

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

Nguyen Tuan Anh,¹ Tran Vu Thieu Nga,² Huynh Minh Tuan,³ Nguyen Si Tuan,⁴ Dao Minh Y,⁵ Nguyen Van Vinh Chau,² Stephen Baker^{2,6,7,*} and Ho Huynh Thuy Duong⁸

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*_{OXA} 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⁻¹) was determined for 109/117 (91.6 %) of the XDR imipenem-nonsusceptible organisms, of which the majority (86.7 %) harboured the *bla*_{OXA-51} and *bla*_{OXA-23} genes associated with an *ISAbal* 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*_{OXA-23} gene with *ISAbal* 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*_{OXA-51} and the acquired *bla*_{OXA-23}, *bla*_{OXA-24/40} and *bla*_{OXA-58} β-lactamases [7, 8]. Furthermore, the presence of specific insertion elements, such as an *ISAbal*, upstream of these *bla*_{OXA} 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

Received 30 October 2016; Accepted 26 December 2016

Author affiliations: ¹Diagnostic Department, Faculty of Nursing and Medical Technology, University of Medicine and Pharmacy, Ho Chi Minh City, Vietnam; ²The Hospital for Tropical Diseases, Wellcome Trust Major Overseas Programme, Oxford University Clinical Research Unit, Ho Chi Minh City, Vietnam; ³Infection Control Department, University Medical Center, Ho Chi Minh City, Vietnam; ⁴Microbiology Department, Thong Nhat-Dong Nai General Hospital, Dong Nai Province, Vietnam; ⁵Microbiology Department, Dong Nai General Hospital, Dong Nai Province, Vietnam; ⁶Centre for Tropical Medicine, Oxford University, Oxford, UK; ⁷The Department of Medicine, The University of Cambridge, Cambridge, UK; ⁸Department of Genetics, Faculty of Biology, University of Science, Ho Chi Minh City, Vietnam.

*Correspondence: Stephen Baker, sbaker@oucru.org

Keywords: nosocomial infections; *Acinetobacter baumannii*; Vietnam; carbapenem resistance; MLVA.

Abbreviations: CC, clonal complex; MDR, multidrug resistant; MLST, multilocus sequence typing; MLVA, multiple-locus variable number tandem repeat analysis; OUCRU, Oxford University Clinical Research Unit; ST, sequence type; XDR, extensively drug 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 *bla*_{OXA-23} and *bla*_{OXA-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 biochemical testing [14], a commercial MALDI Biotyper (software version 3.1) (Bruker Daltonics), the presence of the intrinsic *bla*_{OXA-51} gene [15] and a partial *rpoB* gene sequence, as previously described [16]. From the 252 *Acinetobacter* spp. isolates in this study, 160 were confirmed to be viable *A. baumannii* and were suitable for further characterization. This final collection incorporated 38 isolates from hospital A, 44 isolates from hospital B and 78 isolates from hospital C.

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, ankamycin, ampicillin/sulbactam, ciprofloxacin, ceftazidime, cefpodoxime, cefotaxime, ceftriaxone, cefoperazone/sulbactam, colistin, cefepime, neltimicin, 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 $\mu\text{g ml}^{-1}$ and ≤ 2 $\mu\text{g ml}^{-1}$ were considered as resistant and susceptible, respectively [17].

Identification of carbapenemase genes and ISAbal

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 *bla*_{OXA-51}, *bla*_{OXA-23}, *bla*_{OXA-24} and *bla*_{OXA-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 \times AptaTaq (Roche) master mix and 1 \times 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 *bla*_{OXA-23}, *bla*_{OXA-24}, *bla*_{OXA-51} and *bla*_{OXA-58} PCR amplicons were 82.2 \pm 0.5 °C, 78.0 \pm 0.5 °C, 78.5 \pm 0.5 °C and 80.6 \pm 0.5 °C, respectively. The presence of ISAbal region upstream of *bla*_{OXA-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 \times buffer enzyme, 1.5 mM MgCl₂, 0.2 mM dNTPs, 0.5 \times 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 correlatively using an ABI 3130XL capillary electrophoresis system (Applied Biosystems). For fragment analysis, each PCR amplicon was diluted $\times 80$ and 0.5 μl of the diluted PCR amplicon solution was mixed with 9.32 μl Hi-Di formamide and 0.18 μl GeneScan 1200 LIZ size standard (Applied Biosystems). Fragment analysis data were analysed using Geneious R8.1.6 (Biomatters, demo version). PCR 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/abaumannii/info/primers_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 \times PCR buffer in a total volume of 25 μl . The PCR programme was as follows: 95 °C denaturation for 15 min followed by 40

