



Examination of 388 *Staphylococcus aureus* Isolates from Intensive Care Unit Patients

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ABSTRACT We have examined the draft genomes of 388 methicillin-resistant *Staphylococcus aureus* isolates obtained from intensive care unit patients at three geographically distributed hospitals to determine genomic diversity associated with potential health care worker-associated transmission.

Antimicrobial resistance has been a constantly evolving problem in the hospital setting (1). This study was undertaken to examine the genomic variation of *Staphylococcus aureus* isolates obtained from patients in the intensive care unit (ICU). Between January 2016 and August 2018, ICU patients colonized by methicillin-resistant *Staphylococcus aureus* (MRSA) were enrolled at four hospitals (two in Baltimore, MD, one in Torrance, CA, and one in New York, NY). These patients had a prior surveillance or clinical MRSA culture within 7 days of enrollment. Further details on the patient inclusion criteria and patient demographics are included in a previous publication (2). These isolates were collected as part of a study to examine transmission of *Staphylococcus aureus* isolates to the gloves and gowns of health care workers during patient contact (2). Each swab was enriched overnight in tryptic soy broth (TSB) with 6.5% salt (Becton Dickinson, Sparks, MD) and plated on CHROMagar Staph aureus medium (Becton Dickinson, Sparks, MD). All rose/mauve colonies were confirmed as *S. aureus* by Staphaurex latex agglutination and confirmed as MRSA by susceptibility testing following the Clinical and Laboratory Standards Institute guidelines (3). A total of 388 *Staphylococcus aureus* isolates were collected and examined by whole-genome sequencing.

Genomic DNA was isolated from cultures grown in lysogeny broth overnight. DNA was extracted in 96-well format from 100 µl of sample using the MagAttract Power-Microbiome DNA/RNA kit (Qiagen, Hilden, Germany), automated on a Hamilton MicroLab STAR robotic platform. Bead disruption was conducted on a TissueLyser II (20 Hz for 20 min) instrument in a 96-well deep-well plate in the presence of 200 µl phenol-chloroform. Genomic DNA was eluted in 90 µl water after magnetic bead cleanup. The resulting genomic DNA was quantified by PicoGreen. The sequencing libraries were generated with the Kapa HyperPrep kit (catalog number KK8504) and sequenced on the Illumina HiSeq 4000 using a 2 × 150-bp paired-end kit.

The total number of reads generated for each isolate averaged 3,724,291 bp per genome (Table 1). All software was used with default values. Raw sequencing reads were filtered to remove contaminating phiX reads using BBduk, one of the BBTools software suite (sourceforge.net/projects/bbmap/). The raw reads were also filtered to

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remove contaminating Illumina adaptor sequences and quality trimmed using Trimomatic v. 0.36 (4). The resulting filtered reads were assembled using SPAdes v. 3.13.0 (5). The resulting assemblies were then filtered to contain only contigs longer than 500 bp with a k-mer coverage of $\geq 5\times$. Genomes containing more than 500 contigs or an aberrant GC content were removed from further analysis.

The genomes have a mean sequencing coverage of $198\times$ (standard deviation [SD], $58\times$; minimum [min], $58\times$; maximum [max], $1,008\times$). The final assemblies have a mean contig count of 38 (SD, 16; min, 19; max, 199), a mean genome size of 2,857,897 bp (SD, 56,218 bp; min, 2,682,854 bp; max, 3,009,992 bp), a mean GC content of 32.67% (SD, 0.06%; min, 32.56%; max, 33.22%), and a mean N_{50} value of 271,123 bp (SD, 89,042 bp; min, 47,630 bp; max, 699,731 bp).

Further analysis will reveal the genetic determinants of transmission via health care worker interactions.

Data availability. Relevant statistics, including GenBank and SRA accession numbers for each genome assembly, are included in Table 1.

