Impact of whole genome sequencing in Public Health reference laboratories

Amy V Jennison

Public Health Microbiology,
Forensic and Scientific Services
Queensland Department of Health
Coopers Plains
Brisbane, Qld 4108, Australia
Tel: +61 7 3096 2826
Email:
Amy.Jennison@health.qld.gov.au

Public Health Microbiology reference laboratories fulfil a critical role in providing overarching testing and surveillance for notifiable, emerging and important pathogens. These duties require the laboratory to possess an extensive repertoire of validated assays and the ability to rapidly respond to novel threats and outbreaks. For these, among other reasons, the 'one stop shop' approach of whole genome sequencing (WGS) has been embraced by microbiology reference laboratories. The ability to replace multiple labour-intensive assays with a single technique of superior typeability and discrimination at an often competitive price, although not without its challenges, has already begun to change the workflow of Public Health reference laboratories.

Overseas laboratories such as Public Health England and the US Centers for Disease Control and Prevention (CDC) are exemplars of how WGS has replaced not only conventional molecular typing for the routine surveillance of pathogens but also phenotypic testing such as serotyping[1–3]. The US Food and Drug Administration (FDA) sequencing initiative, GenomeTrakr consists of a network of US and international laboratories performing real time sequencing on foodborne pathogens from food, environmental and human samples, with sequences being made available on the public NCBI database. This rapid and detailed genomic surveillance has already been highly successful in detecting more outbreaks than prior genotyping whilst restricting the number of cases associated with each outbreak (Figure 1)[3]. Participation in GenomeTrakr resulted in an Australian listeriosis case with no known cause being linked to a US stone fruit outbreak, demonstrating the importance of integrated international surveillance to monitor what is now a global food chain[4].

National routine surveillance is beginning to take shape across the Australian State reference laboratories, driven by the Communicable Diseases Genomics Network, of which the reference laboratories belong to. All Australian listeriosis cases are subjected to timely sequencing and national comparison at the Microbiological Diagnostic Unit Public Health Laboratory (MDU PHL) based at The University of Melbourne ensuring outbreaks and disease clusters are detected across state borders[5]. Recently, the nationally

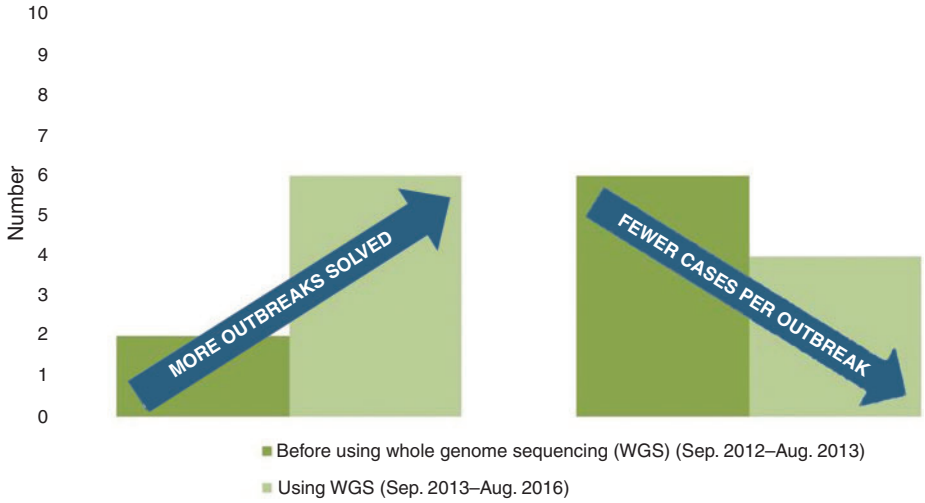

Figure 1. The impact of routine use of WGS for surveillance of *Listeria* cases in the US – improved detection of outbreaks with a reduction in the number of cases per cluster. Figure reproduced with permission from Centers for Disease Control and Prevention, https://www.cdc.gov/listeria/surveillance/whole-genome-sequencing.html.

coordinated surveillance of all invasive *Neisseria meningitidis* infections by genomic sequencing, through a number of state reference laboratories, has commenced. The high-resolution cluster analysis possible from the data has already proven invaluable in understanding the rapid changes to Australian meningococcal disease epidemiology that has occurred over the past two years[6].