Table 1. Primers used for PCR amplification of the *bla*_{OXA} genes

Primers	Sequence (5'→3')	Length (bp)	Temp. (°C)	Product (bp)
OXA-23-F	CACTAGGAGAAGCCATGAAGC	21	55.0	114
OXA-23-R	CAGCATTACCGAAACCAATACG	22	55.0	
OXA-24-F	GCTAAATGCTTTAATCGGGCTAG	24	55.0	141
OXA-24-R	ACTGGAAGTCTGACAATGC	20	55.0	
OXA-51-F	GAAGTGAAGCGTGTGGTTATG	22	55.0	148
OXA-51-R	GCCTCTTGCTGAGGAGTAAT	20	55.0	
OXA-58-F	ATATTTAAGTGGGATGGAAAGCC	23	55.0	110
OXA-58-R	CGTGCCAATTCTTGATATACAGG	23	55.0	
ISAbal-F	CACGAATGCAGAAAGTTG	17	56.0	520
ISAbal-R	CGACGAATACTATGACAC	18	56.0	

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. baumannii* MLST sequence type database (<http://pubmlst.org/abumannii/>) 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 isolates or patient ages. However, there was a significant disparity 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 differing antimicrobials were tested, with only 5 antimicrobials consistently tested between the three sites. The prevalence of antimicrobial resistance in the *A. baumannii* isolates was high, with >70 % of the organisms tested exhibiting resistance against all assayed antimicrobials. The exceptions were colistin (13/116; 11 %), cefoperazon/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 families [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 comparison 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 cefoperazon/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 prevalence of resistance against third- and fourth-generation cephalosporins.

The 119 XDR organisms included 117 (98.3 %) imipenem-resistant organisms (Table 3). The two imipenem-susceptible isolates were alternatively resistant to meropenem. High-level imipenem resistance ($>32 \mu\text{g ml}^{-1}$) 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-nonsusceptible 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 *bla*_{OXA-51}, *bla*_{OXA-23}, *bla*_{OXA-24}, *bla*_{OXA-58} and *ISAbal* in the 160 *A. baumannii* isolates. The resulting combinations of carbapenem resistance genes are shown in Table 4. The proportion of isolates harbouring *bla*_{OXA-51}, with or without the *bla*_{OXA-23} and *bla*_{OXA-58} genes and *ISAbal* displayed no significant difference between the three hospitals ($P=0.629$, $P=0.617$ and $P=0.416$, respectively). No isolates generated a PCR amplicon for the *bla*_{OXA-24} gene. The majority of isolates (128/160; 80 %) carried the *bla*_{OXA-51} and the *bla*_{OXA-23} gene accompanied by an upstream *ISAbal*; 61/78 (78.2 %), 30/38

Table 2. Antimicrobial resistance profiles of 160 *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	100 % (43/43)	98.0 % (50/51)
CRO	100 % (38/38)	97.5 % (39/40)	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, cefoperazone/sulbactam; GM, gentamicin; AMK, amikacin; AK, amikacin; NEL, neltimicin; CIP, ciprofloxacin; LEV, levofloxacin; CO, colistin; SXT, trimethoprim/sulfamethoxazole; NT, not tested.

(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 *bla*_{OXA-51} gene only were susceptible to imipenem. All isolates (130/160, 81.3 %) that carried a *bla*_{OXA-51} and *bla*_{OXA-23} (with or without *ISAbal1*) exhibited high-level resistance against imipenem (Table 4). However, the imipenem susceptibility of the three isolates harbouring the combination of *bla*_{OXA-51} and *bla*_{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 *bla*_{OXA} gene combinations between the three hospitals (Table 4).

