Figure S1. Comparison of the sequences of *qacA4* (MK040360), the 3 previously described *qacA* alleles (*qacA1*, *qacA2*, *qacA3*; Alam et al., 2003), and the 5 novel *qacA* alleles identified in the *qacA*-positive control isolates without elevated CHG MICs sequenced in this study. The associated AluI restriction sites are shown below the nucleotide sequences and the corresponding amino acid sequences are displayed in the boxes above the nucleotide sequences. The nucleotides which distinguish *qacA4* from all other alleles are highlighted in red. Those which distinguish one or more of these alleles from *qacA4* are displayed in green.

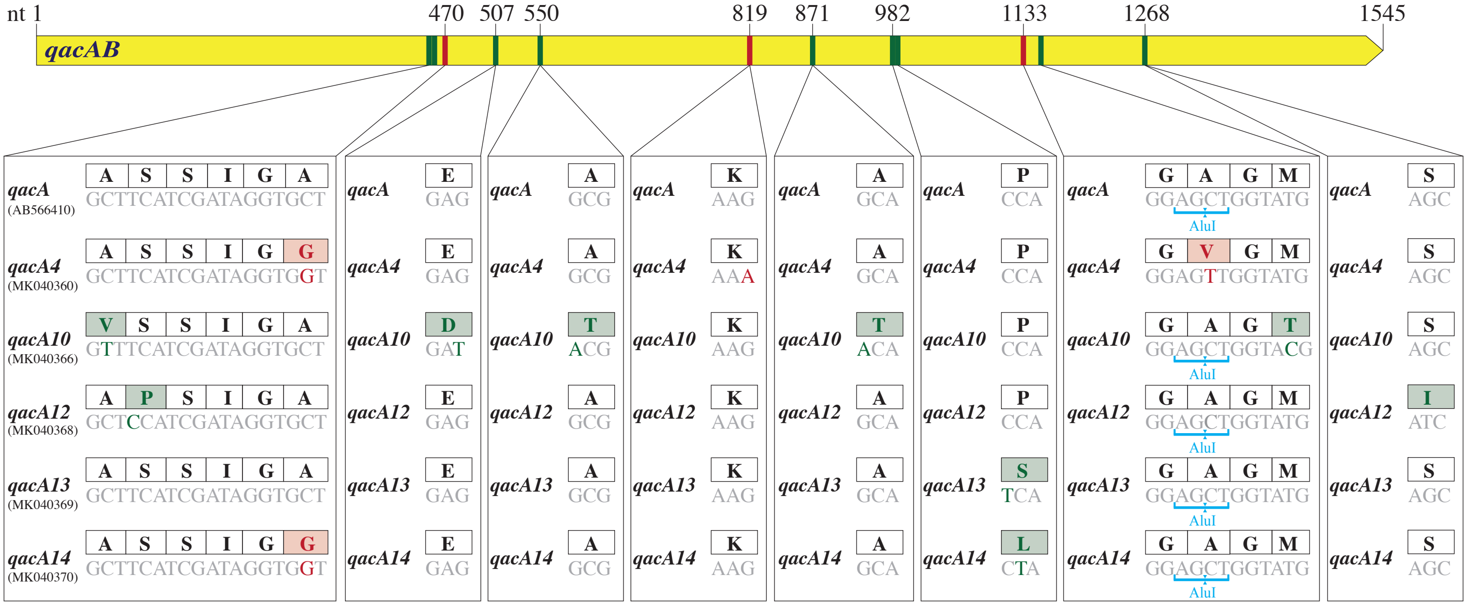

Figure S2. Comparison of the sequences of the reference *qacA* (AB566410), *qacA4* (MK040360), and the 4 novel *qacA* alleles identified in the *qacA*-positive isolates with elevated CHG MICs. The associated AluI restriction sites are shown below the nucleotide sequences and the corresponding amino acid sequences are displayed in the boxes above the nucleotide sequences. The nucleotides which distinguish *qacA4* from the reference *qacA* are highlighted in red. Those which distinguish the novel alleles from *qacA4* and the reference *qacA* are displayed in green.

