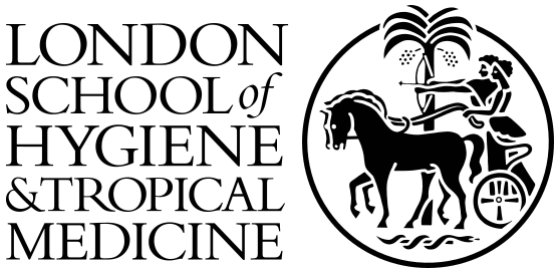

Methodology for whole genome sequencing of MRSA in a routine hospital microbiology laboratory

Running title: Laboratory methods for routine MRSA sequencing

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Abstract

There is growing evidence for the value of bacterial whole genome sequencing in hospital outbreak investigation. Our aim was to develop methods that support efficient and accurate low throughput clinical sequencing of methicillin-resistant *Staphylococcus aureus* (MRSA). Using a test panel of 25 MRSA isolates associated previously with outbreak investigations, we devised modifications to library preparation that reduced processing time by 1 hour. We determined the maximum number of isolates that could be sequenced per run using an Illumina MiniSeq and a 13 hour (overnight) run time, which equated to 21 MRSA isolates and 3 controls (no template, positive and negative). Repeatability and reproducibility assays based on this sequencing methodology demonstrated 100% accuracy in assigning species and sequence type (ST) and detecting *mecA*. Established genetic relatedness between isolates was recapitulated. Quality control (QC) metrics were evaluated over nine sequencing runs. 168/173 (97%) test panel MRSA genomes passed QC metrics based on the correct species assigned, detection of *mecA* and ST, and depth/coverage metrics. An evaluation of contamination in these 9 runs showed that positive and negative controls and test MRSA sequence files contained <0.14% and <0.48% of fragments matching another species, respectively. Deliberate contamination experiments confirmed that this was insufficient to impact on data interpretation. These methods support reliable and reproducible clinical MRSA sequencing with a turnaround time (from DNA extraction to availability of data files) of 24 hours.

Introduction

There is growing evidence of the value of bacterial whole genome sequencing (WGS) in hospital infection control practice and outbreak investigation (1). Numerous retrospective studies have shown that bacterial sequencing provides the discrimination required to distinguish between isolates of the same lineage, overcoming this limitation of previous typing methods (2-7). There is also strong published support for its use to investigate carriage, transmission and suspected outbreaks in high-risk areas such as intensive care units (2, 6). Used early, this could lead to action that limits the size of an outbreak (6, 8). Furthermore, sequencing can exclude outbreaks where a cluster of patients positive for the same pathogenic species has arisen by chance (9), saving unnecessary infection control interventions and outbreak investigations.

The benefit gained from using WGS during outbreak detection is likely to be greatest if the technology is embedded within healthcare institutions and performed with a rapid turnaround time. This has become increasingly feasible through technical advances in sequencing instruments and the availability of commercial kits and liquid handling robots that simplify DNA extraction and library preparation. The laboratory processing aspects of WGS are now within the capabilities of larger diagnostic laboratories. The technical feasibility of sequencing in real-time has been demonstrated previously at a tertiary care hospital in Germany, but the turnaround time was 4.4-5.3 days with a cost of ~£170 (10). Reducing this turn-around time to results and the cost of sequencing will be key to implementing sequencing in the clinical setting and having an impact on infection control. In our clinical microbiology laboratory at Addenbrooke's Hospital in Cambridge, United Kingdom, we are developing the methods and processes to introduce routine WGS of targeted nosocomial pathogens in close to real-time to enhance our infection control practice. Here, we describe the development of laboratory

processing methodology for low throughput clinical sequencing of methicillin-resistant *Staphylococcus aureus* (MRSA).