Genetic relatedness of Vietnamese

A. baumannii isolates

We assessed the genetic relationship of the 160 *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 3. Proportion of MDR/XDR and non-MDR/XDR *A. baumannii* and their corresponding MIC against imipenem

Imipenem MIC (μg ml ⁻¹)	Total n (%)	Non-MDR/XDR n (%)	MDR/XDR n (%)	P value (Fisher's exact test)
Total	160 (100)	41/160 (25.6)	119/160 (74.4)	–
<8	28/160 (17.6)	126/41 (63.4)	2/119 (1.7)	<0.0001
≥8	132/160 (82.4)	15/41 (36.6)	117/119 (98.3)	
<32	37/160 (23.1)	27/41 (65.9)	10/119 (8.4)	<0.0001
≥32	123/160 (76.9)	14/41 (34.1)	109/119 (91.6)	

Table 4. *bla*_{OXA} gene combinations in 160 Vietnamese isolates of *A. baumannii*

<i>bla</i> _{OXA} /IS <i>Aba1</i> gene combinations	Imipenem MIC (µg ml ⁻¹)	Hospital A <i>n</i> (%)	Hospital B <i>n</i> (%)	Hospital C <i>n</i> (%)	Total	<i>P</i> value (Fisher's exact test)
<i>bla</i> _{OXA-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)	
<i>bla</i> _{OXA-51} + <i>bla</i> _{OXA-23}	<8	0	0	0	0	–
	≥8	0	2/38 (5.3)	0	2/160 (1.3)	
<i>bla</i> _{OXA-51} + <i>bla</i> _{OXA-58}	<8	2/78 (2.6)	0	0	2/160 (1.3)	0.333
	≥8	0	0	1/44 (2.3)	1/160 (0.6)	
<i>bla</i> _{OXA-51} + <i>bla</i> _{OXA-23} +IS <i>Aba1</i>	<8	0	0	0	0	–
	≥8	61/78 (78.2)	30/38 (78.9)	37/44 (84.1)	128/160 (80.0)	

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].

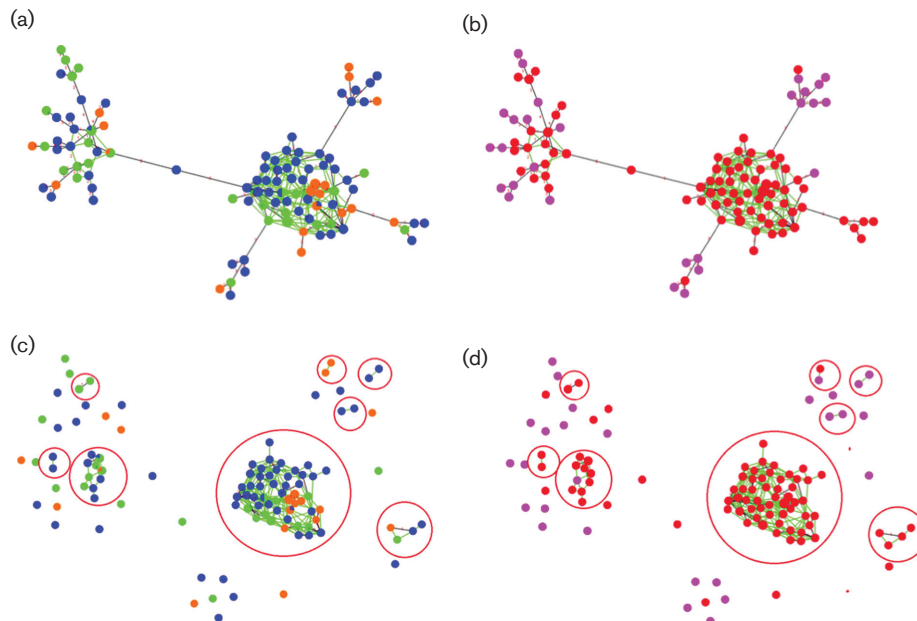


Fig. 1. Population structure of 160 *A. baumannii* isolates by MLVA. Minimum-spanning trees were created using goeBURST algorithm and visualized in PhyloViz. Each MLVA type is represented by a dot with a size proportional to the number of isolates particular to this MLVA type. Green lines link all nodes 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.

Table 5. STs of selected *A. baumannii*

N/A, not amplified.