TABLE 1 Genome assembly characteristics

Sample	Insert size (bp)	Total no. of reads	No. of bases sequenced	Genome coverage (\times)	No. of contigs	Genome size (bp)	GC content (%)	N_{50} (bp)	GenBank accession no.	SRA accession no.
MRSA1	404	2,724,258	411,362,958	147	31	2,791,271	32.62	295,202	WBHV00000000	SRR10179083
MRSA10	493	3,817,952	576,510,752	211	31	2,735,036	32.78	250,902	WBHU00000000	SRR10179082
MRSA100	437	3,630,220	548,163,220	192	35	2,858,562	32.75	170,064	WBHT00000000	SRR10179185
MRSA101	460	3,494,024	527,597,624	186	27	2,838,792	32.62	300,480	WBHS00000000	SRR10179290
MRSA102	420	3,438,980	519,285,980	187	27	2,772,092	32.76	431,335	WBHR00000000	SRR10179017
MRSA103	436	3,348,168	505,573,368	179	31	2,829,018	32.72	443,398	WBHQ00000000	SRR10179006
MRSA104	544	3,590,670	542,191,170	194	29	2,791,200	32.67	699,731	WBHP00000000	SRR10178995
MRSA105	453	3,822,610	577,214,110	200	40	2,887,518	32.65	227,545	WBHO00000000	SRR10178984
MRSA106	545	3,542,228	534,876,428	193	39	2,771,933	32.69	247,058	WBHN00000000	SRR10178973
MRSA107	444	3,791,274	572,482,374	195	28	2,931,006	32.61	284,107	WBHM00000000	SRR10178962
MRSA108	521	5,395,966	814,790,866	284	30	2,873,007	32.73	288,049	WBHL00000000	SRR10178951
MRSA109	516	4,646,288	701,589,488	240	26	2,921,394	32.64	381,525	WBHK00000000	SRR10178940
MRSA11	570	3,440,454	519,508,554	177	29	2,927,157	32.73	287,824	WBHJ00000000	SRR10178929
MRSA110	499	4,174,882	630,407,182	214	36	2,946,853	32.66	283,926	WBHI00000000	SRR10179262
MRSA112	460	3,737,726	564,396,626	195	35	2,887,177	32.65	347,420	WBHH00000000	SRR10179251
MRSA113	490	5,127,932	774,317,732	271	39	2,856,331	32.74	204,938	WBHG00000000	SRR10179240
MRSA114	432	3,427,176	517,503,576	182	34	2,844,520	32.71	243,833	WBHF00000000	SRR10179229
MRSA115	480	2,963,254	447,451,354	153	85	2,924,835	32.70	221,193	WBHE00000000	SRR10179218
MRSA116	464	3,984,944	601,726,544	210	28	2,862,201	32.61	345,460	WBHD00000000	SRR10179207
MRSA117	443	4,100,492	619,174,292	216	32	2,869,996	32.61	333,066	WBHC00000000	SRR10179196
MRSA118	452	3,845,056	580,603,456	202	38	2,877,456	32.64	280,924	WBHB00000000	SRR10179184
MRSA118-1	453	2,658,674	401,459,774	139	39	2,880,266	32.64	198,614	WBHA00000000	SRR10179173
MRSA118-2	465	3,854,178	581,980,878	211	32	2,759,474	32.65	181,622	WBGZ00000000	SRR10179162
MRSA119	542	3,641,384	549,848,984	192	29	2,871,229	32.61	312,042	WBGY00000000	SRR10179151
MRSA12	431	3,292,444	497,159,044	175	38	2,843,273	32.70	242,815	WBGX00000000	SRR10179140
MRSA120	568	3,279,974	495,276,074	174	48	2,851,310	32.73	228,460	WBGW00000000	SRR10179129
MRSA121	530	4,574,424	690,738,024	241	27	2,870,565	32.61	345,387	WBGV00000000	SRR10179118
MRSA122	413	3,287,494	496,411,594	170	58	2,924,929	32.62	158,830	WBGU00000000	SRR10179107
MRSA123	399	2,911,336	439,611,736	158	43	2,783,732	32.69	154,081	WBGT00000000	SRR10179312
MRSA124	445	3,611,020	545,264,020	187	27	2,915,523	32.66	333,124	WBGS00000000	SRR10179301
MRSA125	463	3,311,634	500,056,734	174	34	2,869,237	32.61	304,481	WBGR00000000	SRR10179289
MRSA126	473	3,640,022	549,643,322	190	38	2,897,949	32.56	235,657	WBGQ00000000	SRR10179278
MRSA127	450	3,526,420	532,489,420	183	33	2,912,704	32.65	304,669	WBGP00000000	SRR10179095
MRSA128	502	3,523,528	532,052,728	183	30	2,909,559	32.64	333,118	WBGO00000000	SRR10179084
MRSA129	438	3,439,930	519,429,430	188	32	2,763,701	32.72	226,266	WBGN00000000	SRR10179071
MRSA13	402	5,828,122	880,046,422	301	32	2,925,776	32.62	158,970	WBGM00000000	SRR10179060
MRSA130	447	3,153,906	476,239,806	164	32	2,897,923	32.64	284,122	WBGL00000000	SRR10179049
MRSA131	457	3,798,068	573,508,268	209	37	2,749,664	32.66	153,446	WBGK00000000	SRR10179038
MRSA132	462	4,012,102	605,827,402	218	34	2,776,634	32.73	270,629	WBGJ00000000	SRR10179027
MRSA133	417	3,300,814	498,422,914	184	25	2,713,650	32.69	238,316	WBGJ00000000	SRR10179018
MRSA134	446	3,068,042	463,274,342	161	28	2,881,857	32.66	226,269	WBGH00000000	SRR10179016
MRSA135	496	3,128,940	472,469,940	162	31	2,907,575	32.64	304,501	WBGG00000000	SRR10179015
MRSA136	471	3,625,842	547,502,142	188	27	2,918,501	32.64	381,526	WBGF00000000	SRR10179014

