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Next-Generation Sequencing and Comparative Analysis of Sequential Outbreaks Caused by Multidrug-Resistant *Acinetobacter baumannii* at a Large Academic Burn Center

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Next-generation sequencing (NGS) analysis has emerged as a promising molecular epidemiological method for investigating health care-associated outbreaks. Here, we used NGS to investigate a 3-year outbreak of multidrug-resistant *Acinetobacter baumannii* (MDRAB) at a large academic burn center. A reference genome from the index case was generated using *de novo* assembly of PacBio reads. Forty-six MDRAB isolates were analyzed by pulsed-field gel electrophoresis (PFGE) and sequenced using an Illumina platform. After mapping to the index case reference genome, four samples were excluded due to low coverage, leaving 42 samples for further analysis. Multilocus sequence types (MLST) and the presence of acquired resistance genes were also determined from the sequencing data. A transmission network was inferred from genomic and epidemiological data using a Bayesian framework. Based on single-nucleotide variant (SNV) differences, this MDRAB outbreak represented three sequential outbreaks caused by distinct clones. The first and second outbreaks were caused by sequence type 2 (ST2), while the third outbreak was caused by ST79. For the second outbreak, the MLST and PFGE results were discordant. However, NGS-based SNV typing detected a recombination event and consequently enabled a more accurate phylogenetic analysis. The distribution of resistance genes varied among the three outbreaks. The first- and second-outbreak strains possessed a $bla_{\text{OXA-23-like}}$ group, while the third-outbreak strains harbored a $bla_{\text{OXA-40-like}}$ group. NGS-based analysis demonstrated the superior resolution of outbreak transmission networks for MDRAB and provided insight into the mechanisms of strain diversification between sequential outbreaks through recombination.

Health care-associated infections (HAI) are a substantial cause of morbidity and mortality in acute-care hospitals (1). *Acinetobacter baumannii* is an important opportunistic pathogen causing HAI (2) and has become one of the most common colonizing pathogens in burn patients (3, 4). *A. baumannii* may cause serious outbreaks despite the implementation of rigorous infection prevention interventions and control measures, which occur most commonly in intensive care units (5–8). Furthermore, the emergence of multidrug-resistant *A. baumannii* (MDRAB), especially carbapenem-resistant *A. baumannii* (CRAB), has become a global concern (5, 9). Patients infected by multidrug-resistant *Acinetobacter* strains are likely to have higher mortality rates and longer lengths of hospitalization than those infected by susceptible strains (10).

Pulsed-field gel electrophoresis (PFGE) has been the gold standard approach for bacterial strain typing in hospital outbreak investigations, but several disadvantages have been described, including that it is a time-consuming, labor-intensive, and technically demanding assay and that is has limited reproducibility among laboratories; also, the interpretation of the relative relatedness of strains using this method may be discordant and subjective (11–13). For these reasons, next-generation sequencing (NGS) has emerged as a promising molecular epidemiology method in investigations of health care-associated outbreaks (13). A comparative analysis of single-nucleotide variants (SNVs) in bacterial genomes provides valuable insights into genomic diver-

sity and evolution (e.g., determining the relatedness among epidemiologically linked strains and tracking bacterial strains of interest) (13–15).

Our hospital experienced a prolonged hospital outbreak of MDRAB, mainly among burn patients, which occurred over a 3-year period. Although sequential hospital outbreaks of MDRAB have been reported (16–22), there are very few studies that apply NGS for MDRAB strain typing in this setting. Here, we describe the molecular investigation of this outbreak, showing that this actually represents several sequential outbreaks within the burn unit. Strain typing by PFGE and NGS was conducted on 46 patient

isolates from the course of the outbreak, and NGS data were used to build a transmission network, explore the genetic relatedness of these outbreaks, and understand drug resistance patterns.

MATERIALS AND METHODS

Ethics statement. This retrospective study was approved by the institutional review board (IRB) of the University of North Carolina (UNC) at Chapel Hill (IRB no. 06-0437).

Bacterial isolates. We analyzed a total of 46 clinical isolates of MDRAB, which were collected from sequential outbreaks that occurred between 2007 and 2010 at UNC Hospitals (Chapel Hill, NC, USA), an 853-bed tertiary care academic facility (41 isolates from the North Carolina Jaycee Burn Center, Chapel Hill, NC). This represents 54% (46/85) of the nonduplicate isolates collected in this period. A single isolate from the primary site of infection or colonization was used for each patient (Table 1). *Acinetobacter* species were identified from clinical specimens collected from these sites using standard protocols within UNC's McLendon Clinical Laboratories. Antimicrobial susceptibility testing was performed for clinical isolates from each patient according to the Clinical and Laboratory Standards Institute guidelines (55), and the antimicrobial susceptibility profiles are shown in Table S1 in the supplemental material. *A. baumannii* strains were considered to be multidrug resistant if they met the Centers for Disease Control and Prevention (CDC) criteria for MDRAB (i.e., were intermediate or resistant to at least 1 drug in 3 of the 6 following classes: extended-spectrum cephalosporins, fluoroquinolones, aminoglycosides, carbapenems, piperacillin or piperacillin-tazobactam, and ampicillin-sulbactam) (2).

Patient information. Comprehensive hospital-wide surveillance for HAI is conducted at UNC using a laboratory-based approach verified through chart review. HAI were prospectively ascertained using the CDC definitions (23); the surveillance data were entered into an electronic database.

