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Tn*2008*-Driven Carbapenem-Resistance in *Acinetobacter baumannii* Isolates from a Period of Increased Incidence of Infections in a Southwest Virginia Hospital

Running title: Plasmid-borne $bla_{\text{OXA-23}}$ in MDR *A. baumannii* outbreak isolates

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Highlights

- Plasmid-borne Tn2008 was responsible for carbapenem-resistance in multi-drug resistant Acinetobacter baumannii strains causing an outbreak of infections in a tertiary care hospital in Virginia.
- Similar strains were sporadically encountered in the hospital.
- The other determinant of carbapenem-resistance in Acinetobacter baumannii strains isolated from sporadic infections in the hospital was ISAba1-blaOXA-66.

Abstract

Objectives

The objectives of this study were to determine the genetic basis for carbapenem resistance in multidrug-resistant *Acinetobacter baumannii* strains isolated from patients affected by a sudden increase in the incidence of infections by such organisms in a tertiary care hospital in Virginia, USA, in 2009-2010 and to examine whether such strains are commonly encountered in the hospital setting.

Methods

The whole genomes of one outbreak strain, one carbapenem-resistant strain and one carbapenem-sensitive strain from sporadic infections in 2010-2012 were sequenced and analyzed. Then five outbreak isolates and 57 sporadic isolates of which 39 were carbapenem-resistant were screened via PCR for relevant DNA elements identified in the genomics investigation.

Results

All three strains, for which whole genome sequences were obtained, carried resistance genes linked to multidrug-resistant phenotypes and a ~111 kbp plasmid (pCMCVT*Ab*1) without a drug-resistant gene. Of these, the two carbapenem-resistant strains possessed a ~74 kbp plasmid (pCMCVT*Ab*2) carrying a Tn*2008* transposon that provides high-level carbapenem resistance. The PCR analysis showed that all outbreak isolates carried both plasmids and Tn*2008*, and of the sporadic isolates, 88% carried pCMCVT*Ab*1, 25% contained pCMCVT*Ab*2, and 50% of the latter group carried Tn*2008*.

Conclusions

Carbapenem resistance in outbreak strains and 12% of sporadic isolates was due to the pCMCVT*Ab*2-borne Tn*2008*. This is the first report for a Tn*2008*-driven outbreak of carbapenem-resistant *Acinetobacter baumannii* infections in the Commonwealth of Virginia, which followed similar cases in Pennsylvania and Ohio.

Keywords: *Acinetobacter baumannii*, carbapenem-resistant, whole genome sequencing, MDR, Hospital Acquired Infection, Southwest Virginia

1. Introduction

The multidrug-resistant (MDR) strains of *Acinetobacter baumannii* (MDR-*Ab*) cause 10% of hospital-acquired infections (HAI) that result in patient mortality of up to 70% [1]. *Acinetobacter* species, strictly aerobic, non-motile, Gram-negative coccobacilli, are found in numerous natural niches and occasionally in the clinical setting [2]. MDR-*Ab* strains are the third leading cause of respiratory tract infections among patients in intensive care units (ICU)

[3]. Initial colonization by *Acinetobacter* occurs predominantly in patients with compromised immunity or prolonged hospital stay leading to bacteremia and ventilator-associated pneumonia, particularly in those admitted to an ICU [4]. Often these infections occur in outbreaks or clusters and are tracked to medical support devices [5]. Recently, the emergence of carbapenem-resistant (CR) strains of *Ab* in the hospital setting coupled with limited options of antimicrobial treatments has further aggravated the situation. Most prevalent carbapenem resistance (CR) enzymes of *A. baumannii* belong to a group called Class D or OXA-type β-lactamases or oxacillinases which is frequently encountered in Gram-negative bacteria and provides a wide spectrum of β-lactamase activities [1, 2, 3]. The subsets providing carbapenem resistance are carbapenem-hydrolyzing class D β-lactamases (CHDLs) and in *A. baumannii* these are encoded by bla_{OXA} genes such as $bla_{\text{OXA-23-like}}$, $bla_{\text{OXA-40,}}$ $bla_{\text{OXA-66}}$, $bla_{\text{OXA-51-like}}$ and $bla_{\text{OXA-58-like}}$ [1, 2, 3, 6]. The spread of *bla* genes from one strain to another is facilitated by mobile genetic elements, and through integration within a genome these mobile elements create drug-resistant islands, which for *A. baumannii* are called AbaR [7]. In some cases the expression levels for the bla_{OXA} genes are enhanced by associated insertion sequences (IS) [7]. We report an investigation on the genetic basis for carbapenem resistance in multidrug-resistant *Ab* strains isolated during an outbreak or cluster of infections in a tertiary care teaching hospital in Southwestern Virginia, USA.

2. Materials and Methods

2.1. Phenotypic characterization of *Ab* isolates

We studied 62 isolates of MDR-*Ab*, five from the outbreak period of 2009-2010 and 57 from sporadic infections in 2009-2012 (15 in 2010-2012 and 42 in 2013-2014), obtained from respiratory sources (sputum, tracheal aspirate and bronchoalveolar lavage), wounds, urine or blood; surveillance isolates were from perirectal and nasal swabs. The study followed prior

approval from the local Institutional Review Board. Tests for biochemical properties of the isolates, including antimicrobial susceptibilities, were performed using VITEK 2 (bioMérieux) automated system. Antimicrobial susceptibility readings were recorded according to the Clinical and Laboratory Standards Institute (CLSI) guidelines (http://clsi.org/m100/).

2.2. Clinical data collection and analysis

The complete microbiological characteristics datasets were available for five of the nine outbreak isolates (Table S1) and for 57 sporadic isolates of MDR-*Ab*. Consecutive isolates from a patient during the same admission were not included unless the resistance profile had changed. Recorded variables included the anatomic source of the isolate plus its susceptibility profile, patient comorbidities, as well as calculation of their Charlson comorbidity index score [8], diagnosis at admission, total hospital length of stay (LOS), Intensive Care Unit (ICU)-LOS and discharge disposition. Variables were collected and analyzed in an electronic database. Clinical data were associated with duly de-identified isolates prior to being made available for research.

2.3. Sequencing of the genomic DNA, assembly and analysis of sequence data, and PCR

Genomic DNA isolated with Genomic-tip 20/G (Qiagen, Valencia, CA) was sequenced on the PacBio RS II platform employing Single Molecule Real Time or SMRT™ technology (Pacific Biosciences, Menlo Park, CA) at the University of Delaware Sequencing and Genotyping Center (Newark, DE) using a standard protocol [9]. *De novo* sequence assembly was performed by the SMRT™ analysis version 2.2 system [9]. The resulting polished and assembled sequences were analyzed for circularity using Gepard [10], and the circular sequences were generated by use of Amos [11] and Minimus2 [12]. Automated annotation for gene

functions was performed by the Rapid Annotation using Subsystem Technology (RAST) in the RAST server [13] followed by manual curation. The origin of replication of a plasmid was identified with OriFinder [14]. Identity and Average Nucleotide Identity (ANI) values were calculated using blastn [15] and ANI calculator at the http://enve-omics.ce.gatech.edu/ani/ [16], respectively. PCR was performed with specific primers (Table S2), genomic DNA, and Platinum *Taq* DNA Polymerase Hi Fidelity (Invitrogen, Carlsbad, CA). The PCR observations were validated by determining the DNA sequences of two amplicons per target; sequencing occurred at the Virginia Tech Biocomplexity Institute's Genome Research Laboratory.

2.4. Bioinformatic analyses

2.4.1. Multilocus Sequence Typing (MLST) – The typing was performed following the Pasteur MLST and Oxford schemes using the defined internal segments of *gltA*, *gyrB*, *gdhB*, *recA*, *cpn60*, *gpi*, and *rpoD* genes [17]; the respective sequences were obtained either from the whole genome data or from sequencing of PCR amplicons. The data were analyzed at the Center for Genomic Epidemiology (https://cge.cbs.dtu.dk/services/MLST/) and PubMLST (http://pubmlst.org/abaumannii) webservers.

