Shotgun metagenomics as a One Health tool for better protecting human health

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INTRODUCTION

An open approach towards identifying all biological material in a sample without knowing what to look for a priori, could transform the way specific key public health questions are addressed, in a ONE Health context. Metagenomics is defined as the study of all genetic material within a sample, using sequencing technologies. Shotgun metagenomics is capable of detecting a wide range of microorganisms in a sample, as well as their composing genes such as virulence or antimicrobial resistance (AMR) genes, without prior knowledge, or the need to isolate them. Such an approach could offer efficient and fast solutions to limitations faced by conventional methods e.g. in the field of pathogen detection/characterisation along the food chain, starting from the food production environment (including detection of AMR genes) until foodborne disease outbreak investigation and clinical/veterinary diagnostics. This could also allow a better understanding of infectious diseases and more accurate risk (safety) assessment of food safety issues. However, before it can be used in these settings, this approach should be appropriately developed and validated from sampling and DNA extraction to sequencing and data analysis.

METHODOLOGY

Third generation sequencing technologies have had a breakthrough with the launch of nanopore sequencing, which, through the production of long sequencing reads, portability and real-time data generation, represents a disruptive innovation for microbiology. Compared with short read sequencing (Illumina), this technology allows the user to unambiguously detect and scaffold microbial genes to their host chromosomes, even for complex metagenomics samples, allowing taxonomic classification up to an unprecedented sensitivity, including the identification of linked specific genes, such as AMR or virulence genes. We are developing and optimising the protocols (from DNA extraction to data analysis) for sensitive metagenomics, by analysing various materials collected in food-producing environments, and spiked with defined mock communities, composed of different bacterial species in various concentrations and carrying AMR and/or virulence genes, taking the specific need of each application into account. Moreover, the short read and long read

approaches are being compared to each other, and also to the results obtained with conventional methods.

RESULTS

A commercial kit was selected and optimised for the extraction of sufficient high-molecular weight DNA, to match the needs for long-read sequencing. The analysis of animal and environmental samples collected at a production farm, subsequently spiked with bacterial species present at various concentrations and carrying AMR genes, made it possible to identify the spiked species and to make the link with AMR genes detected in the same sample when found on the same reads used for taxonomic identification, except for species present at low concentrations. Comparable results were obtained using short-read sequencing, without being able to link the identified species with the detected AMR genes. Our approach was also successful on artificially contaminated food samples. It allowed the same characterisation of the foodborne pathogen (serotyping, virulence genes, relatedness to other cases) as the conventional methods used in reference laboratories, however without isolation and in a shorter time-frame. This could be achieved to the strain level even in samples with several E. coli strains. This method was also used to solve a foodborne Salmonella outbreak that occurred in Belgium in 2019.

DISCUSSION

By applying the shotgun metagenomics approach to specific case studies, we delivered proof-of-concepts and demonstrated its added value compared with the conventional methods. We proved that it can characterise a bacterial pathogen to the strain level in spiked food as well as in the context of a real outbreak, and resolve the investigation within a faster time frame. EFSA had called for such proof-of-concepts in a recent opinion about the use of metagenomics for food safety. Additionally, we showed that the use of long-read sequencing can help to achieve a higher level of resolution by identifying specific bacteria and the AMR genes present in their genomes, with only one analysis. Future studies will explore, at the technical level, how this new technology can be transferred for fast, easy and direct use on-site, in a food-producing environment. This will open up ample opportunities for future research in different fields, and beyond, where the metagenomics approach would then be a well-established tool to be used to address specific public health questions at a more detailed and extended/comprehensive level, contributing to better protection of human health.