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# Antibiotic resistance determinants in nosocomial strains of multidrug-resistant *Acinetobacter baumannii*

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***Objectives*: To investigate the presence of resistance genes in nosocomial multidrug-resistant (MDR) *Acinetobacter baumannii* isolated from outbreak and sporadic settings.**

***Methods*: Thirty-two *A. baumannii* isolates were collected, 13 of which were involved in two outbreaks from different hospitals, which resulted in four deaths. The remaining 19 isolates were collected sporadically over 5 years from two other hospitals. The MICs of 25 antibiotics were determined for each isolate. PCR screening was carried out to identify possible genes that contributed to each resistance phenotype. Repetitive extragenic palindromic-PCR (REP-PCR) was performed to assess isolate clonality in conjunction with genotype data.**

***Results*: Between eight and 12 resistance determinants were detected in the 32 MDR *A. baumannii* isolates examined. These resistance determinants included the genes $bla_{OXA-23}$ and *ampC*, with the upstream element IS*Aba1* promoting increased gene expression and subsequent resistance to carbapenems and cephalosporins, respectively. In all isolates, resistance to quinolones and fluoroquinolones was conferred by an S83L mutation in GyrA. Twenty-eight of the 32 isolates were also positive for *tet*(B), a tetracycline resistance determinant, $bla_{TEM-1}$, which contributed to β-lactam resistance, and *strB*, which contributed to aminoglycoside resistance. Class 1 integrons that harboured *aacC1*, *aadA1*, *qacEΔ1* and *sul1* were identified in 10 of the 32 isolates (31%) together with the kanamycin resistance gene, *aphA1*. A putative trimethoprim resistance gene, *folA*, was also identified in all isolates. REP-PCR together with genotyping identified three main clonal types.**

***Conclusions*: Isolates of *A. baumannii* from both outbreak and sporadic cases possess at least eight resistance gene determinants that give rise to the MDR phenotype.**

Keywords: *A. baumannii*, OXA-23, multidrug resistance

## Introduction

*Acinetobacter* spp. have been recognized over the last two decades as important opportunistic pathogens. Extensive use of antimicrobial chemotherapy in clinical environments has contributed to the emergence and dissemination of nosocomial *Acinetobacter baumannii* infections. These infections are difficult to treat due to the organism's multidrug-resistant (MDR) phenotype, which includes resistance to β-lactams, aminoglycosides, fluoroquinolones and more recently, carbapenems. *A. baumannii* in particular is capable of causing a wide spectrum of disease including urinary tract infections, pneumonia and meningitis, especially in intensive care units (ICUs), and is associated with high mortality rates. Lately, several clinical outbreaks associated with carbapenem-resistant *A. baumannii* have been described[1,2] and alternative treatment options are limited.

Currently, there is a relative paucity of data on the number and type of resistance genes and mechanisms that may be present in MDR *A. baumannii* strains. Many studies focus only on a single resistance phenotype and its associated resistance gene. In the current study, four discrete cohorts of *A. baumannii* were investigated. As all these isolates were MDR, we aimed to identify the gene/s responsible for resistance to 25 antibiotics from a range of classes. This is the first study that attempts to determine the resistance gene profile of *A. baumannii* responsible for nosocomial ICU outbreaks and sporadic infections.

