

Whole-Genome Sequencing to Differentiate Relapse From Reinfection in Community-Onset Bacteremic *Acinetobacter baumannii* Pneumonia

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Community-onset bacteremic *Acinetobacter baumannii* pneumonia recurred in 3 of 30 (10%) patients followed prospectively, all with ongoing hazardous alcohol intake, 3–56 months after initial pneumonia. Paired isolates underwent whole-genome sequencing. Phylogenetic analysis showed that recurrence strains were all distinct from preceding strains, indicating reinfection in susceptible individuals rather than relapse.

Keywords. *Acinetobacter baumannii*; community-acquired pneumonia; recurrence; relapse; whole-genome sequencing.

Acinetobacter baumannii is an environmental gram-negative microorganism with worldwide distribution, best known for causing outbreaks of multidrug-resistant infection in hospitals and other health care facilities [1]. *A. baumannii* is also a serious but less commonly reported cause of community-acquired pneumonia (CAP) in tropical and subtropical climates, associated with in-hospital mortality as high as 64% [2, 3]. The typical presentation is with fulminant lobar pneumonia, with hazardous alcohol consumption and diabetes as risk factors [3, 4]. We report the risk of recurrence in survivors of community-onset bacteremic *A. baumannii* pneumonia and use whole-genome sequencing to differentiate between relapse and reinfection. We also sequenced and compared matched sputum and bloodstream isolates from individuals during the same episode of infection to determine the significance of sputum *A. baumannii* isolates.

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METHODS

Clinical Definitions and *A. baumannii* Isolates

We have been conducting a prospective cohort study of community-onset bacteremic *Acinetobacter* pneumonia at Royal Darwin Hospital, the only tertiary referral center in the tropical north of the Northern Territory, Australia, and previously reported 41 cases of community-onset bacteremic *Acinetobacter* pneumonia between January 1, 1997, and December 31, 2012 [4]. The study included all known cases at Royal Darwin Hospital during the study period, and inclusion criteria were as described in detail by Davis et al. [4]. Pneumonia was defined as clinical and radiologic evidence of pneumonia, and community-onset was defined as isolation of *A. baumannii* from the bloodstream within 48 hours of hospital admission. Cases with no recent health care contact were defined as CAP, and cases where there was community onset but recent health care contact were defined as health care-associated pneumonia (HCAP). We identified patients with >1 episode of community-onset bacteremic *A. baumannii* pneumonia between January 1, 1997, and December 31, 2018, and compared whole-genome sequences for bloodstream isolates from these episodes.

In a separate analysis, *A. baumannii* isolates from blood cultures and sputum during the same episode of infection were available from 7 patients, including 1 patient with recurrent infection and 6 patients with single episodes of infection. These blood and sputum paired isolates also underwent sequencing and genomic comparison.

Antimicrobial Susceptibility Testing, Whole-Genome Sequencing, and Bioinformatics Analysis

Antimicrobial susceptibility testing was performed using the Vitek 2 platform on all isolates (BioMerieux, France). Whole-genome sequencing was performed on the Illumina HiSeq2500 with 100-bp paired-end reads. The raw sequence data are available in the Short Read Archive in Bioproject PRJNA478282.

We undertook core-genome phylogenetic analysis using single nucleotide polymorphisms (SNPs) and included an additional 47 *A. baumannii* genomes from northern and central Australia that had been sequenced as part of a previous study for local context [5]. These included 38 bloodstream isolates, 3 sputum isolates, and a pleural fluid isolate collected from 29 patients with CAP, 6 patients with HCAP, and 7 patients with nosocomial bloodstream infection. We also included 5 throat colonization isolates.

We determined the presence or absence of *A. baumannii* virulence genes and determined K and OC locus types for the initial and recurrent isolates. In each instance, the bloodstream and sputum isolate pairs were found to be very closely related.

To maximize the number of variants we could identify between the pairs, we constructed assemblies of the bloodstream isolate genomes and used these as references for mapping of the sputum isolate short reads.

