USE OF THE OXFORD NANOPORE MinION SEQUENCER FOR RAPID MLST GENOTYPING OF *STREPTOCOCCUS PNEUMONIAE*

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INTRODUCTION

Molecular epidemiological surveillance of *Streptococcus pneumoniae* (*S. pneumoniae*) is essential to characterize the serotypes and clones causing invasive pneumococcal disease, an important health problem. Multilocus sequence typing (MLST) consist of characterizing *S. pneumoniae* clones by means of DNA sequencing of 7 highly conserved housekeeping genes (*aroE*, *gdh*, *gki*, *recP*, *spi*, *xpt*, and *ddl)* and their allelic variations.

Conventional MLST methodology, based on Sanger DNA sequencing, has been the preferred method for clonal characterization of *S. pneumoniae* but it is cost and time consuming. MinION, a third-generation sequencer, is being used as a rapid tool for easy strains identification and molecular characterization by using whole genome sequencing (WGS) based process.

OBJECTIVE

To evaluate a rapid and simple MLST analysis of *S.pneumoniae* by using the portable and low cost MinION sequencer.

METHODS

17 clinical isolates: 10 *S. pneumoniae*, 2 Methicillin-resistant *Staphylococcus aureus* (MRSA) 2 *Staphylococcus aureus* (*S. aureus*), *Pseudomonas aeruginosa* (*P. aeruginosa*), *Candida albicans* (*C. albicans*) and *Saccharomyces cerevisiae* (*S. cerevisiae*) strains were tested by MinION to evaluate specie-level identification capacity. Clonal analyses of the ten pneumococcal strains were performed by WGS using MinION and the results were compared by Sanger MLST. Zymobiomics DNA microprep kit and Nanopore Rapid Barcoding kit was used for DNA extraction and WGS, respectively. Fasta sequences were de novo assembled using CANU software. MLST was determined with the online tool www.genomicepidemiology.org. Pneumococcal sequence types (ST) were classified into clonal complexes (CC), whose members must have 5 or 6 of 7 loci that match.

RESULTS

MinION correctly identified all strains at specie level. The concordance of pneumococcal double locus variant (DLV) CC was obtained in all strains: DLV (5 of 7 alleles): 100%, single locus variant (SLV; 6 of 7 alleles) : 80%. ST (7 of 7 alleles) : 0%.

FIGURE 1. MinION and Sanger pipeline time comparison

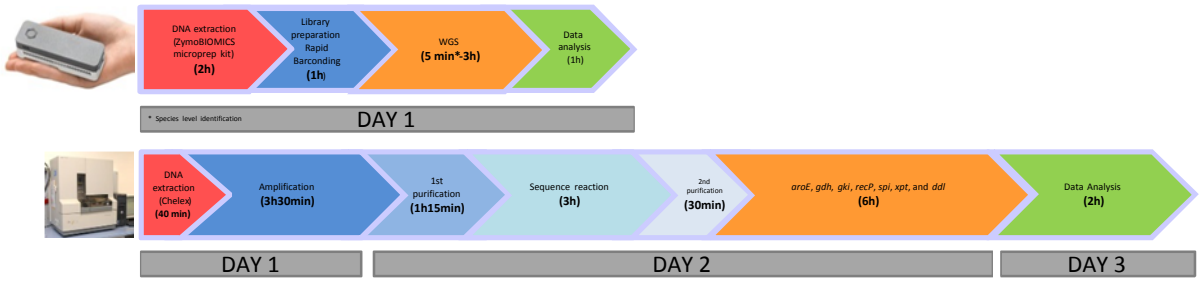

TABLE 1. 17 clinical isolates were correctly identified by MinION

Pathogen	Correct identification
S. pneumoniae	10/10
MRSA	2/2
S. Aureus	2/2
P. Aeruginosa	1/1
C. albicans	1/1
S. cerevisiae	1/1

TABLE 2. Allelic MLST variations and concordances about the 7 housekeeping genes between both methods Sanger and MinION

aroE		*gdh*		*gki*		*recP*		*spi*		*xpt*		*ddl*		ST	
Sanger	MinION	Sanger	MinION	Sanger	MinION	Sanger	MinION	Sanger	MinION	Sanger	MinION	Sanger	MinION	Sanger	MinION
12	12	8	8	13	13	5	5	16	16	4	**338**	20	20	306	SLV-306
7	**421**	11	11	10	10	1	1	6	6	8	8	1	1	156	SLV-156
11	11	19	19	2	2	17	17	6	**100**	22	**605**	223	223	2307	DLV-2307
7	**282**	11	11	10	10	1	1	6	6	8	8	47	47	2944	SLV-2944
7	**282**	11	11	10	10	1	1	6	6	8	8	90	90	838	SLV-838
4	4	4	4	2	2	4	4	4	4	1	**605**	1	1	81	SLV-81
7	**282**	2	2	1	1	1	1	10	10	1	**605**	21	21	113	DLV-113
7	**282**	5	5	10	10	18	18	6	6	8	8	1	1	143	SLV-143
4	4	16	16	19	19	15	15	6	6	20	**605**	1	1	320	SLV-320
5	5	5	**171**	7	7	7	7	8	8	5	5	4	4	87	SLV-87

CONCLUSION

WGS reveals few mismatched bases. MinION proved to be a rapid and sensitive tool for easy identification and molecular characterization of CC of *S. pneumoniae*.

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