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## Materials and methods

### *Bacterial isolates and plasmids*

A total of 32 isolates of *A. baumannii* were collected from four different hospitals in Sydney, Australia. *A. baumannii* was identified by colonial appearance on MacConkey agar, Gram staining, antibiotic susceptibility testing using disc diffusion,[3] growth in oxidative fermentative media, resistance to ampicillin and the presence of $bla_{\text{OXA-51-like}}$. Five isolates (termed cohort A) of *A. baumannii* (A91, A93, A94, A96 and A97) were collected from five patients during an ICU outbreak that resulted in two deaths in May 2005. The second ICU outbreak cohort, termed cohort B, consisted of eight MDR *A. baumannii* (B1–B8) and was obtained from another large Sydney hospital in March 2007. The bacterial infection in three of these latter cases was septicaemia, as a result of which two patients died. Sporadic isolates were defined as strains that were not associated with an outbreak, and the remaining two cohorts fell into this category. Cohort C, obtained from the standard wards of a third hospital, consisted of 10 different isolates (C2–C5, C8, C13–C15, C18 and C20) originating from wound, catheter, blood and urine samples, as well as environmental surfaces including bed railings and air-conditioning vents (isolates C3 and C4). Eight of the cohort C isolates were collected randomly between September 2006 and February 2007, and the remaining two isolates (C18 and C20) were isolated in November 2002. Cohort D consisted of nine MDR *A. baumannii* isolates (D1–D3, D5–D9 and D12) collected sporadically between June 2006 and May 2007. These isolates (none of which were sourced from the ICU) were collected from catheters, wounds and urine samples. A single *A. baumannii* isolate named A95, which was susceptible to many antibiotics, was isolated from the same hospital as cohort A and was used as a control in all procedures. Another control isolate, *Acinetobacter calcoaceticus* (UNSW 031600), was obtained from the University of New South Wales Microbiology Culture Collection (http://www.babs.unsw.edu.au/about/centres/micro_culture.html). *Escherichia coli* strain ACM 5185 was used as a control for MIC determination.

### *Quantitative antibiotic susceptibility testing*

MIC determination of each antibiotic was performed using an agar dilution technique.[4] Susceptibilities of *A. baumannii* to 25 different antibiotics were tested as shown in the Results section. Results were interpreted according to the European Committee on Antimicrobial Susceptibility Testing (EUCAST).[5]

### *Detection of resistance determinants*

Integron detection, characterization and gene cassette amplification were carried out using previously described PCR methods.[6,7] Primers used to detect other non-integron-associated resistance genes are shown in Table 1. Primers for the detection of tetracycline resistance determinants,[8] the β-lactamase gene $bla_{\text{OXA-51-like}}$ [9] and IS*Aba1*[10] were used as previously described. Sequencing and DNA analysis of all PCR products were carried out using methods described elsewhere.[11] To identify mutations associated with quinolone and fluoroquinolone resistance, the 344 bp quinolone resistance determining region (QRDR) of the DNA gyrase gene, *gyrA*, was amplified and restricted using the method of Vila *et al.*,[12] with the primers Vila 95 gyrA-F[12] and PW40 (Table 1). Sequencing was also used to confirm the presence of a mutation in *gyrA*.

**Table 1.** Primer sequences used in this study

| Primer name[a] | Sequence (5′→3′) | Target gene/region |
|---|---|---|
| PW40 | GCCATRCCYACGGCGATACC | *gyrA* |
| PW46 | GGGAATTCTCGGGGAAATGTG | $bla_{\text{TEM-1}}$ |
| PW47 | GGGATCCGAGTAAACTTGGTCTGA | $bla_{\text{TEM-1}}$ |
| PW51 | CGGGCAATACACCAAAAGAC | *ampC* |
| PW52 | CCTTAATGCGCTCTTCATTTGG | *ampC* |
| PW53 | CTTGCTATGTGGTTGCTTCTC | $bla_{\text{OXA-23}}$ |
| PW54 | ATCCATTGCCCAACCAGTC | $bla_{\text{OXA-23}}$ |
| PW166 | CCTATCAGGGTTCTGCCTTCT | IS*Aba1* |
| PW194 | CTCGAGAGTGTTATGCGTGC | pITN84, trimethoprim-resistant clone |
| PW197 | CCGTTCATGCGACCTAACA | pITN84, trimethoprim-resistant clone |
| PW198 | TCGCAATGGACAAAATCACT | pITN84, trimethoprim-resistant clone |
| PW200 | ATGATGTCTAACAGCAAACTG | *strA* |
| PW201 | TCAACCCCAAGTAAGAGG | *strA* |
| PW202 | ATGGGGTTGATGTTCATGCCGC | *strB* |
| PW203 | CTAGTATGACGTCTGTCGCAC | *strB* |
| PW204 | ATGGACAAAAATCACTGTATTGG | *folA* |
| PW205 | TAAGTGGCAAATTCGAATG | *folA* |
| PW208 | TTGCCCATACGCTAGTCAGTT | pITN84, trimethoprim-resistant clone |
| PW216 | ATGAGCCATATTCAACGGG | *aphA1* |
| PW217 | TCAGAAAAACTCATCGAGCATC | *aphA1* |
| PW221 | CGCGAATTCTTTTTTATAAGTGGC | *folA* gene from isolate A91 |
| PW222 | CGCATGGATCCGCAAAATGTA | *folA* gene from isolate A91 |

[a]All primers described in this table were designed in this study.

