Whole-Genome Sequencing Elucidates Epidemiology of Nosocomial Clusters of Acinetobacter baumannii

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We characterized two epidemiologically similar Acinetobacter baumannii clusters from two separate intensive care units (ICU) using core genome multilocus sequence typing. Clonal spread was confirmed in ICU-1 (12 of 14 isolates shared genotypes); in ICU-2, all genotypes (13 isolates) were diverse, thus excluding transmissions and enabling adequate infection control measures.

Acinetobacter baumannii is often reported as a cause of hospital-acquired infections (1, 2) and is associated with respiratory infections, bacteremia, meningitis, and wound infections (1, 3, 4). A. baumannii is transmitted via direct or indirect contact, and its ability to survive for months on inanimate surfaces hampers infection control measures (5). The emergence of multidrug-resistant (MDR) A. baumannii (6, 7) and the frequent association with nosocomial outbreaks (8–10) make MDR A. baumannii a pathogen of serious concern (11, 12).

If bacterial pathogens occur in clusters, epidemiological investigations are triggered and frequently complemented with molecular typing methods to confirm whether or not clusters are due to the simultaneous occurrence of identical bacterial genotypes. Currently, pulsed-field gel electrophoresis and other methods (6, 13–15) are increasingly being replaced by whole-genome sequence (WGS)-based methods, which provide the highest possible discriminatory power (16). WGS-based approaches rely either on the characterization of single nucleotide polymorphisms (SNP) (16, 17) or on gene-by-gene allelic profiling of core genome genes, called core genome multilocus sequence typing (cgMLST) (18–20).

As a published cgMLST scheme for A. baumannii is not yet available, here we investigated the ability of an ad hoc scheme to differentiate two epidemiologically determined clusters of A. baumannii that occurred in two unrelated intensive care units (ICU) and compared our ad hoc cgMLST scheme with SNP typing.

During routine surveillance efforts, which comprise a daily review of patients’ charts and microbiological data, two clusters were detected in two ICUs at the University Hospital Muenster, Muenster, Germany, a 1,457-bed tertiary care hospital, between October 2013 and March 2014. In ICU-1 (cluster I), in addition to intensified disinfection measures to eliminate potential environmental reservoirs, extra training of staff members regarding compliance with hand hygiene measures and contact precautions, and isolation of patients in single rooms, a weekly microbiological screening of patients was established. Moreover, two series of intensified disinfection measures to eliminate potential environmental reservoirs, extra training of staff members regarding compliance with hand hygiene measures and contact precautions, and isolation of patients in single rooms, a weekly microbiological screening of patients was established. The majority of these were rated as MDR A. baumannii, P1b to P10 exhibiting resistance to piperacillin, 3rd/4th generation cephalosporins, and fluoroquinolones but susceptibility to most carbapenems (see the supplemental methods).

Only three isolates (P1a, P11, and P12) were susceptible, i.e., non-MDR. Epidemiological investigations also included one further patient who stayed in ICU-2 in October-November 2013 (P14); however, the isolate of this patient was not available for typing. Since one patient (P18) showed a change from non-MDR A. baumannii (P18a) to MDR A. baumannii (P18b), overall 13 isolates from cluster II were sequenced. In total, 32 A. baumannii isolates were subjected to WGS on a MiSeq platform (Illumina Inc., San Diego, CA, USA), which is described in detail in the supplemental methods, and the resulting reads were quality trimmed and de novo assembled as recently described (21). Using SeqSphere+ software version 2.0 beta (Ridom GmbH, Muenster, Germany), all coding regions (CDS) were extracted and compared in a gene-by-gene approach (cgMLST) using A. baumannii strain ATCC 17978 (GenBank accession number CP000521.1) as the reference sequence. The clonal relationship was displayed in a minimum spanning tree that was generated using the same software (see supplemental methods).

After WGS of the 32 isolates, the 3,319 A. baumannii genes of the ad hoc typing scheme were queried in all genomes and further analyzed (see Table S1 and the supplemental data set in the supplemental material). Of the 3,319 genes, 2,592 to 2,876 genes were present (mean 2,682 genes). In cluster I, 12 of 19 isolates (P1 to P5,
P7 to P9, E1, E2, and E5) were identical, and two isolates showed only a single allelic variation (P6 and P10) indicating clonal spread; the remaining five isolates were only distantly related, exhibiting >2,300 differing genes (Fig. 2). In contrast, in cluster II nearly all isolates were genotypically distantly related (>126 differing genes). Only two isolates (P20 and P25) differed in only two genes (Fig. 2). SNP-based typing of the 32 isolates corroborated both our findings based on cgMLST and the results of a recent study by Fitzpatrick et al. (16), where they determined a maximum distance of two SNPs as a threshold among isolates belonging to an outbreak: of the 19 cluster I isolates, 14 were identical (the 12 isolates identical by cgMLST and the 2 isolates differing only in one allele), the remaining isolates were only distantly related (see Fig. S1 in the supplemental material). A similar situation was detected among cluster II isolates. To investigate whether our cgMLST approach also results in results similar to the data of Fitzpatrick et al., we constructed a minimum spanning tree of the outbreak isolates. Indeed, cgMLST also resulted in a similar clustering of isolates with a maximum difference of 12 alleles in a pairwise comparison (see Fig. S2 in the supplemental material).