The methods for DNA extraction, whole-genome sequencing, and bioinformatic analysis are detailed in full in the Supplementary Data.

Ethics

The study was approved by the Ethics Committee of the Northern Territory Department of Health and Menzies School of Health Research.

RESULTS

Is Recurrent *A. baumannii* CAP Relapse or Reinfection?

Of the 41 cases of bacteremic *Acinetobacter* pneumonia between 1997 and 2012, 35 were confirmed to be *A. baumannii*, with 2 *A. nosocomialis* and 4 identified to the *A. baumannii*-*calcoaceticus* complex only (isolates not stored and therefore not available for further testing). Thirty of 35 patients with confirmed *A. baumannii* infection survived their initial infection, and 3/30 (10%) had a recurrent episode of bacteremic *A. baumannii* pneumonia during the study period (Supplementary Table 1). In addition, 1 of the 4 patients with *A. baumannii*-*calcoaceticus* complex pneumonia had a subsequent episode of *A. baumannii* pneumonia 14 months later.

All 3 patients with >1 episode of confirmed *A. baumannii* pneumonia were of Australian Aboriginal ethnicity. All patients received treatment with intravenous gentamicin and/or meropenem, which in all episodes was commenced within the first 24 hours of presentation to hospital. None of the patients had prolonged bacteremia or prolonged culture positivity from other sites, and all had clinical resolution of infection between episodes. The duration between episodes of bacteremia ranged from 3 to 56 months. All recurrent *A. baumannii* isolates retained susceptibility to gentamicin, meropenem, and ciprofloxacin.

Patient 1

A 37-year-old male from urban Darwin with hazardous alcohol consumption had episodes of bacteremic *A. baumannii* CAP in April 1998 and January 2003, surviving both, with the second episode requiring intensive care unit (ICU) admission.

Patient 2

A 29-year-old female from a remote community had a background of previously repaired tetralogy of Fallot, bronchiectasis, and hazardous alcohol consumption. She had 3 episodes of bacteremic *A. baumannii* CAP requiring ICU admission in March 2010, October 2010, and April 2014, and she survived the final episode. Additionally, she had an episode of *A. nosocomialis* bacteremia associated with severe CAP requiring ICU admission in February 2012.

Patient 3

A 50-year-old female from urban Darwin with hazardous alcohol consumption, cirrhosis, and diabetes had episodes of bacteremic *A. baumannii* CAP requiring ICU admission in December 2011 and March 2012, and died during the second infection.

A phylogenetic tree including the *A. baumannii* strains from each episode is shown in Figure 1 and is based on 141 547 SNPs (consistency index, 0.3528). In all instances, recurrent strains did not cluster with the initial infecting strain. In 1 case, both the initial and subsequent isolates belonged to Pasteur multilocus sequence type (MLST) 10, the commonest sequence type (ST) in northern Australia [5]. However, within the ST10 clade, these strains were only distantly related, being separated by 1996 SNPs. In all other instances, strains associated with recurrence were different STs and were phylogenetically very distant from the preceding infecting strain(s). Additionally, there was discordance in the presence of genes associated with biofilm formation and the type 6 secretion system, and in K and OC locus types between episodes (Supplementary Table 1).

Clinical Significance of *A. baumannii* Isolation From Sputum During Bacteremic Infection

Seven blood and sputum pairs were collected during 7 episodes of community-onset *A. baumannii* pneumonia. Phylogenetic analysis showed that in all 7 cases the blood and sputum strains clustered together in the tree (Figure 1). Furthermore, when sputum sequence reads were mapped to the paired blood isolate assembly, the pairs were found to be almost identical and were separated by a median (range) of 2 (0–6) SNPs and a median (range) of 1 (0–5) indels, confirming that the pairs were the same strain and therefore were most likely derived from the same population of organisms in the patient.