**Table 2.** MICs for the 32 *A. baumannii* isolates as determined by agar dilution

| Isolate | MIC (mg/L)[a] | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | AMP | CAZ | CTX | FOX | TIM | TZP | AMC | MEM | IMP | TIG | AMK | GEN | TOB | KAN | STR | SPT | TET | TMP | PMX |
| Clonal group 1, REP-PCR type I | | | | | | | | | | | | | | | | | | | |
| A91, A93, A94, A96, A97 | >512 | 128 | >128 | >256 | >512 | 128 | >128 | 8 | 8 | 8 | 4 | >64 | 2 | >512 | >128 | >128 | >256 | >64 | 1 |
| C20 | >512 | 128 | >128 | >256 | >512 | 256 | >128 | 16 | 16 | 4 | >128 | >64 | 4 | >512 | >128 | >128 | >256 | >64 | 1 |
| Clonal group 2, REP-PCR type I | | | | | | | | | | | | | | | | | | | |
| B1–B8 | >512 | 128 | >128 | >256 | >512 | 256 | >128 | 16 | 16 | 4 | 2 | 2 | 0.5 | ≤2 | >128 | >128 | >256 | >64 | 1 |
| C3, C5, C8 | >512 | 128 | >128 | >256 | >512 | 256 | >128 | 16 | 16 | 4 | 2 | >64 | 32 | 64 | >128 | >128 | >256 | >64 | 1 |
| C2 | >512 | 128 | >128 | >256 | >512 | 256 | >128 | 16 | 16 | 4 | 16 | >64 | 4 | >512 | >128 | >128 | >256 | >64 | 1 |
| C4 | >512 | 128 | >128 | >256 | >512 | 256 | >128 | 16 | 16 | 2 | 4 | 32 | 16 | 64 | >128 | >128 | >256 | >64 | 1 |
| C13, C14 | >512 | 128 | 64 | 128 | >512 | 256 | >128 | 16 | 16 | 1 | 128 | 1 | ≤0.25 | >512 | >128 | >128 | >256 | >64 | 1 |
| C15, C18 | >512 | 128 | >128 | >256 | >512 | 256 | >128 | 16 | 16 | 4 | 2 | >64 | 32 | 64 | >128 | >128 | >256 | >64 | 1 |
| D1 | >512 | 128 | >128 | >256 | >512 | 256 | >128 | 16 | 16 | 2 | 2 | 1 | ≤0.25 | ≤2 | >128 | >128 | 256 | >64 | 0.5 |
| D5 | >512 | 128 | >128 | >256 | >512 | 256 | >128 | 16 | 16 | 8 | 2 | 2 | 0.5 | ≤2 | >128 | >128 | >256 | >64 | 1 |
| D8, D9 | >512 | 128 | >128 | >256 | >512 | 256 | >128 | 16 | 16 | 4 | 4 | >64 | 64 | 128 | >128 | >128 | >256 | >64 | 1 |
| D12 | >512 | 32 | >128 | >256 | >512 | 256 | >128 | 32 | 16 | 4 | 2 | >64 | 32 | 64 | >128 | >128 | >256 | >64 | 1 |
| Clonal group 3, REP-PCR type II | | | | | | | | | | | | | | | | | | | |
| D1, D3, D6, D7 | 32 | 4 | 8 | 64 | ≤8 | 8 | 16 | ≤0.25 | <0.5 | 4 | 4 | >64 | 0.5 | >512 | 32 | 32 | 16 | 32 | 1 |

AMP, ampicillin; CAZ, ceftazidime; CTX, cefotaxime; FOX, cefoxitin; TIM, ticarcillin/clavulanate; TZP, piperacillin/tazobactam; AMC, amoxicillin/clavulanate; MEM, meropenem; IPM, imipenem; TIG, tigecycline; AMK, amikacin; GEN, gentamicin; TOB, tobramycin; KAN, kanamycin; STR, streptomycin; SPT, spectinomycin; TET, tetracycline; TMP, trimethoprim; PMX, polymyxin B.

