Bacteriology

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

Patrick McGann a,⁎, Jessica L. Bunin b, Erik Snesrud a, Seema Singh c, Rosslyn Maybank a, Ana C. Ong a, Yoon I. Kwak a, Scott Seronello c, Robert J. Clifford a, Mary Hinkle a, Stephen Yamada d, Jason Barnhill c, Emil Lesho a

a Multidrug-resistant Organism Repository and Surveillance Network, Walter Reed Army Institute of Research, Silver Spring, MD, USA
b Department of Critical Care, Tripler Army Medical Center, Honolulu, HI, USA
c Department of Pathology, Tripler Army Medical Center, Honolulu, HI, USA
d Infectious Diseases Service, Tripler Army Medical Center, Honolulu, HI, USA

ARTICLE INFO

Article history:
Received 16 February 2016
Received in revised form 30 March 2016
Accepted 27 April 2016
Available online 30 April 2016

Keywords:
Whole genome sequencing
Enterococcus faecium
Turnaround time

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.

Published by Elsevier Inc. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).

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
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 (Biomerieux) 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 Kycler (KAPA Biosciences) on a CP96 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 with a minimum standard deviation of 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 Bioproject 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>
<thead>
<tr>
<th>Table 1</th>
<th>Characteristics of VRE isolates used in this study.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Barcode</td>
<td>Date</td>
</tr>
<tr>
<td>33033</td>
<td>1</td>
</tr>
<tr>
<td>33034</td>
<td>12</td>
</tr>
<tr>
<td>33032</td>
<td>28</td>
</tr>
<tr>
<td>14241</td>
<td>11/2012</td>
</tr>
<tr>
<td>14246</td>
<td>01/2013</td>
</tr>
<tr>
<td>14513</td>
<td>01/2013</td>
</tr>
</tbody>
</table>

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.
peritoneal fluid culture on Day 31 grew Enterobacter cloacae complex, E. coli, Klebsiella oxytoca and VRE. A blood culture was positive for VRE on Day 36, and on Day 37 the patient died due to septic shock with multi-organ dysfunction. (See Fig. 2.)

Patient #2 was a 69 y.o. female with toe gangrene. A transmetatarsal amputation culture grew Corynebacterium species, Pantoea species, E. faecalis, E. casseliflavus, Peptostreptococcus sp., and an unidentified non-spore forming Gram-positive rod. The patient underwent further amputation and developed transaminitis, coagulopathy, leukocytosis, and a persistently elevated serum lactate. An exploratory laparotomy revealed duodenal perforation and blood cultures grew VRE. The patient died two days later from septic shock with multi-organ failure due to a perforated duodenal ulcer.

Patient 3 was a 57 y.o. male with a history of chronic heart failure, atrial fibrillation/flutter, and chronic kidney disease. He was admitted for management of septic shock with multi-organ failure from metastatic MRSA of unknown etiology. VRE was cultured from the patient’s pleural fluid on Day 63, and the patient died the following day of cardio-pulmonary arrest associated with MRSA bacteremia.

3.2. Evaluation of turnaround time (TAT)

A breakdown of the total TAT, including overnight culturing using the 2 × 75 bp and 2 × 150 bp kits is presented in Table 2. Though the instrument run time was 3 h shorter with the 2 × 75 bp kits, this was offset by the 16-fold increase in the time required to assemble and quality check (QC) the sequences when compared to the 2 × 150 bp kit (8 h versus 0.5 h). This was attributable to the smaller read lengths generated by the 2 × 75 bp kit, which complicated the assembly and subsequent analysis (5.5 h versus 4.5 h; Table 2). Overall, the 2 × 150 bp kit was able to provide a faster TAT than the 2 × 75 bp kit, despite the increased run time. In addition to the longer assembly analysis time, the final genome assembly of MRSN 33033 consisted of 534 contigs and 154 scaffolds when using the 2 × 75 bp kits, and 323 contigs and 147 scaffolds with the 2 × 150 bp kits. This greater fragmentation of the final genomes with the 2 × 75 bp kit was also observed in all other sequenced genomes (data not shown). As all of the reagents used for sequencing remain the same, the only additional cost incurred is the price difference between the 2 × 75 bp and 2 × 150 bp kits. Assuming 24 samples per run, this is just $8.90 per genome at current prices (March 2016).

