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## Inaccurate Multilocus Sequence Typing of *Acinetobacter baumannii*

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Multilocus sequence typing has been useful for genotyping pathogens in surveillance and epidemiologic studies. However, it cannot reflect the true relationships of isolates for species with very dynamic genomes. Using a robust genome phylogeny, we demonstrated the limitations of this method for typing *Acinetobacter baumannii*.

An adequate genotyping system is of paramount importance for infectious disease epidemiology. Two decades ago, the multilocus sequence typing (MLST) scheme was proposed as a genotyping method (1), and today, because of its reproducibility and portability, MLST schemes are available for many human pathogens (2). MLST has been instrumental in increasing understanding of the epidemiology and population structure of many bacteria.

*Acinetobacter baumannii*, a major source of nosocomial infections, is no exception, and 2 MLST schemes (Oxford and Pasteur) have been established for this species (3,4). Each scheme uses just 7 loci and, therefore, only samples a small fraction of the chromosome, which could be a serious issue for genotyping species with highly variable genomes. Some studies have shown that *A. baumannii* has both high gene content variation (5) and substantial levels of recombination (6).

We revisited one of the most comprehensive genome datasets of *A. baumannii* (5) to construct a robust phylogeny to show that sequence type (ST) assignment in both MLST schemes does not reflect true relationships among isolates of this species. This dataset of >80 genomes covers 36 different STs according to the Oxford scheme (STox) and 19 different STs according to the Pasteur scheme (STp) (Appendix Table 1, <https://wwwnc.cdc.gov/EID/article/25/1/18-0374-App1.pdf>). We constructed a concatenated alignment of 574 orthologous genes and conducted statistical model selection as in a previous study (5) and, on that alignment, constructed a maximum-likelihood phylogeny by PhyML (7).

The 2 schemes showed different levels of resolution. Although in many instances a single STp had just 1 equivalent STox, 2 STs exist in the Pasteur scheme that encompass many Oxford STs (Appendix Figure, red branches). For instance, under the Pasteur scheme, STp2 represents >15 STs in the Oxford scheme and STp1 encompasses 5 STox. Thus, the Pasteur scheme seems to have considerably less resolution than the Oxford scheme to distinguish isolates. The Pasteur scheme's lack of resolution was not insignificant, however. STp2 comprises 43 isolates (approximately half of our dataset) showing considerable levels of genetic variation according to our phylogeny, but according to this MLST scheme, they constitute just 1 genotype.

Many of the STs in either scheme formed coherent (monophyletic) groups in our phylogeny. However, we recorded some clear exceptions in which isolates from some STs did not form monophyletic groups, that is, isolates with the same ST did not cluster. The most striking case is STox208 (orange tips in the phylogeny), where there are 2 well-defined groups with several isolates each and an extra isolate not close to either of those well-defined groups. We also noted that the 2 STox455 isolates did not cluster and are located far apart on the tree (green tips). Additionally, 1 of the STox369 isolates did not fall within the ST369 group (blue tips). These 3 examples show that the Oxford MLST does not accurately reflect the relationships among the isolates. Also notable is that, although for the Oxford scheme 36 STs are represented in this dataset, only 16 of them have  $\geq 2$  isolates and therefore only in these STs could we detect problems with the clustering within any given ST. Thus, 3 of these 16 STs did not cluster the isolates properly inasmuch as these STs were polyphyletic. In summary, for the Oxford scheme we demonstrated that some STs form polyphyletic groups

because 4 of the 7 loci have signals of recombination (Appendix Table 2), whereas for the Pasteur scheme, we noted a serious lack of resolution for some STs because the loci used only by this scheme have the lowest levels of genetic diversity (Appendix Table 2). Two previous studies noted problems with the MLST schemes for this species (8,9); nonetheless, neither was as extensive as our study, nor did they benchmark both schemes against a genome-based phylogeny.

In conclusion, we showed that the correct relationships among isolates cannot be recovered using either of the MLST schemes for *A. baumannii*. In addition, we highlighted the importance of using more powerful genotyping strategies when analyzing bacteria with highly dynamic genomes; in this regard, the ever-decreasing cost of genome sequencing will make this technology the perfect tool for genotyping bacterial species.