[a]The MICs of the following antibiotics were identical for all 32 isolates: cefazolin, >128 mg/L; cefalexin, >128 mg/L; chloramphenicol, >64 mg/L; ciprofloxacin ≥64 mg/L; nalidixic acid, >256 mg/L; and sulfafurazole, >128 mg/L.

**Table 3.** Resistance determinants detected by PCR screening in 32 *A. baumannii* isolates

| Isolates with the phenotype | Resistance determinants in *A. baumannii* | | | | | | | | | | | | | | | | REP-PCR type |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Class 1 integron | IntI1-associated genes: *aacC1*-orfX-orfX'-*aadA1*, *sul1*, *qacEΔ1* | *ampC* | *ampC*+ ISA*ba1* | $bla_{TEM-1}$ | $bla_{OXA-23}$ | $bla_{OXA-23}$+ ISA*ba1* | $bla_{OXA-51}$ | Ser83-Leu83 *gyrA* mutation | *tet*(B) | *strB* | *aphA1* | *folA* | |
| A91, A93, A94, A96, A97, C20 | + | + | + | + | + | + | + | + | + | + | + | + | + | I |
| B1-B8, C2, C3, C4, C5, C8, C13, C14, C15, C18, D1, D5, D8, D9, D12 | – | – | + | + | + | + | + | + | + | + | + | – | + | I |
| D2, D3, D6, D7 | + | + | + | – | – | – | – | + | + | – | – | + | + | II |

+ denotes the presence of a particular resistant determinant. – denotes the absence of a particular resistant determinant.

### *DNA extraction and repetitive extragenic palindromic-PCR (REP-PCR) for clonality assessment*

Genomic DNA extractions were carried out using the Bio-Rad AquaPure Genomic DNA Extraction Kit (Bio-Rad) according to the manufacturer's instructions. REP-PCR was performed using the method and primers of Bou *et al.*[13]

### *Trimethoprim resistance determination*

In order to identify the trimethoprim resistance gene, genomic DNA was extracted from a representative isolate, *A. baumannii* A91, and then restricted with *Eco*RI (Promega, WI, USA). The restricted fragments were then ligated with *Eco*RI cut pUC18. Resultant transformants were then patched onto LB agar containing 25 mg/L trimethoprim. Plasmids conferring trimethoprim resistance were extracted using the Bio-Rad Quantum Plasmid Miniprep Kit (Bio-Rad) and sequenced using vector primers[14] and those listed in Table 1. Shotgun cloning led to the isolation of the putative trimethoprim resistance gene, *folA*, which was then amplified from the genomic DNA of A91 using primers PW221 and PW222 (Table 1), cloned into pTrcHis2A and transformed into *E. coli* BL21. Subsequent transformants were selected on trimethoprim and subjected to MIC determination. Phylogenetic analysis was carried out as previously described.[11]

## Results

### *Antibiotic susceptibility testing*

All 32 isolates of MDR *A. baumannii* were tested for antibiotic susceptibility by determining MICs of a range of 25 antibiotics (Table 2). The most resistant isolates (C3, C5, C8, C15, C18, C20, D8, D9 and D12) were resistant to 23 of the 25 antibiotics tested (Table 2). All 32 isolates showed high levels of resistance to cefalexin, cefazolin, chloramphenicol, sulfafurazole and nalidixic acid (Table 2). Twenty-eight isolates demonstrated an additionally high level of resistance to ampicillin, ticarcillin/clavulanate, amoxicillin/clavulanate, streptomycin and spectinomycin (Table 2). The majority of isolates were also resistant to tigecycline, with only four isolates (C4, C13, C14 and D1) and the control isolate A95 susceptible to the drug. Of the 25 antimicrobials tested, the most effective was polymyxin B, followed by amikacin and tobramycin (Table 2).