2.4.2. Searches for antibiotic resistance genes and IS elements – The resistance genes were identified by using the RAST platform via built-in Blastp searches in the NCBI Reference Sequence Database for proteins as well as the ResFinder and Antibiotic Resistance Gene Database (ARDB) webservers (https://cge.cbs.dtu.dk//services/ResFinder/; https://ardb.cbcb.umd.edu/) with the default parameters. The IS elements were identified by

RAST and annotated by PSI-BLAST, as well as the IS finder tool (http://www-is.biotoul.fr) with the standard parameters.

3. Results

3.1. A sudden rise in CR-MDR-*Ab* infections

The incidence of MDR-*Ab* and CR-MDR-*Ab* infections at Carilion Medical Center in Roanoke, Virginia, was zero per 10,000 patient days prior to August 2009 (Fig. 1). Then, in August 2009 - January 2010 period, coinciding with an H1N1 influenza epidemic in Southwestern Virginia, an acute spike and clustering of nine CR-MDR-*Ab* cases were identified in an ICU (Table S1). Three of the nine affected patients died. Using retrospective analysis, the index case was traced to a nursing home patient admitted in August 2009. These frequent infections were halted by strict cohorting of patients and staff and heightened general infection control measures. The result of this action is reflected in the observation that in the March 2010-Ausgust 2012 period the hospital encountered two more periods of increased infections by MDR-*Ab* strains where the incidences of carbapenem-resistance were relatively low (Fig. 1).

3.2. Demographic and clinical features of MDR-*Ab* isolates

In total, we studied 62 MDR-*Ab* isolates, of which five were from the outbreak (2009-2010) and 57 from sporadic infections (2010-2014) (Table 1). All outbreak isolates and 57 sporadic isolates were considered carbapenem-resistant (CR) per the CLSI definition [18], as the respective CLSI breakpoint for imipenem and/or meropenem were ≥ 8 μg/ml [19]; for 18 sporadic isolates the CLSI breakpoint value was <2 μg/ml. In analyzing the clinical characteristics of patients from whom these isolates were obtained, we excluded seven patients

with incomplete data and another 11 patients whose cultures were obtained due to surveillance screening (nasal, perirectal, rectal). The remaining 44 patients had the following clinical characteristics. Seventy percent of the isolates were from a respiratory source, while 15.9% represented *Ab*-bacteremia. Sixteen percent of the patients died during hospitalization; about two-thirds (65.9%) were discharged to a long-term care facility (LTCF) and 18% were discharged home. Mean total LOS was 23.7 days (SD 28.3, range 0-157 days), and ICU-LOS was 14.7 days (SD 11, range 1-35 days).

3.3. Genomic analysis of *A. baumannii* (*Ab*) strains

All five outbreak strains and two sporadic strains for which whole genome sequences were analyzed exhibited identical Multilocus Sequence Typing (MLST) profiles and were classified to Cluster 2 or ST2 of the Pasteur scheme and ST208 of Oxford scheme [17]. To determine the molecular basis for carbapenem resistance in the *Ab* isolates (Table 1) in a cost-effective manner, we used a combination of whole genome sequencing and PCR analysis. One of the strains isolated from an outbreak patient (CMC-CR-MDR-*Ab*4), a carbapenem-sensitive (CMC-MDR-*Ab*59), and a carbapenem-resistant strain (CMC-CR-MDR-*Ab*66) of *Ab* from sporadic infections (Table 1) were targeted for the genome sequencing on the PacBio RS II platform. The sequence for all three strains readily assembled to fully complete chromosomes and one or two plasmids (Table S3). The assembled sequences have been submitted to the GenBank with the following accession numbers: CMC-CR-MDR-*Ab*4 chromosome, CP016295; CMC-CR-MDR-*Ab*59 chromosome, CP016298; CMC-CR-MDR-*Ab*66 chromosome, CP016300; pCMCVT*Ab*1-4, CP016296; pCMCVT*Ab*1-59, CP016299; pCMCVT*Ab*1-66, CP016301;

pCMCVT*Ab*2-4, CP016297; pCMCVT*Ab*2-66, CP016302. Presented below is a comparative analysis of the relevant features of these genomes.

3.4. Chromosomal antibiotic resistance genes

The antibiotic resistance genes found in whole genome sequences are shown in Table 2. Both CMC-CR-MDR-*Ab*4 and CMC-CR-MDR-*Ab*66 carried the following three chromosomally encoded *bla* genes [7] (class, *bla* gene): class A, $bla_{\text{TEM-1F}}$; class C, $bla_{\text{ADC-25}}$; class D, $bla_{\text{OXA-66}}$. These genes, except $bla_{\text{TEM-1F}}$, were also present in CMC-MDR-*Ab*59, which is sensitive to carbapenem. The following additional antibiotic resistance genes were found in all three chromosomes (drug (*gene name*)) (Table 2): sulfonamide (*sul1* and *sul2*); tetracyclines (*tet*B); choloramphenicol (*catB8*); aminoglycosides (*str*B: *aph(6)-Id*; *str*A: *aphA(3")-Ib*, *aadA1*, *aacA4*, *aphA1*, and *aph(3')-Ic*, *armA*); and fosmidomycin (*fsr*). They also carried the multidrug resistance genes (gene name, (drug names): *MFS_1* (fluoroquinolone, macrolides, tetracyclines and chloramphenicol); *Bcr/CflA*, (bicyclomycin, chloramphenicol and florfenicol); *mph*(E) and *msr*(E) (macrolides, lincosamide and streptogramin B).

3.5. Plasmids

The carbapenem-resistant strains, CMC-CR-MDR-*Ab*4 and CMC-CR-MDR-*Ab*66, carried two plasmids, named pCMCVT*Ab*1 (~111 kbp) and pCMCVT*Ab*2 (~74 kbp) (Fig. 2), while carbapenem-sensitive CMC-MDR-*Ab*59 carried pCMCVT*Ab*1 and lacked pCMCVT*Ab*2. The plasmid pCMCVT*Ab*1 (Fig. 2A) did not carry an obvious drug resistance gene, but several metabolic genes such as those for alcohol dehydrogenase and heme biosynthesis. It also contained remnants of genes for phage structural proteins, which have been found in plasmids of

MDR-*Ab* isolates from China, such as pABTJ2 (GenBank accession number: CP004359.1). The pCMCVT*Ab*1 plasmids from CMC-CR-MDR-*Ab*4, CMC-CR-MDR-*Ab*66, and CMC-CR-MDR-*Ab*59 exhibited sequence identities higher than 99% among themselves and the differences mapped to a few SNPs in a phage tail protein gene (ORF numbers AOT16_19195 in pCMCVTAb1-4). Similar plasmids are frequently encountered in *Ab* isolates. For example, in nucleotide sequences the following plasmids were 99% identical to pCMCVTAb1-4 [GenBank accession number]: pAB386 [CP010780.1], pABTJ2 [CP004359.1]; pORAB01-1 [CP015484.1]; and p6200 [CP010400.1].

The plasmid pCMCVT*Ab*2 contained *tra*, a conjugative transfer gene (Figs. 2B and C). The pCMCVT*Ab*2 of CMC-CR-MDR-*Ab*4, called pCMCVT*Ab*2-4 (Fig. 2B), carried two transposons: Tn*2008* or IS*Aba1*-*bla*$_{\text{OXA-23}}$ and Tn*aphA6* [20]; *bla*$_{\text{OXA-23}}$ and *aphA6* impart resistance to carbapenem and aminoglycoside antibiotics, respectively. The plasmid pCMCVT*Ab*2-4 carried an additional copy of IS*Aba1*, situated downstream of a *telA* gene that provides resistance to tellurite and certain other toxic anions [21]. The CMC-CR-MDR-*Ab*66 carried a variant of pCMCVT*Ab*2, called pCMCVT*Ab*2-66, that lacked Tn*aphA6* but contained remnants of Tn*aphA6*, including two IS*Aba125* units (Fig. 2C). Plasmids related to pCMCVT*Ab*2 have been found in clinical isolates of *Ab*. For example, the backbone and the Tn*aphA6* element of pCMCVT*Ab*2 constitute most of the pAb-G7-2 and pACICU-2, and both plasmids lack Tn*2008* [22]. When compared with respect to homologous sections, pCMCVT*Ab*2-4 exhibit 91-94% sequence identities to the following plasmids [GenBank accession number]: p1AB5075 [CP008707.1]; pAb-G7-2 [KF669606]; pAba3207b [CP015366.1]; and p2ABTCDC0715 [CP002524.1].