Pulsed-field gel electrophoresis. PFGE was performed on a 1% PFGE agarose gel with a CHEF-DR II system (Bio-Rad Laboratories, Richmond, CA, USA) using the SmaI restriction enzyme (New England BioLabs, Inc., Ipswich, MA, USA). Relatedness among *Acinetobacter* strains was determined as described previously (24).

Next-generation sequencing. Bacterial isolates were grown overnight in LB broth at 37°C. DNA from each isolate was extracted and purified using an UltraClean microbial DNA isolation kit (Mo Bio, Carlsbad, CA). Sequencing libraries were prepared using the Nextera XT library prep kit (Illumina, San Diego, CA) with indexed adapters, according to the manufacturer's instructions. This approach uses a modified transposase to prepare an adapter-ligated sequencing library. All short-read libraries were pooled and sequenced on a single Illumina MiSeq run, using 150-base paired-end chemistry at the UNC High-Throughput Sequencing Facility, Chapel Hill, NC.

In order to provide a reference genome specific for this outbreak against which to compare other isolates, we defined the isolate with the earliest detection date as the outbreak index case (*A. baumannii* strain A03). A whole-genome long-read sequencing library was prepared for the PacBio RSII system and was run on two single-molecule real-time (SMRT) cells (Pacific Biosciences, Menlo Park, CA) at the UNC High-Throughput Sequencing Facility.

Sequence assembly and mapping. PacBio sequencing reads were assembled *de novo* into an outbreak reference genome using Hierarchical Genome Assembly Process (HGAP) 2.0, part of the SMRT Analysis version 2.3 software package (PacBio, Menlo Park, CA), deployed on the Amazon Elastic Cloud (EC2). Illumina reads from all samples were then mapped to the PacBio-generated outbreak reference genome using bwa-mem reference-guided assembly (25). We then applied GATK version 3.3 (26) indel realignment and removed duplicate sequences. After sequence mapping, 4 of the 46 isolates were excluded from further analysis, because these isolates had low genome-wide coverage when aligned to the index case reference genome (see Fig. S1 in the supplemental material), leaving

42 isolates for further analysis. Using short reads from all samples, species identification was confirmed using Kraken (27).

Variant calling. SNV discovery was performed by simultaneously applying the GATK UnifiedGenotyper across all reference-aligned MDRAB isolates. Variants were filtered stringently using cutoffs responsive to the underlying distribution of quality scores. The following parameters were used for filtering: quality-by-depth, $\geq$20; mapping quality, $\geq$55; Fisher score, $\leq$10; map quality rank sum, -5.0 or higher; and read position rank sum, -5.0 or higher. In addition, only genomic positions with at least 5$\times$ coverage in 100% of the isolates were used for further comparative analysis.

Genetic analysis. Acquired resistance genes were identified using ResFinder version 2.1 (28), with a threshold of 95% identity and a minimum length of 40% on unaligned sequencing reads. Multilocus sequence typing (MLST) was performed, and sequence types (STs) were determined from the unaligned sequence data using MLST version 1.7 (29) and the Pasteur MLST database (http://pubmlst.org/abaumannii) (30, 31). eBURST analyses were performed under stringent (minimum of six shared alleles) grouping parameters using eBURST version 3 (32), based on STs identified from the Pasteur scheme, to investigate evolutionary relationships between the founder and the other STs. Each clonal complex (CC) was formed by the founder ST and its single-locus variants (30). Recombination analysis was undertaken using Gubbins (33). Potential blocks of horizontal gene transfer were identified by at least three base substitutions, and recombinogenic regions were masked during construction of a maximum-likelihood phylogenetic tree, using RAxML (34). Additionally, a neighbor-joining tree was constructed on the basis of SNVs from the core mapped genome and bootstrapped 100 times in R using the phangorn and ape softwares (35, 36). A corresponding map representing genome-wide SNVs was calculated in R using the adegenet software (37). Variants were also used to calculate principal component analysis using adegenet (see Fig. S2 in the supplemental material). Bayesian transmission chain reconstruction was performed in R using the outbreaker software (38). Case infectivity probability density followed a log-normal distribution, with σ of 0.8 and μ of 3 (see Fig. S3 and S4 in the supplemental material). Inferred transmission chains were then used to inform mutation rate calculations (see Fig. S5 in the supplemental material). Pairwise F_{ST} (a fixation index indicating the degree of shared alleles) between outbreaks was calculated in R using PopGenome (39) and visualized using circlize (40).

Nucleotide sequence accession numbers. All sequence data reported in this study are deposited at the DDBJ/EMBL/GenBank Sequence Read Archive (SRA) under the accession numbers DRX029904 to DRX029951 (short reads for A01 to A48), DRX029952 (long reads for A03, the index case), and DRZ007436 (A03 long-read reference sequence). See Table 1 for the accession numbers.

RESULTS

The epidemiological and clinical characteristics of patients with MDRAB infection or colonization in the sequential outbreaks are provided in Fig. 1 and Table 1. The cases included 34 (74%) patients with burns, 6 (13%) patients with toxic epidermal necrolysis or Stevens-Johnson syndrome, and 6 patients with other diseases (13%). Thirty-four of the 46 patients (74%) were diagnosed with more than one HAI, including respiratory tract infections ($n =$ 25), bloodstream infections ($n = 8$), urinary tract infections ($n =$ 8), surgical site infections ($n = 1$), and other infections ($n = 8$). Twenty-three patients (50%) died during their hospitalization.