Materials and Methods

Test panel isolates

Twenty-nine bacterial isolates (27 *S. aureus* and 2 *E. coli*) were assembled into a test panel for the study (Table 1). The majority of *S. aureus* (n=25) were MRSA from two evaluations of sequencing at the Cambridge University Hospital NHS Foundation Trust hospital (CUH) (6,7). Twenty-one MRSA were selected from a 12-month study of MRSA-positive patients (7) to provide representation of the dominant clonal complexes in our setting (CC22, CC30 and CC5), combined with a range of genetic relatedness. A further 4 MRSA (all sequence type (ST) 22) were from an outbreak in a special care baby unit (6). Also included were 4 reference isolates: MRSA HO 5096 0412, methicillin-susceptible *S. aureus* NCTC 6571, *E. coli* NCTC 12241 and *E. coli* NCTC 10418. For sequencing, isolates were cultured from frozen stocks onto Columbia Blood Agar (CBA, Oxoid), incubated in air at 37°C overnight, and single colonies picked for DNA extraction and further processing. Table 1 indicates the isolates used in each sequencing run.

Positive and negative controls

Three controls were included in every sequencing run to monitor the ongoing performance of the entire testing process. These were a no template control, a positive control (MRSA MPROS0386) that is 115 core genome SNPs different from the MRSA HO 5096 0412 mapping reference, and a negative control (*E. coli* NCTC12241). The no template control contained all assay components except for DNA and was used to verify the lack of contamination across reagents and samples. The positive control was used to control the entire

assay process and analytical accuracy. The negative control was used to assess cross-contamination during processing and represented the non-target DNA sample to verify analytical specificity. In the first two runs an alternative *E. coli* control (NCTC10418) was used, but this had a low match to *E. coli* in Kraken (~22%) and was replaced by NCTC12241 (>50% match). Fresh stocks of molecular grade water and phosphate-buffered-saline were opened each week. Other 'reuse' reagents were checked for bacterial contamination weekly by sub-culturing using a 1µl loop onto CBA and incubating overnight in air at 37°C.

DNA extraction, library preparation and sequencing

DNA was extracted using the QIAgen DNA mini extraction kit (https://www.qiagen.com/gb/shop/sample-technologies/dna/genomic-dna/qiaamp-dna-mini-kit/#resources) following 'Appendix D: Protocols for Bacteria' 'Isolation of genomic DNA from Gram-positive bacteria' with the following amendments: the incubation with proteinase K was performed at 56°C for 30 minutes; and in the final elutions, 50ul distilled water was added with the full 5 minutes incubation. DNA was quantified using a Qubit fluorometer. Sequencing libraries were made using the Illumina Nextera DNA flex kit based on the manufacturer's instructions (11), with several modifications to reduce processing time (see results). In the first 3 runs, the input DNA to library preparation was normalized to ~100ng, but thereafter we used a range of up to 500ng DNA. Libraries were sequenced on an Illumina MiniSeq with a run time of 13 hours (overnight) using the high output 150 cycle MiniSeq cartridge and the Generate Fastq workflow. Genomes were demultiplexed using the Generate Fastq workflow and the data transferred to an external 1TB USB-connected hard drive for further analysis. Ten sequencing runs were performed during this evaluation; the objective of each run is summarized in Table S1.

Sequence data analysis

Multilocus sequence types (ST) of the MRSA isolates were identified using ARIBA version 2.12.1 as described at https://github.com/sanger-pathogens/ariba/wiki/MLST-calling-with-ARIBA. Species were determined using Kraken version 1 (https://ccb.jhu.edu/software/kraken/) with the miniKraken database available at https://ccb.jhu.edu/software/kraken/dl/minikraken_20171019_8GB.tgz. The presence of *mecA* (accession number HE681097, position 2790560-2792566) was determined using ARIBA, with a minimum percentage identity of 70% required based on Ito *et al.* (12) , and a minimum of 90% of the gene length covered. All isolates were mapped to the MRSA HO 5096 0412 CC22 reference (accession number HE681097) using SMALT (https://www.sanger.ac.uk/science/tools/smalt-0) with mapping and base calling performed as described previously (13) with the following modifications: kmer size 13, step size 6. The depth and percentage coverage of the mapping reference were determined using the script available at https://github.com/sanger-pathogens/vr-codebase/blob/master/modules/VertRes/Pipelines/Mapping.pm.