3.6. Insertion sequences (IS), transposons and antibiotic resistance islands

The genomes of all three *Ab* strains studied here contained numerous IS elements representing the IS*26*, ISCR2, IS*Ab24*, and IS4 families [7], and we discuss here those associated with antibiotic resistance genes creating transposons, or large islands in the chromosome (Tables 2, S4 and S5 and Fig. 3); the plasmid borne IS elements have been discussed above (Table S5 and Fig. 3). The inverted repeats, IRL and IRR, for the IS elements and transposons are shown in Table S6.

We identified three AbaR type [7] chromosomal antibiotic resistance islands (I-III) flanked by direct repeats, which likely resulted from transposition into the chromosome (Table S4 and Fig. 3). These elements or their parts have been found previously in *Ab* genomes [7, 20, 23]. In addition to these islands, the isolate CMC-CR-MDR-*Ab*4 carried a combination of $bla_{\text{ADC-25}}$ (ORF AOT16_05315) and two IS*Aba1*, and this arrangement seemed to be unique to this strain. A version with only one IS*Aba1* was found in CMC-CR-MDR-*Ab*59 and CMC-CR-MDR-*Ab*66 that caused sporadic infections and it also occurs in the following *Ab* strains (GenBank accession number): ORAB01 (CP015483.1) and Ab11111 (Permanent Draft) (NZ_AKAQ00000000.1). The position of IS*Aba1* in the latter case mimics that found in Tn*2008* [23] and an association of $bla_{\text{ADC-25}}$ with IS*Aba1* in clinical isolates of *Ab*, and consequent up-regulation of this *bla* gene and higher MIC for cephalosporins have been observed previously [24]; $bla_{\text{ADC-25}}$ without an associated IS*Aba1* has also been found in certain CR outbreak strains of *Ab* [25].

Two forms of Island I were found (Fig. 3). In CMC-CR-MDR-*Ab*4 and *Ab*66, it was Tn*6297*, a common *Ab* transposon and composite of Tn*6020b-1* and Tn*1548-like-1* that contains

class I integron with aminoglycosides and sulphonamides resistance genes [26]; each transposon was bounded by two IS26 elements. The form found in CMC-CR-MDR-*Ab*59 carried an extra *aph3* gene linked with a preceding IS*26* element (Fig. 3).

The Island II was found in CMC-CR-MDR-*Ab*4 and CMC-CR-MDR-*Ab*66 and in two forms (Fig. 3). In CMC-CR-MDR-*Ab*4, it was identical to AbGRI2-1 of *Ab* isolate A1, an Australian global clone 2 [20]. In CMC-CR-MDR-*Ab*66 it contained a truncated form of *aphA1*, an aminoglycoside resistance gene (Fig. 3). Both forms of the island carried $bla_{\text{TEM-1F}}$ (ORF AOT16_12495 in CMC-CR-MDR-*Ab*4) (Fig. 3), encoding a β-lactamase that does not degrade carbapenems [27]. The formation for $bla_{\text{TEM-1F}}$ with truncated resolvase gene (*tnp*R') and IS*26*-*tnp*A in island II partly mimicked that of Tn*3* and has been reported previously [28].

Island III is typical of many clinical isolates of CR-MDR-*Ab* [20, 29]. However, unlike many of these previously reported islands, such as AbGRI1-2 [20], Island III lacked a β-lactamase gene (Fig. 3). Island III carried genes for resistance to sulphonamides (*sul2*), tetracycline (*tetA*(B)), and streptomycin (*strA*-*strB*); *sul2* was preceded by a IS*Aba1* and in analogy to the case of Tn*2008*, it could enhance the expression of this resistance gene. These features make Island III a new type of AbaR. Island III is present in *Ab* strains XH386 and AF673 (Accession numbers CP010779 and CP018256, respectively), and elements with sequence similarities of 99% to Island III are found in *Ab* MDR-TJ and TYH (Accession numbers CP003500.1 and CP003856.1, respectively).

3.7. PCR-based analysis of the *A. baumannii* isolates

The 16S rRNA gene sequences of all strains analyzed fully (100%) matched that of *Acinetobacter baumannii* and consequently identified the organisms as *Ab*. The *ligK* and *parA* were selected as markers for pCMCVT*Ab*1 and pCMCVT*Ab*2, respectively (Fig. 2), for the following reasons. The DNA sequence of *ligK* (DNA ligase gene) of pCMCVT*Ab*1 (Fig. 2A) was identical to that of the following pCMCVT*Ab*1-type *A. baumannii* plasmids (GenBank accession number): pORAB01-1 of strain ORAB01 (CP015484.1); pAB386 of strain XH386 (CP010780.1); p6200-114.848kb of strain 6200 (CP010398.1); pABTJ2 of strain MDR-TJ (CP004359.1). Often *parA*, an ATPase involved in the partition of P1 type plasmids, is found as part of a *parAB* operon [30] and in pCMCVT*Ab*2 the *parA* and *parB* genes formed an operon-like arrangement (Figs. 2B and C).

The *ligK* gene was present in all five outbreak strains and in 50 of the 57 sporadic isolates (Table 1), suggesting a wide presence of pCMCVT*Ab*1 in local *Ab* strains. This plasmid likely provides certain useful metabolic capabilities, such as alcohol oxidation and the heme biosynthesis. The *parA* gene was found in all five outbreak isolates and only 14 of 57 sporadic isolates (Table 1), and suggested a limited presence for pCMCVT*Ab*2. Tn*2008* element was present in all five outbreak strains and seven of the 57 sporadic isolates (Table 1). All five outbreak isolates and two of 57 sporadic isolates carried Tn*aphA6* (Table 1). Five sporadic isolates carried *parA* and Tn*2008* but not Tn*aphA6*, whereas seven sporadic isolates carried *parA* but neither Tn*2008* nor Tn*aphA6* (Table 1). Such variations are seen with other pCMCVT*Ab*2 type plasmids of *Ab* [31] and these could be driven by the flanking insertion sequences.

Of the β-lactamase genes of *Ab* [7], *bla*$_{\text{OXA-58}}$, *bla*$_{\text{NDM-1}}$, *bla*$_{\text{VIM-2}}$, *bla*$_{\text{IMP-1}}$, and *bla*$_{\text{OXA-143}}$ and *bla*$_{\text{SIM-1}}$ were absent from all isolates, whereas *bla*$_{\text{OXA-40}}$ was widespread in sporadic isolates (33 of 57 being positive), irrespective of whether they were carbapenem-resistant or not (Table 1). The most prevalent β-lactamase gene was *bla*$_{\text{OXA-66}}$, a *bla*$_{\text{OXA-51}}$–like gene, covering all five outbreak isolates and 54 of 57 sporadic isolates (Table 1). In 25 sporadic isolates, *bla*$_{\text{OXA-66}}$ was part of an IS*Aba1-bla*$_{\text{OXA-66}}$ element that has been found in *Ab* isolates [32]. The absence of *bla*$_{\text{OXA-66}}$ in three isolates was surprising as *bla*$_{\text{OXA-51}}$ is intrinsic in *Ab* [7]. The *bla*$_{\text{TEM}}$ gene was detected in all outbreak isolates, and only three of the 57 sporadic isolates carried this gene.