For the long-read *de novo* reference assembly of the first-outbreak index case (A03), two PacBio SMRT cells yielded a total of 92,781 postfiltered reads, with an N_{50} of 21,638. After *de novo* assembly, the resulting reference genome was composed of 31 contigs with a maximum contig length of 2,128,013 bp, an N_{50} of 698,352 bp, and a total genome size of 4,461,520 bp. Illumina short reads for 46 isolates produced an average of 615,000 read

TABLE 1 Epidemiological and clinical characteristics of patients with multidrug-resistant *A. baumannii* infection or colonization in the sequential outbreaks

Strain ID[a]	Specimen source[b]	Accession no.	Age range (yr)	Sex[c]	Outcome	Underlying disease[d]	HAI[e]	Type of HAI[f]
A01	BAL fluid	DRX029904	60–69	M	Died	60% TBSA burn	Yes	RTI
A02	Blood	DRX029905	60–69	F	Died	30% TBSA burn	No	
A03	Tracheal aspirate	DRX029906	40–49	M	Died	60% TBSA burn	Yes	UTI
A04	Catheter tip	DRX029907	10–19	M	Survived	60% TBSA burn	Yes	RTI
A05	Tracheal aspirate	DRX029908	40–49	F	Died	50% TBSA burn	Yes	RTI, other
A06	Swab (back wound)	DRX029909	30–39	F	Died	43% TBSA burn	Yes	RTI, UTI, other
A07	Urine	DRX029910	30–39	F	Died	Meningococcal sepsis	Yes	RTI
A08	Autopsy, myocardium	DRX029911	60–69	M	Died	60% TBSA burn	Yes	RTI
A09	Throat	DRX029912	40–49	M	Survived	10% TBSA burn	No	
A10	BAL fluid	DRX029913	50–59	F	Survived	26% TBSA burn	Yes	RTI, UTI, other
A11	Sputum	DRX029914	60–69	M	Died	35% TBSA burn	No	
A12	Throat	DRX029915	20–29	M	Survived	Inhalation injury without cutaneous burn	Yes	RTI
A13	Tracheal aspirate	DRX029916	40–49	M	Survived	25% TBSA burn	Yes	RTI
A14	BAL fluid	DRX029917	50–59	M	Survived	17% TBSA burn	Yes	RTI
A15	Urine	DRX029918	80–89	M	Survived	Withdrawal from benzodiazepines complicated by aspiration pneumonia	No	
A16	Drainage (pus from nose)	DRX029919	20–29	F	Died	12.5% TBSA burn	Yes	UTI
A17	Swab (surface)	DRX029920	50–59	M	Died	End-stage liver disease, admit for liver transplantation	Yes	SSI
A18	Swab (surface)	DRX029921	70–79	M	Survived	Stevens-Johnson syndrome with TENS	No	
A19	Swab (thigh)	DRX029922	40–49	M	Survived	22% total surface area full-thickness burn	No	
A20	Tracheal aspirate	DRX029923	50–59	F	Survived	8% TBSA burn	Yes	RTI
A21	Sputum	DRX029924	0–9	M	Survived	25% TBSA burn	Yes	RTI
A22	Tracheal aspirate	DRX029925	70–79	M	Died	47% TBSA 2nd/3rd degree burn	Yes	RTI
A23	Swab (axilla)	DRX029926	60–69	M	Survived	18% burn	No	
A24	Catheter tip	DRX029927	40–49	M	Survived	75% TBSA 2nd/3rd degree burn	No	
A25	Sputum	DRX029928	60–69	M	Died	Hepatic lobe wedge resection for septic embolism	Yes	UTI
A26	Tracheal aspirate	DRX029929	0–9	M	Survived	60% TBSA burn	Yes	RTI
A27	Catheter tip	DRX029930	50–59	F	Died	Stevens-Johnson syndrome	Yes	Other
A28	Swab (axilla)	DRX029931	40–49	M	Survived	9% TBSA burn	No	
A29	Urine	DRX029932	40–49	M	Died	72% TBSA burn	Yes	RTI, UTI, other
A30	Swab (leg)	DRX029933	50–59	M	Survived	22% TBSA burn	Yes	RTI
A31	Swab (thigh)	DRX029934	40–49	F	Survived	61% TBSA burn	Yes	RTI, other
A32	Swab (abdomen)	DRX029935	20–29	M	Survived	Pyoderma gangrenosum	No	
A33	Swab (thigh)	DRX029936	50–59	F	Died	Stevens-Johnson syndrome	No	
A34	Swab (leg)	DRX029937	30–39	F	Survived	63% TBSA burn	Yes	BSI, RTI
A35	Tracheal aspirate	DRX029938	40–49	F	Died	TENS	Yes	RTI
A36	Sputum	DRX029939	80–89	F	Died	Ventral hernia repair, sepsis	Yes	BSI, RTI
A37	Swab (back wound)	DRX029940	50–59	M	Died	45% TBSA burn	Yes	RTI
A38	Throat	DRX029941	50–59	F	Died	40% TBSA burn	Yes	UTI, BSI, RTI, other
A39	Blood	DRX029942	30–39	M	Died	50% TBSA burn	Yes	BSI, RTI, UTI, other
A40	Throat	DRX029943	60–69	M	Survived	18% TBSA burn	Yes	RTI
A41	Catheter tip	DRX029944	70–79	F	Survived	TENS	Yes	BSI
A42	Throat	DRX029945	40–49	M	Survived	35% TBSA burn	Yes	BSI
A43	Swab (chest)	DRX029946	30–39	F	Died	Stevens-Johnson syndrome	Yes	BSI
A45	Throat	DRX029948	80–89	M	Survived	Inhalational injury	No	
A46	Blood	DRX029949	40–49	M	Died	30% TBSA burn	Yes	BSI
A48	Swab (neck)	DRX029951	70–79	M	Died	19% TBSA burn	Yes	RTI