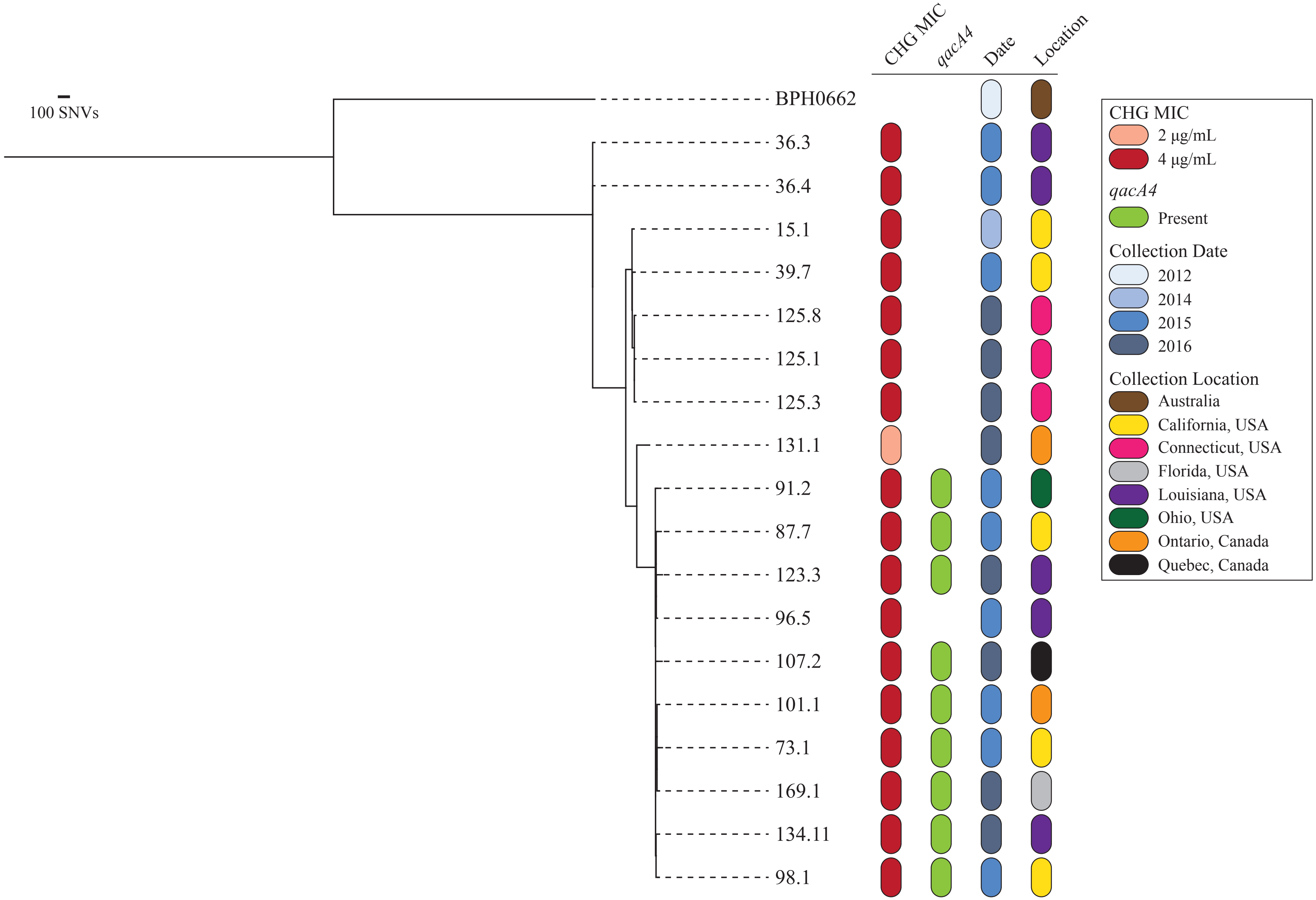

Figure S3. Phylogenetic tree produced from a core genome analysis of 9 *qacA4*-carrying and 9 non-*qacA4*-carrying *S. epidermidis* ST2 isolates.