4. **Discussion**

The goal of this investigation was to identify the genomic basis for a high-level carbapenem resistance in MDR *A. baumannii* strains isolated during an outbreak in a tertiary care hospital in Virginia and to examine if such strains are sporadically encountered in this hospital. The majority of the MDR-*Ab* isolates that were analyzed were obtained from a respiratory source (tracheal, bronchial, sputum). This observation is consistent with earlier reports on *Ab* infection [33]. Discharge to a long-term care facility (LTCF) was common with this infection as has been previously described [34]. Although this could be due to the high burden of chronic comorbidities and acute medical conditions in these patients, it emphasizes the need for continued appropriate infection control precautions in LTCF in order to prevent spread of these MDR organisms. As noted earlier, our index case was admitted from a nursing home.

Infections with MDR-*Ab* have generally been associated with prolonged LOS and impaired functional status in survivors [34]. This was evident in our study as well. The main

cause of admission in our series was sepsis (~1/3 of patients), and the mean Charlson comorbidity index score was 3, reflecting a high burden of chronic comorbidities and acute medical conditions in these patients, which could have played a role in their prognosis. Data regarding mortality in this study were limited to the discharge disposition and thus could underestimate death occurring after discharge. The mortality rate for our patients with outbreak MDR–*Ab* was 33%. Overall mortality rate in our series is 16%. In other studies, mortality rates in *Ab* infections have been reported as high as >30%, depending on factors such as site of infections, drug susceptibility, adequacy of antibiotic therapy and the presence of severe underlying diseases [33, 35].

One of the outbreak strains and one carbapenem-sensitive and one carbapenem-resistant strain from sporadic infections were analyzed at the whole genome level. The sequencing was performed using the PacBio RS II platform as it provides longer sequence reads (up to 30 kbp) and thereby allows facile assembly of sequences with repeat elements that are common in *Ab* [8]. The sequence data for each strain were readily assembled to respective fully complete chromosomes and plasmids, and the analysis that followed suggested all these strains belonged to a coherent group (Table S3). The ANI values for the chromosomes fell in the 99.88 - 99.98% range. Similarly, these three strains and additional four outbreak isolates were members of the Cluster 2 or ST2 of the Pasteur scheme and ST 208 of Oxford scheme [17].

The whole genome sequences showed that the MDR phenotypes of the strains that were analyzed were due to 19 chromosomal antibiotic resistance genes (Table 2) [7, 36] and 15 of these were located within genomic islands and often linked to IS elements or parts of transposons

(Tables 2 and S4 and Fig. 3). All three carried pCMCVT*Ab*1 and the two carbapenem-resistant strains contained pCMCVT*Ab*2. A PCR analysis detected both plasmids in all five outbreak isolates, and 88% of the 57 sporadic isolates carried pCMCVT*Ab*1 and 25% contained pCMCVT*Ab*2 (Table 1). But only about 12.28% of the sporadic isolates carried pCMCVT*Ab*2 with Tn*2008* (Table 1). Thus, pCMCVT*Ab*1 possibly serves important housekeeping roles through activities such as alcohol oxidation and heme biosynthesis, whereas pCMCVT*Ab*2 is not essential and has been selected through exposure to beta-lactams. The data indicate that an association of Tn*2008*, IS*Aba1*-*bla*$_{\text{OXA-23}}$ with pCMCVT*Ab*2 was responsible for enhanced carbapenem resistance (Table 1). IS*Aba1*, an IS4 family member, offers strong promoter activity to associated genes and is common in *Ab* isolates, and IS*Aba1*-*bla*$_{\text{OXA-23}}$ imparts a high level of carbapenem resistance [6, 20]. In the 39 carbapenem-resistant isolates, the following distribution for carbapenemase producing *bla* genes or associated transposons was observed (number of isolates, gene): 5, Tn*2008* and *bla*$_{\text{OXA-66}}$; 2, Tn*2008*, IS*Aba1*-*bla*$_{\text{OXA-66}}$; 19, IS*Aba1*-*bla*$_{\text{OXA-66}}$ and *bla*$_{\text{OXA-40}}$; 9, *bla*$_{\text{OXA-66}}$ and *bla*$_{\text{OXA-40}}$; 1, IS*Aba1*-*bla*$_{\text{OXA-66}}$; 3, *bla*$_{\text{OXA-66}}$. Clearly, for 27 isolates, resistance was due to Tn*2008* or IS*Aba1*-*bla*$_{\text{OXA-66}}$, both of which are known to provide carbapenemase activities; for the nine isolates, the resistance was likely due to an overexpression of *bla*$_{\text{OXA-40}}$, and for the three strains it was due to an unknown mechanism. Of the 18 carbapenem-sensitive isolates, three were negative for all *bla* genes tested for, 10 had only *bla*$_{\text{OXA-66}}$, two were with IS*Ab*A1-*bla*$_{\text{OXA-66}}$, two carried *bla*$_{\text{OXA-66}}$ and *bla*$_{\text{OXA-40}}$, and one possessed IS*Ab*A1-*bla*$_{\text{OXA-66}}$ and *bla*$_{\text{OXA-40}}$. The carbapenem sensitivity in three strains with IS*Ab*A1-*bla*$_{\text{OXA-66}}$ was surprising and the possible reasons for this phenotype include mutations in the promoters in IS*AbA1* and/or in the *bla* gene. Promoter and/or coding sequence mutations could also be reasons for an observed lack of carbapenem resistance in strains carrying *bla*$_{\text{OXA-40.}}$

5. Conclusion

The pCMCVT*Ab*2-borne Tn*2008* (Figs. 2B and C) was responsible for a high-level carbapenem resistance in the outbreak strains of *A. baumannii*. This is the first such report in the Commonwealth of Virginia. A PCR-based assay targeting Tn*2008* and other parts of pCMCVT*Ab*2 would allow epidemiological tracking and control of CR-MDR-*Ab* infections.

Competing interests

None declared.

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Ethical approval

The study followed prior approval from the local Institutional Review Board.

References

[1] Peleg AY, Hooper DC. Hospital-acquired infections due to gram-negative bacteria. N Engl J Med 2010;362:1804-13.
[2] Peleg AY, Seifert H, Paterson DL. *Acinetobacter baumannii*: emergence of a successful pathogen. Clin Microbiol Rev 2008;21:538-82.
[3] MacVane SH. Antimicrobial Resistance in the Intensive Care Unit: A Focus on Gram-Negative Bacterial Infections. J Intensive Care Med 2017;32:25-37.
[4] Wilks M, Wilson A, Warwick S, Price E, Kennedy D, Ely A, et al. Control of an outbreak of multidrug-resistant *Acinetobacter baumannii-calcoaceticus* colonization and infection in an intensive care unit (ICU) without closing the ICU or placing patients in isolation. Infect Control Hosp Epidemiol 2006;27:654-8.
[5] Shete VB, Ghadage DP, Muley VA, Bhore AV. Multi-drug resistant *Acinetobacter* ventilator-associated pneumonia. Lung India 2010;27:217-20.
[6] Evans BA, and Amyes SG . 2014. OXA beta-lactamases. *Clin. Microbiol Rev.* **27** (**2**): 241-263.
[7] Pagano M, Martins AF, Barth AL. Mobile genetic elements related to carbapenem resistance in *Acinetobacter baumannii*. Braz J Microbiol 2016;47:785-92.
[8] Charlson ME, Pompei P, Ales KL, MacKenzie CR. A new method of classifying prognostic comorbidity in longitudinal studies: development and validation. J Chronic Dis 1987;40:373-83.
[9] Chin CS, Alexander DH, Marks P, Klammer AA, Drake J, Heiner C, et al. Nonhybrid, finished microbial genome assemblies from long-read SMRT sequencing data. Nat Methods 2013;10:563-9.
[10] Krumsiek J, Arnold R, Rattei T. Gepard: a rapid and sensitive tool for creating dotplots on genome scale. Bioinformatics 2007;23:1026-8.
[11] Treangen TJ, Sommer DD, Angly FE, Koren S, Pop M. Next generation sequence assembly with AMOS. Curr Protoc Bioinformatics 2011;Chapter 11:Unit 11 8.
[12] Sommer DD, Delcher AL, Salzberg SL, Pop M. Minimus: a fast, lightweight genome assembler. BMC Bioinformatics 2007;8:64.
[13] Overbeek R, Olson R, Pusch GD, Olsen GJ, Davis JJ, Disz T, et al. The SEED and the Rapid Annotation of microbial genomes using Subsystems Technology (RAST). Nucleic Acids Res 2014;42:D206-14.
[14] Gao F, Zhang CT. Ori-Finder: a web-based system for finding oriCs in unannotated bacterial genomes. BMC Bioinformatics 2008;9:79.
[15] Altschul SF, Gish W, Miller W, Myers EW, Lipman DJ. Basic local alignment search tool. J Mol Biol 1990;215:403-10.
[16] Goris J, Konstantinidis KT, Klappenbach JA, Coenye T, Vandamme P, Tiedje JM. DNA-DNA hybridization values and their relationship to whole-genome sequence similarities. Int J Syst Evol Microbiol 2007;57:81-91.
[17] Rafei R, Kempf M, Eveillard M, Dabboussi F, Hamze M, Joly-Guillou ML. Current molecular methods in epidemiological typing of *Acinetobacter baumannii*. Future Microbiol 2014;9:1179-94.
[18] Markelz AE, Mende K, Murray CK, Yu X, Zera WC, Hospenthal DR, Beckius ML, Calvano T, and Akers KS. 2011. Carbapenem susceptibility testing errors using three automated systems, disk diffusion, Etest, and broth microdilution and carbapenem resistance genes in