Our results shown here enabled us to concentrate infection control measures on patients and the environment of the affected ward and indicated that the detected cluster I was localized rather than a hospital-wide problem with transmissions on several wards. This is further underlined by the fact that from discharge of the last patient of cluster I until the day of writing this paper no further A. baumannii isolate with the cluster I genotype has been found among the 14 MDR A. baumannii isolates detected up to mid-2016. When the first isolates of cluster II were detected, the WGS workflow for A. baumannii typing had already been established. This enabled an immediate response with respect to infection con-

![FIG 1 Linelist of all 26 patients and 5 environmental samples positive for A. baumannii for the two clusters in ICU-1 and ICU-2 during September 2013 and March 2014. P, patient; E, environment; MDR, multidrug-resistant phenotype (for details, see Table 1).]
trol procedures, avoiding extensive measures that would have been withdrawn after typing data were available. Since, starting from the first \textit{A. baumannii} of cluster II determined, the isolates exhibited a genotype different from those of both the cluster I isolates and among the cluster II isolates, no additional infection control procedures were implemented to prevent nosocomial spread; only one single putative transmission (patients P20 and P25) of non-MDR \textit{A. baumannii} within cluster II was recognized.

Despite efforts to elucidate the origin of cluster II, a final explanation for this temporary increase in \textit{A. baumannii} detections was not determined. Moreover, we could not finally explain the detection of two genotypically different isolates of P18. The most likely explanation is that at the time of the first strain’s isolation, P18 already carried more than one \textit{A. baumannii} variants that were unrecognized. Furthermore, the two environmental isolates, E3 and E4, warrant some comments. We assume that E3 and E4 originated from a previous patient in this ward as \textit{A. baumannii} in general is not an environmental bacterium but is able to survive for a long time in the environment.

Our approach clearly demonstrates the technical evolution in our laboratory of the two clusters, enabling prospective WGS typing to rapidly confirm or refute clonal spread of the pathogens as recently described by Roach et al. (22). Spurred on by these results, we have been constantly monitoring the molecular epidemiology of MDR \textit{A. baumannii} using WGS. The cgMLST approach allows immediate comparisons of newly determined genotypes with his-

### Table 1: Antibiotic susceptibility patterns of the \textit{A. baumannii} isolates investigated

<table>
<thead>
<tr>
<th>Antibiotic substance</th>
<th>P1 a, P1 b, P2, P9, E1, E2, E5</th>
<th>P3</th>
<th>P4 - P6, P8, P10</th>
<th>P7</th>
<th>P11, P16</th>
<th>P12, P22, P25, P26</th>
<th>P13, E3</th>
<th>E4</th>
<th>P14</th>
<th>P15</th>
<th>P17, P18 a, P18 b</th>
<th>P19</th>
<th>P20</th>
<th>P21</th>
<th>P23</th>
<th>P24</th>
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<td>R</td>
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<td>R</td>
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</tr>
</tbody>
</table>
| Trimethoprim-
| T sulfamethoxazole| S                               | R  | R                | R  | R        | R                 | R     | R  | R   | R   | S                 | R   | R   | R   | R   | R   |

Overall rated as Non-MDR MDR MDR MDR MDR Non-MDR Non-MDR MDR Non-MDR Non-MDR Non-MDR Non-MDR Non-MDR MDR Non-MDR Non-MDR

*Antibiotic susceptibility was determined using a Vitek II system (bioMérieux, Marcy-l’Étoile, France); testing was performed in accordance with EUCAST guidelines (versions 3.1 to 5.0). S, susceptible; I, intermediate; R, resistant; NT, not tested.*
Some studies have suggested that transmission from one individual to another is facilitated by high diversity of clonal lineages, possibly as a result of the limited genetic diversity that is available to facilitate transmission. However, high diversity of clonal lineages might not necessarily indicate high transmission potential, as was highlighted by a recent study demonstrating that high diversity among isolates of a single strain can still result in low transmission potential.


detection of transmissions as suggested by Salipante et al. (24). For a calcium submitted to the European Nucleotide Archive (http://www.ebi.org.uk/ena/) under the study accession number PRJE7302.

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