Rapid identification of bacterial and fungal pathogens and resistance determinants directly from positive blood culture bottles using long-read sequencing

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INTRODUCTION

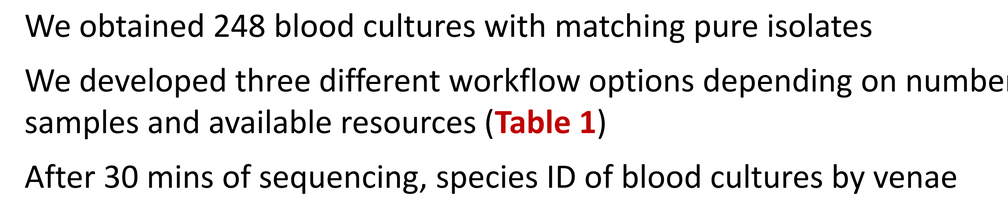
- The gold standard for diagnostics of bloodstream infections (BSI) are blood cultures followed by phenotypic methods for organism ID and antimicrobial susceptibility testing (AST), which can take multiple days[1]
- Early pathogen detection and appropriate antimicrobial therapy for BSIs reduce mortality, morbidity, cost of treatment, length of hospital stay, and development of antimicrobial resistance (AMR) [2]
- **We developed a laboratory and bioinformatic workflow to rapidly and accurately determine bacterial and fungal species identity and predict AMR phenotypes from positive blood cultures**

METHODS

- DNA from blood culture bottles was extracted within several hours of flagging positive using the QIAGEN QIAamp BiOstic Bacteremia DNA kit
- DNA was sequenced using Oxford Nanopore Technologies (SQK-RBK114-24, R10.4.1, Dorado v5.0.0 SUP basecalling)
- Corresponding pure cultures isolated using the gold standard method were characterized using MALDI-TOF for species ID, Sensititre for AST, and Illumina sequencing (NextSeq2000, Nextera XT DNA)
- Analysis was completed with the **venae** pipeline (**Figure 1**) which uses sylph[3] and kraken2[4] for species ID, and StarAMR[5] and KmerResistance[6] for AMR detection (www.github.com/phac-nml/venae)

RESULTS

- We obtained 248 blood cultures with matching pure isolates
- We developed three different workflow options depending on number of samples and available resources (**Table 1**)
- After 30 mins of sequencing, species ID of blood cultures by venae matched 97.6 % (n=242/248) of pure isolates. Mismatches are likely due to technical errors.
- *Staphylococcus, Escherichia, Streptococcus,* and *Klebsiella* were the most common organisms detected (**Figure 2**), and blood culture sequencing produced more specific IDs and more polymicrobial IDs than MALDI-TOF
- Overall categorical agreement between clinically-relevant AMR phenotypes (based on CLSI Table 1 Tiers 1 & 2 antimicrobials) predicted in blood cultures and lab AST of pure isolates was 86.3 % (assembly-based) and 82.8 % (read-based) by 5 h (**Figure 3**)
- For assembly-based AMR prediction, results varied by organism but longer sequencing time resulted in more clinically-relevant genes being detected (**Figure 4**)
- We generated a clinician-friendly **HTML results report:**



CONCLUSIONS

- **This proof-of-concept project shows it is possible to reduce the turnaround time for BSI diagnostics from days to hours**
- Species ID and prediction of AMR phenotypes from sequencing blood cultures accurately matches the corresponding pure isolates

Table 1: Workflow overview and time for each step

		Fast workflow 1 sample	Standard workflow 2+ samples	Higher computational resource workflow 2+ samples
	Features	Organism ID, preliminary AMR	Organism ID, confident AMR	Organism ID, confident AMR
Hands-on time	DNA extraction	1.5 h	1.5 h	1.5 h
	Sample cleanup (dependent on sample quality)	0.5 h	0.5 h	0.5 h
	Quantification	0.2 h	0.2 h	0.2 h
	Library prep and loading	0.25 h (RAD114 on Flongle)	2 h (RBK114 on MinION)	2 h (RBK114 on MinION)
	Total prep time	**2.5 h**	**4.2 h**	**4.2 h**
	Sequencing time	2 h	5 h	4 h (adaptive)
	Total time	**4.5 h**	**9.2 h**	**8.2 h**