[a] ID, identification.
[b] BAL, bronchoalveolar lavage.
[c] M, male; F, female.
[d] TBSA, total body surface area; TENS, toxic epidermal necrolysis.
[e] HAI, health care-associated infection.
[f] RTI, respiratory tract infection; UTI, urinary tract infection; SSI, surgical site infection; BSI, bloodstream infection.
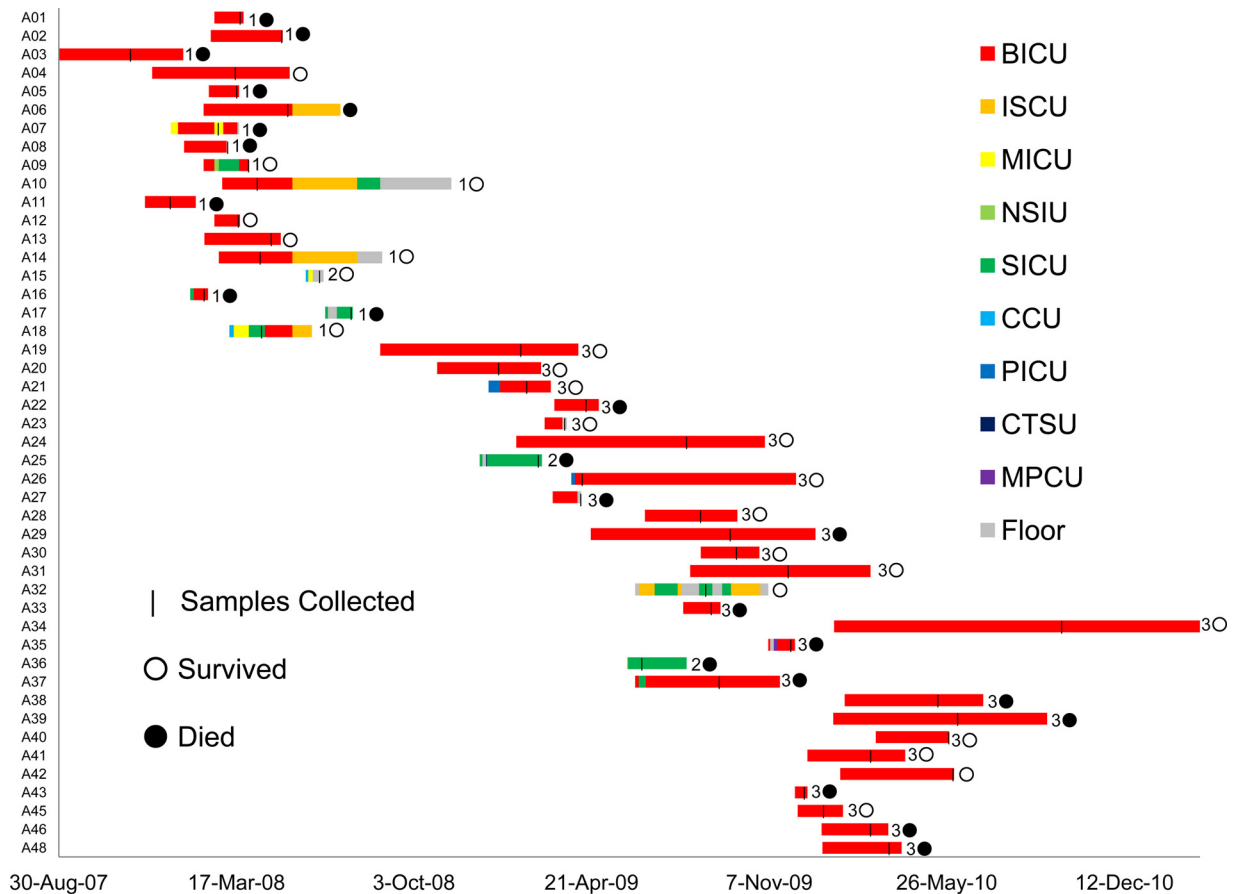
FIG 1 Timeline of sequential outbreaks caused by multidrug-resistant *Acinetobacter baumannii*. BICU, burn intensive care unit; ISCU, intermediate surgical care unit; MICU, medicine intensive care unit; NSIU, neuroscience intensive care unit; SICU, surgery intensive care unit; CCU, coronary care unit; PICU, pediatric intensive care unit; CTSU, cardiothoracic stepdown unit; MPCU, medicine progressive care unit; floor, general ward. Each case corresponds to a case described in Table 1. The numbers (1, 2, and 3) designate the outbreak to which an isolate is predicted to belong.

pairs per isolate. Kraken metagenomic sequence classification confirmed that all 46 sequenced isolates were *A. baumannii*. After reference-guided assembly to the A03 index case genome, four of 46 *A. baumannii* alignments did not reach ≥10× coverage at 40% of the reference genome (see Fig. S1 in the supplemental material) and were excluded from further analysis. Among the remaining 42 isolates, 41 isolates had ≥60% of the genome accessible for variant calling with at least 5× coverage. Variant calling identified 35,551 high-quality single-nucleotide variants that were used for NGS-based analysis.

