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Whole genome sequencing: an efficient approach to ensuring food safety

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Abstract. Whole genome sequencing is an effective, powerful tool that can be applied to a wide range of public health and food safety applications. A major difference between WGS and the traditional typing techniques is that WGS allows all genes to be included in the analysis, instead of a well-defined subset of genes or variable intergenic regions. Also, the use of WGS can facilitate the understanding of contamination/colonization routes of foodborne pathogens within the food production environment, and can also afford efficient tracking of pathogens' entry routes and distribution from farm-to-consumer. Tracking foodborne pathogens in the food processing-distribution-retail-consumer continuum is of the utmost importance for facilitation of outbreak investigations and rapid action in controlling/preventing foodborne outbreaks. Therefore, WGS likely will replace most of the numerous workflows used in public health laboratories to characterize foodborne pathogens into one consolidated, efficient workflow.

1. Introduction

Food safety is a global concern, and consumers have the right to safe and nutritious food. However, the estimated burden of foodborne diseases at global level, i.e. 600 million foodborne illnesses and 420,000 deaths from 31 major food safety hazards in 2010 [1] as well as at EU level, with around 400,000 confirmed human illnesses and 463 deaths [2] from 5 major food safety hazards (causing listeriosis, salmonellosis, campylobacteriosis, STEC infections, yersiniosis) and the related social and economic costs (hospitalization, loss of income, employment and market access) remain unacceptably high [3]. Tracking foodborne pathogens in the food processing – distribution – retail – consumer continuum is of utmost importance for facilitation of outbreak investigation and rapid action in controlling/preventing foodborne disease outbreaks. Whole genome sequencing (WGS), using next generation sequencing (NGS) technology, has the ability to sequence entire genomes [4] and provides rapid, comprehensive information and characterization of microorganisms with a level of precision not previously possible. WGS, now internationally recognized as the new revolution in microbiology food safety, is a technology that likely will transform public health microbiology in a few years. Field applications of WGS have garnered success in multiple contexts in recent years: from transmission studies and outbreak tracking of pathogens in hospitals to traceback investigations and source attribution in foodborne outbreaks at both national and multinational levels [5]. Therefore, WGS likely will replace most of the numerous workflows used in public health laboratories to characterize foodborne pathogens into one consolidated workflow [6].

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2. Traditional techniques for distinguishing bacteria

At present, several different techniques are used to identify and characterize bacteria, and to distinguish between different strains of a species. The method of choice varies depending on the species in question and on precisely what information is required. Some of the commonly used methods are:

- Serotyping – different bacterial strains have different sets of molecules coating them; antibodies are used to determine which molecules are present on the outer surface.
- Phage typing – distinguishes between strains based on their susceptibility to infection by various types of bacteriophage (a group of viruses).
- Pulsed Field Gel Electrophoresis (PFGE) – the bacterial genome is chopped up at specific points, to create fragments that are then electrophoretically separated on a gel according to their size. Different strains have different patterns of fragments.
- Multilocus sequence typing (MLST) – sequencing 400-500 base pair fragments of DNA at seven different conserved genes allows small variations within a species to be detected. Quite time consuming and costly, but can be highly discriminatory if the genes are correctly chosen.
- Multilocus variable number tandem repeat analysis (MLVA) – particular regions of DNA are very repetitive, and the number of repeats of a sequence in a particular region varies between different bacterial strains. A few such areas are analysed to determine how many repeats there are in each. MLVA is faster and easier to perform than MLST, but there are issues with reproducibility and validation.

All of the genotyping methods mentioned above have their limitations and ideally the entire genome of a microbial isolate would be sequenced to provide definitive typing. Until quite recently, this would have been very costly and taken years to complete, but the advent of NGS technology, such as the Ion TorrentTM platform from Life Technologies and the Illumina sequencing system, make WGS within days a practical option. Now that high-throughput bench-top NGS instruments like Life Technologies' Ion PGMTM and the Illumina NextSeq 500 are available, WGS is within the reach of smaller clinical microbiology and research laboratories. The costs of NGS are falling, and an entire bacterial genome can now be sequenced for about the same price as MLST typing using conventional PCR/Sanger sequencing. Otherwise, WGS in principle offers one simplified approach, easily reproducible across different laboratories [7]. In addition, WGS is different from current typing methods because it requires – in addition to the actual laboratory work – substantial data processing, storage and analysis to extract useful information from the large amount of generated data. Collaboration and data sharing between organizations and countries is required due to the international dimension of food and waterborne bacterial (FWD) pathogens and food trade in particular. National public health reference laboratories form the first line of such collaboration, as they are usually the first to have sufficient information, i.e. the microbiological typing data, to allow linkage of cases at national level and subsequent detection of human clusters of outbreaks [8].