Sequence metrics for controls

Controls were required to pass the following quality metrics. MRSA positive control: highest match to *S. aureus* using Kraken, assigned to ST22, *mecA* detected, minimum mean sequence depth of 20x and minimum 80% coverage of the mapping MRSA reference genome (HO 5096 0412). *E. coli* negative control: highest species match to *E. coli* in Kraken, *mecA* not detected, no *S. aureus* ST assigned. No template control: contamination from any bacterial DNA of less than 95,000 fragments in Kraken. MRSA isolates from the test panel were required to pass the following metrics: highest match to *S. aureus* using Kraken, assigned to the correct ST, *mecA*

detected, minimum sequence depth of 20x and minimum 80% coverage of the mapping MRSA reference genome (HO 5096 0412).

Optimizing the number of isolates per sequencing run

We estimated that the maximum number of MRSA isolates in a single sequencing run was 24 based on an expected total data output of 3.3-3.8Gb, an average MRSA genome size of 2.8 MB (https://www.ncbi.nlm.nih.gov/genome/?term=Staphylococcus%20aureus[Organism]&cmd=DetailsSearch) and a target of ~50x coverage (24 isolates would provide ~49x coverage). We estimated that 21 test MRSA isolates and three controls (*E. coli*, MRSA and no template) could be included per sequence run. This was evaluated by performing sequencing runs that contained either 14, 18 or 21 test MRSA isolates from the study panel plus the 3 controls. One MRSA isolate from the 21-test isolate run failed to produce sufficient DNA during extraction and the *E. coli* control was included twice.

Repeatability and reproducibility

Repeatability was evaluated by sequencing six MRSA isolates (HO 5096 0412, MPROS0386, SASCBU17, SASCBU18, SASCBU25 and SASCBU35) in triplicate in a single sequencing run. For each isolate, frozen stock was sub-cultured onto CBA, incubated in air at 37°C overnight, and three separate colonies taken forward for individual DNA extraction, library preparation and sequencing. Reproducibility was evaluated by sequencing 21 MRSA isolates from the test panel in three independent runs. Each isolate was sub-cultured onto CBA and incubated in air at 37°C overnight, after which three individual colonies were taken forward for DNA extraction, library preparation and sequencing, one for each sequence run. The entire process was performed by different laboratory staff on three different days. The resulting fastq

files were analysed as above. Isolates that failed QC metrics were excluded from further analysis (3/18 and 1/63 test isolates failed the repeatability and reproducibility assays, respectively, based on low depth/coverage.

Definition of a correct result was based on species identification, ST assignment, detection of *mecA*, and identification of genetic relatedness based on the detection of single nucleotide polymorphisms (SNPs) in the core genome compared to the original sequence and the within-run or between run-replicates. Genetic relatedness was determined based on mapping to a clonal complex (CC)-specific references, excluding positions denoted as 'N' because of failure to call a base. Each repeat and the original sequence data were mapped to a CC-specific mapping reference using SMALT (MRSA HO 5096 0412 (CC22) for ST22 and ST2371; MRSA252 (CC30, BX571856) for ST30; and N315 (CC5, BA000033) for ST5). Mobile genetic elements were removed using the files available at https://figshare.com/authors/Francesc_Coll/5727779 and the script available at https://github.com/sanger-pathogens/remove_blocks_from_aln. Single nucleotide polymorphisms (SNPs) were identified using the script available at https://github.com/sanger-pathogens/snp-sites. SNPs were identified based on the following parameters: minimum number of reads matching the SNP = 4; minimum number of reads matching the SNP per strand = 2; ratio of SNP base to alternative base >0.75; variant quality >50; mapping quality >30.

Diagnostic sensitivity and specificity were calculated, using the following definitions: true positives, the number of genetically related isolates based on the original data that cluster together based on the test data; false negatives, the number of genetically related isolates based on the original data that do not cluster together in the test data; true negatives, the number of

genetically unrelated isolates based on the original data that do not cluster together in the test data; and false positives, the number of genetically distant isolates based on the original data that cluster together based on the test data (14). Clustering was defined based on three SNP classifications: (i) Recent transmission highly likely, 0-10 SNPs different (based on a median within host diversity of 6 SNPs over a year (7) and an estimated mutation rate of 4 SNPs/core genome/year (15), (ii) Recent transmission likely, 11-25 SNPs, and (iii) Recent transmission possible, 26-50 SNPs different (based on the definition of a cluster described by Coll et al. (7)). Isolates >50 SNPs different were classified as genetically unrelated.