isolates of *Acinetobacter baumannii-calcoaceticus* complex. Antimicrobial Agents and Chemotherapyvol. 55 (10): 4707-4711.
[19] CLSI. Performance Standards for Antimicrobial Susceptibility Testing. 27 th ed. CLSI Supplement M100. Wayne, PA: Clinical and Laboratory Standards Institute; 2017.
[20] Nigro SJ, Hall RM. Loss and gain of aminoglycoside resistance in global clone 2 *Acinetobacter baumannii* in Australia via modification of genomic resistance islands and acquisition of plasmids. J Antimicrob Chemother 2016;71:2432-40.
[21] Amin IM, Richmond GE, Sen P, Koh TH, Piddock LJ, Chua KL. A method for generating marker-less gene deletions in multidrug-resistant A*cinetobacter baumannii*. BMC Microbiol 2013;13:158.
[22] Hamidian M, Kenyon JJ, Holt KE, Pickard D, Hall RM. A conjugative plasmid carrying the carbapenem resistance gene *bla*$_{\text{OXA-23}}$ in AbaR4 in an extensively resistant GC1 *Acinetobacter baumannii* isolate. J Antimicrob Chemother 2014;69:2625-8.
[23] Nigro S, Hall RM. Distribution of the *bla*$_{\text{OXA-23}}$-containing transposons Tn*2006* and Tn*2008* in Australian carbapenem-resistant *Acinetobacter baumannii* isolates. J Antimicrob Chemother 2015;70:2409-11.
[24] Boo TW, Walsh F, Crowley B. Molecular characterization of carbapenem-resistant *Acinetobacter* species in an Irish university hospital: predominance of *Acinetobacter* genomic species 3. J Med Microbiol 2009;58:209-16.
[25] Zong Z, Lu X, Valenzuela JK, Partridge SR, Iredell J. An outbreak of carbapenem-resistant *Acinetobacter baumannii* producing OXA-23 carbapenemase in western China. Int J Antimicrob Agents 2008;31:50-4.
[26] Karah N, Dwibedi CK, Sjostrom K, Edquist P, Johansson A, Wai SN, et al. Novel Aminoglycoside Resistance Transposons and Transposon-Derived Circular Forms Detected in Carbapenem-Resistant *Acinetobacter baumannii* Clinical Isolates. Antimicrob Agents Chemother 2016;60:1801-18.
[27] Wu PJ, Shannon K, Phillips I. Effect of hyperproduction of TEM-1 beta-lactamase on in vitro susceptibility of *Escherichia coli* to beta-lactam antibiotics. Antimicrob Agents Chemother 1994;38:494-8.
[28] Blackwell GA, Hamidian M, Hall RM. IncM Plasmid R1215 Is the Source of Chromosomally Located Regions Containing Multiple Antibiotic Resistance Genes in the Globally Disseminated *Acinetobacter baumannii* GC1 and GC2 Clones. mSphere 2016;1.
[29] Fang Y, Quan J, Hua X, Feng Y, Li X, Wang J, et al. Complete genome sequence of *Acinetobacter baumannii* XH386 (ST208), a multi-drug resistant bacteria isolated from pediatric hospital in China. Genom Data 2016;7:269-74.
[30] Hayes F. The partition system of multidrug resistance plasmid TP228 includes a novel protein that epitomizes an evolutionarily distinct subgroup of the ParA superfamily. Mol Microbiol 2000;37:528-41.
[31] Wright MS, Haft DH, Harkins DM, Perez F, Hujer KM, Bajaksouzian S, et al. New insights into dissemination and variation of the health care-associated pathogen *Acinetobacter baumannii* from genomic analysis. MBio 2014;5:e00963-13.
[32] Lin YC, Hsia KC, Chen YC, Sheng WH, Chang SC, Liao MH, et al. Genetic basis of multidrug resistance in *Acinetobacter* clinical isolates in Taiwan. Antimicrob Agents Chemother 2010;54:2078-84.

[33] Villar M, Cano ME, Gato E, Garnacho-Montero J, Miguel Cisneros J, Ruiz de Alegria C, et al. Epidemiologic and clinical impact of *Acinetobacter baumannii* colonization and infection: a reappraisal. Medicine (Baltimore) 2014;93:202-10.
[34] Abbo A, Carmeli Y, Navon-Venezia S, Siegman-Igra Y, Schwaber MJ. Impact of multi-drug-resistant *Acinetobacter baumannii* on clinical outcomes. Eur J Clin Microbiol Infect Dis 2007;26:793-800.
[35] Kim UJ, Kim HK, An JH, Cho SK, Park KH, Jang HC. Update on the Epidemiology, Treatment, and Outcomes of Carbapenem-resistant *Acinetobacter infections*. Chonnam Med J 2014;50:37-44.
[36] Lee Y, D'Souza R, Yong D, Lee K. Prediction of Putative Resistance Islands in a Carbapenem-Resistant *Acinetobacter baumannii* Global Clone 2 Clinical Isolate. Ann Lab Med 2016;36:320-4.
[37] Nigro SJ, Farrugia DN, Paulsen IT, Hall RM. A novel family of genomic resistance islands, AbGRI2, contributing to aminoglycoside resistance in *Acinetobacter baumannii* isolates belonging to global clone 2. J Antimicrob Chemother 2013;68:554-7.
[38] Nigro SJ, Hall RM. Tn*6167*, an antibiotic resistance island in an Australian carbapenem-resistant *Acinetobacter baumannii* GC2, ST92 isolate. J Antimicrob Chemother 2012;67:1342-6.
[39] Sung JY, Koo SH, Cho HH, Kwon KC. AbaR7, a genomic resistance island found in multidrug-resistant *Acinetobacter baumannii* isolates in Daejeon, Korea. Ann Lab Med 2012;32:324-30.

Figure Legends

Figure 1. Prevalence of multidrug-resistant and carbapenem-resistant *Acinetobacter baumannii* infections encountered at Carilion Medical Center (CMC), Roanoke, VA, from August 2009-February 2012. Multidrug-resistant or MDR, blue line; carbapenem-resistant or CR, red dotted line.