Public Health Laboratories have also taken the opportunity to replace multiple complex and specialised phenotypic and genotypic tests with the more streamlined process of genomic sequencing. Like other Australian Public Health reference laboratories, the Queensland Public Health Microbiology (PHM) laboratory has moved away from the technical Elek test to testing *C. diphtheriae* and *C. ulcerans* by PCR for the diphtheria toxin gene. However, international studies have suggested between 5–10% of PCR positive strains are actually carrying non-functional toxin genes, referred to as non-toxigenic toxin gene bearing (NTTB) isolates. Genetic mutations conferring non-toxigenic status cannot be determined from the screening PCR. By applying WGS, PHM has been able to infer functionality in diphtheria toxin gene positive strains, to not only report the first NTTB isolates from cases tested in Australia but to also characterise the genetic mutations associated with these isolates[7]. This sequencing is now routinely performed with appropriate timeliness to contribute to the public health response to toxin gene positive cases.

The use of whole genome sequencing to predict antibiotic resistance is still in its infancy. Overall, correlation between the extrapolated genotype and phenotype is promising and the epidemiological information generated on genetic mechanisms is valuable[8,9]. Reports suggest that inferring antibiotic resistance from genotype results in an overestimation of resistance, possibly due to the presence of silent genes or poorly understood mechanisms[9,10]. Certainly, there is no doubt that phenotypic testing is still incredibly important and that a greater body of work is necessary and rapid turn-around time established before WGS inferred antimicrobial resistance will be suitable for standalone use in clinical decision-making.

The application of genomics in public health microbiology laboratories is probably best known for its convenience and performance in resolving disease outbreaks and clusters. The ability to respond rapidly, regardless of the organism's identity, with a technique that offers superior discrimination to conventional typing methods has begun to transform and simplify reference laboratories testing regimes. Public Health Microbiology laboratories are beginning to move away from multi-locus variable number tandem repeat analysis (MLVA), multi-locus sequence typing (MLST) and binary typing based schemes for cluster analysis, instead applying core genome (cgMLST) or whole genome (wgMLST) and single nucleotide polymorphism (SNP)-based typing. The scientific literature contains numerous examples of the retrospective application of WGS to examine outbreaks, which demonstrate the improved discrimination that wgMLST or SNP-based typing offers to either delineating linked cases from sporadic infections or identifying related cases unsuspected through epidemiological data[11–13]. An increasing number of reports in the literature are also showing that WGS can be utilised to generate real time data able to inform earlier and with increased confidence on ongoing outbreaks[3,14]. A recent spate of *Burkholderia cenocepacia* bacteraemia in Australian patients was identified as a point source cluster caused by contaminated ultrasound gel used in central line insertion by whole genome sequencing. The cluster analysis prompted a real time actionable response even as cases were still being identified, including a TGA recall of the product and an international publication calling for healthcare facilities to perform retrospective patient investigations[15].

It is also evident that the application of genomics to investigating the transmission of antimicrobial resistant organisms through clinical centres and tracking inter-institutional spread is critical for surveillance and control of resistance. Institutional genomics surveillance programs have demonstrated that WGS based phenotypic tracking can be economically and clinically feasible[16,17]. Furthermore, WGS has been able to accurately pinpoint not only the ongoing transmission of a resistant strain within the clinical setting but identify the likely point source. An investigation into an MRSA outbreak in a special care baby unit revealed that a baby became infected with the same strain, post deep cleaning and 64 days after the last positive patient. Rapid genomic-based screening revealed a MRSA outbreak strain carrier amongst staff. After relocation and decolonisation therapy of this staff member, no further cases were identified[18].

While whole genome sequencing performed on bacterial isolates is well established, metagenomic or deep sequencing directly on clinical specimens has more recently emerged as a valuable technique in public health microbiology. Deep sequencing is particularly useful for the generation of antibiotic resistance data for slow growing or difficult to culture organisms such as *Mycobacterium tuberculosis*. While PCR based testing is limited in the number of genetic mutations it can target, there is no such limitation for genomic sequencing. A recent paper has reported that they were able to predict antibiotic resistance mechanisms for *Mycobacterium tuberculosis* by direct deep sequencing on respiratory

specimens in less than 48 hours turnaround time, generating results for which standard testing would usually require weeks[19].

For other microorganisms, deep sequencing may fill the gap in epidemiological data caused by the increase in culture independent diagnostic testing (CIDT) in pathology laboratories. Molecular diagnosis has many advantages for patient care, however the absence of isolates subsequently available for public health surveillance is concerning. For certain pathogens, such as *Neisseria gonorrhoeae*, molecular diagnoses can represent up to 80% of disease notifications, meaning resistance and cluster analysis surveillance traditionally only performed on isolates, is fragmented. A recent publication reported success in generating genotyping and antibiotic resistance markers direct from *N. gonorrhoeae* PCR positive clinical specimens, indicating that epidemiological surveillance is feasible in the absence of culture[20].