Organism ID	<i>gltA</i>	<i>gyrB</i>	<i>gdhB</i>	<i>recA</i>	<i>cpn60</i>	<i>gpi</i>	<i>rpoD</i>	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	6-0-7-1-11-8-0-1	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 ¹	2	Undefined	8-0-6-0-19-5-0-2	c
DN012	1	1	13	12	4	N/A ¹	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.

However, we did detect several interesting insights into anti-microbial-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 *ISAbal* 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}, *ISAbal* and *bla*_{OXA-23}. Studies from TaiwanPRChina, Republic of Korea and Italy also found that *ISAbal* 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 *ISAbal* had *ISAbal* associated with *bla*_{OXA-23} gene. These data were in agreement with previous observations that *ISAbal* is consistently associated with *bla*_{OXA-23} when *bla*_{OXA-51} and *bla*_{OXA-23} co-exist [10, 19]. The predominance of *ISAbal*–*bla*_{OXA-23} in imipenem-nonsusceptible isolates in this study indicates the persistence and spread of

this carbapenemase gene. All isolates possessing *bla*_{OXA-51} as the sole carbapenemase gene, apart from a single isolate, were imipenem susceptible [10]. We additionally found three isolates bearing the *bla*_{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 IS*Aba*3 [34]; we did not characterize other associated insertion sequences. *A. baumannii* can possess other resistance mechanisms which work in concert with *bla*_{OXA-58} to reach clinically significant levels of resistance [3]. The absence of isolates carrying *bla*_{OXA-24} gene in this study may be due to the small sample size; the prevalence of *bla*_{OXA-24} gene is low in Vietnam [13].

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 *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 *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 *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*Aba*1. 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 *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 *A. baumannii* causing infections in Vietnamese hospitals.

In conclusion, our study indicates a very high prevalence of MDR *A. baumannii* causing clinically significant infections in three hospitals in southern Vietnam. These organisms commonly harboured IS*Aba*1 with *bla*_{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 *A. baumannii* in Vietnam and across Asia and should inform infection control policies and antimicrobial cycling regimes.

Funding information

This study was supported by a grant from the Department of Science and Technology Ho Chi Minh City (DOST HCMC) for Youth Science and Technology Innovation Incubator Program (2014–2016) and a grant from the Khoa Thương Biotechnology Company for PhD training. S. B. is a Sir Henry Dale Fellow, jointly funded by the Wellcome Trust and the Royal Society (100087/Z/12/Z).

Acknowledgements

We thank Christine J. Boinett for the critical reading and editing of this article.

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.

References

1. Fournier PE, Vallenet D, Barbe V, Audic S, Ogata H *et al.* Comparative genomics of multidrug resistance in *Acinetobacter baumannii*. *PLoS Genet* 2006;2:e7.
2. Abbott I, Cerqueira GM, Bhuiyan S, Peleg AY. Carbapenem resistance in *Acinetobacter baumannii*: laboratory challenges, mechanistic insights and therapeutic strategies. *Expert Rev Anti Infect Ther* 2013;11:395–409.
3. Evans BA, Amyes SG. OXA β -lactamases. *Clin Microbiol Rev* 2014;27:241–263.
4. Fishbain J, Peleg AY. Treatment of *Acinetobacter* infections. *Clin Infect Dis* 2010;51:79–84.
5. Higgins PG, Dammhayn C, Hackel M, Seifert H. Global spread of carbapenem-resistant *Acinetobacter baumannii*. *J Antimicrob Chemother* 2010;65:233–238.
6. Poirel L, Leviandier C, Nordmann P. Prevalence and genetic analysis of plasmid-mediated quinolone resistance determinants QnrA and QnrS in *Enterobacteriaceae* isolates from a French university hospital. *Antimicrob Agents Chemother* 2006;50:3992–3997.
7. Brown S, Amyes S. OXA β -lactamases in *Acinetobacter*: the story so far. *J Antimicrob Chemother* 2006;57:1–3.