3.3. Outbreak analysis

MRSN 33033 was cultured from the blood of patient #1, and was used as the reference sequence for generating a single nucleotide polymorphism (SNP) phylogenetic tree (Fig. 1). All isolates belonged to ST-78, and WGS revealed that MRSN 33032 and MRSN 33034 were genetically identical to MRSN 33033 with the exception of a single, unique, non-synonymous mutation in both isolates. MRSN 33032 had a Met92Ile substitution in a putative transcriptional regulator, while MRSN 33034 had an Ile190Leu substitution in a putative multidrug ABC transporter. Hence, nosocomial transmission between the patients was the most probable cause of infection. Notably, rather than a transmission from patient #1 to #2 to #3, as might be suspected from the timeline, the distinct SNPs in the patient #2 and #3 isolates instead suggested transmission from patient #1 (MRSN 33033) to patient #2 and #3 in two separate events. As no patient was housed in the same room concurrently, it was suspected that transmission was due to environmental contamination. However, subsequent swabbing of the ICU and attendant equipment failed to isolate VRE from any source. Thorough cleaning of the implicated ICU room and sterilization of all equipment used was performed and as of this writing (4 months), no further outbreaks of VRE have occurred.

Table 2
Sequencing run metrics.

<table>
<thead>
<tr>
<th>Procedure</th>
<th>150 bp kit</th>
<th>300 bp kit</th>
</tr>
</thead>
<tbody>
<tr>
<td>Culture</td>
<td>12</td>
<td>12</td>
</tr>
<tr>
<td>DNA isolation</td>
<td>0.5</td>
<td>0.5</td>
</tr>
<tr>
<td>Library preparation</td>
<td>7</td>
<td>7</td>
</tr>
<tr>
<td>Run time</td>
<td>21</td>
<td>24</td>
</tr>
<tr>
<td>Assembly and QC</td>
<td>8</td>
<td>8</td>
</tr>
<tr>
<td>Analysis and Report generation</td>
<td>5.5</td>
<td>4.5</td>
</tr>
<tr>
<td><strong>Total time</strong></td>
<td><strong>54</strong></td>
<td><strong>48.5</strong></td>
</tr>
</tbody>
</table>
3.4. Correlation between phenotypic antibiotic susceptibilities and gene content

Antibiotic susceptibility data indicated resistance to a broad range of antibiotics (Table 1). As part of the MRSN analysis pipeline, the assembled genome is automatically searched for known antibiotic resistance genes using an updated database of resistance genes rooted in ResFinder (Zankari et al., 2012). The outbreak strains carried genes known to encode resistance to the aminoglycosides, macrolides, tetracyclines and vancomycin (Table 3), in accordance with *in vitro* susceptibilities (Table 1). Though no genes encoding resistance to β-lactams and fluoroquinolones were detected, *in vitro* susceptibility data indicated that the isolates had high level resistance to these antibiotics. Both β-lactam and fluoroquinolone resistance in enterococci have been linked to mutations in genes encoding the targets of these drugs, specifically *pbp5* with β-lactams (Rice et al., 2004) and *gyrA* and *parC* with fluoroquinolones (Werner et al., 2010). Analysis of our VRE showed that the S83Y *gyrA* and S80R *parC* mutations described by Werner et al. (2010) and the 466+S, M485A, I499T, and E629V mutations in *pap* described by Rice et al. were present (Rice et al., 2004). Finally, the outbreak strain carries *aac(6′)-aph(2′)*, which encodes a bi-functional aminoglycoside (Rouch et al., 1987) that confers resistance to gentamicin, kanamycin and tobramycin (Ramirez and Tolmasky, 2010). Antibiotic susceptibility profiles indicated that all isolates were resistant to gentamicin, but remained sensitive to gentamicin combination therapy (Table 1).

