Long-read 16S rRNA sequencing for rapid bacterial identification in infective endocarditis

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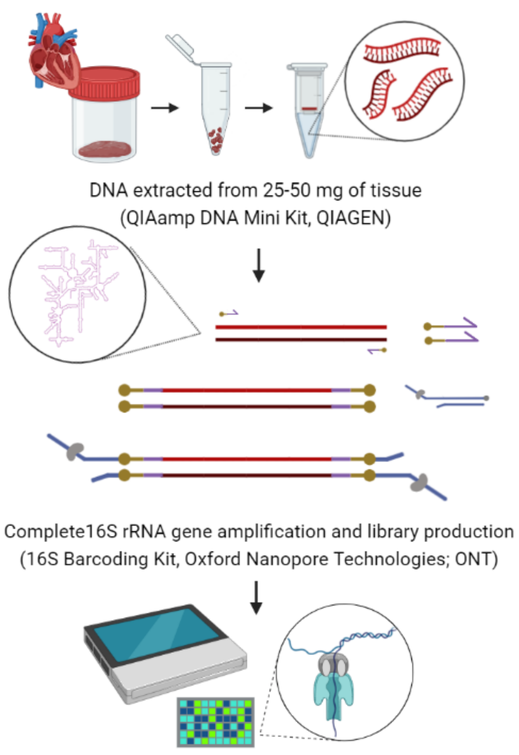
Background

- Identification of the causative agent is crucial for accurate antimicrobial therapy administration in infective endocarditis (IE).
- Although positive culture remains the gold standard for microbiological identification, its sensitivity is limited after antibiotic administration. Thus, molecular characterization is useful to complement pathogen identification.
- Long-read sequencing allows the analysis of the full-length 16S rRNA gene, in order to accurately identify bacteria at the species level. In this sense, nanopore-based sequencing is fast, flexible and easy-to-use.

We aimed to validate long-read sequencing for rapid identification of bacterial species causing IE.

Methods

- Frozen heart biopsies from IE cases were retrospectively (May 2020-December 2022) and prospectively (December 2022-January 2023) selected.
- Gold standard: conventional culture (blood and/or tissue) followed by isolate identification by MALDI biotyper (Bruker Daltonics).
- Experimental procedure for long-read 16S rRNA sequencing:



- Bacterial sequencing reads were classified using the EPI2ME 16S workflow (ONT), and the following criteria were used to validate an identification: ≥10 reads/assignation and ≥3% of total reads. The EMU pipeline (Curry KD. *et al*., Nature Methods 2022) was used to settle on a decision when multiple species of the same genus were identified by EPI2ME.
- Concordance between 16S rRNA sequencing and culture identification was evaluated at the species level.

Results

- The turnaround time for bacterial identification by conventional culture was 3-9 days, while the sequencing approach required 1-3 days.
- In retrospective samples (*n*=14), a 100% concordance in bacterial identification was observed between conventional culture and 16S rRNA sequencing (Table 1).

Table 1. Concordance between 16S rRNA sequencing and culture identification methods.

Sample classification	Sample ID	Blood culture	Heart biopsy culture	Identification by sequencing in heart tissue (% quality reads identified)
Positive blood and tissue cultures (n=8)	1	*Enterococcus faecalis*	*Enterococcus faecalis*	*Enterococcus faecalis* (99%)
	2	*Streptococcus equi*	*Streptococcus equi*	*Streptococcus equi* (99.7%)
	3	*Streptococcus bovis* *	*Enterococcus faecalis*	*Streptococcus lutetiensis* *(80.0%) / *Enterococcus faecalis* (4.8%)
	4	*Streptococcus gallolyticus*	*Streptococcus gallolyticus*	*Streptococcus gallolyticus* (79.0%)
	5	*Staphylococcus aureus*	*Staphylococcus aureus*	*Staphylococcus aureus* (94.2%)
	6	*Streptococcus anginosus*	*Streptococcus gallolyticus*	*Streptococcus anginosus* Group (91.6%)
	7	*Streptococcus equi*	*Streptococcus equi*	*Streptococcus equi* (98.1%)
	8	*Staphylococcus epidermidis*	*Staphylococcus epidermidis*	*Staphylococcus epidermidis* (91.9%)
Negative blood and positive tissue cultures (n=2)	9	Negative	*Streptococcus gallolyticus*	*Streptococcus gallolyticu* s (99.5%)
	10	Negative	*Staphylococcus epidermidis*	*Staphylococcus epidermidis* (94.4%)
Positive blood and negative tissue cultures (n=4)	11	*Streptococcus mutans*	Negative	*Streptococcus mutans* (94.5%)
	12	*Streptococcus gallolyticus*	Negative	*Streptococcus gallolyticus* (95.8%)
	13	*Streptococcus viridans*	Negative	*Streptococcus sanguinis* ** (92.6%)
	14	*Streptococcus viridans*	Negative	*Streptococcus salivarius* (89.7%)

**Streptococcus bovis* Group; **Viridans group streptococci.

- Two heart biopsies were prospectively included with negative tissue and blood cultures; *Mycoplasma hominis* (84.5%) and *Streptococcus pneumoniae* (78.7%) were detected by sequencing, respectively.

Conclusions

Full-length 16S rRNA sequencing with the ONT methodology is a sensitive method for bacterial identification in heart biopsies with a rapid turnaround time, critical on IE management.

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