Analysis of contamination

The impact on quality metrics from varying levels of DNA contamination during clinical MRSA sequencing was evaluated using intentional spiking experiments. One MRSA isolate from the test panel (MPROS1839 (ST22)) and *E. coli* NCTC 12241 were cultured and DNA extracted and quantified as described above. Donor DNA was inoculated into the recipient sample to achieve a final spiked concentration of 0%, 0.1%, 1%, 10% or 20% (see results for details of donor and recipient). Contamination with the spike was defined based on the number and proportion of fragments matching to *S. aureus* or *E. coli* based on Kraken. The effect of contamination was evaluated using this metric together with the proportion of the *S. aureus* CC22 reference covered during mapping, depth of coverage of the mapping reference, and *mecA* and ST detected by Ariba. Unintentional contamination from internal controls or external sources was evaluated based on the number and proportion of reads matching to other species in Kraken.

Data availability

Sequence data generated during this study are available from the European Nucleotide Archive (https://www.ebi.ac.uk/ena) under the accession numbers listed in Table 1.

Results

Our aim was to develop methods that would support efficient and accurate low throughput MRSA sequencing in a routine microbiology laboratory in less than 24 hours (from DNA extraction to availability of sequence data). Key goals were to maximize the number of isolates sequenced per run, reduce processing time of DNA preparation, and evaluate quality controls, precision (reproducibility and repeatability), and contamination.

Maximizing the number of isolates per sequencing run was evaluated by performing sequencing runs that contained either 14, 18 or 21 test MRSA isolates from the study panel plus the 3 controls, which were sequenced using the Illumina MiniSeq with a run time of 13 hours. Median (range) sequence depth for the test MRSA isolates was 92x (33-247x), 63x (45-77x) and 65x (18-107x), respectively, with a minimum of 87% of the genome covered (Table S2). One isolate in the 21 test MRSA run failed the QC metrics based on depth of coverage (17.9x), which on further evaluation could be explained by low input DNA (Table S2). Based on this, we used 21 test isolates plus 3 controls per run during the remainder of the study.

We sought modifications to the manufacturer's protocol for library preparation (Illumina Nextera DNA flex kit) that would reduce processing time while maintaining performance. We proposed that two steps could be changed: (i) the tagmentation (TAG program) and tagmentation stop (TSB incubation) steps each require 15 minutes incubation, which were reduced to 5 minutes each; (ii) Pooling of libraries is recommended after bead clean-up and size selection, but we pooled libraries after PCR and before the bead-cleanup and size

selection. Two sequencing runs of 21 test panel MRSA + 3 controls were compared, one of which used the original protocol and the other made both changes to the protocol. Data were compared for quantity of DNA added to the library preparation versus the size of the resulting fastq files and depth of coverage, as surrogates for the individual DNA quantity outputs from library preparation, which are unavailable with the modified protocol. Detailed results are provided in Table S3. In summary, comparison of original versus modified protocol showed negligible difference. The median (range) fastq size for the original versus modified protocols were 171MB (77-208MB) following 174-480ng DNA input versus 112MB (90-133MB) following 90-384ng DNA input. The median (range) depth of coverage for the original versus modified protocols were 87x (37-99x) versus 56x (43-70x). Together, these resulted in a reduction in processing time from 3.5 to 2.5 hours for library preparation, taking the combined time for DNA preparation and library preparation to 4.5 hours. Subsequent runs used these modifications.

