Comparison of long read vs short read sequencing data for rapid sample-to-genotype MRSA in clinical diagnostics research

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Aim

To compare long read Oxford Nanopore Technology sequencing with short read sequence data from methicillin-resistant *Staphylococcus aureus* (MRSA) to shorten the sequencing time with culture-based ONT sequencing in clinical diagnostics research.

Methods

Subcultured MRSA isolates (n=18) with different genetic characteristics were handled and analyzed according to fig 1.

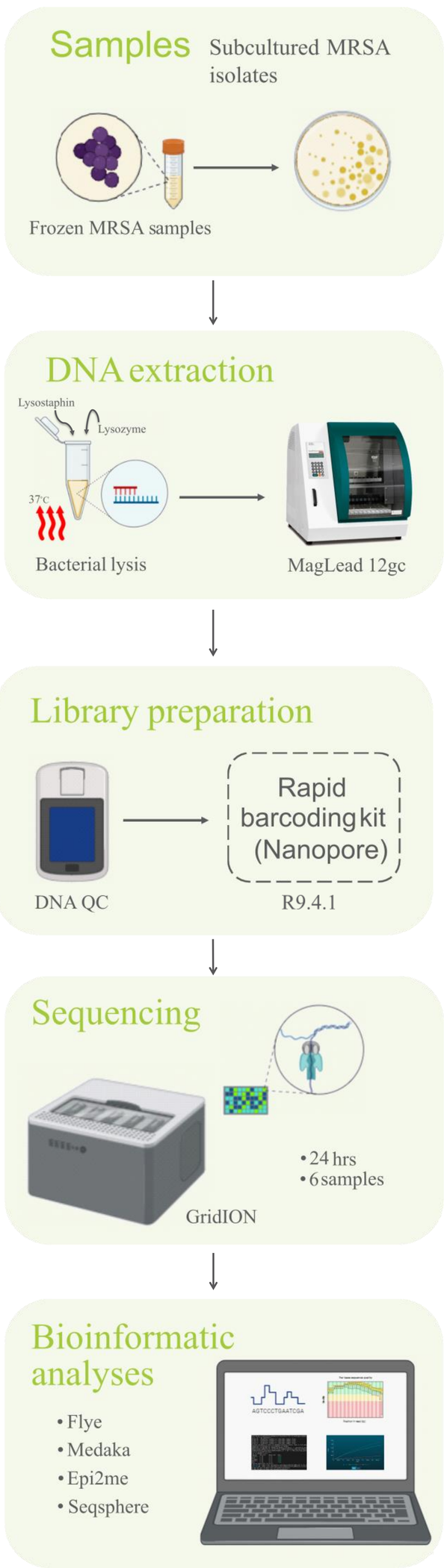

Fig 1. Entire method flow from cultivation to genetic analysis.

Background

Under the pandemic caused by SARS-CoV-2, long read sequencing with Oxford Nanopore Technology (ONT) has been widely used in clinical research laboratories. ONT sequencing is less expensive compared to short read sequencing, but the technology has been associated with higher error rates and concerns regarding homopolymeric regions. The development of ONT sequencing is however, advancing rapidly and the error rate has been improving over time.