3.5. Comparison with non-outbreak associated VRE

Three additional VRE with similar antibiotic susceptibility profiles (Table 1) and isolated from the same facility during the preceding 2 years were included in the analysis. Two of the 3 had been cultured from patients housed in the same ICU where the outbreak took place. *In silico* Multi-locus Sequence type (MLST) revealed that all isolates belong to ST’s within *E. faecium* Clonal Complex 17 (CC-17), a hospital-adapted clade with a global distribution (Willems et al., 2005). One isolate (MRSN 14241) was assigned to ST-78, the same ST as the outbreak strains, but grouped closer to MRSN 14246, which belongs to ST-192, a single-locus variant of ST-78 (Fig. 1). SNP-based analysis of the core genome revealed that a large 249 kb recombination event occurred in both isolates, and was responsible for the majority of the SNP differences (556 SNPs) between these and the outbreak strains. This accumulation of SNPs in a limited genetic region can be considered “pseudo-SNPs” for investigating relatedness, as they do not reflect gradual accumulation of SNPs by random mutation and can skew subsequent evolutionary analysis. A second recombination event occurred in MRSN 14246, which added several hundred additional pseudo-SNPs and hence a longer branch length on the phylogenetic tree (Fig. 1). When these pseudo-SNPs were removed from the analysis, MRSN 14241 and MRSN 14246 differed from MRSN 33033 by just 11 and 15 SNP’s respectively. In contrast, MRSN 14513 had hundreds of SNP differences randomly distributed across the core genome. The similarity in antibiotic resistance profiles among isolates could be explained by the antibiotic resistance gene content; the three non-outbreak strains had an almost identical resistance gene content as the outbreak strains but MRSN 14240 and MRSN 14246 lacked the bi-functional aminoglycoside-modifying enzyme AAC(6′)-APH(2′). In contrast, MRSN 14513 carried *aac(6′)-aph(2′)* but it lacked *tet(M)* and *tet(U)* which correlated with the sensitivity of this strain to tetracycline *in vitro* (Table 1).

4. Discussion

A suspected outbreak of VRE in the ICU of a large healthcare facility provided the opportunity to determine a realistic TAT for generating clinically relevant data in real-time using WGS. Including overnight culture, a comprehensive report based on core genome phylogeny was readily achievable in just over 48 h, providing a level of resolution that cannot be provided by current typing methods such as PFGE and MLST. The value of WGS went beyond simply determining relatedness, with SNP-based analysis generating additional epidemiological data that gave greater insights into the potential route of transmission as well as providing valuable data on antibiotic susceptibilities.

The 48-hour TAT reported herein included every aspect of the process, from overnight culture to report generation (Table 2). This was achieved by utilizing the most recent advances in DNA extraction and library construction procedures, which generated pooled libraries ready for sequencing in less than 8 h. The two most time-consuming aspects of the procedure were overnight culturing and instrument run time. Though genome assembly and sequence QC times were excessively high with the shorter read kits (Table 2). These times could be shortened further. For example, fast growing organisms could be cultured for just 4–6 h, which would provide sufficient material for DNA extraction and library construction. Alternatively, cultured bacteria could be submitted via overnight courier, which would allow DNA extraction to be performed immediately upon arrival. Furthermore, as DNA sequencing technologies continue to advance, the time to perform a sequencing run is expected to fall significantly.

These advances are remarkable when one considers that just eight years ago a genomic analysis of *Francisella tularensis* using pyrosequencing took over 6 weeks to complete, with DNA extraction alone taking 2 days (La Scola et al., 2008). In the ensuing years, TAT has steadily decreased, with Eyre et al. performing WGS and analysis on *Staphylococcus aureus* and *Clostridium difficile* in just 5 days (Eyre et al., 2012). More recently, a TAT of just 48 h from culture to sequencing was demonstrated during an outbreak of MRSA, but as sample preparation and DNA sequencing were performed off-site (Illumina), it is unclear if this was achieved in real-time (Harris et al., 2013; Koser et al., 2012b).

Unlike other typing methods, NGS can be used to identify potential antibiotic resistance genes (*Diene and Rolain*, 2013; *Dunne et al.*, 2012), as well as point mutations responsible for increased resistance (*Chin et al.*, 2013). As this technology evolves, the implementation of NGS to determine the antibiotic resistance phenotype in near-real time in a culture-independent manner is rapidly approaching (*Diene and Rolain*, 2013; *Dunne et al.*, 2012). Recent studies have shown the utility of this approach (*Gordon et al.*, 2014; *Sertizawa et al.*, 2010; *Stoesser et al.*, 2013), and have highlighted the barriers still remaining to effectively translate this technology to the clinical laboratory (*Koser et al.*, 2014). In this study, an automated pipeline for detecting antibiotic resistance genes based on the Resfinder database (Zankari et al., 2012), identified eight genes encoding resistance to multiple antibiotic classes (Table 3). These correlated with *in vitro* susceptibility data, but were unable to account for the high-level resistance to penicillin and the fluoroquinolones (Table 1). Further analysis revealed point mutations in the targets of these drugs that have previously been shown to confer high-level resistance to these antibiotics (Rice et al., 2004; Werner et al., 2010). This highlights one of the remaining challenges for effectively

---

**Table 3**

Antibiotic resistance genes carried by VRE outbreak strains.