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## Severe Disseminated Infection with Emerging Lineage of Methicillin-Sensitive *Staphylococcus aureus*

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We report a case of severe disseminated infection in an immunocompetent man caused by an emerging lineage of methicillin-sensitive *Staphylococcus aureus* clonal complex 398. Genes encoding classic virulence factors were

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## Appendix

**Appendix Table 1.** Genome dataset employed for this study\*

Strain name	Assembly ID	Oxford scheme ST assignment	Pasteur scheme ST assignment
D1279779	GCA_000186665.4	942	267
D36	GCA_001399655.1	498	81
A85	GCA_002210065.1	781	1
ATCC-17978	GCA_000015425.1	112	437
AB031	GCA_000746605.1	1000	638
AB030	GCA_000746645.1	758	79
Ab04-mff	GCA_001077655.1	447	10
MDR-TJ	GCA_000187205.4	369	2
MDR-ZJ06	GCA_000226275.1	643	2
BJAB07104	GCA_000419385.1	368	2
BJAB0715	GCA_000419405.1	642	23
BJAB0868	GCA_000419425.1	218	2
ZW85-1	GCA_000505685.2	378	639
XH386	GCA_001026965.1	208	2
XH860	GCA_001573065.1	457	2
XH859	GCA_001573085.1	368	2
XH857	GCA_001573105.1	806	215
XH856	GCA_001573125.1	381	2
XH858	GCA_001578145.1	642	23
HRAB-85	GCA_001887305.1	208	2
XDR-BJ83	GCA_001902375.1	368	2
Ab6200	GCA_000814345.1	1161	464
AYE	GCA_000069245.1	231	1
R2090	GCA_001261895.2	942	267
CIP70-10	GCA_001457535.1	819	126
R2091	GCA_001517645.1	819	126
B8342	GCA_001077555.2	NA	NA
B8300	GCA_001077965.2	NA	NA
ACICU	GCA_000018445.1	437	2
NCGM-237	GCA_000828795.1	455	2
AB042	GCA_001941765.1	112	437
AC29	GCA_000695855.2	195	2
AC30	GCA_000307975.2	195	2
Ab3207	GCA_001636235.1	1321	422
AF-401	GCA_001896005.1	new	79
IOMTU-433	GCA_000828935.1	919	622
Ab1656-2	GCA_000188215.1	423	2
KBN10P02143	GCA_001514375.1	191	2
YU-R612	GCA_001543995.1	191	2
DU202	GCA_000498375.2	423	2
KAB01	GCA_001806345.1	451	2
KAB02	GCA_001806365.1	369	2
KAB03	GCA_001806385.1	451	2
KAB04	GCA_001806405.1	191	2
KAB05	GCA_001806425.1	369	2
KAB06	GCA_001806445.1	369	2
KAB07	GCA_001806465.1	191	2
KAB08	GCA_001806485.1	208	2
JBA13	GCA_002082625.1	191	2
CBA7	GCA_002082645.1	208	2
15A34	GCA_002082685.1	872	2
15A5	GCA_002082705.1	191	2
USA2	GCA_002082725.1	357	2
SSA6	GCA_002082745.1	357	2