Figure 2. Circular maps of plasmids of *A. baumannii* clinical isolates. A. pCMCVTAb1, a plasmid containing phage elements and several transposons (*tnp*); **B and C.** pCMCVTAb2-4 and pCMCVTAb2-66, respectively. pCMCVTAb2 contains genes encoding conjugative transfer protein (*tra*), Tn*2008*, *tel*A (tellurite resistance gene) and a complete or partial components of Tn*aph*A6. *lig*K and *par*A, genes encoding DNA ligase and partition protein (ATPase), respectively, were selected for plasmid backbone screening (Table 1). White arrows without label, hypothetical proteins; OriC, origin of replication.

Figure 3. Antibiotic resistance islands in *A. baumannii* isolates (CMC-CR-MDR-*Ab*4, 59 and 66**).** Three AbaR type [7] islands, I-III, carrying antibiotic resistance genes are shown. Each was flanked by direct repeats, which likely resulted from transposition into the chromosome. The names of the strains bearing these elements have been indicated. The structures of the islands I and II varied among isolates. Abbreviations: IS, Insertion sequence; Tn, transposon. The IS and Tn elements have been identified with their previously described names [23, 37, 38]. AbGRI, *A. baumannii* genomic resistance island [37]. The inverted repeats at 5'- and 3-ends (left and right ends), IRL and IRR, for the IS elements and transposons are shown in Table S6. The arrows

represent the ORFs and their orientations. Arrow color: white, hypothetical protein; black, IS element; pink, β-lactamase (*bla*); red, antibiotic resistance proteins except β-lactamases; and green, magnesium chelatase (*comM*) [39]. Other colors are gene-specific. In most cases previously described gene names have been used and the functions of these genes have been discussed in the text. The parts of disrupted or truncated genes have been named according to their relative location (5' or 3' part) in the intact gene. WP_000736777.1-5' and WP_000736777.1-3' in Island I (ORFs AOT16_06285 and AOT16_06265 in CMC-CR-MDR-*Ab*4) represent parts of an intact ORF of an *Acinetobacter baumannii* strain (accession number, WP_000736777.1). It is not clear whether WP_000736777.1, a putative DNA-binding protein, has a role in DNA replication and/or transcriptional control. In Island III, the terminal direct repeats as shown belong to the insertion site in the *comM* gene that is shown right below the Island. The information presented in this figure to a large extent has relied on published literature that has been cited in the text.

Figure 1.

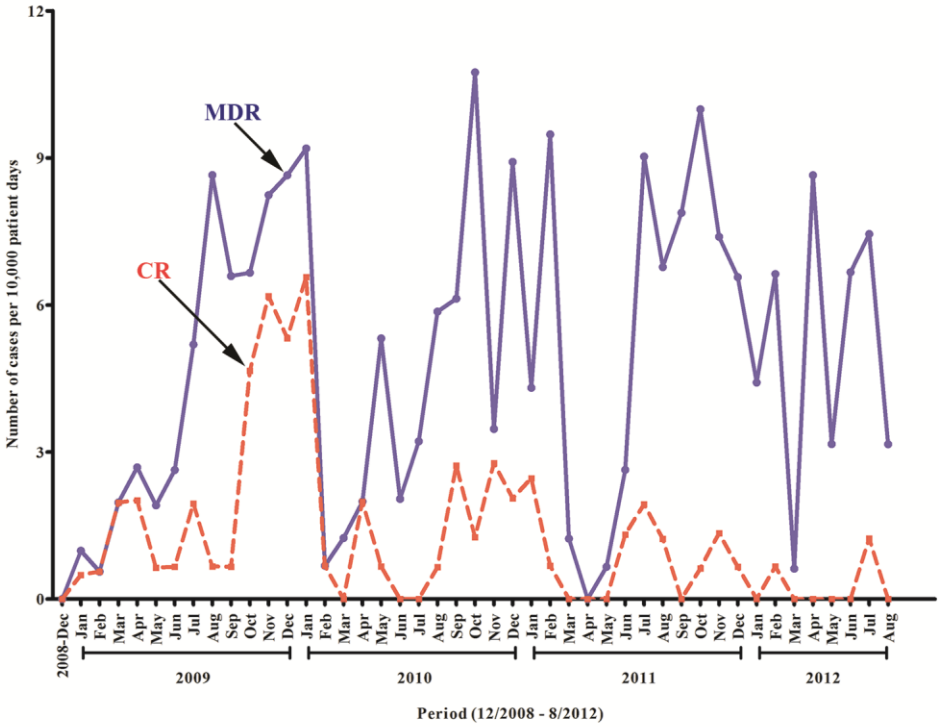

Figure 2.

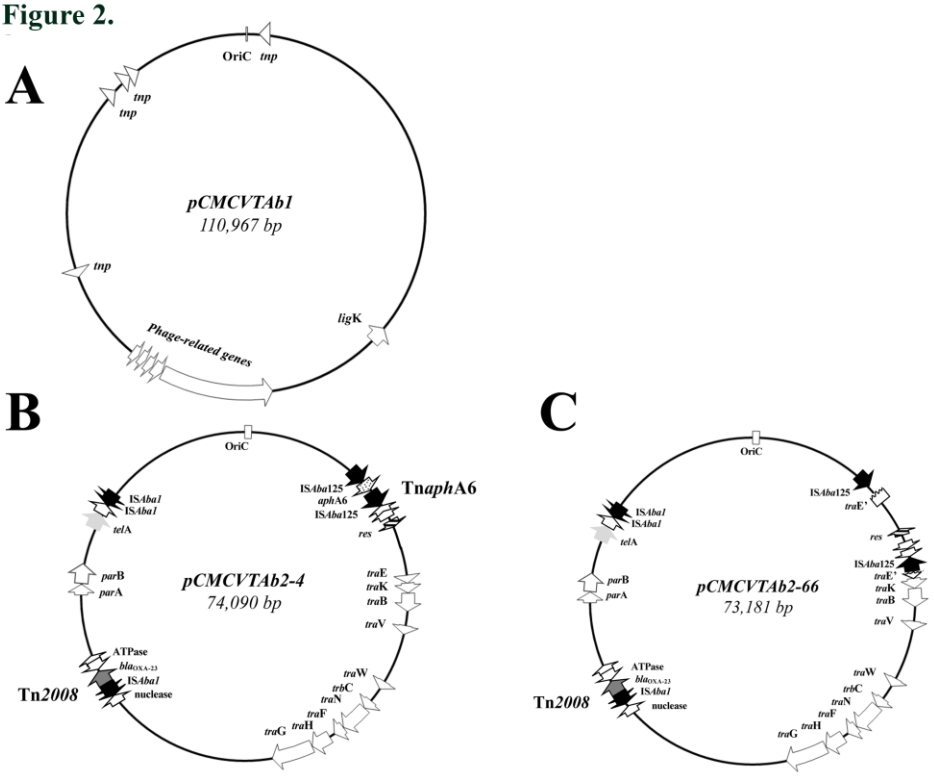

Figure 3.