Repeatability was based on concordance of assay results and quality metrics for six MRSA isolates sequenced in triplicate in a single sequencing run. This demonstrated 100% concordance in assigning species, ST and detecting *mecA*. Four of the six isolates were drawn from a study that investigated a single outbreak on an intensive care unit (6) and were previously identified as being 0 SNPs different (SASCBU17 and SACBU18), 5 SNPs different (SASCBU25), or unrelated to the outbreak (SASCBU35, >1,500 SNPs different from other isolates). The remaining two isolates were MRSA HO 5096 0412 and the positive MRSA control (MPROS0386). Zero SNPs were identified between the within-run replicates for all isolates, equating to a repeatability per replicate of 100%. Using the original published sequence mapped to the CC22 reference (HO 5096 0412) as the gold standard, all 6 isolates in triplicate had identical base calls to the original sequence (excluding positions denoted as

261 'N' because of failure to call a base), equating to a repeatability per replicate and per base pair
262 of 100%.
263
264 Reproducibility was evaluated by sequencing 21 test panel MRSA isolates in three
265 independent runs. This demonstrated 100% accuracy in assigning species, ST and detecting
266 *mecA*. Eighteen of the 21 isolates represented six distinct outbreaks encompassing four
267 different STs (ST22, ST30, ST5 and ST2371) identified during 12 months of genomic
268 surveillance (n=15) (7) or a single outbreak in an intensive care unit (n=3) (6) . Of the
269 remainder, 2 isolates were not involved in these outbreaks based on low relatedness, and 1
270 isolate was the mapping reference MRSA HO 5096 0412. There were 0 SNPs identified
271 between between-run replicates, providing a reproducibility per replicate of 100%. Using the
272 original published sequence when mapped to the CC22 reference as the gold standard, 18
273 isolates were identical to the original sequence across replicates. The remaining three isolates
274 showed a difference in SNPs compared with the original sequence: MPROS0292 (ST22) had
275 1-2 SNPs different, one of which was reproduced in all three repeats and the other was
276 reproduced in two repeats with an N base call in the remaining repeat. MPROS1125 (ST22)
277 had 1 SNP different in one repeat with an N base call in the same position in the remaining
278 two repeats. MPROS2335 was identical for two replicates but the third replicate had 10 SNPs.
279 In comparison to the original sequence this provides an assay accuracy of 92.3% (60/65
280 repeats), although the true accuracy is likely be higher as the majority of SNPs may be genuine
281 based on their presence among repeats.
282
283 We next sought to determine the diagnostic sensitivity and specificity for outbreak detection
284 in each of the three reproducibility runs, using the genetic relatedness established previously
285 (6,7) as the gold standard (Table S4). All test isolate pairs within each run were in the same

genetic relatedness category (0-10 SNPs, 11-25 SNPs, 26-50 SNPs, >50 SNPs) as isolate pairs in the original data. This was reproducible across all three runs and represents a diagnostic sensitivity and specificity for outbreak detection of 100%, which was retained across a range of definitions for genetic relatedness. The majority of isolate pairs were within 1 SNP of the expected SNP difference based on the gold standard. The exceptions were Cluster 3 (2 SNPs different between MPROS0046 and MPROS1125 in two runs relating to failure to call a base at one position, and a SNP in a region that was absent across the replicates but present at low coverage in the original sequence); and Cluster 4 (MPROS0688 and MPROS2335; 2 SNPs different in two runs due to two positions at which a base failed to be called; and 6 SNPs different in the final run. The isolate sequence MPROS2335 was genetically identical to a second isolate from the same patient sequenced by Coll *et al*. (7) but not included here. From this, we suspect within-host diversity of MRSA in this case, and sequencing of different colonies of the same lineage.

Quality control metrics were evaluated for the assay controls and MRSA isolates from the test panel over nine sequencing runs (Table S5 provides further details). All three controls in each sequence run passed the required QC metrics. Of 173 *S. aureus* test panel MRSA isolates sequenced, 168 (97%) passed the QC metrics. The five failures were based on insufficient depth/coverage associated with low input DNA (n=2) or potential loss of DNA during library preparation (n=3). Excluding these 5 failed isolates and the control isolates, *S. aureus* was the top match in Kraken in all cases (median (range) 85.8% (77.2-89.3%), the median (range) depth was 59x (21–247x), and the median (range) proportion of the reference genome covered was 94.6% (86.3-100%).