NGS-based SNV typing suggests these sequential MDRAB outbreaks over a 3-year period were caused by three clones with distinct genetic backgrounds; the first outbreak of 13 isolates originated with A03 (August 2007 to November 2008), the second outbreak of 3 isolates originated with A15 (June 2008 to August 2009), and the third outbreak of 24 isolates originated with A20 (August 2008 to March 2011) (Fig. 2; see also Fig. S6 in the supplemental material). Within each of these outbreak clusters, the samples did not vary by >27 SNVs (see Fig. S7 in the supplemental material). The mean mutation rates (95% confidence interval [CI]) of the MDRAB strains in the first and third outbreaks were estimated to be 10.2 mutations (7.2 to 24.5 mutations) and 27.1 mutations (5.3 to 139.4 mutations) per genome per year, respectively (see Fig. S5 in the supplemental material). This is in line with

previous findings that the estimated rate of SNV accumulation in *A. baumannii* strains was approximately 2 to 10 mutations per year (13, 14). Modeling of genetic differences between the 42 genotyped infections/colonizations predicted that half of all infections/colonizations were sampled (see Fig. S4 in the supplemental material), which corresponds well with epidemiological data (42/85 infections/colonizations were sequenced and used for analysis).

NGS-based SNV typing showed that two strains (A32 and A42) collected during the outbreak period were not related to these sequential outbreaks and likely represent sporadic cases within the burn unit. Interestingly, the second outbreak (strains A15, A25, and A36) represents a strain that likely underwent recombination with the strain responsible for the initial outbreak and an additional strain that was not found among the isolates we sampled (Fig. 2; see also Fig. S8 in the supplemental material). There was 89.6% identity between A15 (second outbreak) and A18 (first outbreak) (3,687 differences/35,551 SNVs). Moreover, the SNVs that differentiate the first outbreak from the second outbreak are clustered in a few recombination events, with the vast majority of these variants (3,481/3,687 variants [94.4%]) occurring in a single 697,976-nucleotide recombination block (11.8% of the genome). The majority of the remaining variants were also clustered into recombination blocks.
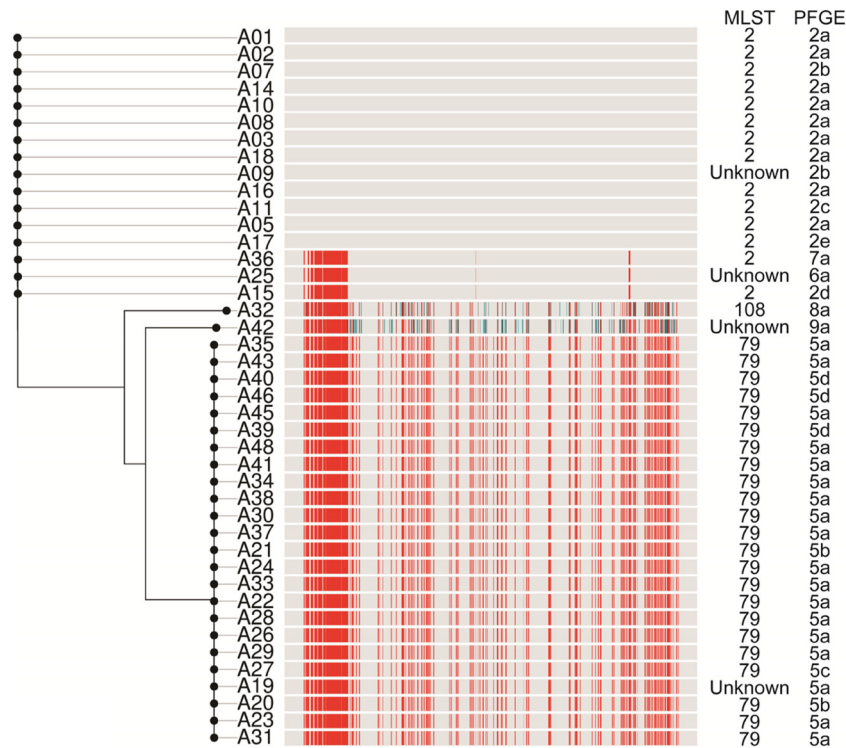
FIG 2 Maximum-likelihood (ML) phylogram (left) and recombination map (center) show relatedness between outbreak isolates. The ML phylogeny was constructed from genetic variation in nonrecombinant genomic regions. Recombination events were inferred from the >30,000 variants identified among individual isolates. In the horizontal tracks, each column represents a single nucleotide in the reference genome, and each row represents an individual clinical isolate. Red blocks mark recombination events occurring on an internal branch of the phylogeny, while blue blocks mark potential recombination events or extensive mutations occurring on a terminal node. Pasteur MLST and PFGE types are included in the first and second columns on the right, respectively.