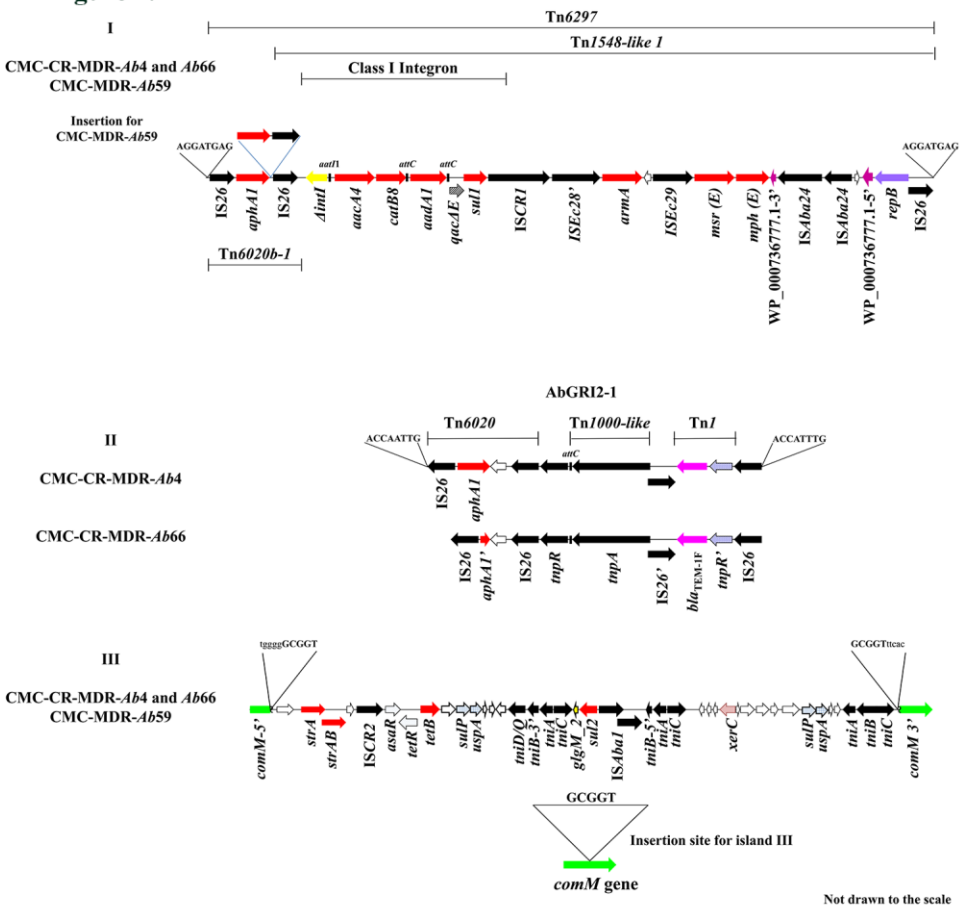

Table 1. Phenotypes and genotypes of *A. baumannii* strains

Strain designation[a]	Source	Antibiotic Sensitivity[b]			PCR detection of pCMCVTAb1, pCMCVTAb2, selected transposons and a beta-lactamase gene (+, present; -, absent)							Charlson comorbidity index score	Discharge[g] disposition
		Gentamicin	Tobramycin	Carbapenem[c]	*ligK*[d]	*parA*[d]	Tn*aphA6*[e]	Tn*2008*[e]	bla_{OXA-66}	*ISAba1*-bla_{OXA-66}	bla_{OXA-40}[f]		
CMC-CR-MDR-*Ab*1	Respiratory	R	R	R/I	+	+	+	+	+	-	-	5	Expired
CMC-CR-MDR-*Ab*2	Respiratory	R	R	R/I	+	+	+	+	+	-	-	0	NA
CMC-CR-MDR-*Ab*3	Wound	R	R	R/I	+	+	+	+	+	-	-	0	SNF/Rehab
CMC-	Respir	R	R	R/I	+	+	+	+	+	-	-	1	Home

CR-MDR-*Ab*4	atory												
CMC-CR-MDR-*Ab*5	Urine	R	R	R/I	+	+	+	+	+	-	-	0	NA
CMC-CR-MDR-*Ab*7	NA	S	S	R/I	+	+	+	+	+	-	-	NA	NA
CMC-*Ab* 8	NA	S	S	S/I	+	-	-	-	-	-	-	NA	NA
CMC-MDR-*Ab*9	NA	R	R	S/I	+	-	-	-	+	-	-	NA	NA
CMC-MDR-*Ab*11	Wound	R	R	S/I	+	-	-	-	+	-	+	2	NA
CMC-CR-MDR-*Ab*13	Blood	R	R	R/I	+	+	-	-	+	-	+	3	Expired
CMC-MDR-*Ab*16	Respiratory	R	R	S/I	+	-	-	-	+	-	-	3	SNF/Rehab
CMC-MDR-	Respiratory	R	R	S/I	+	-	-	-	-	-	-	1	SNF/Rehab

*Ab*21													
CMC-CR-MDR-*Ab*41	Respiratory	R	R	R/I	-	-	-	-	+	-	+	3	Expired
CMC-CR-MDR-*Ab*47	Respiratory	R	R	R/I	+	-	-	-	+	-	+	3	Expired
CMC-MDR-*Ab*59	Respiratory	R	R	S/I	+	-	-	-	+	-	-	0	SNF/Rehab
CMC-MDR-*Ab*61	Respiratory	R	R	S/I	+	-	-	-	+	-	-	4	NA
CMC-CR-MDR-*Ab*62	Respiratory	R	R	R/I	+	+	+	+	+	-	-	0	Home
CMC-CR-MDR-*Ab*66	Wound	R	R	R/I	+	+	-	+	+	-	-	2	SNF/Rehab
CMC-MDR-*Ab*72	Respiratory	R	R	S/I&M	+	-	-	-	+	-	partial	3	Expired
CMC-CR-	Respiratory	R	R	R/I	+	-	-	-	+	-	partial	0	SNF/Rehab

MDR-*Ab*73													
CMC-CR-MDR-*Ab*1001	Respiratory	R	R	R/M	+	-	-	-	+	+	+	2	SNF/Rehab
CMC-CR-MDR-*Ab*1003	Perirectal	S	S	R/M	+	+	-	+	+	-	-	9	SNF/Rehab
CMC-CR-MDR-*Ab*1004	Nasal	S	S	R/M	+	-	-	-	+	-	+	0	SNF/Rehab
CMC-*Ab*1005	Blood	S	S	S/I	+	-	-	-	+	-	-	0	SNF/Rehab
CMC-CR-MDR-*Ab*1006	Nasal	R	R	R/M	+	-	-	-	+	+	+	4	Home
CMC-CR-MDR-*Ab*1007	Respiratory	S	R	R/M	+	+	-	+	+	+	+	9	SNF/Rehab
CMC-CR-MDR-*Ab*1008	Urine	R	S	R/M	+	+	-	-	+	+	+	11	SNF/Rehab

CMC-*Ab*1010	Respiratory	S	S	S/M	+	-	-	-	-	-	partial	1	SNF/Rehab
CMC-CR-MDR-*Ab*1011	Respiratory	R	R	R/M	+	-	-	-	+	-	+	0	SNF/Rehab
CMC-CR-MDR-*Ab*1012	Urine	S	R	R/M	+	-	-	-	+	+	+	3	SNF/Rehab
CMC-CR-MDR-*Ab*1013	Nasal	R	R	R/M	+	-	-	-	+	+	+	0	SNF/Rehab
CMC-CR-MDR-*Ab*1014	Nasal	R	R	R/M	+	-	-	-	+	+	+	7	Home
CMC-CR-MDR-*Ab*1015	Urine	S	S	R/M	+	-	-	-	+	+	+	4	Home
CMC-CR-MDR-*Ab*1016	Blood	S	R	S/M	+	-	-	-	+	+	-	7	Home
CMC-CR-	Nasal	R	R	R/I	+	+	-	-	+	+	+	2	SNF/Rehab

MDR-*Ab*1017													
CMC-CR-MDR-*Ab*1018	Nasal	S	S	R/M	+	+	-	+	+	+	+	9	SNF/Rehab
CMC-CR-MDR-*Ab*1019	Respiratory	R	R	R/M	+	+	-	-	+	+	+	1	Expired
CMC-*Ab*1020	Respiratory	S	S	S/I	+	+	-	-	+	-	+	5	Expired
CMC-CR-MDR-*Ab*1021	Urine	R	R	R/M	+	-	-	-	+	+	+	4	Home
CMC-MDR-*Ab*1022	Respiratory	R	S	S/M	+	-	-	-	+	+	+	4	SNF/Rehab
CMC-CR-MDR-*Ab*1023	Respiratory	R	R	R/M	+	-	-	-	+	+	-	4	SNF/Rehab
CMC-*Ab*1024	Respiratory	S	S	S/M	+	-	-	-	+	+	-	2	SNF/Rehab
CMC-CR-MDR-	Perirectal	R	R	R/M	+	-	-	-	+	+	+	1	SNF/Rehab