### *Detection and characterization of integrons*

In order to assess the presence of integrons in *A. baumannii*, PCR screening for *intI* genes was performed. Class 1 integrons were found in 10 of the 32 isolates (31%); including the five isolates from cohort A, a single positive isolate from cohort C (C20) and four of nine isolates from cohort D (D2, D3, D6 and D7) (Table 3). No integron positive isolates were found in the outbreak cohort B and no class 2 or 3 integrons were found within the cohorts. Subsequent PCR amplification and sequencing of the cassette arrays revealed that all 10 integron-positive *A. baumannii* isolates contained a 2.6 kb gene cassette array containing *aacC1*, orfX, orfX' and *aadA1*, which conferred resistance to gentamicin, streptomycin and spectinomycin.

### *Streptomycin resistance*

The *aadA1* gene cassette was only present in 10 integron positive isolates, yet all 32 isolates were resistant to streptomycin. Thus, other determinants conferring resistance to this antibiotic were sought. The presence of two genes, *strA* and *strB*, recently implicated in streptomycin resistance in *A. baumannii*,[15] was assessed by PCR. Twenty-eight of the 32 isolates contained the single *strB* gene (Table 3), while the *strA* gene was not found in any isolate. The control isolate A95 was fully susceptible to both spectinomycin and streptomycin and lacked *aadA1*, *strA* and *strB* resistance genes.

### *Kanamycin resistance*

Another aminoglycoside resistance gene, *aphA1*, encodes kanamycin resistance in *A. baumannii*.[16] The *aphA1* resistance gene was identified in 10 of the 22 kanamycin resistant isolates (A91, A93, A94, A96, A97, C20, D2, D3, D6 and D7), the same 10 isolates that harboured the class 1 integron (Table 3). These 10 isolates were highly resistant to kanamycin (MIC >512 mg/L) (Table 2).

### *Genetic basis of β-lactam resistance*

Resistance to β-lactams was observed in all isolates. The intrinsic *ampC* β-lactamase gene was identified in all 32 isolates (Table 3). The presence of an insertion element, IS*Aba1*, immediately upstream of *ampC* has been attributed to increased *ampC* expression resulting in an extended hydrolysis profile and high-level ceftazidime resistance.[17,18] This insertion element was found upstream of the *ampC* gene in 28 of 32 isolates (88%) (Table 3). These 28 isolates were resistant to a range of cephalosporins, including ceftazidime (Table 2). The four isolates that lacked the insertion sequence (D2, D3, D6 and D7) had reduced MICs of ceftazidime, cefotaxime, cefoxitin and ticarcillin/clavulanate but were still highly resistant to cefalexin and cefazolin (Table 2).

The gene $bla_{\text{TEM-1}}$ was detected in 28 of the 32 isolates (88%) (Table 3). The product, TEM-1, hydrolyses penicillin and ampicillin, but does not confer resistance to β-lactamase inhibitors.[19] The same four isolates (D2, D43, D6 and D7) that lacked IS*Aba1* upstream of *ampC* also lacked the $bla_{\text{TEM-1}}$ gene (Table 3).

### *Carbapenem resistance is conferred by $bla_{OXA-23}$ with an upstream* IS*Aba1*

In *A. baumannii*, carbapenem-hydrolysing β-lactamases of Ambler class B and D play a significant role in providing resistance to carbapenems.[20–22] Two oxacillinases were identified in this study, $bla_{\text{OXA-23}}$ and $bla_{\text{OXA-51-like}}$. The gene $bla_{\text{OXA-23}}$ was amplified from 28 isolates, all of which harboured an upstream insertion element, IS*Aba1* (Table 3). This results in an increase in carbapenemase activity and leads to imipenem and meropenem resistance,[21] which was reflected in the MDR phenotype (Table 2). The four susceptible isolates from cohort D (D2, D3, D6 and D7) lacked both the gene and the insertion sequence (Table 3) and were susceptible to the carbapenems (Table 2). The intrinsic β-lactamase gene $bla_{\text{OXA-51-like}}$ was amplified in all 32 *A. baumannii* isolates (Table 3). However, $bla_{\text{OXA-51-like}}$ confers insufficient carbapenemase activity for resistance without the presence of an upstream insertion sequence element.[23] No upstream IS*Aba1* was identified through PCR screening; therefore, it is unlikely that the $bla_{\text{OXA-51-like}}$ gene was involved in carbapenem resistance.