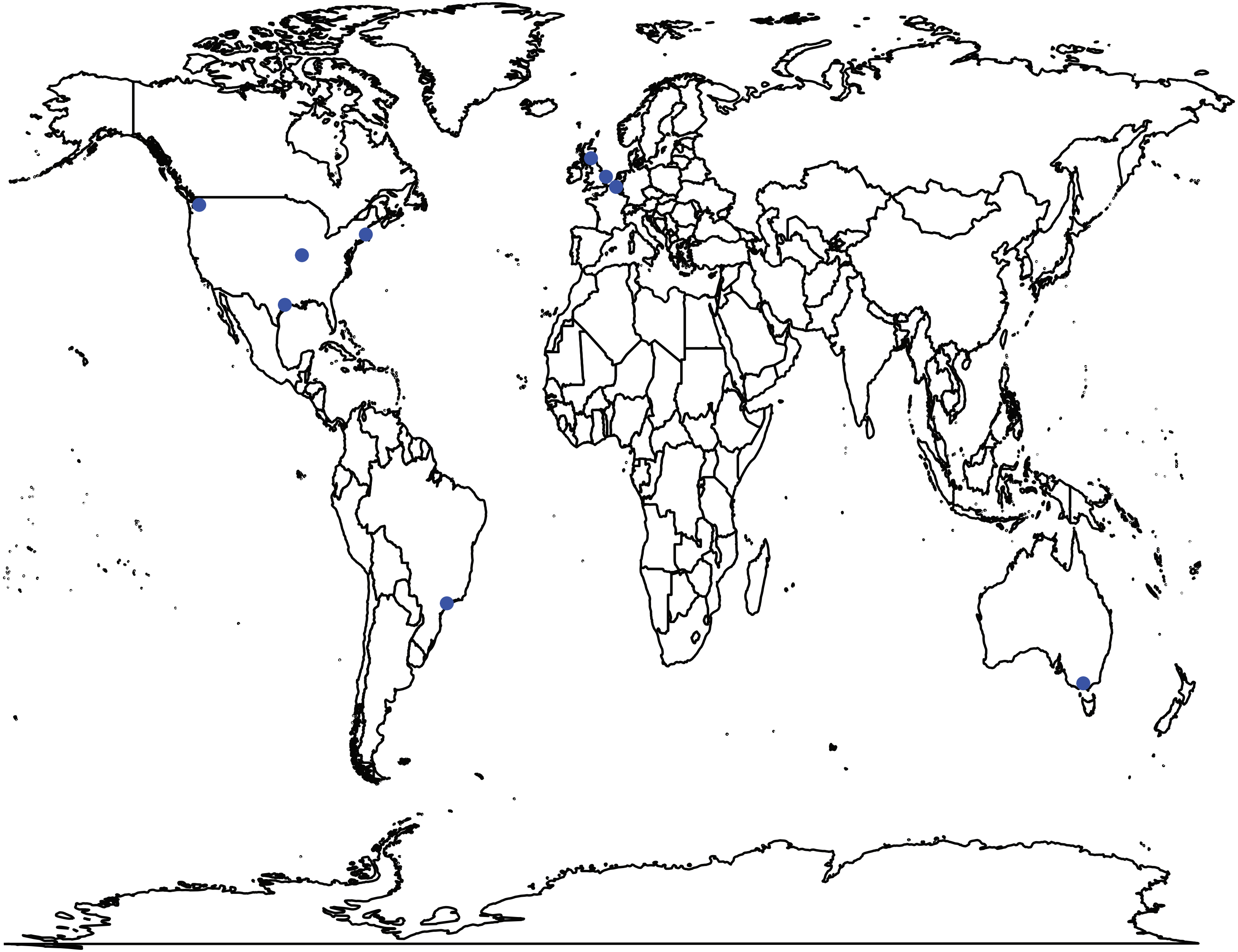

Figure S4. Geographic distribution of *qacA4*-carrying *Staphylococcus epidermidis* isolates collected outside of our study. A blue dot represents a center from which a *qacA4*-carrying *S. epidermidis* was identified.