A comparison of the NGS-based SNV typing data to the MLST and PFGE data is shown in Fig. 2. The sequenced MDRAB strains represented 3 STs, including 14 strains belonging to ST2, 23 strains of ST79, 1 strain of ST108, and 4 strains of unknown ST. The first and second outbreaks were caused by ST2. In the second outbreak, A25, which was classified as unknown due to coverage reasons, had eight SNV differences compared to A36 and three SNV differences compared to A15, both of which belong to ST2. In contrast, the third outbreak was led by ST79. PFGE differentiated the 42 isolates into 13 types. Of them, 2b, 2c, 2d, and 2e were categorized as being closely related to 2a, and 5b, 5c, and 5d were closely related to 5a by PFGE using the Tenover et al. criteria (24). However, the three strains from the second outbreak (A15, A25, and A36, which were highly similar by SNV typing) were classified into different strains by PFGE. Furthermore, PFGE showed some isolates to be closely related, with 2 to 3 band differences, despite no SNV difference between two strains (e.g., A3 and A9, and A19 and A20).

The Bayesian transmission network of the 42 MDRAB strains in sequential outbreaks is provided in Fig. 3. Most transmission events in the outbreaks were predicted with a high degree of certainty (posterior probabilities near 1.0) and may represent direct transmissions, given their genetic relatedness (see Fig. S7 in the supplemental material) and timeline (Fig. 1). In contrast, there was uncertainty with respect to certain transmission events during the first outbreak (A08, A17, and A18) and the third outbreak (A21 to A23, A26, A27, A34, and A40), with posterior probabilities for the numerous potential transmission scenarios of <0.5. Al-

though these exact transmission events were difficult to predict, these strains, except A17, shared ward space (burn intensive care unit [BICU]) and were closely grouped in time and genetic distance, suggesting epidemiologic connections between the isolates. Although A42 and some strains from the third outbreak (e.g., A34, A41, and A48) shared the same place (BICU) and time (Fig. 1), both NGS-based SNV typing and PFGE typing uncovered profound unrelatedness between A42 and strains of the third outbreak. The three isolates that comprised the second outbreak (A15, A25, and A36) had no direct mutual contact, despite convincing genetic relatedness by typing, possibly due to transmission through environmental or unsampled sources. A15 did not share a location with any other cases in this study but may have had contact with contaminated environment or health care personnel who cared for cases from the first outbreak, as some of these cases (e.g., A10, A14, and A18) were transferred outside the BICU. The SNV differences between A15 and strains from the first outbreak suggest that gene transfer may have occurred between the strain responsible for the first outbreak and another MDRAB strain within the hospital. In addition, A36 from the second outbreak shared a location (surgery intensive care unit [SICU]) and time with A37 from the third outbreak, but these strains were highly genetically divergent from one another.

Thirty-eight MDRAB isolates from this study belonged to three different STs (ST2, ST79, and ST108), while the STs of 4 strains remained undetermined. The eBURST analysis based on the Pasteur MLST scheme revealed that these 38 MDRAB strains belonged to two *A. baumannii* CCs (CC2 and CC79), while ST108
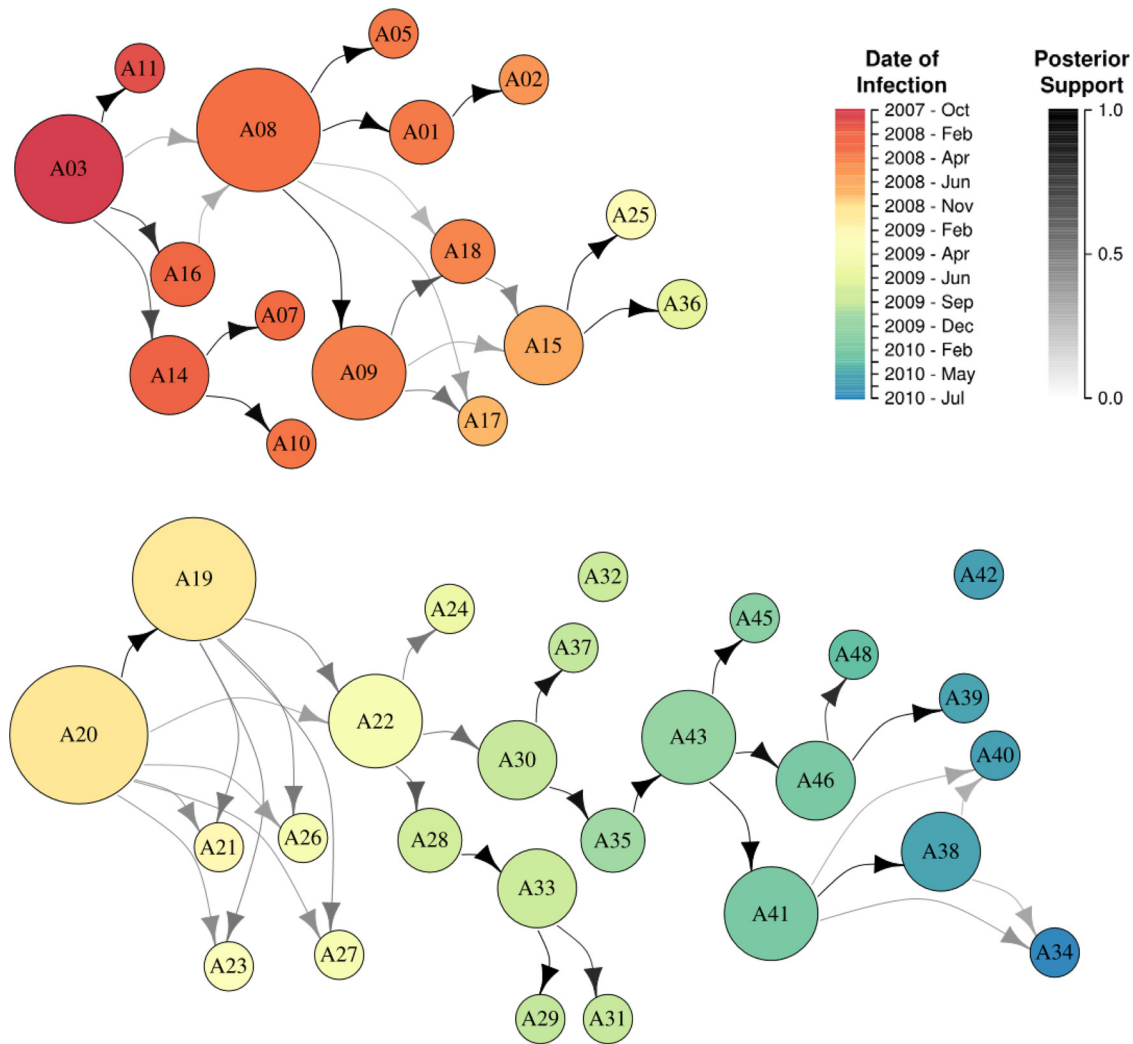
FIG 3 Bayesian reconstruction of transmission chains for all 42 multidrug-resistant *A. baumannii* outbreak isolates. The nodes are colored by isolate collection date, and the node sizes are proportional to the number of inferred outward transmission events originating from each node. Vector direction (indicated by arrows) reflects transmission sequence, and vector transparency indicates the degree of posterior support for the transmission link. Two distinct and successive outbreaks are clearly seen. Three recombinant strains (A15, A25, and A36) branching from the earliest outbreak are seen. Additionally, there were no inferred inward or outward transmission events for the two isolated non-outbreak strains (A32 and A42).

