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Multilocus Sequence Typing Scheme for *Staphylococcus aureus*: Revision of the *gmk* Locus

Multilocus sequence typing (MLST) is a sequence-based genotyping method based on polymorphisms (each variant is termed an allele) in seven housekeeping genes (loci) (*arcC*, *aroE*, *glpF*, *gmk*, *pta*, *tpi*, and *yqiL*) in *Staphylococcus aureus* providing unique allelic profiles known as sequence types (STs) (1). The data obtained by MLST are highly reproducible and are available in the *S. aureus* MLST database (http://saureus.mlst.net/). Since the publication of Enright and coworkers' article (1), MLST has been extensively used to study evolution and population dynamics among *S. aureus* lineages (our search identified >1,000 citations of this paper indexed at ISI Web of Knowledge as of 16 April 2012).

We occasionally have had difficulties assigning allele numbers for the *gmk* locus due to suboptimal DNA sequence quality in the 3′ end of the *gmk* amplicons, which prompted us to investigate the problem. DNA sequence analysis revealed a 12-bp overlap between the 3′ end of the 429-bp region that is used to define the *gmk* alleles and the 3′ end of the primer *gmk*-Dn (5′-TCATTAACTAC AACGTAATCGTA-3′ [the overlapping 3′ end is underlined]). This means that the 12 bp (TACGATTACGTT) in the 3′ end of the 429-bp *gmk* region should always be conserved given that the *gmk*-Dn primer is integrated into the amplicons.

Nevertheless, among the 183 *gmk* alleles available in the *S. aureus* MLST database as of 16 April 2012, we identified 12 *gmk* alleles with a polymorphism(s) in the 12-bp region corresponding to 48 STs (Table 1). The most common polymorphism (8/12) was a G instead of a T at position 429 (Table 1).

Based on our experience, many of the polymorphisms in the 12-bp region are likely to be "false" due to suboptimal DNA sequence quality toward the end of the *gmk* amplicons. On the other hand, we acknowledge that some of the polymorphisms may be "true" if they have been identified by PCR amplification using alternative downstream primers or by analysis of whole-genome sequence data.

We argue that exclusion of the 12 bp from the 429-bp *gmk* region is the most straightforward procedure to ensure the robustness of *S. aureus* MLST in the future. The required changes in the *S. aureus* MLST database and the software used to assign *gmk* alleles based on the new 417-bp region have recently been made, and the issue identified here has been highlighted on the homepage (http://saureus.mlst.net/). Where changes of the ST have oc-

TABLE 1 Changes in the *S. aureus* MLST database due to revision of the *gmk* locus

Original *gmk* allele	Polymorphism(s)	Position(s)	New *gmk* allele	Original ST[a]	New ST	CC change
50	G	429	44	377	152	
				1471	1471[a]	
64	G	429	107	1066	1277	
				1067	1067[a]	
68	T	426	2	686	489	
				1697	121	
81	G	429	129	856	1643	
88	G	429	1	931	8	
				1283	239	
				1441	15	
				1476	97	
				1494	1465	
				1595	25	
				1738	1	
				2129	2129[a]	
				2136	9	
				2156	199	
				2157	2157[a]	
				2163	2163[a]	
				2278	2278[a]	
121	T	420	2	1452	133	
136	G	429	136[a]	1727	1727[a]	
137	G	429	8	1765	72	
				2094	188	
149	T, A	420, 429	101	1823	1223	
150	T, A	420, 429	34	1824	1824[a]	
				1848	1848[a]	
				1849	2198	
				1850	1850[a]	Singleton to CC75
				2043	2043[a]	
				2044	2044[a]	
154	G	429	18	1868	89	
155	G	429	2	1869	30	
				1952	59	
				2012	2012[a]	Singleton to CC522
				2073	121	
				2076	2057	
				2077	398	
				2080	433	
				2095	1768	
				2097	2097[a]	Singleton to CC398
				2098	2098[a]	
				2099	2099[a]	
				2100	2100[a]	
				2161	2155	
				2307	2307[a]	
				2308	2308[a]	
				2348	2348[a]	

[a] New *gmk* alleles or STs that were unique in the database retained their original allele or ST numbers.

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curred, these are noted in the "comments" field of the isolate entry in the database. Those whose isolates were affected by these changes have been notified.

The consequences of these changes are described in Table 1. Where the revision of the *gmk* locus resulted in a new allele or ST that was unique in the database (1 of 12 affected alleles and 20 of 48 affected STs), the original allele or ST number was maintained. Accordingly, although 12 alleles and 48 STs were affected, only 11 allele numbers and 28 ST numbers had to be changed. In total, 68 of the 4,526 isolates present in the *S. aureus* MLST database on 16 April 2012 (or 1.5% of all isolates deposited) were affected by these changes. Since the new STs are, by definition, single-locus variants of the 28 original STs, we would expect only slight changes in the population structure. Using the stringent group definition (6/7 shared alleles), eBURST (version 3; http://eburst.mlst.net) analyses of the entire *S. aureus* MLST database before and after the revision of the *gmk* locus (conducted on 16 April 2012) showed that the new STs remained within the same clonal complex (CC) as the original STs and that three singletons (ST1850, ST2012, and ST2097) can now be assigned to existing CCs (Table 1).

REFERENCE

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