Table S1. BioSample accession numbers and phenotypic data for the isolates sequenced in this study. The CHG MIC is measured in µg/mL. “R” denotes the isolate is resistant to the specified antimicrobial and “S” indicates the isolate is susceptible to the specified antimicrobial.

Isolate ID	**BioSample Accession No.**	***qacA/B* RFLP**	***qacA* allele**	***smr***	**CHG MIC**	**FOX**	**ERY**	**CIP**	**GEN**	**SXT**	**LZD**	**RIF**	**VAN**	**ST**
The initial 10 qacAB$_{273}$*-positive isolates with elevated CHG MICs randomly selected for whole-genome sequencing*														
53.1	SAMN07839754	*qacAB*$_{273}$	*qacA4**	No	4	R	R	R	R	R	S	S	S	2
73.1	SAMN07839755	*qacAB*$_{273}$	*qacA4*	Yes	4	R	R	R	R	R	S	S	S	2
87.7	SAMN07839756	*qacAB*$_{273}$	*qacA4*	Yes	4	R	R	R	R	R	S	S	S	2
91.2	SAMN07839757	*qacAB*$_{273}$	*qacA4*	Yes	4	R	R	R	S	R	S	S	S	2
98.1	SAMN07839758	*qacAB*$_{273}$	*qacA4*	Yes	4	R	R	R	R	R	S	S	S	2
101.1	SAMN07839759	*qacAB*$_{273}$	*qacA4*	Yes	4	R	R	R	R	R	S	S	S	2
107.2	SAMN07839760	*qacAB*$_{273}$	*qacA4*	No	4	R	R	R	R	R	S	S	S	2
123.3	SAMN07839761	*qacAB*$_{273}$	*qacA4*	Yes	4	R	R	R	R	R	S	S	S	2
134.11	SAMN07839762	*qacAB*$_{273}$	*qacA4*	Yes	4	R	R	R	R	R	S	S	S	2
169.1	SAMN07839763	*qacAB*$_{273}$	*qacA4*	Yes	4	R	R	R	R	R	S	S	S	2
The 10 qacA-positive isolates without elevated CHG MICs randomly selected as positive controls														
20.1	SAMN07839764	*qacA*	*qacA3*	No	1	R	R	S	S	R	S	S	S	101
66.3	SAMN07839765	*qacA*	*qacA7*	No	2	R	S	R	S	R	S	S	S	83
68.5	SAMN07839766	*qacA*	*qacA8*	No	2	S	R	S	S	R	S	S	S	7
97.1	SAMN07839767	*qacA*	*qacA8*	No	2	S	R	S	S	S	S	S	S	59

99.1	SAMN07839768	*qacA*	*qacA9*	No	1	R	R	S	S	R	S	S	S	290
106.1	SAMN07839769	*qacA*	*qacA9*	No	2	S	R	S	S	S	S	S	S	846
110.3	SAMN07839770	*qacA*	*qacA10*	No	2	R	S	R	R	R	S	S	S	130
128.1	SAMN07839771	*qacA*	*qacA10*	No	1	S	S	S	S	S	S	S	S	88
131.1	SAMN07839772	*qacA*	*qacA9*	No	2	R	R	R	R	R	S	S	S	2
135.2	SAMN07839773	*qacA*	*qacA11*	No	1	R	R	S	R	R	S	S	S	88
The 4 qacB-positive isolates randomly selected as positive controls														
54.2	SAMN07839774	*qacB*		No	1	S	S	S	S	S	S	S	S	358
81.4	SAMN07839775	*qacB*		No	1	R	S	S	S	S	S	S	S	5
156.3	SAMN07839776	*qacB*		Yes	2	S	R	S	S	S	S	S	S	358
174.1	SAMN07839777	*qacB*		No	1	R	R	S	S	S	S	S	S	224
The 2 qacAB$_{273}$*-positive isolates without elevated CHG MICs*														
36.5	SAMN10232702	*qacAB*$_{273}$	*qacA5*	No	1	R	S	R	S	R	S	S	S	83
125.10	SAMN10232703	*qacAB*$_{273}$	*qacA6*	No	0.5	S	R	S	S	S	S	S	S	†
The 9 qacA-positive isolates with elevated CHG MICs														
15.1	SAMN10232693	*qacA*	*qacA12*	No	4	R	R	R	R	R	S	S	S	2
36.3	SAMN10232698	*qacA*	*qacA13*	No	4	R	R	R	R	R	S	S	S	2
36.4	SAMN10232699	*qacA*	*qacA13*	No	4	S	R	R	R	R	S	S	S	2
39.7	SAMN10232694	*qacA*	*qacA12*	No	4	R	R	R	R	R	S	S	S	2
86.4	SAMN10232700	*qacA*	*qacA10*	No	4	R	R	S	S	S	S	S	S	130

