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Real time application of whole genome sequencing for outbreak investigation – What is an achievable turnaround time?

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ABSTRACT

Whole genome sequencing (WGS) is increasingly employed in clinical settings, though few assessments of turnaround times (TAT) have been performed in real-time. In this study, WGS was used to investigate an unfolding outbreak of vancomycin resistant *Enterococcus faecium* (VRE) among 3 patients in the ICU of a tertiary care hospital. Including overnight culturing, a TAT of just 48.5 h for a comprehensive report was achievable using an Illumina Miseq benchtop sequencer. WGS revealed that isolates from patient 2 and 3 differed from that of patient 1 by a single nucleotide polymorphism (SNP), indicating nosocomial transmission. However, the unparalleled resolution provided by WGS suggested that nosocomial transmission involved two separate events from patient 1 to patient 2 and 3, and not a linear transmission suspected by the time line. Rapid TAT's are achievable using WGS in the clinical setting and can provide an unprecedented level of resolution for outbreak investigations.

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1. Introduction

Over the past decade, the advent of next generation sequencing technology (NGS) has revolutionized the field of whole genome sequencing (WGS) and molecular biology (Mardis, 2011). It has been incorporated into diverse disciplines (Ankala and Hegde, 2014; van Dijk et al., 2014), and lower costs combined with higher throughput and accuracy has greatly expanded its accessibility to many laboratories (Goldberg et al., 2015). WGS is particularly suited to clinical microbiology, where relatively small genomes allow multiple isolates to be sequenced simultaneously, and with high coverage (Didelot et al., 2012; Pak and Kasarskis, 2015). Indeed, it has been speculated that WGS could be "the most significant advance in diagnostic microbiology and surveillance since the advent of *in vitro* culture" (Koser et al., 2012a).

Despite the obvious advantages of WGS for clinical microbiology, adoption has been slow (Goldberg et al., 2015). This can be attributed to outstanding challenges in both the "wet" (i.e. sample preparation, library construction, and sequencing) and "dry" (i.e. bioinformatic support) aspects of NGS (Aziz et al., 2015), including the lack of standards and reference materials (Gargis et al., 2012), the need for greater automation (Gargis et al., 2012), and inadequate bioinformatics infrastructure (Fricke and Rasko, 2014).

While some applications, such as single cell microbiology and clinical metagenomics, are currently confined to translational research (Pallen et al., 2010), WGS is increasingly being employed for outbreak investigation and bacterial genomic epidemiology, species identification, and to a lesser extent, culture independent microbiology and susceptibility testing (Koser et al., 2012a). In particular, WGS has been instrumental in tracking outbreaks and unravelling the epidemiology of carbapenem-resistant *Klebsiella pneumoniae* (Lopez-Camacho et al., 2014; Snitkin et al., 2012), methicillin-resistant *Staphylococcus aureus* (MRSA) (Eyre et al., 2012; Harris et al., 2010, 2013; Harrison et al., 2013; Koser et al., 2012b), *Clostridium difficile* (Eyre et al., 2012; He et al., 2013), enterohemorrhagic *Escherichia coli* (Rasko et al., 2011; Underwood et al., 2013), and *Chlamydia trachomatis* (Harris et al., 2012). These studies have been invaluable in expanding our knowledge of bacterial epidemiology, and provide striking examples of the transformative potential of WGS.

Current typing methods lack comprehensive resolution and can be time-consuming and expensive (Fournier et al., 2007). WGS has the potential to revolutionize this paradigm by providing the ultimate level of resolution. A critical component of these investigations is

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turnaround time (TAT), and justifiable concerns remain on whether WGS can meet this challenge. Previous studies have shown that short TAT (48–96 h) can be achieved using WGS (Eyre et al., 2012; Harris et al., 2013; Koser et al., 2012b), but the majority of these TAT's have been artificial and not conducted in real-time. In this study, we demonstrate that WGS can readily provide accurate and comprehensive feedback on potential outbreaks in just 2 days, allowing real-time feedback to the requesting facility and resulting in an appropriate and comprehensive response by Infection Control (IC) departments.

2. Methods

2.1. Bacterial isolates

Three vancomycin-resistant *Enterococcus faecium* (VRE) with identical antibiotic susceptibilities were isolated from 3 patients housed at the ICU of a 206-bed hospital offering patient care to 800,000 active duty, family members, and retiree population of all four armed services (Table 1). Three additional VRE cultured from patients at the same facility but from an earlier time period were also included in the analysis (Table 1). Identification and antibiotic susceptibilities were performed at a CAP-accredited clinical microbiology laboratory on a Vitek 2 automated susceptibility platform (Biomeriuex) using the Gram Positive (GP) identification panel and the GP AST71 panels, respectively.

2.2. Outbreak investigation pipeline and Whole genome sequencing

Isolates were shipped overnight on TSA slants, and upon arrival were cultured overnight at 37 °C on blood agar plates (BAP). DNA was extracted using the Ultra-Clean Microbial DNA Isolation kit (MoBio Inc. Carlsbad, CA, USA) and libraries were constructed using the KAPA Hyperplus Library preparation kit (KAPA Biosciences, Wilmington, MA, USA). Libraries were quantified using the KAPA Library Quantification Kit – Illumina/Bio-Rad iCycler (KAPA Biosciences) on a CFX96 real-time cycler (Biorad, Hercules, CA, USA). Libraries were normalized to 2 nM, pooled, denatured, and diluted to 20 pM. The pooled samples were further diluted to a final concentration of 13 pM. Samples were sequenced using MiSeq Reagent Kit v3 (150 cycle; 2 × 75 bp) (Illumina, San Diego, CA, USA).