Figure 1: Outline of steps in the **venae** analysis pipeline

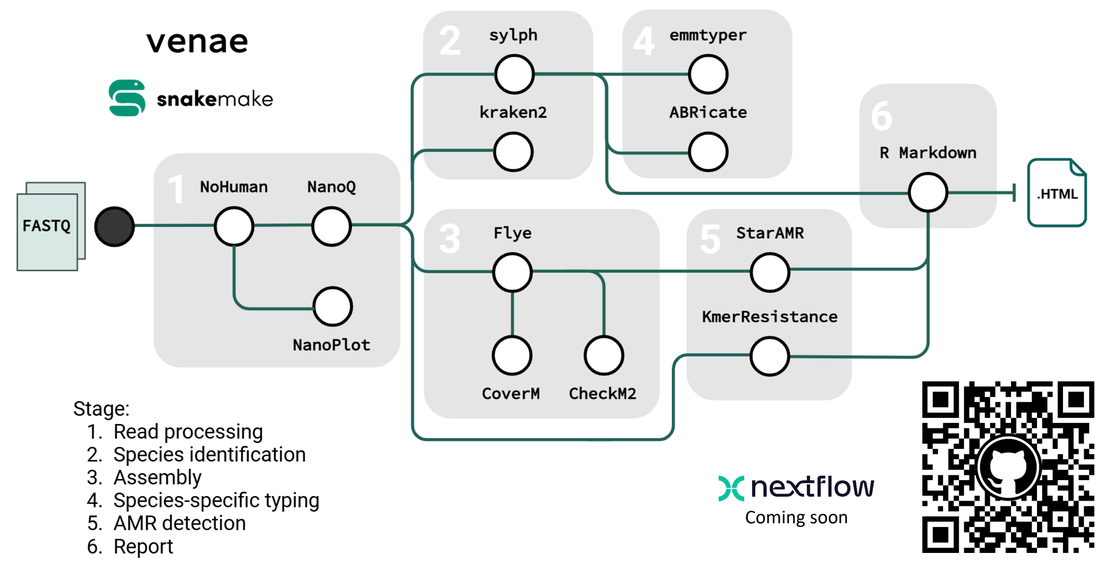


Figure 2: Top organisms identified in blood cultures

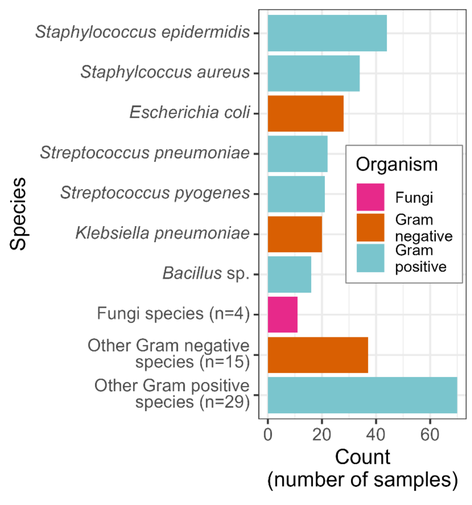


Figure 3: Performance of sequencing-based AMR prediction compared to AST of pure cultures

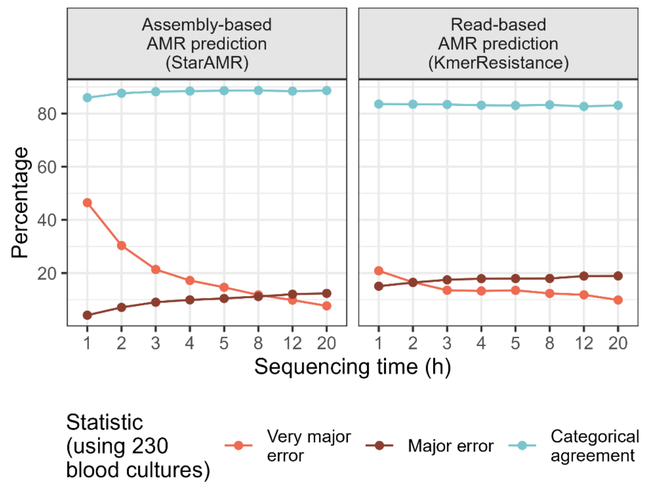
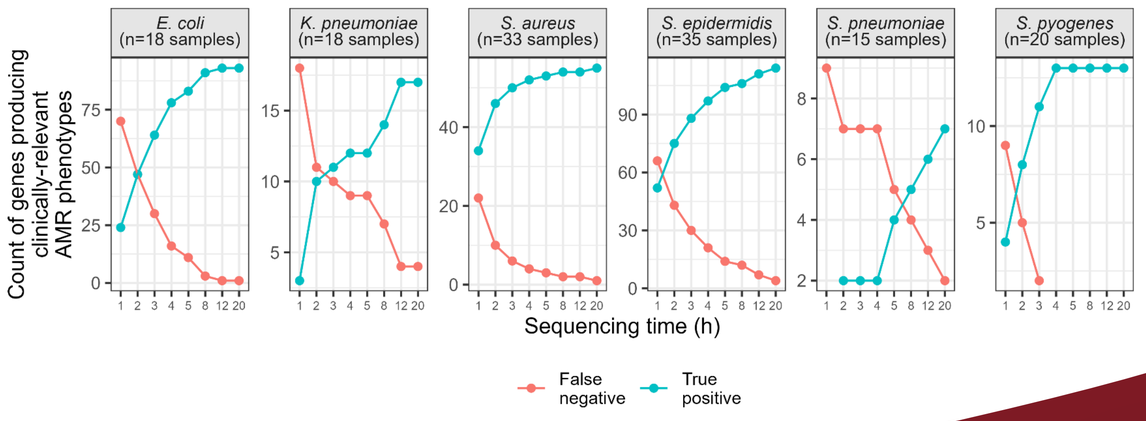


Figure 4: Time to detection of clinically-relevant AMR genes in blood culture sequencing data (assembly-based) for top organisms



References: [1]DOI:10.1038/s41579-024-01105-2; [2]DOI:10.1056/NEJMoa1703058; [3]DOI:10.1038/s41587-024-02412-y; [4]DOI:10.1186/s13059-019-1891-0; [5]DOI:10.3390/microorganisms10020292; [6]DOI:10.1128/jcm.02981-13

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