96.5	SAMN10232701	*qacA*	*qacA14*	Yes	4	R	R	R	R	R	S	S	S	2
125.1	SAMN10232695	*qacA*	*qacA12*	No	4	R	R	R	R	R	S	S	S	2
125.3	SAMN10232696	*qacA*	*qacA12*	No	4	R	R	I	R	R	S	S	S	2
125.8	SAMN10232697	*qacA*	*qacA12*	No	4	R	R	R	R	R	S	S	S	2
The isolate cured of qacA4														
107.2_{cured}	SAMN10490861	Negative		No	1	R	R	R	R	R	S	S	S	2
The 5 qacAB$_{273}$*-positive isolates with discordant susceptibility patterns (susceptible to cefoxitin, gentamicin, or erythromycin)*														
73.5	SAMN10232704	*qacAB*$_{273}$	*qacA4*	No	4	R	R	I	S	R	S	S	S	2
134.15	SAMN10232705	*qacAB*$_{273}$	*qacA4*	Yes	4	S	R	R	R	R	S	S	S	2
171.1	SAMN10232706	*qacAB*$_{273}$	*qacA4*	Yes	4	R	S	R	R	R	S	S	S	2
171.10	SAMN10232707	*qacAB*$_{273}$	*qacA4*	Yes	4	R	S	R	R	R	S	S	S	2
173.6	SAMN10232708	*qacAB*$_{273}$	*qacA4*	Yes	4	R	S	R	R	R	S	S	S	2

* Isolate 53.1 contained two *qacA* alleles, with one being *qacA4*.

† MLST was not performed on isolate 125.10 as it identified as *Staphylococcus hominis*.

Table S2. GenBank accession numbers of the previously characterized *qacA* alleles and the 11 novel *qacA* alleles identified in this study.

***qacA* Allele**	**GenBank Accession No.**	**First reported**
qacA1	GU565967	(Alam et al., 2003). Previously described as "qacA prototype."
qacA2	N/A	(Alam et al., 2003). Previously described as "qacA-V1."
qacA3	MK040371	(Alam et al., 2003). Previously described as "qacA-V2."
qacA4	MK040360	This study.
qacA5	MK040361	This study.
qacA6	MK040362	This study.
qacA7	MK040363	This study.
qacA8	MK040364	This study.
qacA9	MK040365	This study.
qacA10	MK040366	This study.
qacA11	MK040367	This study.
qacA12	MK040368	This study.
qacA13	MK040369	This study.
qacA14	MK040370	This study.

Table S3. SNV matrix obtained from a core genome analysis of *S. epidermidis* ST2 isolates sequenced in our study using BPH0662 as the reference genome.

		Isolate ID																		
		BPH0662	169.1	131.1	73.1	87.7	91.2	98.1	101.1	107.2	123.3	134.11	125.1	125.3	125.8	15.1	36.3	36.4	39.7	96.5
Isolate ID	BPH0662																			
	169.1	2163																		
	131.1	2093	272																	
	73.1	2186	63	295																
	87.7	2177	78	286	101															
	91.2	2172	77	281	100	91														
	98.1	2161	62	270	85	76	75													
	101.1	2163	40	272	63	78	77	62												
	107.2	2182	85	291	108	99	96	83	85											
	123.3	2184	87	293	110	91	100	85	87	108										
	134.11	2163	64	272	87	78	77	54	64	85	87									
	125.1	2005	330	248	353	344	339	328	330	349	351	330								
	125.3	2005	330	248	353	344	339	328	330	349	351	330	0							
	125.8	2004	333	251	356	347	342	331	333	352	354	333	3	3						
	15.1	2001	326	244	349	340	335	324	326	345	347	326	34	34	37					
	36.3	1904	491	419	514	505	500	489	491	510	512	491	315	315	314	311				
	36.4	1905	492	420	515	506	501	490	492	511	513	492	316	316	315	312	1			
	39.7	2012	337	255	360	351	346	335	337	356	358	337	41	41	44	41	322	323		
	96.5	2135	38	244	61	42	51	36	38	59	49	38	302	302	305	298	463	464	309	