was a singleton (see Fig. S9 in the supplemental material). Fourteen MDRAB isolates (ST2) belonged to CC2, corresponding with international clone II, for which ST2 was the founder (9, 41). The remaining 23 MDRAB isolates (ST79) belonged to CC79, for which ST79 was the founder.

The antimicrobial resistance genes in these 42 multidrug-resistant *A. baumannii* strains are summarized in Table 2. Strains from the first and second outbreaks possessed *bla*$_{OXA-23-like}$ group and *bla*$_{OXA-51-like}$ group genes, while strains from the third outbreak harbored *bla*$_{OXA-40-like}$ group and *bla*$_{OXA-51-like}$ group genes. All isolates with *bla*$_{OXA-23-like}$ group genes were *bla*$_{OXA-23}$ and were classified into ST2 (except one isolate with an undetermined ST). Similarly, all isolates with *bla*$_{OXA-40-like}$ group genes were *bla*$_{OXA-24}$ (renamed *bla*$_{OXA-40}$) and were assigned to ST79 (except one isolate with undetermined ST). One isolate (A42) had the *bla*$_{OXA-58}$ gene, belonging to the *bla*$_{OXA-58-like}$ group. The isolates with *bla*$_{OXA-51-like}$ group genes included *bla*$_{OXA-65}$, *bla*$_{OXA-66}$, *bla*$_{OXA-75}$, *bla*$_{OXA-92}$, *bla*$_{OXA-116}$, and *bla*$_{OXA-117}$. The distribution of

genes resistant to other antibiotics also varied among the three outbreaks.

DISCUSSION

NGS-based analysis revealed transmission pathways during three sequential outbreaks over a 3-year period, demonstrating the longevity of MDRAB strains in this health care setting and the difficulty of infection control at a large academic burn center. Several *Acinetobacter* outbreaks in burn units were described previously and were partially associated with extensive environmental contamination (42–45). *A. baumannii* survives in the hospital environment for a prolonged period, even on dry surfaces and the hands of health care personnel, and the carriage of MDRAB in human hosts may also be prolonged (10, 46, 47), which can facilitate transmission and outbreaks of *A. baumannii*.

This study revealed some discrepancies between genotyping methods. Isolates from the second outbreak (A15 and A36) were from the same sequence type (ST2) as clones from the first out-

TABLE 2 Distribution of acquired antimicrobial resistance genes in 42 multidrug-resistant *A. baumannii* strains