3. *Listeria monocytogenes* and whole genome sequencing

Listeria monocytogenes is a predominantly foodborne pathogen capable of causing a range of clinical illnesses, including invasive disease such as bacteraemia and meningoencephalitis in humans [9], and is commonly monitored by public health facilities for the emergence of outbreaks [10]. A number of serotypes of *L. monocytogenes* can be isolated from environmental and food sources, but most outbreaks of human disease are due to serotypes 1/2a, 1/2b and 4b [11]. It has been confirmed that listeriosis carries one of the highest hospitalization rates among known foodborne pathogens – up to 91% [12, 13]. Altogether, 1,642 listeriosis cases in humans were reported in the European Union (EU) in 2012 with high fatality rate (17.8%) among the confirmed cases; this was a 10.5% increase of reported listeriosis cases compared with 2011 [14]. *L. monocytogenes* was found in 10.3% of fishery

products, 2.1% of heat-treated meat products and 0.5% of soft and semi-soft cheeses collected from supermarkets and shops across the EU, from January 2010 to January 2012. Fermented sausages contaminated with *L. monocytogenes* have rarely been implicated in critical listeriosis outbreaks [14]. WGS has emerged as a powerful technology for the comparison of isolates in outbreak analysis [15], because the *Listeria* genome is fairly small and relatively easy to sequence and analyse [16]. With WGS, *Listeria* outbreaks can be detected when as few as two people have fallen ill. Determining that the same strain of *Listeria* is making people ill is an indication that these illnesses may have come from the same source – for example, the same contaminated food processing facility. By combining real time WGS with data from patients about the foods they ate and data about *Listeria* in foods, public health officials can: detect more cluster (possible outbreaks) of *Listeria* infections, link cases of *Listeria* to a likely source, identify unrecognized sources of *Listeria* and stop *Listeria* outbreaks while they are still small.

4. Food Safety and WGS

WGS is a significant new tool in the area of food safety, including foodborne disease surveillance, food inspection (testing) and monitoring, outbreak detection and investigation (including food attribution studies) and food technology developments. In comparison to the plethora of molecular identification and characterization technologies available to date, WGS is conceptually speaking, quite simple, regardless of the platform used. WGS is universal for all organisms and provides virtually the entire genome, which facilitates targeted exchange and comparison of its data. The results are not only useful for food monitoring, disease surveillance, and outbreak investigation and response, but also for addressing broader questions that are critical for food safety improvements and preventive measures, through source tracking, source attribution and the identification of transmission pathways [17, 18]. Since the data comprise the genetic code, WGS results can be used for more than one purpose simultaneously – such as identification, subtyping, virulence marker detection, antimicrobial resistance (AMR) predictions, and genome-wide association studies. In food monitoring, WGS is used as forensic evidence for source tracking and to inform regulatory action. Since food is a global commodity, global use of this common technology facilitates sharing and collaboration across sectors, and greatly increases the availability of contextual data when interpreting results and recommending regulatory actions on a scientific basis. It is, however, important to emphasize that WGS cannot stand alone. It is just one source of information among the complex systems that comprise the whole food supply chain. The technology requires that clinical, food and environmental isolates/samples from routine testing, inspection and surveillance, and associated data are made available, and that infrastructure is in place to utilize the data for regulatory food safety and public health action. Thus, the implementation of WGS should be accompanied by the establishment of an integrated national food control system and relevant food safety programmes that assimilate information from different sources [17].

5. Conclusion

WGS is poised to become standard methodology in food safety for identification and characterization of foodborne pathogens, including antimicrobial resistant organisms, with a level of precision not previously possible. WGS provides the most extensive analysis for isolate comparison and is superior to current typing methods for pathogens. Also, WGS as an incredibly powerful technique is less labour-intensive and more cost-effective than current typing methods for surveillance. It is already being utilised by Public Health England (PHE) and the Food Standards Agency (FSA) to aid outbreak investigation; but it has a multitude of other potential applications in relation to food.

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