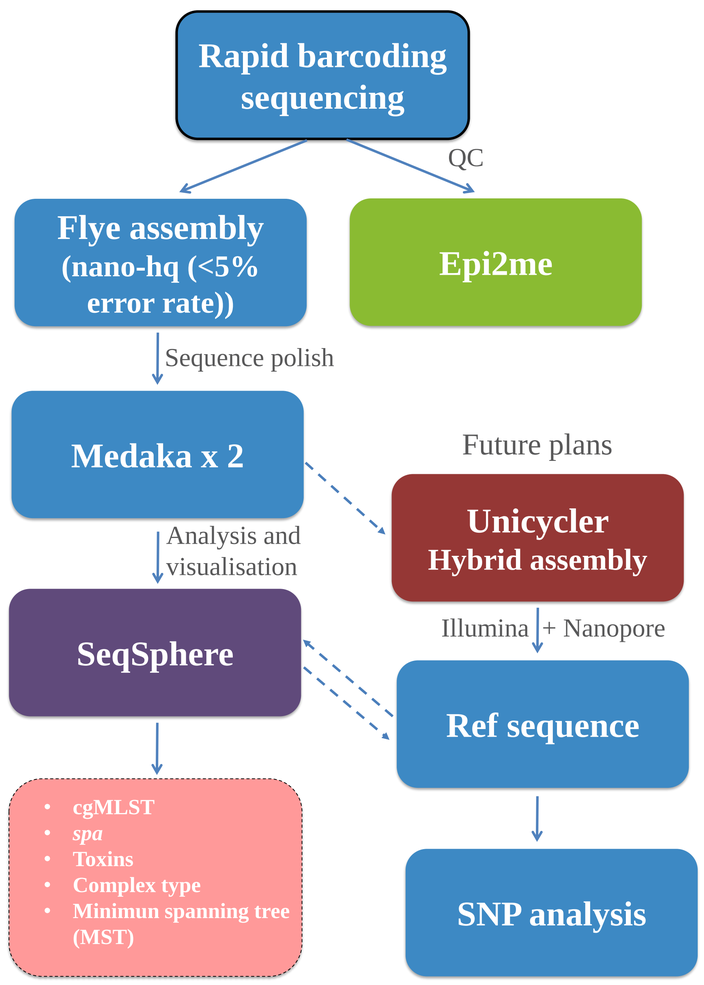

Fig 2. Bioinformatic analysis flow chart. The majority of the pipelines are run in command line and are publicy available for downloading.

Results

All 18 isolates were successfully sequenced with ONT (Table 1). The majority of the isolates contained 1-2 contigs. ONT sequencing displayed the same results in core genome MLST analysis (cgMLST), complex type, *spa*, and toxins (PVL, TSST-1, ETA/B) when compared to results obtained with Illumina. The sequencing was not affected by homopolymeric regions. In addition, the MST displayed core allele differences in 1861 core genes between Illumina and ONT in 14 isolates, with one difference seen in 9 isolates, 2 in 3 and 3 differences in 2 isolates (Fig 3), however the differences did not result in any shift in complex type. In four of the isolates, the MST results for ONT and Illumina were identical (Fig 3, C).

Table 1. Quality parameters obtained with ONT sequencing

Parameters	Mean	Range
Sequence length	3.272	1.955-5.070
Quality score	13.2	12.92-13.59
Coverage	123	58-295
Total reads	112.604	37.568-258.613

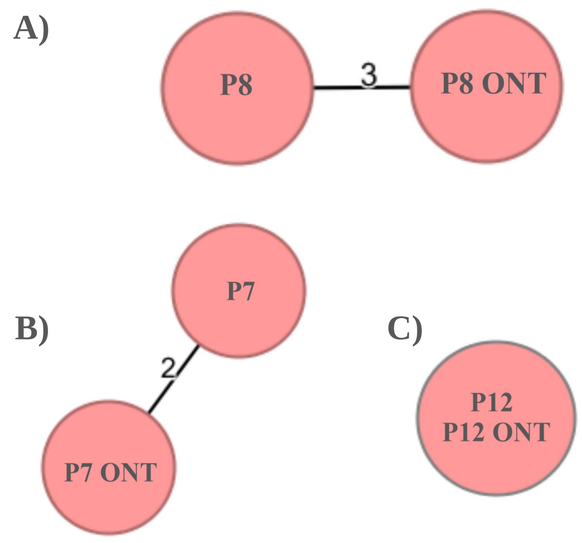

Fig 3. Minimum spanning tree for three isolates P8, P12 and P7. A) three core allele differences between Illumina (P8) and Nanopore (P8 ONT). B) MST of two core allele differences between the methods and C) shows a core alllele difference of null meaning that the sequences were identical.

Conclusion

According to our results, ONT meet the requirements for typing of MRSA in clinical research laboratories and shorten the analysis time from 5 to 3 days (sample to final results). ONT sequencing shows the capability for rapid sample-to-genotype of MRSA to facilitate surveillance and reveal outbreak situations.

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