We then undertook deliberate contamination experiments to allow us to estimate the impact of varying levels of DNA contamination from internal controls or external sources on quality metrics. Details of the donor and recipient DNA, the concentrations of spiked DNA and our findings are summarized in Table 2. Contamination of the no template control with increasing concentrations of MRSA DNA did not lead to the control erroneously passing the QC metrics for MRSA until the final spiked concentration reached 10% or greater. This indicates that contamination of the no template control at 1% (which equated to 96,671 fragments matching *S. aureus* in Kraken) can be tolerated. Contaminating the positive MRSA control with increasing concentrations of *E. coli* DNA demonstrated that this could tolerate up to 10% contamination (which equated to 4.04% fragments matching *E. coli* in Kraken) before the MRSA QC metrics were not achieved.

We also evaluated unintentional contamination in nine runs (excluding the deliberate contamination assay). All *E. coli* and MRSA control sequence data files contained less than 0.14% of fragments matching another species (Table S6). For the test MRSA sequence files, matches to other staphylococcal species were identified in over half of samples (109/173, median 0.05%, range 0.01-0.48% of fragments). Very low-level matches (0.01-0.13%) to other species were also identified in specific files (Table S6). All isolates had less than 0.2% of fragments matching to anther species, with the exception of a single reference isolate of MSSA that had a match of up to 0.48% to *Staphylococcus nepalensis*. Based on the number of fragments in Kraken for the no template controls, and the proportion of fragments in Kraken for the remaining sequences, this demonstrates that, with the exception of the isolate above, all controls and test isolates had levels of contamination below 1% (0.4% of fragments) across the nine sequencing runs (Table S6).

Discussion

Our aim was to develop and describe methods for low throughput clinical sequencing of MRSA using commercial kits and manual methods. Our rationale was that this could support wider uptake in smaller diagnostic laboratories that are not equipped to undertake high volume sequencing using automated robots. Whilst liquid handling robots are essential for high volume sequencing such as that increasingly performed by public health reference laboratories, the majority of routine clinical laboratories have yet to invest in sequencing pipelines with their associated capital and maintenance costs.

An important objective was to enable a 24-hour turnaround time from DNA extraction to availability of sequence data. The combined time for DNA preparation and library preparation is 4.5 hours, followed by a 13-hour (overnight) sequencing run on the Illumina MiniSeq. This would support a pipeline of clinical sequencing in which relevant cultures were identified in a routine laboratory and processed including sequencing within a day. The methods described here are based on a single colony, which when implemented in routine practice could be obtained from the original diagnostic clinical plate. This turnaround time, in combination with a rapid automated analysis pipeline, would allow infection control to determine whether patients were involved in an outbreak or not the day after a positive culture. This could allow rapid instigation of enhanced infection control procedures when an outbreak is detected to prevent further spread of the outbreak, and prevention of infection control actions such as ward closures if a suspected outbreak could be refuted.

We also maximised the number of MRSA per sequencing run to minimize the cost per isolate. Based on 21 clinical isolates per run with three controls, the price per clinical isolate is currently £70 for DNA extraction, library preparation and sequencing. Whilst individual

hospitals are unlikely to frequently reach 21 clinical MRSA isolates suspected to be involved in an outbreak, we suggest a paradigm shift whereby all patients identified as MRSA positive have an isolate sequenced, and whole-genome sequencing leads infection control actions. This would reduce the turnaround time for action since current outbreak detection relies on multiple time-consuming steps including manually identifying patients that have been in the same ward at the same time. Using whole-genome sequencing combined with automated analysis would rapidly pinpoint which patients are involved in an outbreak or not, defining the cases that infection control need to act on, and those that require no action. The combination of turnaround and cost are critical measures for clinical translation. Alternative sequencing instruments such as the Oxford Nanopore Technologies provide the option for further reductions in sequencing time (16), and over time the cost and turnaround time of sequencing will undergo further reductions. As costs fall, lower-throughput technologies such as the Illumina iSeq 100 may become viable for routine clinical laboratories with smaller sample numbers.