8. Woodford N, Ellington MJ, Coelho JM, Turton JF, Ward ME *et al*. Multiplex PCR for genes encoding prevalent OXA carbapenemases in *Acinetobacter* spp. *Int J Antimicrob Agents* 2006;27:351–353.
9. Héritier C, Poirel L, Nordmann P. Cephalosporinase over-expression resulting from insertion of IS*Aba1* in *Acinetobacter baumannii*. *Clin Microbiol Infect* 2006;12:123–130.
10. Turton JF, Ward ME, Woodford N, Kaufmann ME, Pike R *et al*. The role of IS*Aba1* in expression of OXA carbapenemase genes in *Acinetobacter baumannii*. *FEMS Microbiol Lett* 2006;258:72–77.
11. Antunes LC, Visca P, Towner KJ. *Acinetobacter baumannii*: evolution of a global pathogen. *Pathog Dis* 2014;71:292–301.
12. Pendleton JN, Gorman SP, Gilmore BF. Clinical relevance of the ESKAPE pathogens. *Expert Rev Anti Infect Ther* 2013;11:297–308.
13. Nhu NT, Lan NP, Campbell JI, Parry CM, Thompson C *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.
14. Bouvet PJ, Grimont PA. Identification and biotyping of clinical isolates of *Acinetobacter*. *Ann Inst Pasteur Microbiol* 1987;138:569–578.
15. Turton JF, Woodford N, Glover J, Yarde S, Kaufmann ME *et al*. Identification of *Acinetobacter baumannii* by detection of the bla_{OXA-51-like} carbapenemase gene intrinsic to this species. *J Clin Microbiol* 2006;44:2974–2976.
16. La Scola B, Gundi VA, Khamis A, Raoult D. Sequencing of the rpoB gene and flanking spacers for molecular identification of *Acinetobacter* species. *J Clin Microbiol* 2006;44:827–832.
17. Clinical and Laboratory Standards Institute. *Performance Standards for Antimicrobial Susceptibility Testing: Twenty-Fourth Informational Supplement*, CLSI document M100-S24. Wayne, PA: CLSI;2014.
18. Huang XZ, Cash DM, Chahine MA, Nikolich MP, Craft DW. Development and validation of a multiplex TaqMan real-time PCR for rapid detection of genes encoding four types of class D carbapenemase in *Acinetobacter baumannii*. *J Med Microbiol* 2012;61:1532–1537.
19. Segal H, Garny S, Elisha BG. Is IS*ABA-1* customized for *Acinetobacter*? *FEMS Microbiol Lett* 2005;243:425–429.
20. Pourcel C, Minandri F, Hauck Y, D'Arezzo S, Imperi F *et al*. Identification of variable-number tandem-repeat (VNTR) sequences in *Acinetobacter baumannii* and interlaboratory validation of an optimized multiple-locus VNTR analysis typing scheme. *J Clin Microbiol* 2011;49:539–548.
21. Francisco AP, Vaz C, Monteiro PT, Melo-Cristino J, Ramirez M *et al*. PHYLOViZ: phylogenetic inference and data visualization for sequence based typing methods. *BMC Bioinformatics* 2012;13:87.
22. Jolley KA, Maiden MC. BIGSdb: Scalable analysis of bacterial genome variation at the population level. *BMC Bioinformatics* 2010;11:595.
23. Magiorakos AP, Srinivasan A, Carey RB, Carmeli Y, Falagas ME *et al*. Multidrug-resistant, extensively drug-resistant and pandrug-resistant bacteria: an international expert proposal for interim standard definitions for acquired resistance. *Clin Microbiol Infect* 2012;18:268–281.
24. Abbo A, Navon-Venezia S, Hammer-Muntz O, Krichali T, Siegman-Igra Y *et al*. Multidrug-resistant *Acinetobacter baumannii*. *Emerg Infect Dis* 2005;11:22–29.
25. Liu Q, Li W, Du X, Li W, Zhong T *et al*. Risk and prognostic factors for multidrug-resistant *Acinetobacter baumannii* complex bacteremia: a retrospective study in a tertiary hospital of West China. *PLoS One* 2015;10:e0130701.
26. Trang NH, Nga TV, Campbell JI, Hiep NT, Farrar J *et al*. The characterization of ESBL genes in *Escherichia coli* and *Klebsiella pneumoniae* causing nosocomial infections in Vietnam. *J Infect Dev Ctries* 2013;7:922–928.
