A Comparison of Whole Genome Sequencing and Pulsed-Field Gel Electrophoresis for Bacterial Outbreak Surveillance

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Abstract

The tracking of antibiotic resistant organisms is integral to controlling their spread and the safety of the patient population. This study investigates the comparison between pulsed-field gel electrophoresis (PFGE) and whole genome sequencing (WGS) for use in outbreak surveillance of carbapenem-resistant Enterobacterales (CRE). The possible advantages of newer WGS methods were considered in contrast to PFGE's previous gold-standard for bacterial typing. This study was conducted as a literature review that consulted articles within the last decade. The clinical impact of CRE was a point of interest for narrowing the scope of the situation and providing suitable context. Articles discussing limitations or complications to either methodology were then the focus of research for use in comparison. It was found that PFGE had many limitations imposed on it by both the technology and methodology used. PFGE works well for local outbreak typing that does not consider possible uncertain banding patterns from non-related samples. In contrast, the newer WGS methods do not demonstrate this limitation due to their ability to resolve isolates down to the single nucleotide. WGS allows for both the typing of isolates in outbreak surveillance and the ability to produce libraries of genomic data for use in further studies. Future research into making WGS technology more commercially available has the potential to revolutionize the clinical laboratory through improvements to both patient safety and treatment.

Introduction

Antimicrobial resistance is the adaptation of a microorganism to grow in the presence of antibiotics that previously were effective. Resistance has become an important target of research in recent years due to its detrimental impact on aspects such as healthcare costs, patient risks associated with procedures, lengths of hospital stays, and morbidity/mortality rates (Founou et al., 2017). It is estimated that by 2050 resistant organisms will be responsible for upwards of 300 million deaths and drain up to $100 trillion from the world's gross domestic product (Thaden et al., 2017). A significant concern are the gram-negative CREs, as they are resistant to our strongest carbapenem-class antibiotics.

The most common mechanism of resistance for CREs is the production of carbapenemases that hydrolyze the carbapenems (Mariappan et al., 2017). The carbapenemases of the biggest concern are those of Class A Klebsiella pneumoniae carbapenemase (KPC) enzymes; Class B metallo-beta-lactamases (MBL) such as New Delhi metallo-β-lactamase (NDM), Verona integron-encoded metallo-β-lactamase (VIM), and Imipenemase (IMP); and Class D oxacillinase (OXA)-48 and its variants (Mariappan et al., 2017). These gram-negative organisms are seen in a wide variety of infection locations including respiratory, urinary, bloodstream, and wound sites. In a healthcare setting, CREs can easily spread between patients through contact with contaminated surfaces, patient care equipment, and healthcare personnel ("About CRE/KPC," n.d.). The monitoring of CRE prevalence is essential in outbreak control and ensuring patient safety within the hospital setting.

There are two common methods for outbreak surveillance of CRE organisms: PFGE and WGS. For both methods, similarities between isolate samples indicate a close relation between strains and may suggest a common route of infection. Our primary concern is the safety of our patients, so the identification of a potential infection source is a must to prevent the persistence of an outbreak.

In PFGE, large DNA molecules from digested bacterial genomes are separated on an agarose gel matrix under an electric field that periodically changes direction to assist in movement. PFGE provides a representation of a bacterial isolate with a highly reproducible restriction profile using clearly distinct and well-resolved DNA fragments (Sharma-Kuinkel et al., 2016). Each DNA "fingerprint" produced by their specific fragment pattern allows for comparison between clinical isolates that may lead to identification of a common strain of infection.

In WGS, the entire genetic code of an organism is mapped out to produce a unique pattern. Organisms of the same species will have a common set of genes called the core genome. By mapping the entire genome of clinical isolates, multiple strains of organism can be analyzed for similarity within these core regions. There is a multitude of options for comparison, with the most notable being the single nucleotide polymorphism (SNP) approach (Schürch et al., 2018). In SNP analysis, sequence reads are typically mapped against a reference genome and the number of nucleotide differences is counted.

