susceptible [27]. In a study conducted in two Vietnamese hospitals between 2008 and 2011, the authors noted higher resistance rates against amikacin, gentamicin, ciprofloxacin and piperacillin and lower resistance rates against ceftazidime, imipenem, meropenem and piperacillin/tazobactam than observed here [28]. This discordance could be explained by the presence of differing populations of resistant isolates that are specific to each hospital. A further explanation may be the sampling locations of the clinical strains. The majority of isolates in the previous report were recovered from aspirates of patients undergoing mechanical ventilation; our samples were obtained from other (and unknown) sampling locations.

We found a high proportion of imipenem-resistant organisms within the three sampled hospitals, which supports a previous work that found a substantial annual increase in carbapenem-resistant *Acinetobacter* spp. in Vietnamese patients during 2008–2011 [13]. The distribution of *bla*OXA genes and ISAba1 combinations and the corresponding imipenem resistance rates were not significantly different between the three hospitals. In our study, approximately 80% of the imipenem-nonsusceptible isolates carried the combination of *bla*OXA-51, ISAba1 and *bla*OXA-23. Studies from Taiwan PR China, Republic of Korea and Italy also found that ISAba1 with a *bla*OXA-23 was the determinant associated with carbapenem resistance [29–33]. Furthermore, all strains harbouring *bla*OXA-51, *bla*OXA-23 and ISAba1 had ISAba1 associated with *bla*OXA-23 gene. These data were in agreement with previous observations that ISAba1 is consistently associated with *bla*OXA-23 when *bla*OXA-51 and *bla*OXA-23 co-exist [10, 19]. The predominance of ISAba1-*bla*OXA-23 in imipenem-nonsusceptible isolates in this study indicates the persistence and spread of

### Table 5. STs of selected *A. baumannii*

| Organism ID | gltA | gyrB | gdhB | recA | cpn60 | gpi | rpoD | ST  | MLVA profile* | CC |
|------------|------|------|------|------|-------|-----|------|-----|---------------|----|
| YD031      | 1    | 3    | 3    | 2    | 2     | 16  | 3    | 136 | 9-2-6-11-18-6-18-3 | d  |
| YD072      | 1    | 3    | 3    | 2    | 2     | 16  | 3    | 136 | 9-2-6-12-18-6-19-3 | d  |
| YD085      | 1    | 3    | 3    | 2    | 2     | 16  | 3    | 136 | 9-2-6-12-18-6-19-3 | d  |
| YD086      | 1    | 3    | 3    | 2    | 2     | 16  | 3    | 136 | 9-2-6-12-18-6-13-3 | d  |
| YD110      | 1    | 3    | 3    | 2    | 2     | 16  | 3    | 136 | 9-2-6-12-18-6-19-3 | d  |
| YD112      | 1    | 3    | 3    | 2    | 2     | 16  | 3    | 136 | 9-2-6-13-18-6-16-3 | d  |
| TN113      | 1    | 3    | 3    | 2    | 2     | 142 | 3    | 451 | 9-2-6-13-18-6-16-3 | d  |
| DN009      | 1    | 15   | 3    | 2    | 2     | 106 | 3    | 493 | 6-2-6-16-20-6-15-3 | d  |
| DN017      | 1    | 15   | 3    | 2    | 2     | 106 | 3    | 493 | 6-2-6-16-20-6-15-3 | d  |
| YD029      | 51   | 31   | 49   | 11   | 48    | 103 | 4    | 605 | 13-0-6-1-19-5-18-2 | Singleton |
| YD024      | 1    | 15   | 3    | 70   | 2     | 106 | 3    | 795 | 5-2-6-15-18-6-15-3 | d  |
| DN061      | 1    | 15   | 3    | 70   | 2     | 202 | 3    | 804 | 6-2-6-16-18-6-16-3 | d  |
| DN035      | 1    | 102  | 59   | 28   | 4     | N/A | 45   | Undefined | 15-2-7-1-20-5-0-2 | Singleton |
| YD047      | 21   | 12   | 59   | 11   | 32    | 271 | 4    | 1310| 6-0-7-11-8-0-1-1 | e |
| YD131      | 1    | 81   | 11   | 48   | 18    | 272 | 43   | 1322| 8-0-5-18-13-6-0-2 | Singleton |
| DN050      | 1    | 62   | 80   | 28   | 1     | 178 | N/A  | Undefined | 6-0-7-1-17-5-0-3 | Singleton |
| YD046      | 1    | 102  | 12   | 6    | 28    | 264 | 40   | 1311| 9-0-7-11-20-8-0-1 | e |
| YD108      | 21   | 12   | 2    | 28   | 1     | 203 | 5    | 1263| 6-0-7-12-10-5-0-2 | Singleton |
| DN008      | 1    | 19   | 2    | 43   | 93    | 140 | 114  | 1323| 7-2-7-22-15-5-0-2 | Singleton |
| TN100      | 1    | 1    | 13   | 12   | 4     | N/A | 2    | Undefined | 8-0-6-0-19-5-0-2 | c |
| DN012      | 1    | 1    | 13   | 12   | 4     | N/A | 2    | Undefined | 8-0-6-0-19-5-0-2 | c |
| YD126      | 1    | 1    | 13   | 12   | 4     | 203 | 2    | 805 | 9-0-6-0-20-5-0-2 | c |
| DN043      | 1    | 1    | 13   | 12   | 4     | 203 | 2    | 805 | 9-0-6-0-20-5-0-2 | c |