27. Van TD, Dinh QD, Vu PD, Nguyen TV, Pham CV *et al*. Antibiotic susceptibility and molecular epidemiology of *Acinetobacter calcoaceticus-baumannii* complex strains isolated from a referral hospital in northern Vietnam. *J Glob Antimicrob Resist* 2014;2:318–321.
28. Tada T, Miyoshi-Akiyama T, Kato Y, Ohmagari N, Takeshita N *et al*. Emergence of 16S rRNA methylase-producing *Acinetobacter baumannii* and *Pseudomonas aeruginosa* isolates in hospitals in Vietnam. *BMC Infect Dis* 2013;13:251.
29. Brigante G, Migliavacca R, Bramati S, Motta E, Nucleo E *et al*. Emergence and spread of a multidrug-resistant *Acinetobacter baumannii* clone producing both the carbapenemase OXA-23 and the 16S rRNA methylase ArmA. *J Med Microbiol* 2012;61:653–661.
30. Dai W, Huang S, Sun S, Cao J, Zhang L. Nosocomial spread of carbapenem-resistant *Acinetobacter baumannii* (types ST75 and ST137) carrying bla_{OXA-23-like} gene with an upstream IS*Aba1* in a Chinese hospital. *Infect Genet Evol* 2013;14:98–101.
31. Lee K, Kim MN, Choi TY, Cho SE, Lee S *et al*. Wide dissemination of OXA-type carbapenemases in clinical *Acinetobacter* spp. isolates from South Korea. *Int J Antimicrob Agents* 2009;33:520–524.
32. Lee HY, Huang CW, Chen CL, Wang YH, Chang CJ *et al*. Emergence in Taiwan of novel imipenem-resistant *Acinetobacter baumannii* ST455 causing bloodstream infection in critical patients. *J Microbiol Immunol Infect* 2015;48:588–596.
33. Lin YC, Hsia KC, Chen YC, Sheng WH, Chang SC *et al*. Genetic basis of multidrug resistance in *Acinetobacter* clinical isolates in Taiwan. *Antimicrob Agents Chemother* 2010;54:2078–2084.
34. Poirel L, Marqué S, Héritier C, Segonds C, Chabanon G *et al*. OXA-58, a novel class D β -lactamase involved in resistance to carbapenems in *Acinetobacter baumannii*. *Antimicrob Agents Chemother* 2005;49:202–208.
35. Maâtallah M, Bakhrouf A, Habeeb MA, Turlej-Rogacka A, Iversen A *et al*. Four genotyping schemes for phylogenetic analysis of *Pseudomonas aeruginosa*: comparison of their congruence with multi-locus sequence typing. *PLoS One* 2013;8:e82069.
36. Tada T, Miyoshi-Akiyama T, Kato Y, Ohmagari N, Takeshita N *et al*. Emergence of 16S rRNA methylase-producing *Acinetobacter baumannii* and *Pseudomonas aeruginosa* isolates in hospitals in Vietnam. *BMC Infect Dis* 2013;13:251.
37. Kim DH, Choi JY, Kim HW, Kim SH, Chung DR *et al*. Spread of carbapenem-resistant *Acinetobacter baumannii* global clone 2 in Asia and AbaR-type resistance islands. *Antimicrob Agents Chemother* 2013;57:5239–5246.
38. Diancourt L, Passet V, Nemec A, Dijkshoorn L, Brisse S. The population structure of *Acinetobacter baumannii*: expanding multi-resistant clones from an ancestral susceptible genetic pool. *PLoS One* 2010;5:e10034.
39. Zarrilli R, Pournaras S, Giannouli M, Tsakris A. Global evolution of multidrug-resistant *Acinetobacter baumannii* clonal lineages. *Int J Antimicrob Agents* 2013;41:11–19.

Five reasons to publish your next article with a Microbiology Society journal

1. The Microbiology Society is a not-for-profit organization.
2. We offer fast and rigorous peer review – average time to first decision is 4–6 weeks.
3. Our journals have a global readership with subscriptions held in research institutions around the world.
4. 80% of our authors rate our submission process as 'excellent' or 'very good'.
5. Your article will be published on an interactive journal platform with advanced metrics.

Find out more and submit your article at microbiologyresearch.org.