Methods

Several strategies were employed to ensure high-quality review of the current literature. The literature search focused primarily on electronically sourced material published in the last decade. Key search terms included whole genome sequencing, pulsed-field gel electrophoresis, outbreak surveillance, CRE/KPC genes, PFGE/WGS limitations, and clinical impacts of CRE/KPC organisms. Various primary databases were searched including the CDC database, PubMed, and EBSCOhost. The Journal of Clinical Microbiology and Microbial Genomics are independent journals that were also explored. The reference sections of these resources were searched for additional articles. Presentations from the Minnesota Department of Health were also referenced from personal communications. The search uncovered 32 peer-reviewed sources from 2012 to 2021, which were consolidated after consideration of repeated information.

Results

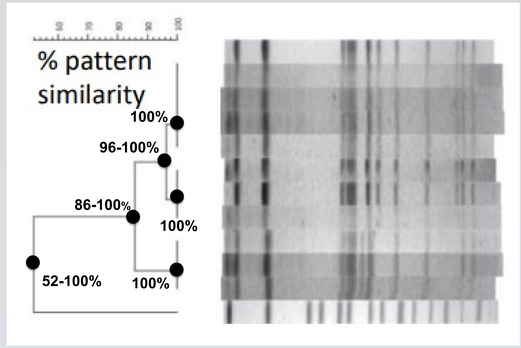

Figure 1.* PFGE with phylogenic tree comparison of bacterial isolates. The agarose gel contains the restriction profiles for digested genomes of isolates. The phylogenic tree on the left has a scale that shows an isolate's similarity from its counterparts. The nodes at the beginning of a split indicate the minimum to maximum similarity of all isolates stemming from that node.

-	1	2	0	1	3	8	47447
1	-	1	2	3	5	11	47551
2	1	-	3	4	6	11	46429
0	2	3	-	1	3	8	47496
1	3	4	1	-	4	9	47482
3	5	6	3	4	-	11	47550
8	11	11	8	9	11	-	47594
47447	47551	46429	47496	47482	47550	47594	-

Figure 2.* SNP analysis comparison of bacterial isolates following WGS. The table compares the number of SNP differences between bacterial isolates. Isolates that are a part of the same outbreak strain will be distinguished via their low count. The number of SNPs that determine relatedness or outbreak isolates is set by the researcher(s) following analysis of the population. It is typical to include a divergent isolate from the possible outbreak population to help gauge relatedness.

*S. Namugenyi (personal communication, September 7, 2022) *Next Generation Sequencing Overview* [PowerPoint slides]

Discussion

The capability of microbes to transfer resistance genes horizontally makes it crucial to control the spread of hospital-acquired infections. Both discussed methods have seen use in recent years due to increases in antibiotic resistance from clinical isolates. PFGE has long since been considered the gold standard for bacterial typing using restriction patterns as seen in Figure 1. However, WGS methods surpass this by being able to resolve differences down to the single nucleotide as seen in Figure 2.

Besides the improvement to the quality of analysis using WGS, PFGE on its own suffers from limitations imposed by its methodology. PFGE is associated with non-reproducible results, bias use of specific enzymes and/or band interpretations, and long turn-around times (Chen et al., 2019). PFGE can also suffer from uncertain clustering without an obvious epidemiological link and requires isolates to be in a well-defined spatiotemporal context such as a local outbreak (Martak et al., 2020). WGS does not suffer in these regards and its analysis to the single nucleotide level of a genome allows for large scale comparison of isolate typing. The sheer portability of the data allows for the formation of genomic libraries and investigation of large populations between multiple regions.

Despite WGS' perceived benefits, its widespread implementation in clinical and public health microbiology laboratories is limited by the need for effective semi-automated workflows, standardized quality control/data interpretation, and bioinformatics expertise (Kwong et al., 2015). Besides the more technical aspects of the limitations, one of the easily perceived roadblocks will likely be the infrastructure cost for many smaller and medium sized laboratories. For those labs that can use this technology, they will see a superior ability to confirm isolates and have access to repositories of genomic data that can be shared worldwide.

Further research into the cost and suitability of WGS methods in the clinical laboratory would further our dream of ensuring patient safety. In conclusion, although PFGE has long since been the gold-standard for bacterial isolate typing, newer WGS methods will logically become the standard for reference and clinical laboratories in outbreak surveillance.

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