*MLVA profile according to the surveyed loci: 3468-1988-3002-845-2396-5350-826-2240.*
this carbapenemase gene. All isolates possessing \( \text{bla}_{\text{OXA-51}} \) as the sole carbapenemase gene, apart from a single isolate, were imipenem susceptible [10]. We additionally found three isolates bearing the \( \text{bla}_{\text{OXA-58}} \) gene; one was imipenem resistant and two were imipenem susceptible. This could be explained by the presence (or absence) of an alternative insertion sequence such as ISAb\(_{a3} \) [34]; we did not characterize other associated insertion sequences. \( \text{A. baumannii} \) can possess other resistance mechanisms which work in concert with \( \text{bla}_{\text{OXA-58}} \) to reach clinically significant levels of resistance [3]. The absence of isolates carrying \( \text{bla}_{\text{OXA-24}} \) gene in this study may be due to the small sample size; the were imipenem susceptible [10]. We additionally found as the sole carbapenemase gene, apart from a single isolate, insertion sequence such as IS\(_{\text{Aba1}}\).

The majority of the STs we identified here were concordant with MLVA types. Some variant MLVA loci observed in ST136 isolates may be caused by mutations arising during the persistence of this ST within the sampled hospitals. Our data suggest that MLVA has higher discriminatory ability than MLST for short-term \( \text{A. baumannii} \) epidemiological studies; this has been previously suggested for other organisms [35]. The 107 MLVA types within the 160 isolates reflect substantial genetic diversity in the sampled Vietnamese \( \text{A. baumannii} \); this was apparent even among the closely related isolates belonging to the same CC. The presence of three delineated sub-clusters, essentially consisting of imipenem-resistant organisms, isolated from three different hospitals within the biggest major group indicates the persistence of well-established clones. The distribution of both imipenem-resistant and -susceptible isolates from three hospitals into the four less common groups implies the emergence of new imipenem-resistant variants. Imipenem-resistant isolates recovered from differing hospitals but belonging to the same MLVA types and ST [CC(c) and CC (d)] suggest strain transfer events between hospitals. Notably, ST136, which included six \( \text{A. baumannii} \) isolates from hospital A, has been circulating in one hospital in Ho Chi Minh City since 2011 [36]. ST136 is a member of the CC92, which is a highly prevalent clone across Asia and the most broadly distributed CC globally [3, 37].

Our study has some limitations. We did not have access to adequate patient information to trace the history of antimicrobial usage, the length of hospital stay and ward location within the hospital or whether the patient was involved in an ongoing outbreak. As an ad hoc retrospective analysis of available hospital strains and routine data, the reported information, including the tested antimicrobials, was not standardized. Additionally, we did not characterize other carbapenem resistance associated determinants such as NDM-1 gene or insertion sequences other than the IS\(_{\text{Aba1}}\). Indeed, our study and data interpretation would be greatly improved through the use of whole genome sequencing, which was not available in the context of this investigation. The use of next-generation sequencing allows a standardized approach for genotyping \( \text{A. baumannii} \) [38, 39]. Exploring the phylogenetic structure of our isolates in a global context would greatly improve the interpretation of the results and add insight into the international transfer of successful clones such as GC2. However, the strength of this study was that the organisms were isolated longitudinally from three different hospitals in southern Vietnam, providing a current snapshot into antimicrobial-resistant \( \text{A. baumannii} \) causing infections in Vietnamese hospitals.

In conclusion, our study indicates a very high prevalence of MDR \( \text{A. baumannii} \) causing clinically significant infections in three hospitals in southern Vietnam. These organisms commonly harboured IS\(_{\text{Aba1}}\) with \( \text{bla}_{\text{OXA-23}} \) and were consequently carbapenem resistant; their resistance phenotype may explain their continued selection and transfer within the Vietnamese healthcare system. The phylogenetic analyses identified the clonal spread of XDR strains within and among hospitals. This study contributes to the pool of data relating to local antimicrobial susceptibility patterns for clinical \( \text{A. baumannii} \) in Vietnam and across Asia and should inform infection control policies and antimicrobial cycling regimes.

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**Conflicts of interest**
The authors declare that there are no conflicts of interest.

**Ethical statement**
The ethics committees of the University Medical Centre, Thong Nhat-Dong Nai General Hospital and Dong Nai General Hospital, Vietnam, approved this study. The individual identity of the hospitals could not be identified as a consequence of the ethical approvals and have been randomly designated hospitals A, B and C. Patient data were anonymized; therefore individual patient consent was not required.

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