Table S4. *Staphylococcus epidermidis* carrying *qacA4* sequenced in other projects.

Isolate ID	BioSample Accession No.	BioProject	Collection Location	Collection Date	Source	*qacA* Allele	pAQZ1	ST	Reference
13A1	SAMN12037431	PRJNA415995	Seattle, Washigton, United States	2010	Skin	*qacA4*	Yes	2	Soma et al., 2012
AUS16	SAMN09093621	PRJNA470534	Melbourne, Australia	2017	Blood	*qacA4*	Partial†	2	Lee et al., 2018
UK01	SAMN09103953	PRJNA470752	East England, United Kingdom	2015	Blood	*qacA4*	Yes	2	Lee et al., 2018
BEL14	SAMN09103870	PRJNA470752	Brussels, Belgium	2017	Blood	*qacA4*	Partial†	2	Lee et al., 2018
MB503	SAMN08558266	PRJNA434275	Houston, Texas, United States	2014	Blood	*qacA4*	Yes	2	Li et al., 2018
MB591	SAMN08558277	PRJNA434275	Houston, Texas, United States	2014	Blood	*qacA4**	Yes	2	Li et al., 2018
MB1595	SAMN08558194	PRJNA434275	Houston, Texas, United States	2015	Blood	*qacA4*	Partial†	2	Li et al., 2018
MB1617	SAMN08558196	PRJNA434275	Houston, Texas, United States	2015	Blood	*qacA4*	Yes	2	Li et al., 2018
MB1708	SAMN08558205	PRJNA434275	Houston, Texas, United States	2015	Blood	*qacA4*	Yes	2	Li et al., 2018
MB2095	SAMN08558235	PRJNA434275	Houston, Texas, United States	2016	Blood	*qacA4***	Yes	2	Li et al., 2018
MB4623	SAMN08558260	PRJNA434275	Houston, Texas, United States	2014	Blood	*qacA4*	Yes	2	Li et al., 2018
HMSC058E12	SAMN04498569	PRJNA296153	St. Louis, Missouri, United States	Unknown	Gastrointestinal Tract	*qacA4*	Yes	2	
273	SAMN06015656	PRJNA278886	Boston, Massachusetts,	2016	Blood	*qacA4*	Yes	2	

			United States						
NGS-ED-1107	SAMN03396260	PRJNA255947	Edinburgh, United Kingdom	2013	Blood	*qacA4*	Unknown	2	Walsh et al., 2015
NGS-ED-1110	SAMN03396286	PRJNA255947	Edinburgh, United Kingdom	2013	Blood	*qacA4*	Unknown	2	Walsh et al., 2015
NGS-ED-1111	SAMN03396287	PRJNA255947	Edinburgh, United Kingdom	2013	Blood	*qacA4*	Unknown	2	Walsh et al., 2015
NGS-ED-1117	SAMN03396288	PRJNA255947	Edinburgh, United Kingdom	2013	Blood	*qacA4*	Unknown	2	Walsh et al., 2015
NGS-ED-1118	SAMN03396289	PRJNA255947	Edinburgh, United Kingdom	2013	Blood	*qacA4*	Unknown	2	Walsh et al., 2015
SH03_17	SAMN08095611	PRJNA419705	São Paulo, Brazil	2017	Blood	*qacA4*	Unknown	2	
6_SEPI	SAMN03197799	PRJNA267549	Seattle, Washington, United States	2012 - 2013	Urine	*qacA4*	Yes	2	Roach et al., 2015
8_SEPI	SAMN03198012	PRJNA267549	Seattle, Washington, United States	2012 - 2013	Urine	*qacA4*	Yes	2	Roach et al., 2015
15_SEPI	SAMN03197339	PRJNA267549	Seattle, Washington, United States	2012 - 2013	Bronchoalveolar Lavage	*qacA4*	Yes	2	Roach et al., 2015
16_SEPI	SAMN03197351	PRJNA267549	Seattle, Washington, United States	2012 - 2013	Bronchoalveolar Lavage	*qacA4*	Yes	2	Roach et al., 2015
21_SEPI	SAMN03197402	PRJNA267549	Seattle, Washington, United States	2012 - 2013	Bronchoalveolar Lavage	*qacA4*	Yes	2	Roach et al., 2015
92_SEPI	SAMN03198134	PRJNA267549	Seattle, Washington, United States	2012 - 2013	Bronchoalveolar Lavage	*qacA4*	Yes	2	Roach et al., 2015
101_SEPI	SAMN03196971	PRJNA267549	Seattle, Washington, United States	2012 - 2013	Intracutaneous Line Segment	*qacA4*	Yes	2	Roach et al., 2015