*Ab*1025													
CMC-CR-MDR-*Ab*1026	Respiratory	R	R	R/M	+	-	-	-	+	+	+	0	SNF/Rehab
CMC-CR-MDR-*Ab*1027	Perirectal	R	R	R/M	+	-	-	-	+	-	-	4	SNF/Rehab
CMC-CR-MDR-*Ab*1028	Respiratory	S	S	R/M	+	-	-	-	+	-	partial	1	SNF/Rehab
CMC-CR-MDR-*Ab*1029	Urine	R	S	R/M	+	+	-	+	+	-	partial	9	SNF/Rehab
CMC-CR-MDR-*Ab*1030	Urine	R	R	R/M	+	+	-	-	+	-	+	4	SNF/Rehab
CMC-*Ab*1031	Respiratory	S	S	S/M	+	+	-	-	+	-	partial	1	Home
CMC-CR-MDR-*Ab*1075	Respiratory	R	R	R/M	+	-	-	-	+	+	+	7	Home
CMC-	Blood	R	R	R/M	+	-	-	-	+	+	+	0	SNF/R

CR-MDR-*Ab*1077													ehab
CMC-MDR-*Ab*1079	Blood	R	R	S/M	+	-	-	-	+	-	pratial	1	SNF/Rehab
CMC-CR-MDR-*Ab*1084	Respiratory	R	R	R/M	+	-	-	-	+	+	+	2	SNF/Rehab
CMC-CR-MDR*Ab*1089	Respiratory	R	R	R/I	+	-	-	-	+	+	+	2	Home
CMC-MDR-*Ab*1120	Respiratory	R	R	S/M	-	-	-	-	+	-	pratial	2	SNF/Rehab
CMC-CR-MDR-*Ab*1122	Perirectal	S	S	R/M	-	-	-	-	+	+	+	2	SNF/Rehab
CMC-CR-MDR-*Ab*1123	Blood	R	R	R/M	-	-	-	-	+	+	+	5	SNF/Rehab
CMC-*Ab*1124	Respiratory	S	S	S/M	-	-	-	-	+	-	pratial	5	SNF/Rehab
CMC-	Perirec	S	S	R/M	+	-	-	-	+	-	+	1	SNF/R

CR-MDR-*Ab*1126	tal												ehab
CMC-CR-MDR-*Ab*1128	Respiratory	S	S	R/M	-	-	-	-	+	-	+	3	SNF/Rehab
CMC-CR-MDR-*Ab*1130	Respiratory	R	R	R/M	+	-	-	-	+	+	+	3	SNF/Rehab
CMC-CR-MDR-*Ab*1131	Blood	S	S	R/M	-	-	-	-	+	-	+	4	SNF/Rehab

Contd/-

Table 1, continued

[a]Strain designations were based on Carilion Clinic's de-identified numbers; [b]R, Resistant, S, Sensitive (according to the Clinical and Laboratory Standards Institute (CLSI) guidelines) [19]; [c]I or M, Resistance or sensitivity was tested, respectively, with Imipenem or Meropenem, two commonly used Carbapanems; [d]*lig*K and *par*A are markers for pCMCVT*Ab*1 and pCMCVT*Ab*2, respectively (Fig. 2); [e]details for the transposons Tn*2008* and Tn*aphA6* are in Fig. 2; [f]the qualification "Partial" for some of the strains states that PCR analysis detected a truncated form or a part of $bla_{\text{OXA-40}}$ gene; [g]SNF, Skilled nursing facility. The strains CMC-MDR-*Ab*16, 62 and 66 carried $bla_{\text{TEM-1}}$. Shades: grey, isolates from the period of increased incidence of infection; blue, PCR positive. Note: PCR results for $bla_{\text{OXA-143}}$, $bla_{\text{OXA-58}}$, $bla_{\text{NDM-1}}$, $bla_{\text{VIM-2}}$, $bla_{\text{SIM-1}}$ and $bla_{\text{IMP-1}}$ were negative for all the isolates.

Table 2. Chromosomally-encoded antibiotic resistance genes in clinical isolates of *Acinetobacter baumannii* (*Ab*)

Resistance Gene[a] (Remarks)	**Antibiotic resistance island number (Fig. 3)**	**Accession number of the reference gene[b]**	**Antibiotic class**	**ORF numbers for resistance gene in the indicated strains (amino acid substitutions with respect to the reference[c])**		
				***Ab*4** (CP016295)[d]	***Ab*59** (CP016298)[d]	***Ab*66** (CP016300)[d]
bla$_{TEM-1F}$	II	AF188200	Betalactams (Pencillins, Carbapenems)	AOT16_12495	-	AOT18_12480
bla$_{ADC-25}$	-	EF016355		AOT16_05315 (A245E; N341T)	AOT17_05280 A245E	AOT18_05275 A245E N341T
bla$_{OXA-66}$	-	FJ360530		AOT16_10390	AOT17_10365	AOT18_10370
*sul*2	III	GQ421466	Sulfonamide	AOT16_17635	AOT17_17595	AOT18_17750
*sul*1	I	AY224185		AOT16_06225	AOT17_06200	AOT18_06195
MFS_1 (Major Facilitator Superfamily)	I	EEX04381	Multidrug (Fluoroquinolone, Macrolides, Tetracyclines and Chloramphenicol)	AOT16_06300	AOT17_06275	AOT18_06270
*tet*B	II	AP000342	Tetracycline	AOT16_17575	AOT17_17535	AOT18_17690
*cat*B8	I	AF227506	Phenicol	AOT16_06210	AOT17_06185	AOT18_06180
Bcr/CflA (Drug resistance efflux transporter)	-	WP_031980383.1	Multidrug protein (Bicyclomycin, Chloramphenicol and Florfenicol)	AOT16_03290 I15V	AOT17_03280 I15V	AOT18_03275 I15V

Continued/-

Table 2.Continued/-

Resistance Gene[a] (Remarks)	**Antibiotic resistance island number (Fig. 3)**	**Accession number of the reference gene[b]**	**Antibiotic class**	**ORF numbers for resistance gene in the indicated strains (amino acid substitutions with respect to the reference[c])**		
				***Ab*4** (CP016295)[d]	***Ab*59** (CP016298)[d]	***Ab*66** (CP016300)[d]
mph(E)	I	EU294228	Macrolide, Lincosamide and Streptogramin B resistance	AOT16_06260	AOT17_06235	AOT18_06230
msr(E)	I	EU294228		AOT16_06255	AOT17_06230	AOT18_06225
*str*B: *aph(6)-Id*	III	M96392	Aminoglycoside	AOT16_17550	AOT17_17510	AOT18_17665
*str*A: *apha(3")-Ib*	III	M96392		AOT16_17545	AOT17_17505	AOT18_17660
aadA1	I	JQ414041		AOT16_06215	AOT17_06190	AOT18_06185
aacA4	I	KM278199		AOT16_06205 L102S D183V	AOT17_06180 L102S D183V	AOT18_06175 L102S D183V
*aph*A1	I & II	X62115		AOT16_06185[f] AOT16_12465	AOT17_06150[f]	AOT18_06155[f]
armA	I	AY220558		AOT16_06240	AOT17_06215	AOT18_06210
fsr	-	SBS22948.1	Fosmidomycin	AOT16_13530	AOT17_13465	AOT18_13510

[a]Antibiotic resistance genes were identified by analyzing assembled individual genomes at the ResFinder 2.1 web server (https://cge.cbs.dtu.dk//services/ResFinder) with the threshold for 98% ID and minimum length of 60% and the Antibiotic Resistance Gene Database (ARDB) webserver (https://ardb.cbcb.umd.edu/) with threshold E-value and percent identity, $1e^{-25}$ and 40%, respectively.
[b]The amino acid sequence identities with the respective query sequence, > 99.6%.
[c]Amino acid replacement with respect to the reference.
[d]The Genbank accession number for the genomes.
[e]These are generic names and experimental studies are needed for assigning the true functional names.
[f]*aphA1*carries a silent mutation at a Threonine codon, C249T.