### *Quinolone and fluoroquinolone resistance is conferred by an S83L gyrA mutation*

All 32 isolates studied were resistant to nalidixic acid and ciprofloxacin. RFLP analysis and sequencing revealed all the isolates of *A. baumannii* contained a nucleotide mutation in the QRDR of *gyrA* (Table 3). The mutation results in an amino acid change from S83L and is a well-recognized resistance mutation.[12] The control, A95, which did not contain this mutation, was susceptible to the quinolones.

### *Tetracycline resistance*

In clinical isolates of *A. baumannii*, *tet*(A) and *tet*(B) confer tetracycline resistance; however, *tet*(B) is more prevalent.[8] All 32 isolates were resistant to tetracycline, and *tet*(B) was identified by PCR in 28 isolates (Table 3). The remaining four isolates (D2, D3, D6 and D7) did not contain *tet*(A) or *tet*(B) genes (Table 3); however, they were still resistant, albeit with lower MIC values of tetracycline (16 mg/L), when compared with the rest of the isolates (MIC >256 mg/L) (Table 2). The control isolate, A95, was susceptible to tetracycline and also did not contain *tet*(A) and *tet*(B).

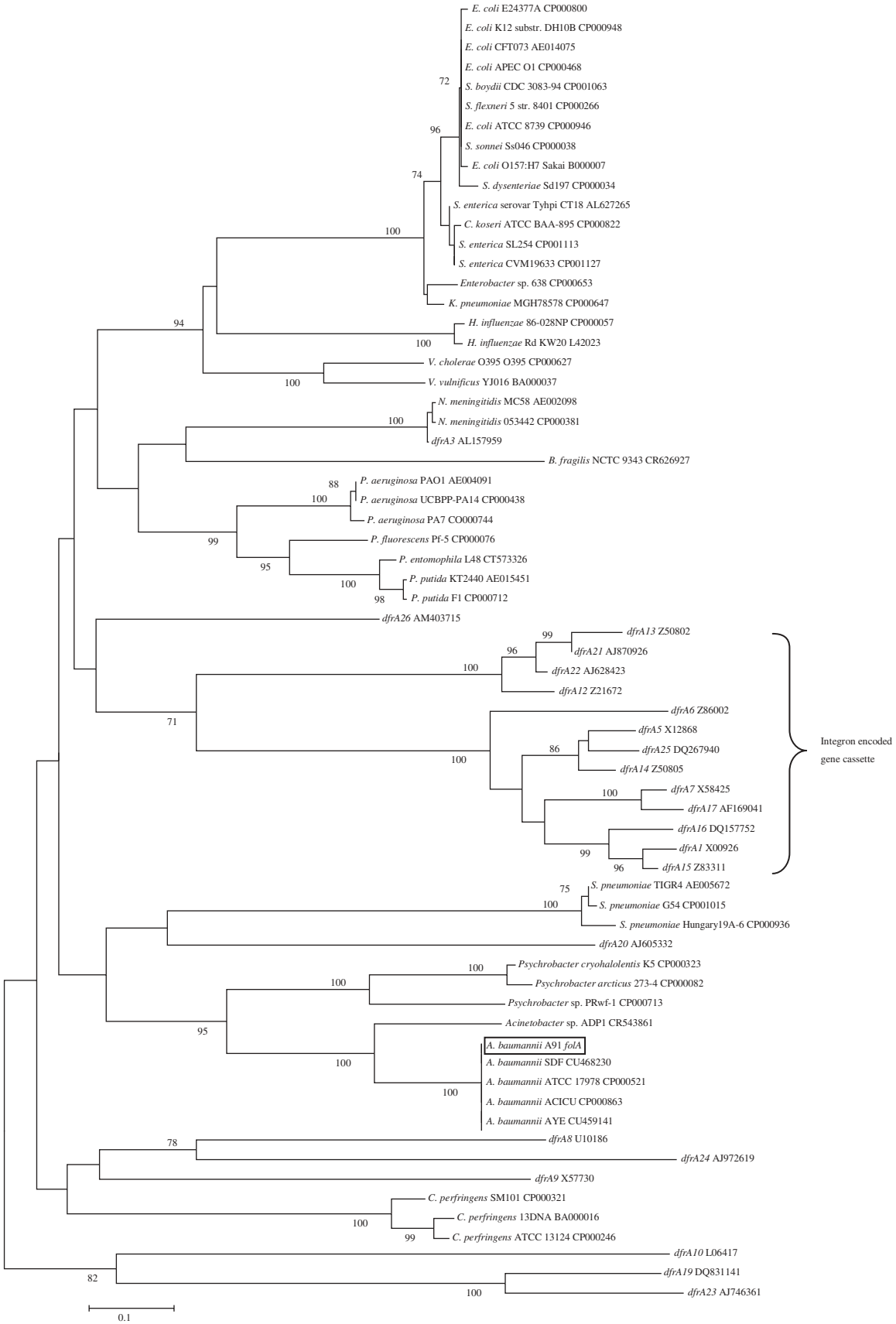
### *folA is a putative trimethoprim resistance determinant in MDR A. baumannii*