DISCUSSION

Among survivors of bacteremic community-onset *A. baumannii* pneumonia, 10% had at least 1 recurrence, with each recurrence occurring with ongoing hazardous alcohol consumption. Whole-genome sequencing showed that these recurrent *A. baumannii* infections were new infections rather than relapse. Few previous studies have investigated recurrence of *A. baumannii* infection, with none investigating recurrent community-onset infection. A study from Taiwan reported recurrence of nosocomial *Acinetobacter* bacteremia in 6% of patients, with 12/25 patients suspected to have relapse rather than reinfection on the basis of pulsed-field gel electrophoresis comparisons [6]. Among those with relapse, half had underlying diabetes. Diabetes was also found to be a risk factor for recurrent episodes of *A. baumannii* isolation in respiratory specimens in a Taiwanese ventilation weaning unit; however, molecular typing was not performed [7]. Although diabetes was present in 27% of patients in our cohort study [4], including

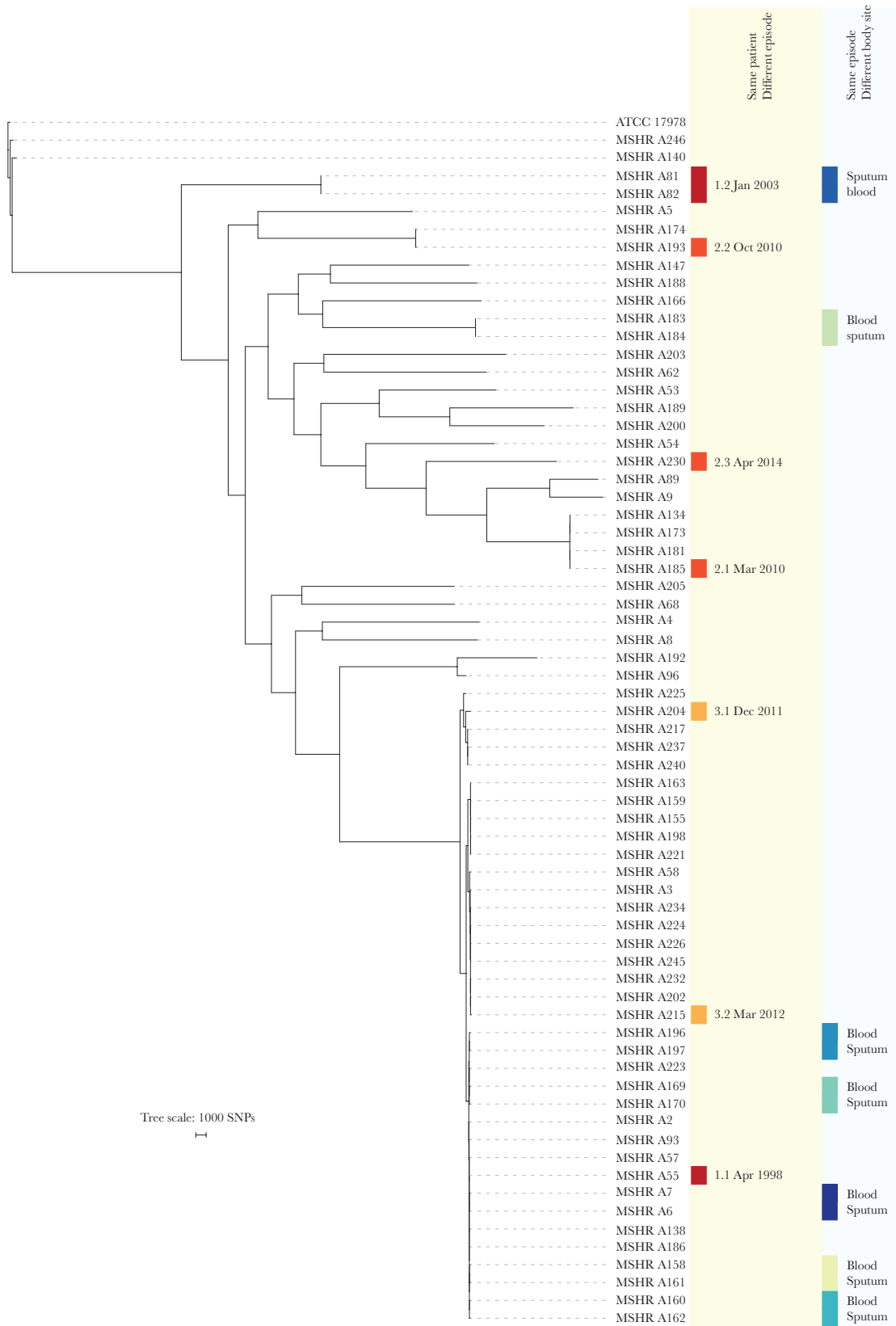


Figure 1. Maximum parsimony phylogenetic tree. Strains from the same individual are highlighted in the same color in column 1, with patient and episode number and date of isolation. Bloodstream and sputum isolate pairs are highlighted in column 2, with isolates from the same individual highlighted with the same color. The tree includes 47 additional local *A. baumannii* strains. Abbreviation: SNP, single nucleotide polymorphism.

1 patient with recurrent infection reported here, hazardous alcohol intake was the predominant host risk factor for both initial (80%) and recurrent *A. baumannii* infections in our cohort [4].

The pathogenesis of *A. baumannii* CAP is incompletely understood, and the relative contributions of the host immune response and bacterial virulence gene profile are uncertain [1]. An association between bacterial virulence genes and patient outcomes in community-onset *A. baumannii* infection has not been demonstrated [5]. In this study, we found that patients presented with a similar disease phenotype in each episode despite discordant virulence gene profiles. Alcohol intoxication, the major risk factor in our cohort [4], may contribute to *A. baumannii* pathogenesis via impairment of phagocytosis [8, 9] and by upregulating bacterial virulence gene expression [10]. Further work investigating host–pathogen interactions, including in the setting of alcohol intoxication, hyperglycemia, and their combination, is required.