The use of deep sequencing for direct diagnostics in clinical microbiology is still very much a burgeoning technology, although examples in the literature do showcase the potential[21–23]. While cost and validity of results are still at times difficult to warrant, the clear advantages of hypothesis-free testing with no prior knowledge of the causative agent required or issues around mutational changes in primer regions offers tantalising prospects to the clinical microbiology field[24]. As sequencing costs decrease and long read technologies become more accessible, it is likely that current issues around financial justification, sensitivity, validation and clinical interpretation will be addressed.

Despite the many advantages WGS brings to public health microbiology, the integration into laboratories does not come without a suite of challenges. Traditionally wet lab-based laboratories must become *au fait* with high power computing infrastructure, generate solutions for the handling and storage of big data, acquire bioinformatics skills and establish interpretation and management of complex data. Laboratory staff must not only become familiar with these new analyses and solve reporting and LIMS challenges around the use of constantly changing dendrograms and SNP differences, but must engage in the re-education of clinicians, government bodies and public health officers. Accreditation to ensure robustness of WGS analysis is essential but is still in early phases for most laboratories and with the respective regulatory bodies, acceptance criteria and analysis still far from standardised at a national, let alone international level, and to date only a handful of international QAPs are available. It is essential that accreditation is appropriate and handled by subject matter experts as it is not amenable to simply apply established human clinical genetics requirements to public health microbiology genomics. While standardisation of SNP typing is still complex and requires centralised analysis, wgMLST with the development of online, curated, freely accessible databases may fill the specifications for stable standardised analogous analysis for at least some bacterial species.

The current challenges, however, are far from untenable and it is clear that as the cost continues to decrease, so that replacement of more standard microbiology tests becomes feasible, and standardisation efforts at both the national and international level progress, that WGS will only continue to revolutionise testing strategies employed in public health microbiology.

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Biography

Dr Amy Jennison is the Supervising Scientist of Molecular Epidemiology, Public Health Microbiology, which is the Queensland reference laboratory responsible for the molecular surveillance of notifiable bacterial pathogens and characterisation of public health related outbreaks. She is leading a team in the application of WGS to routine molecular surveillance and heads numerous research projects aimed at utilising WGS for improving molecular epidemiological investigation and addressing culture independent diagnostic testing through deep sequencing approaches.

The role of microbiology in gonococcal control in the West: helping to understand the enemy

David J Speers

Department of Microbiology
PathWest Laboratory Medicine WA
Queen Elizabeth II Medical Centre
Hospital Avenue
Nedlands, WA 6009, Australia
Tel: +61 8 9346 2197
Fax: 08 9346 3960
Email:
david.speers@health.wa.gov.au

Western Australia (WA), Australia's largest state by area, has one of the highest notification rates of gonorrhoea in the world. This is likely a reflection of the challenges of providing health services over a vast remote area combined with a unique set of sociocultural aspects. Despite this, microbiology can play a pivotal role in the public health management of gonorrhoea even if the primary health services are thousands of kilometres away from the laboratory. However, it requires new approaches to how diagnostic testing and laboratory surveillance are conducted and the repurposing of existing technologies to cater for novel demands. In this article I describe some of the microbiological approaches that have been undertaken in WA to help address the public health challenge of gonorrhoea. That is, facilitating the appropriate antimicrobial management of gonorrhoea in an era of increasing resistance to prevent treatment failure, timely provision of an accurate diagnosis to inform appropriate treatment, and providing molecular insights to better understand gonococcal transmission (Table 1).

Mitigating antimicrobial resistance

Neisseria gonorrhoeae has shown a remarkable capacity to become resistant to the antimicrobial agents employed to control it. With an estimated 78 million new cases each year and the emergence of ceftriaxone and azithromycin resistance in many countries around the world[1], including Australia[2,3], *N. gonorrhoeae* has earned its World Health Organization and Centers for Disease Control and Prevention designation as an urgent antimicrobial resistance threat. It is critical to delay the introduction of multi-resistant *N. gonorrhoeae* strains into remote WA, with its high rate of gonorrhoea and more limited access to healthcare. Australia, like most of the world, has transitioned to a combination of ceftriaxone and azithromycin[4,5] for empiric gonorrhoea therapy but the emerging resistance to extended spectrum cephalosporins[6] and the increasing prevalence of low level azithromycin resistance in Australia[3] is a reminder that resistance is an inevitable consequence of widespread use of these agents. The incursion of ceftriaxone-