Acinetobacter baumannii is an important opportunistic pathogen causing healthcare-associated infections and has become one of the most common colonizing pathogens in burn patients.

A. baumannii may cause serious outbreaks despite implementation of rigorous infection prevention strategies and antibiotic stewardship.

The emergence of multidrug-resistant A. baumannii (MDRAB) has become a global concern.

Whole-genome sequencing (WGS) has emerged as a promising method for molecular epidemiology investigations of healthcare-associated outbreaks. However, WGS data are labor-intensive and as outbreaks of MDRAB during a three-war period at a large academic burn center and examined transmission dynamics of MDRAB strains through WGS and comparative analysis.

Methods

Forty-six non-duplicate clinical isolates of MDRAB between 2007 and 2010 at the burn center were analyzed (four did not reach 10X coverage or deeper at a 40% of reference genome and were excluded).

Short-read libraries from DNA of all samples were pooled and sequenced on a single Illumina MiSeq run, then were mapped to a PathoGenome-generated reference genome of the outbreak index case (A01).

Acquired resistance genes were identified using ResFinder v2.1.

Multilocus sequence typing (MLST) was performed and sequence types (STs) were determined from the WGS data using MLST v1.7 and the Pasteur MLST database.

eBURST analyses were performed under stringent (minimum of six shared alleles) grouping parameters using the eBURST tool to illustrate evolutionary relationships between the founder and the other STs.

A neighbor-joining tree was constructed on the basis of single-nucleotide variants (SNVs) from the core mapped genome and generated 100 times in R using phangorn and ape software.

A corresponding mapping representing genome-wide SNVs was calculated in R using ggtree and igvR software.

Bayesian transmission chain reconstruction was performed using Outbreaks software.

Figure 1. Timeline of sequential outbreaks caused by MDRAB. (A): burn (ICU/ICG), interventional surgical care unit (MCIC), medicine ICU, neurosurgery ICU, trauma ICU, CCU, coronary care unit (MCIC), pediatrics ICU, cardiology ward, orthopedic ward, NICU, medicine progressive care unit. (B): first, general ward

Figure 2. Neighbor-joining phylogram and SNV map showing relationships between outbreak isolates. (A) Color bar: black, white; (B) Size of nodes: proportionate to the number of samples collected.

Figure 3. Bayesian reconstruction of transmission chains for MDRAB outbreak isolates:

- Color of nodes: Sample collection date.
- Sizes of nodes: No. of secondary cases from each infected case.
- Direction of arrows: Who transmitted to whom.
- Shading of arrows: Degree of posterior support for transmission link.

Most transmission events in the outbreaks were predicted with a high degree of certainty (posterior probabilities near 1.0), and may represent direct transmissions, recombination events and the number of A. baumannii strains.

The relativeness of STs within two-clonal complexes is shown.

The remaining 23 MDRAB isolates (ST79) belonged to another CC79, for which ST79 was the founder.

Table 1. Distribution of acquired antimicrobial resistance genes in MDRAB strains

Conclusions

- WGS and comparative analysis enabled us to conduct outbreak tracing and determine transmission pathways as well as detect recombination events and resistance genes among MDRAB strains.

- WGS revealed transmission pathways during three sequential outbreaks over a three-year period, suggesting the longevity of MDRAB strains in this healthcare setting and the difficulty of infection control at a large academic burn center.

- The second outbreak occurred following a recombinant event between a bacterium of the first outbreak and an occult MDRAB clone that was likely circulating within the hospital.

- WGS can provide valuable information concerning the onset, course and size of hospital outbreaks, and on possible transmission networks.