A putative trimethoprim resistance gene was found by shotgun cloning a representative isolate, A91. Selection of clones on agar plates supplemented with trimethoprim resulted in the isolation of a resistant plasmid, named pITN84. Sequencing 4.5 kb of the 16 kb insert revealed the presence of a 510 bp ORF, *folA*, which encoded a dihydrofolate reductase (Dfr), an enzyme that can confer trimethoprim resistance. This gene was present in all 32 trimethoprim-resistant *A. baumannii* isolates (Table 3). The 510 bp *folA* gene from one isolate, A91, was amplified and cloned into pTrcHis2A. Expression of the enzyme in *E. coli* strain BL21 resulted in high-level trimethoprim resistance, with a MIC >1024 mg/L, while the *E. coli* host strain showed an MIC of ≤0.5 mg/L.

The 169 residue *folA* product demonstrated 99.4% identity with the Dfr found in *A. baumannii* ACICU,[24] and 98.8% identity with the Dfrs in *A. baumannii* ATCC 17978, [25] *A. baumannii* AYE and *A. baumannii* SDF[26] (Figure 1). The Dfr in this study was also related to other *folA*-encoded Dfrs, including those from *Acinetobacter* sp. ADP1 (72%, 122/169 amino acids) and *Psychrobacter* sp. PRwf-1 (43%, 81/188 amino acids), as well as known trimethoprim resistance genes, DfrA26 (35.9%, 66/184 amino acids) and DfrA16 (35.7%, 61/171 amino acids) (Figure 1).

### *REP-PCR and clonality of isolates*

In order to determine the clonality of the 32 *A. baumannii* isolates, REP-PCR was carried out on genomic DNA. Two REP-PCR patterns or 'genomic fingerprints' were observed [see Figure S1, available as Supplementary data at *JAC* Online



(http://jac.oxfordjournals.org/)]. The patterns of 28 of the 32 isolates were indistinguishable and designated REP-PCR type I. These isolates consisted of all of cohorts A, B and C, as well as five isolates from cohort D (D1, D5, D8, D9 and D12). Four isolates from cohort D (D2, D3, D6 and D7) displayed a separate pattern, designated REP-PCR type II. This technique lacked the power to discriminate the six isolates (grouped as type I) that possessed an integron and *aphA1* from those that did not contain these elements. Therefore, a combination of the genotypic data and the REP-PCR types was used to group all 32 isolates into three clonal groups (Table 3). The control isolates *A. baumannii* A95 and *A. calcoaceticus* produced an REP-PCR pattern distinctly different from all the other isolates (Figure S1).

## Discussion

*A. baumannii* causes a significant number of nosocomial outbreaks worldwide, which commonly occur in settings with high antibiotic selective pressures, such as ICUs. Therapeutic options for the treatment of MDR *A. baumannii* are becoming increasingly limited. All the isolates of *A. baumannii* from the current study exhibited an MDR phenotype, with the nine most resistant isolates susceptible to only two of the 25 antibiotics tested. There were no significant differences in the genotypic and phenotypic profiles, as the 19 sporadic and environmental surface (C3 and C4) *A. baumannii* isolates possessed the same resistance genes as the 13 outbreak isolates.