Outbreak (no. of strains)	Strain ID(s)	Resistance gene type(s)				
		Aminoglycoside	β-Lactam(s)	Sulfonamide	Tetracycline	Other
First (13)	A1, A2, A3, A5, A7, A8, A9, A10, A11, A14, A16, A17, A18	*strA, strB, aac(3)-IIa, aph(3′)-Ic, aph(3′)-Ic, aph(3′)-VIa*	bla_{OXA-23}, bla_{OXA-65}, bla_{OXA-66}, $bla_{OXA-117}$, bla_{ADC-25}	*sul2*	*tet*(B)	
Second (3)	A15, A25, A36	*strA, strB, aadA1, aac(3)-Ia, aph(3′)-Ic*	bla_{OXA-23}, bla_{OXA-65}, bla_{OXA-92}, bla_{ADC-25}, bla_{TEM-1D}	*sul1, sul2*	*tet*(B)	
Third (24)	A19, A20, A21, A22, A23, A24, A26, A27, A28, A29, A30, A31, A33, A34, A35, A37, A38, A39, A40, A41, A43, A45, A46, A48	*strA, strB, aac(3)-IIa*	bla_{OXA-24}, bla_{OXA-65}, bla_{OXA-75}, $bla_{OXA-116}$, $bla_{OXA-117}$, bla_{ADC-25}, bla_{TEM-1B}	*sul2*		
Unrelated (1)	A32	*aadB, aph(3′)-VIa*	bla_{ADC-25}	*sul1, sul2*		*msr*(E), *mph*(E), *catB3*
Unrelated (1)	A42	*aadA1, aadB, aph(3′)-Ic, strA, strB*	bla_{OXA-10}, bla_{OXA-58}, bla_{ADC-25}, bla_{CARB-8}	*sul1, sul2*		*msr*(E), *mph*(E), *floR, dfrA1*

break, despite there being a difference of >3,000 SNVs between these outbreak strains. A recent study showed that unlike vancomycin-resistant *Enterococcus faecium* and methicillin-resistant *Staphylococcus aureus*, genomically identical *A. baumannii* isolates might be misclassified as different by PFGE, leading to incorrectly reconstructed transmission trees and an underestimation of the true extent of *A. baumannii* outbreaks (13). A large-scale sequencing study of *A. baumannii* strains was more discriminatory than MLST (48). These previous reports and the results from our study suggest that for *A. baumannii* in particular, NGS-based SNV typing can more informatively distinguish strains than can MLST or PFGE.

Our NGS-based analysis suggested that the second outbreak occurred following a recombination event between a bacterium of the first outbreak and an occult MDRAB clone that was likely circulating within the hospital (Fig. 2; see also Fig. S8 in the supplemental material). This is the first time that sequential outbreaks within a single hospital have been reported in which the second outbreak represents a recombinant with the original outbreak strain as a parent. Previously, several studies described that *A. baumannii* hospital outbreaks can be polyclonal, and a variety of recombination and horizontal gene transfer events occurred in *A. baumannii* strains and contributed to the genetic diversity in the microorganism, even among colonized patients (49–52). Horizontal gene transfer that occurs between sampled and unsampled *A. baumannii* isolates may explain discrepancies between NGS-based SNV typing and PFGE typing results, as was seen here (13).

Global epidemic CCs of *A. baumannii* (e.g., international clones I, II, and III), consisting of a subset of different STs, have been described (9). ST2 harboring bla_{OXA-23} was the most frequent in the first outbreak. bla_{OXA-23} was the most prevalent acquired carbapenemase-encoding gene among *A. baumannii* strains in many countries worldwide, including the United States, especially among international clone II (9, 41, 53). ST79 strains were introduced in the burn center and caused the third outbreak. To our knowledge, this was a novel outbreak due to ST79 possessing $bla_{OXA-24/40}$, although $bla_{OXA-24/40}$ was mostly detected among CC2 (9). None of the MDRAB isolates belonged to ST10, which has been associated with enhanced virulence among fatal outbreak strains (54).

This study has several potential limitations. First, sequencing failed to cover the complete genome of every isolate, which re-

duced the sensitivity of sequencing for detecting genetic variation among strains. This uneven coverage is likely due to the transposase-mediated library preparation approach used, which, despite the lack of a strong GC or AT bias in the *A. baumannii* genome, created libraries with genomic regions of high and low read representation. In this scenario, additional sequencing runs would not substantially increase coverage quality. Furthermore, the sensitivity we observed for detecting genetic variation may have been decreased, because we elected a conservative variant-calling approach in which all SNVs had to meet a minimum coverage depth in all isolates to be used for this study. Despite the shortcoming of incomplete sequence coverage, the available data enabled us to analyze >50% of the reference sequence space in these isolates. Second, there were a few discrepancies between NGS-based typing and PFGE typing. This might be explained by (i) genetic variation occurring in genomic regions where we achieved low sequence coverage, (ii) PFGE experiments being performed on different gels, or (iii) horizontal gene transfer into or out of isolates, which was not reflected in the reference genome. To realize the full potential of NGS-based analysis, reference-free methods that enable us to capture and represent the entire repertoire of genetic variation within and between samples should be developed. Finally, our transmission network analysis is likely impacted by isolates not detected or analyzed during the study. The posterior probabilities of some transmission events are likely low due to this issue.

In conclusion, our data support previous claims that NGS-based SNV typing is superior to both MLST and PFGE for the strain typing of *A. baumannii*. The use of this technique allowed us to understand at a finer level the likely transmission dynamics of the first outbreak within the hospital and to determine the nature of sequential outbreaks more thoroughly, including the previously undescribed recombinant strain responsible for the second outbreak. NGS-based analysis also allows for a better understanding of underlying resistance genes among MDRAB strains. However, challenges remain for WGS, specifically the cost, complexity of bioinformatic analysis, and lack of standard criteria for determining the number of genome variants that constitute an outbreak (13). Further WGS studies on hospital outbreak investigations and infection control are needed to help overcome these challenges. WGS can provide valuable information concerning the onset, course, and size of hospital outbreaks and can posit a

transmission network. This information can help hospital epidemiologists and infection preventionists design and implement more efficient interventions for outbreak control.

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