We described the use and evaluation of assay controls, examined the impact of contamination on data interpretation and determined the extent to which we inadvertently contaminated the assay. All three controls passed the required QC metrics in every run, together with 97% of test panel MRSA isolates sequenced. High levels of contamination were required before the controls failed QC metrics, and levels of inadvertent contamination were low. Evaluation of precision showed 100% repeatability and reproducibility in assigning species and ST and detecting *mecA*. SNP detection was 100% repeatable, but reproducibility was less than 100% because of the detection of a small number of SNPs that were not present in the original sequence. These can be explained by minor heterogeneity in colonies prepared for independent sequencing, with similar findings reported previously based on sequencing of a range of

bacterial species (14). Importantly, diagnostic sensitivity and specificity for outbreak detection were 100%, indicating that the data generated accurately determined MRSA relatedness, which supports use of this assay during outbreak investigation. The parameters evaluated in this study were in line with the workflow for validation of whole-genome sequencing in clinical laboratories described previously, and obtained comparable results (14).

Our findings indicate that the methods evaluated here can provide high quality data. The single largest impediment to clinical sequencing is lack of fully automated data interpretation software that has a rapid turn-around time and is suitable for use by non-experts. This will need to be addressed for routine clinical sequencing to become viable, and is currently being investigated by numerous groups and investigators.

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Conflict of interest

SJP and JP are consultants to Specific Technologies and Next Gen Diagnostics. PK-G, DP, LF and JB are employees of and hold stock in Illumina, Inc. The other authors have no conflicts of interest.

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Table 1. Panel of bacterial isolates used in the study.

Sample name	Accession number	Control or Test isolate	Species	ST	Original study	Transmission clusters	Run 1	Run 2	Run 3	Run 4	Run 5	Run 6	Run 7	Run 8	Run 9	Run 10
SASCBU35	ERR131801	Test isolate	*Staphylococcus aureus*	22	Harris et al.	Unrelated to Cluster 1	x	x		x	x	x	x	x	x	
SASCBU17	ERR72246	Test isolate	*Staphylococcus aureus*	2371	Harris et al.	Cluster 1	x	x	x	x	x	x	x	x	x	
SASCBU18	ERR72247	Test isolate	*Staphylococcus aureus*	2371	Harris et al.	Cluster 1	x	x	x	x	x	x	x	x	x	
SASCBU25	ERR108054	Test isolate	*Staphylococcus aureus*	2371	Harris et al.	Cluster 1	x	x	x	x	x	x	x	x	x	
MPROS0386	ERR212946	Control isolate	*Staphylococcus aureus*	22	Coll et al.	Unrelated to Cluster 2	x	x	x (2)	x	x	x	x	x	x	
MPROS1839	ERR715142	Test isolate	*Staphylococcus aureus*	22	Coll et al.	Cluster 2	x	x	x	x	x		x	x	x	x
MPROS2508	ERR715397	Test isolate	*Staphylococcus aureus*	22	Coll et al.	Cluster 2	x	x	x	x	x		x	x	x	
MPROS2264	ERR715156	Test isolate	*Staphylococcus aureus*	22	Coll et al.	Cluster 2	x	x	x	x	x		x	x	x	
MPROS2239	ERR715240	Test isolate	*Staphylococcus aureus*	22	Coll et al.	Cluster 2			x	x	x		x	x	x	
MPROS0292	ERR212846	Test isolate	*Staphylococcus aureus*	22	Coll et al.	Cluster 2	x	x		x	x		x	x	x	
MPROS2066	ERR702160	Test isolate	*Staphylococcus aureus*	30	Coll et al.	Cluster 3	x	x	x	x	x		x	x	x	
MPROS1560	ERR737278	Test isolate	*Staphylococcus aureus*	30	Coll et al.	Cluster 3			x		x		x	x	x	
MPROS0947	ERR714803	Test isolate	*Staphylococcus aureus*	30	Coll et al.	Cluster 3	x	x		x (2)	x		x	x	x	
MPROS2402	ERR715316	Test isolate	*Staphylococcus aureus*	30	Coll et al.	Unrelated to Cluster 3	x	x	x		x		x	x	x	
MPROS0541	ERR702114	Test isolate	*Staphylococcus aureus*	5	Coll et al.	Cluster 4	x		x	x	x		x	x	x	
MPROS1125	ERR737419	Test isolate	*Staphylococcus aureus*	5	Coll et al.	Cluster 4	x		x	x	x		x	x	x	
MPROS0046	ERR212783	Test isolate	*Staphylococcus aureus*	5	Coll et al.	Cluster 4			x	x	x		x	x	x	
MPROS0238	ERR204190	Test isolate	*Staphylococcus aureus*	5	Coll et al.	Cluster 4			x							
MPROS2412	ERR715326	Test isolate	*Staphylococcus aureus*	5	Coll et al.	Cluster 4	x		x							
MPROS0158	ERR211966	Test isolate	*Staphylococcus aureus*	5	Coll et al.	Unrelated to Cluster 4	x		x (2)							
MPROS0688	ERR701921	Test isolate	*Staphylococcus aureus*	22	Coll et al.	Cluster 5				x	x		x	x	x	
MPROS2335	ERR736981	Test isolate	*Staphylococcus aureus*	22	Coll et al.	Cluster 5				x	x		x	x	x	
MPROS0659	ERR701905	Test isolate	*Staphylococcus aureus*	22	Coll et al.	Cluster 6				x	x		x	x	x	
MPROS2044	ERR702173	Test isolate	*Staphylococcus aureus*	22	Coll et al.	Cluster 6				x	x		x	x	x	
MPROS1689	ERR737479	Test isolate	*Staphylococcus aureus*	22	Coll et al.	Not applicable	x	x	x	x						
H050960412	HE681097	Test isolate	*Staphylococcus aureus*	22	Reference strain	Not applicable	x	x	x	x	x	x	x	x	x	
NCTC 6571	ERR1100774	Test isolate	*Staphylococcus aureus*	Not available	Reference strain	Not applicable	x	x								
NCTC 12241	ERR718772	Control isolate	*Escherichia coli*	Not applicable	Reference strain	Not applicable			x (2)	x	x	x	x	x	x	x
NCTC 10418	ERS523599	Control isolate	*Escherichia coli*	Not applicable	Reference strain	Not applicable	x	x								