A. baumannii colonizes or is carried in the skin and throat in healthy individuals [11], so there is potential for contamination of sputum cultures with this organism [12]. We have previously required positive blood cultures with *A. baumannii* before we diagnose definite *A. baumannii* CAP. When *A. baumannii* is grown from sputum but not blood in a patient with CAP, it is less clear if this is definitely the causative organism. The *A. baumannii* isolates from blood and sputum were almost identical in all 7 instances in our study, suggesting that the *A. baumannii* in sputum was the cause of severe pneumonia in these cases. Identification of variants is influenced by the choice of reference genome and the methodology used for alignment and variant calling, so it can be difficult to compare SNP distances reported in different studies, and there are no definitive thresholds for attributing isolates to a common source. However, the number of variants we identified between the pairs was within the range reported by others where a common source was considered likely. Among 20 *A. baumannii* isolates from different body sites and at different time points in a burns patient, 9/20 isolates were identical with a maximum pairwise difference of 8 SNPs [13]. In the context of a hospital outbreak, a threshold of <2.5 core SNPs was previously proposed to rule patients into the outbreak [14].

In conclusion, in this study, recurrent episodes of CAP due to *A. baumannii* were reinfections rather than relapses. Along with the finding that each of the recurrences occurred with ongoing hazardous alcohol intake, the molecular discordance is in keeping with the hypothesis that host factors rather than bacterial factors are the key drivers of the severe phenotype of *A. baumannii* CAP. Blood and sputum pairs were almost

identical, suggesting that growth of *A. baumannii* from purulent sputum is etiologically significant in a patient with severe CAP in this setting.

Supplementary Data

Supplementary materials are available at *Open Forum Infectious Diseases* online. Consisting of data provided by the authors to benefit the reader, the posted materials are not copyedited and are the sole responsibility of the authors, so questions or comments should be addressed to the corresponding author.

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