<table>
<thead>
<tr>
<th>Gene</th>
<th>Target</th>
<th>Accession</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>aac(6′)-aph(2′)</em></td>
<td>Aminoglycosides – gentamicin, kanamycin and tobramycin</td>
<td>M13771</td>
</tr>
<tr>
<td><em>ant(6)-Ia</em></td>
<td>Aminoglycosides – streptomycin</td>
<td>AF330699</td>
</tr>
<tr>
<td><em>aph(3′)-III</em></td>
<td>Aminoglycosides – kanamycin and streptomycin</td>
<td>M26832</td>
</tr>
<tr>
<td><em>erm(B)</em></td>
<td>Macrolides</td>
<td>X66468</td>
</tr>
<tr>
<td><em>msr(C)</em></td>
<td>Macrolides</td>
<td>AY084350</td>
</tr>
<tr>
<td><em>tet(M)</em></td>
<td>Tetracyclines</td>
<td>FN433986</td>
</tr>
<tr>
<td><em>tet(U)</em></td>
<td>Tetracyclines</td>
<td>U01917</td>
</tr>
<tr>
<td><em>vanA</em></td>
<td>Vancomycin</td>
<td>M97297</td>
</tr>
</tbody>
</table>

1. Based on closest match to NCBI database. All strains carry vanH, S, X, Y, and Z in addition to vanA.
2. Predicted antibiotic resistance phenotype.
3. Corresponding NCBI accession number of the gene sequence.
implementing culture-independent susceptibility testing, namely the creation and curation of databases describing point mutations that increase antibiotic resistance (Gordon et al., 2014; Stoesser et al., 2013). Recent studies have shown high concordance between genotype and phenotype, with very major error and major error rates comparable to that of culture-based techniques (Gordon et al., 2014; Koser et al., 2012b; Stoesser et al., 2013), but validation on larger datasets is essential (Stoesser et al., 2013).

A unique aspect of whole genome sequencing is the ability to infer transmission dynamics. In this study, initial epidemiological evidence, based on the timeframe of the outbreak, suggested a linear transmission from patient #1 to patient #2, and then from patient #2 to patient #3. However, this paradigm was challenged by the SNP-based analysis; MRSN 33032 and 33034 from patient #2 and patient #3 differed from that of patient #1 (MRSN 33033) by just a single SNP, but this SNP was different in both isolates. To support the initial assessment of transmission, this would have required the SNP from MRSN 33032 to have reverted to the original nucleotide in MRSN 33034, which then acquired was different in both isolates. To support the initial assessment of transmission dynamics, in this study, initial epidemiological evidence, based on the timeframe of the outbreak, suggested a linear transmission. In this study, 3 additional VRE from the same medical facility, but collected years earlier were also sequenced (Table 1). Two isolates Acinetobacter baumannii, though potential hypermutators can skew the analysis, as reported for MRSA and Acinetobacter baumannii (Komp Lindgren et al., 2015; Koser et al., 2012b). In this study, 3 additional VRE from the same medical facility, but collected years earlier were also sequenced (Table 1). Two isolates were closely related to the outbreak strain but recombination events had introduced hundreds of pseudo-SNPs. When these pseudo-SNPs were removed, the two isolates differed from the outbreak strains by just 11 and 15 SNPs, respectively. Though no SNP threshold has been established for VRE, these numbers are consistent with the mutation rate in other species (Didelot et al., 2012). This close relationship with the outbreak strains could be due to a single clone circulating within the hospital, but the lack of consistent outbreaks over the last three years does not support this hypothesis. As the isolates belong to the hospital-adapted and globally distributed clonal complex 17 (Willems et al., 2005), another possibility is that this clone is endemic within the community and may reflect the success of this clone through convergent evolution (Woodford et al., 2011). Continued surveillance of VRE from this location is currently ongoing, with the aim of continually refining and updating the epidemiology of isolates within the facility.

5. Conclusions
We provide the first assessment of the utility of WGS to provide data in a clinically relevant timescale. The wealth of data provided by WGS exceeds any other typing method currently available, including optical mapping (Grad et al., 2012), particularly when trying to elucidate the mechanism of transmission. In this report, culturing, sequencing, analysis, and report generation were achieved in just 48 h, demonstrating that an actionable TAT is not confined to large sequencing centers (Harris et al., 2013; Koser et al., 2012b) but can be accomplished by small-scale laboratories.

Acknowledgements
This study was funded by the U.S. Army Medical Command, the Global Emerging Infections Surveillance and Response System, and the Defence Medical Research and Development Program. Material has been reviewed by the Walter Reed Army Institute of Research. There is no objection to its presentation. The opinions or assertions contained herein are the private views of the authors and are not to be construed as official, or reflecting the views of the Department of the Army or the Department of Defense.

References