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Question	Recipient	Contaminant	Evaluation of impact	Interpretation
Determine the effect of contaminating the no template control with increasing concentrations of MRSA DNA	No template control	Spiked with MRSA MPROS1839 DNA at a final concentration of 0, 0.01 or 0.1%	- Number of fragments matching *S. aureus* were 6, 6 & 20, respectively - Coverage of mapping reference 36.8-46.7% - Average depth 0x - Did not pass QC metric for MRSA	No template control can tolerate up to 1% contamination with MRSA DNA
	No template control	Spiked with MRSA MPROS1839 DNA at final concentration of 1%	- Number of fragments matching *S. aureus* were 96,671 - Coverage of mapping reference 99.3% - Average depth 7.5x - Did not pass QC metric for MRSA	
	No template control	Spiked with MRSA MPROS1839 DNA at final concentration of 10% or 20%	- Number of fragments matching *S. aureus* were 363,031 and 623,855, respectively. - Coverage of mapping reference 99.3% (at both concentrations) - Average depth 28.3x and 48.6x, respectively - Both passed QC metrics for MRSA based on depth/coverage, species identification, assignment to ST22 and detection of *mecA*	
Determine the effect of contaminating the MRSA control with increasing concentrations of *E. coli* DNA	MRSA control	Spiked with serial *E. coli* NCTC12241 DNA at final concentration of 0, 0.01, 0.1, 1, 10 or 20%	- MRSA control passed QC metrics at all spikes except 20%, when the proportion of *S. aureus* genome covered fell from 84.6-91.6% (0-10% contamination) to 77.8% (20% contamination) - Proportion of fragments matching *E. coli* was 0.44, 4.02 and 8.19 at 1%, 10% & 20%, respectively	Positive control can tolerate up to 10% contamination with *E. coli* DNA

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