Insertion sequences containing promoters that alter the levels of gene expression have been documented in various Gram-negative bacteria, including Acinetobacters.[10,17] Carbapenem resistance in *A. baumannii* is often mediated through increased oxacillinase gene expression, which is driven by the promoter region of an upstream IS*Aba1*.[23] In this study, IS*Aba1* was found upstream of both *ampC* and $bla_{\text{OXA-23}}$, thus giving rise to cephalosporin and carbapenem resistance. The finding of IS*Aba1* upstream of two different genes in the same bacteria suggests that under a selective pressure it is able to transpose, providing a strong promoter sequence for multiple resistance genes. The $bla_{\text{OXA-23}}$ gene has previously been isolated in conjunction with the same integron described in the present study from *A. baumannii* outbreak strains from pan-Europe,[27] Greece[28] and previously from Australia.[2] The integron array found in this study, together with the gene $bla_{\text{OXA-23}}$, has also been described in an *A. baumannii* outbreak clone in the UK, designated OXA-23 clone 2.[29] This clone, first isolated in July 2003, continues to be isolated in hospitals around the UK.[30] Therefore, the OXA-23 clone 2 may also contain the other resistance determinants such as IS*Aba1*/*ampC*, $bla_{\text{TEM-1}}$, *tet*(B), *strB*, *folA* and the *gyrA* mutation as the isolates found in this study.

In *A. baumannii* strains AYE and ACICU, *aphA1* was located between two IS*26* elements just upstream of a class 1 integron on large 'resistance islands'.[15,25] Isolates in this study containing an integron also possessed the kanamycin resistance gene *aphA1*, and the co-occurrence of these two elements has been shown previously.[27] Therefore, we hypothesize that the *aphA1* gene and the integron are associated with one another.

A gene conferring putative trimethoprim resistance was found by shotgun cloning. This gene was present in all 32 trimethoprim-resistant isolates and differed in only one nucleotide from *folA* genes from four clinical isolates of *A. baumannii*.[24–26] While trimethoprim resistance seems to be intrinsic in clinical *A. baumannii* isolates, no gene has yet been described that mediates this resistance. The role of FolA and other housekeeping Dfrs in conferring trimethoprim resistance has previously been reported.[31,32] Overexpression of chromosomal Dfrs is also a recognized mechanism of trimethoprim resistance.[33,34] When overexpressed in *E. coli*, the *folA* gene identified in this study confers high-level trimethoprim resistance; however, more evidence is needed to confirm its ability to confer trimethoprim resistance in its natural context.

It has been shown in this study that MDR *A. baumannii* can possess at least eight resistance determinants that give rise to its MDR phenotype. Although resistance was observed against amikacin, tobramycin, chloramphenicol and tigecycline, a genetic basis for the resistance was not accounted for. In addition, there were a number of isolates in which a resistance phenotype was observed, but no specific resistance determinant was identified. This is an indication that there are as yet unidentified resistance genes present in these cohorts of MDR *A. baumannii*.

The resistance determinants MDR *A. baumannii* possess, such as an integron, the β-lactam resistance genes $bla_{\text{OXA-23}}$, *ampC* and $bla_{\text{TEM-1}}$, aminoglycoside, tetracycline and trimethoprim resistance genes, and a *gyrA* mutation conferring quinolone resistance, contribute to the MDR profile of nosocomial strains within hospitals in Australia. These resistance determinants were present in outbreak as well as sporadic isolates, which emphasizes the multitude of resistance genes *A. baumannii* is capable of possessing.

**Figure 1.** Phylogenetic tree of the *dfrA* genes that confer trimethoprim resistance and those termed *folA*, which are intrinsic Dfr genes in many species of bacteria. The bacterial name, strain and GenBank accession numbers are shown. The percentage bootstrap values in which the major groupings were observed among 10 000 replicates are indicated at each branch point. The putative trimethoprim resistance gene identified in this study named *A. baumannii* A91 *folA* is boxed. The branch lengths are proportional to the evolutionary distance between sequences, and the distance scale in nucleotide substitutions per position is shown. The integron-encoded Dfr genes are contained within the bracket.

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## Transparency declarations

None to declare.

## Supplementary data

Figure S1 is available as Supplementary data at *JAC* Online (http://jac.oxfordjournals.org/).

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