(Continued on next page)

TABLE 1 (Continued)

Sample	Insert size (bp)	Total no. of reads	No. of bases sequenced	Genome coverage (×)	No. of contigs	Genome size (bp)	GC content (%)	N_{50} (bp)	GenBank accession no.	SRA accession no.
MRSA749	461	2,881,656	435,130,056	151	40	2,890,590	32.66	197,752	WATD00000000	SRR10179024
MRSA75	490	4,630,334	699,180,434	242	27	2,883,659	32.66	333,122	WAXL00000000	SRR10179106
MRSA750	457	3,051,362	460,755,662	162	40	2,840,221	32.72	279,245	WATC00000000	SRR10179023
MRSA751	498	3,811,110	575,477,610	201	28	2,863,501	32.69	288,405	WATB00000000	SRR10179022
MRSA752	453	4,324,122	652,942,422	230	26	2,837,974	32.62	345,460	WATA00000000	SRR10179021
MRSA753	477	3,367,518	508,495,218	178	33	2,863,634	32.61	201,596	WASZ00000000	SRR10179020
MRSA754	431	4,180,350	631,232,850	217	199	2,909,153	32.68	47,630	WASY00000000	SRR10179019
MRSA76	404	3,201,396	483,410,796	169	29	2,866,225	32.62	345,460	WAXK00000000	SRR10179105
MRSA77	535	3,342,204	504,672,804	177	30	2,844,557	32.56	304,502	WAXJ00000000	SRR10179104
MRSA78	469	18,938,328	2,859,687,528	1,008	25	2,836,910	32.65	299,314	WAXI00000000	SRR10179103
MRSA79	505	4,755,926	718,144,826	259	32	2,770,266	32.70	200,135	WAXH00000000	SRR10179102
MRSA8	566	3,874,494	585,048,594	201	32	2,917,786	32.63	283,744	WAXG00000000	SRR10179101
MRSA80	490	4,449,382	671,856,682	231	31	2,913,262	32.63	345,459	WAXF00000000	SRR10179100
MRSA81	580	4,095,304	618,390,904	212	75	2,910,841	32.69	153,760	WAXE00000000	SRR10179315
MRSA83	546	4,524,148	683,146,348	240	34	2,844,125	32.64	304,681	WAXD00000000	SRR10179314
MRSA85	527	4,457,926	673,146,826	237	29	2,839,253	32.62	304,752	WAXC00000000	SRR10179313
MRSA86	550	3,954,150	597,076,650	213	41	2,804,242	32.76	199,663	WAXB00000000	SRR10179311
MRSA87	536	3,929,800	593,399,800	203	61	2,928,066	32.69	219,253	WAXA00000000	SRR10179310
MRSA88	544	3,411,670	515,162,170	188	27	2,742,253	32.68	234,118	WAWZ00000000	SRR10179309
MRSA89	511	4,417,348	667,019,548	242	20	2,755,367	32.67	247,479	WAWY00000000	SRR10179308
MRSA9	595	3,779,022	570,632,322	201	37	2,845,063	32.75	216,299	WAWX00000000	SRR10179307
MRSA90	409	2,959,362	446,863,662	159	39	2,818,569	32.71	387,589	WAWW00000000	SRR10179306
MRSA91	527	5,203,138	785,673,838	271	45	2,896,174	32.76	221,197	WAWV00000000	SRR10179305
MRSA92	570	2,741,320	413,939,320	142	31	2,923,613	32.62	304,505	WAWU00000000	SRR10179304
MRSA93	574	3,563,988	538,162,188	187	30	2,880,303	32.66	333,118	WAWT00000000	SRR10179303
MRSA94	512	4,462,482	673,834,782	231	35	2,922,102	32.62	377,842	WAWS00000000	SRR10179302
MRSA95	520	3,276,014	494,678,114	172	60	2,872,762	32.75	212,998	WAWR00000000	SRR10179300
MRSA96	445	3,755,668	567,105,868	194	43	2,917,478	32.64	191,715	WAWQ00000000	SRR10179299
MRSA97	405	3,122,276	471,463,676	173	27	2,731,544	32.70	234,329	WAWP00000000	SRR10179298
MRSA98	464	3,570,268	539,110,468	185	36	2,908,087	32.74	419,319	WAWO00000000	SRR10179297
MRSA99	484	3,843,378	580,350,078	203	26	2,863,314	32.64	307,780	WAWN00000000	SRR10179296
MRSAS284	460	6,213,650	938,261,150	328	30	2,856,374	32.62	333,124	WBAG00000000	SRR10179188

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