364_SEPI	SAMN03197554	PRJNA267549	Seattle, Washington, United States	2012 - 2013	Bronchoalveolar Lavage	*qacA4*	Yes	2	Roach et al., 2015
366_SEPI	SAMN03197556	PRJNA267549	Seattle, Washington, United States	2012 - 2013	Bronchoalveolar Lavage	*qacA4*	Yes	2	Roach et al., 2015
386_SEPI	SAMN03197577	PRJNA267549	Seattle, Washington, United States	2012 - 2013	Wound	*qacA4*	Partial[††]	2	Roach et al., 2015
642_SEPI	SAMN03197843	PRJNA267549	Seattle, Washington, United States	2012 - 2013	Blood	*qacA4*	Yes	2	Roach et al., 2015
795_SEPI	SAMN03198008	PRJNA267549	Seattle, Washington, United States	2012 - 2013	Blood	*qacA4*	Partial[†]	847	Roach et al., 2015
798_SEPI	SAMN03198010	PRJNA267549	Seattle, Washington, United States	2012 - 2013	Blood	*qacA4*	Partial[†]	847	Roach et al., 2015
802_SEPI	SAMN03198016	PRJNA267549	Seattle, Washington, United States	2012 - 2013	Blood	*qacA4*	Yes	847	Roach et al., 2015
803_SEPI	SAMN03198017	PRJNA267549	Seattle, Washington, United States	2012 - 2013	Blood	*qacA4*	Yes	847	Roach et al., 2015
821_SEPI	SAMN03198036	PRJNA267549	Seattle, Washington, United States	2012 - 2013	Bronchoalveolar Lavage	*qacA4*	Yes	2	Roach et al., 2015
872_SEPI	SAMN03198079	PRJNA267549	Seattle, Washington, United States	2012 - 2013	Tissue	*qacA4*	Yes	2	Roach et al., 2015
961_SEPI	SAMN03198181	PRJNA267549	Seattle, Washington, United States	2012 - 2013	Fluid	*qacA4*	Yes	2	Roach et al., 2015
1040_SEPI	SAMN03197006	PRJNA267549	Seattle, Washington, United States	2012 - 2013	Blood	*qacA4*	Yes	2	Roach et al., 2015
1295_SEPI	SAMN03197267	PRJNA267549	Seattle, Washington, United States	2012 - 2013	Urine	*qacA4*	Yes	2	Roach et al., 2015

[*] The *qac* gene in isolate MB591 contained the two coding changes but not the synonymous mutation of *qacA4*.

**Isolate MB2095 appeared to contain two *qacA* alleles, with one being *qacA4*.

[†] Five isolates (AUS16, BEL14, MB1595, 795_SEPI and 798_SEPI) appeared to lack a 4.6 kb segment of the pAQZ1 plasmid.

[††] Isolate 386_SEPI appeared to lack a 10.4 kb segment of the pAQZ1 plasmid.

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