In addition, after the outbreak investigation was completed new libraries from fresh overnight cultures were prepared and sequenced using the MiSeq Reagent Kit v2 (300 cycle; 2 × 150 bp) kits. These kits take an additional 3 h to complete a sequencing run, but yield longer reads.

2.3. Analysis of WGS data

Sequencing reads were quality and adapter trimmed and then *de novo* assembled using Newbler (V2.7). The draft assembly of a single outbreak strain was used as the reference for read mapping and SNP detection of the remaining strains. Reads from the reference strain were also mapped, and SNP detection performed to detect any false SNPs that could arise from errors in the draft assembly. Finally, the data was reanalyzed using each strain as the reference to ensure SNPs were valid. A combination of PanSeq (Laing et al., 2010) and Gegenees (Agren et al., 2012) was also used to ensure sequence accuracy and to detect any large scale rearrangements and/or deletions.

Sequencing at 2 × 75 bp resulted in 100% coverage of contigs greater than 200 bp in length with a minimum coverage of 36× (Average 78.6×, Standard deviation 12.7). Sequencing at 2 × 150 bp resulted in 100% coverage of contigs greater than 200 bp, with a minimum coverage of 42×. (Average 88×, Standard deviation 13.9). Both kits gave average read coverage that exceeded the threshold for obtaining the maximum N50 (Junemann et al., 2013).

Comparative genomic analyses were performed using Geneious (Biomatters, Auckland, New Zealand) (Kearse et al., 2012). Antimicrobial resistance genes were annotated using ResFinder 2.0 (Zankari et al., 2012). Bowtie V2.2.4 (http://bowtie-bio.sourceforge.net/bowtie2/, last accessed March 2016) was used for read mapping. Samtools/BCFtools V1.2.1 (http://www.htslib.org/download/, last accessed March 2016), and bedtools V2.23.0 (http://bedtools.readthedocs.org/en/stable/, last accessed March 2016) were used for SNP calling and detection of regions without read coverage

2.4. Nucleotide sequences

The Whole Genome Shotgun (WGS) sequence of MRSN 33033 has been deposited at DDBJ/EMBL/Genbank WGS database with accession number LPWB00000000. The SRA files for MRSN 33032, 33033, and 33034 have been deposited to the NCBI Bioprojects database (http://www.ncbi.nlm.nih.gov/bioproject/306525) with accession numbers SRR3306347, SRR3306348, and SRR3306349, respectively.

3. Results

3.1. Description of outbreak

A timeline of the outbreak, spanning 65 days, can be found in Fig. 1. Patient 1 was a 71 year-old (y.o.) male with multiple medical comorbidities, including gastrointestinal bleeding and ischemic colitis. A

Table 1
Characteristics of VRE isolates used in this study.

Barcode[1]	Date[2]	Source[3]	Location[4]	MLST[5]	MIC's (μg/ml) and susceptibility call of[6]							
					CIP	ERY	GEN	LZD	PEN	TET	TGC	VAN
33033	1	Blood	ICU	78	≥8 R	≥8 R	SYN-S	2 S	≥64 R	≥16 R	≤0.12 S	≥32 R
33034	12	Blood	ICU	78	≥8 R	≥8 R	SYN-S	2 S	≥64 R	≥16 R	≤0.12 S	≥32 R
33032	28	Pleural fluid	ICU	78	≥8 R	≥8 R	SYN-S	2 S	≥64 R	≥16 R	≤0.12 S	≥32 R
14241	11/2012	Peritoneal fluid	ICU	78	≥8 R	≥8 R	SYN-S	2 S	≥64 R	≥16 R	≤0.12 S	≥32 R
14246	01/2013	Blood	ICU	192	≥8 R	≥8 R	SYN-S	2 S	≥64 R	≥16 R	≤0.12 S	≥32 R
14513	01/2013	Catheter	Ward	584	≥8 R	≥8 R	SYN-S	2 S	≥64 R	≤1 S	≤0.12 S	≥32 R

Abbreviations used: MLST, Multi-locus sequence type; MIC, Minimum inhibitory concentration; CIP, Ciprofloxacin; ERY, Erythromycin, GEN, Gentamicin; LZD, Linezolid; PEN, Penicillin; TET, tetracycline; TGC, Tigecycline; VAN, vancomycin; SYN, Synergy.

[1] Strain number, de-identified designation used to track isolates throughout the MRSN. Outbreak strains are highlighted in bold font.

[2] Outbreak strains: Expressed in days after the first isolate was recovered from Patient 1; All outbreak strains were cultured in 2015. Non-outbreak strains: The month and year when isolates were cultured.

[3] Clinical site where the isolate was recovered.

[4] Hospital location of the patient when isolate was recovered.

[5] Based on *in silico* analysis of the whole genome sequence.

[6] Antibiotic susceptibilities were determined using the Vitek 2 automated susceptibility platform with Gram Positive (GP) AST71 panels. Susceptibility call is based on CLSI guidelines.

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