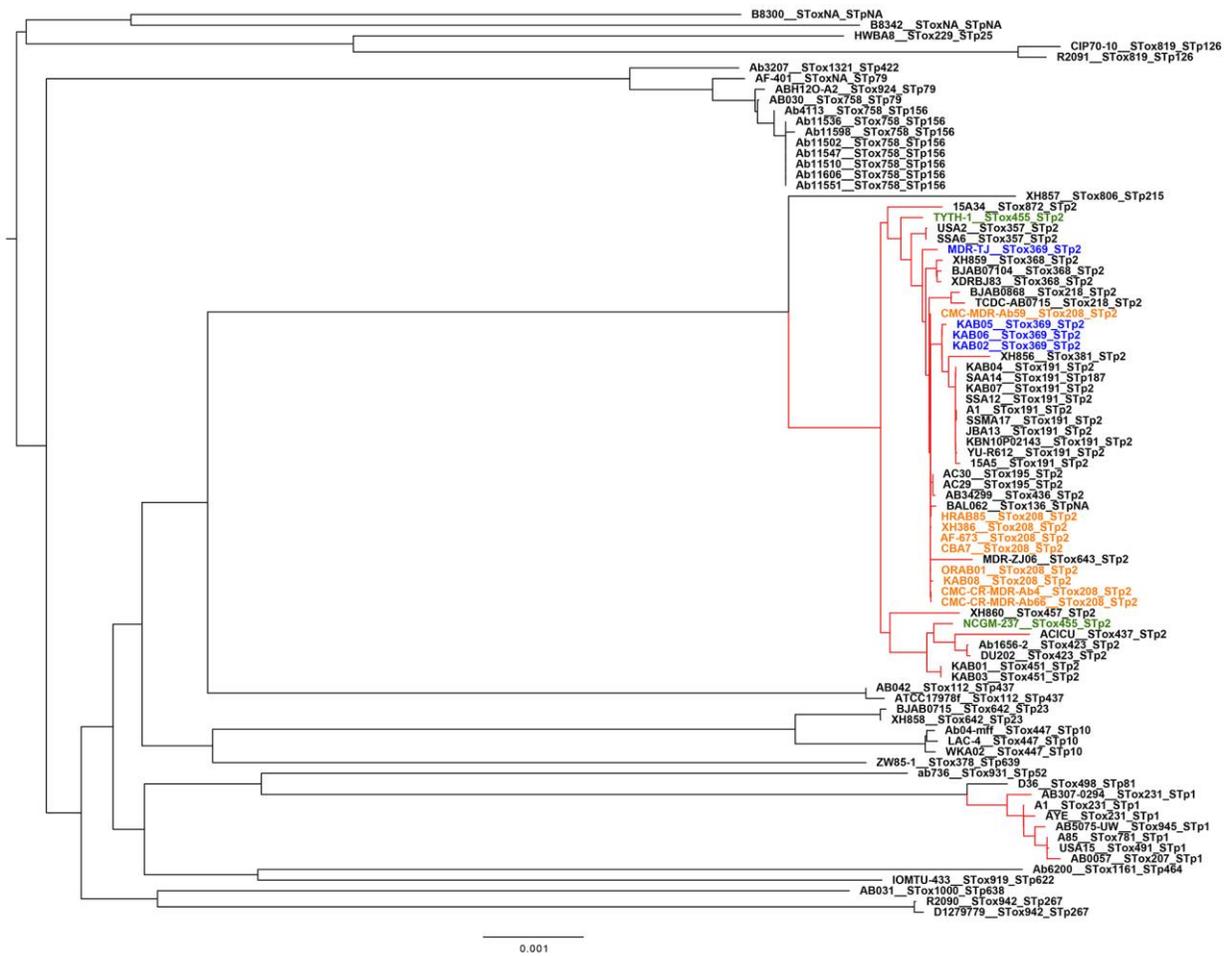
Strain name	Assembly ID	Oxford scheme ST assignment	Pasteur scheme ST assignment
HWBA8	GCA_002082785.1	229	25
WKA02	GCA_002082805.1	447	10
USA15	GCA_002082825.1	491	1
SAA14	GCA_002082845.1	191	187
SSA12	GCA_002082865.1	191	2
SSMA17	GCA_002082885.1	191	2
ABH12O-A2	GCA_000761175.1	924	79
TYTH-1	GCA_000302575.1	455	2
TCDC-AB0715	GCA_000189735.2	218	2
A1	GCA_000830055.1	231	2
A1	GCA_000830055.1	231	2
AB307-0294	GCA_000021145.1	231	1
AB0057	GCA_000021245.1	207	1
LAC-4	GCA_000786735.1	447	10
ORAB01	GCA_000939415.2	208	2
AB5075-UW	GCA_000963815.1	945	1
AF-673	GCA_001895985.1	208	2
CMC-CR-MDR-Ab4	GCA_001922205.1	208	2
CMC-MDR-Ab59	GCA_001922225.1	208	2
CMC-CR-MDR-Ab66	GCA_001922245.1	208	2
ab736	GCA_002116925.1	931	52
BAL062	GCA_900088705.1	136	NA
AB34299	GCA_002009115.1	436	2
Ab11598	MSCZ00000000	758	156
Ab11536	MSCY00000000	758	156
Ab4113	MSDA00000000	758	156
Ab11502	MSCX00000000	758	156
Ab11551	MSDC00000000	758	156
Ab11547	MSDB00000000	758	156
Ab11606	MSDD00000000	758	156
Ab11510	CP018861/CP018862	758	156

\*ID, identification; ST, sequence type.

**Appendix Table 2.** Measures of genetic diversity and recombination test for the loci of both multilocus sequence typing schemes for *Acinetobacter baumannii*

Gene	Scheme	Proportion of variable sites	Nucleotide diversity	PhiTest p value*
gdhB	Oxford	0.263	0.1060244	<b>2.83e-02</b>
gpi	Oxford	0.431	0.0798361	<b>0.00e+00</b>
gyrB	Oxford	0.047	0.0096987	<b>1.44e-09</b>
recA	Both	0.029	0.0044261	5.74e-02
cpn60	Both	0.024	0.0043936	<b>1.46e-02</b>
gltA	Both	0.022	0.0036340	2.15e-01
pyrG	Pasteur	0.025	0.0031657	<b>1.34e-03</b>
rpoD	Oxford	0.016	0.0031083	1.26e-01
rpoB	Pasteur	0.021	0.0029657	<b>3.50e-04</b>
rplB	Pasteur	0.010	0.0019185	1
fusA	Pasteur	0.010	0.0009114	1.89e-01

\*Bold indicates statistical significance.



**Appendix Figure.** Maximum-likelihood phylogeny depicting the relationships among *Acinetobacter baumannii* isolates considered in this study. The phylogeny was constructed on the concatenated alignment of all the single gene families not showing recombination signals. Statistical model selection was conducted to determine the most adequate model, which was GTR+R+I. When possible, the sequence types in accordance with the Oxford scheme (STox) and the Pasteur scheme (STp) are shown. The red branches show cases of single STp that encompass many STox. Colored tips show polyphyletic groups under the Oxford scheme: blue, STox369; orange, STox208; green, STox455. Scale bar indicates nucleotide substitutions per site.