

European Metrology
Programme for Innovation
and Research

Delivering Impact



Combating infections with advanced DNA sequencing

Antimicrobial resistance is a serious threat to patient care. Modern molecular methods such as whole genome sequencing (WGS) have the potential to identify the type of resistance present to help guide treatment plans. However, in order for these methods to be widely implemented more robust approaches are required.

Europe's National Measurement Institutes working together

The European Metrology Programme for Innovation and Research (EMPIR) has been developed as part of Horizon 2020, the EU Framework Programme for Research and Innovation. EMPIR funding is drawn from 28 participating EURAMET member states to support collaborative research between Measurement Institutes, academia and industry both within and outside Europe to address key metrology challenges and ensure that measurement science meets the future.

Challenge

Resistance to antibiotic treatments are responsible for approximately 33,000 deaths a year in Europe and infectious diseases were the leading cause of death among children under five. This includes bacteria that have developed resistance to Carbapenems, considered drugs of 'last resort' for Antimicrobial Resistance (AMR) infections. A 2016 UK Government review has predicted that AMR could contribute to 45% of all global deaths by 2050 with an associated global loss of economic output of US\$100 trillion. This increased financial burden may also limit advances in other medical areas such as routine surgery or cancer treatment.

Whole genome sequencing (WGS), that can identify the presence of pathogen DNA, has become an important tool for AMR outbreak management. With a markedly higher resolution than conventional methods it has the potential of identifying a wide range of potential resistance mutations, indicating which treatments are most suited to combat a particular infection. However, WGS requires a complex stepwise procedure that can generate large amounts of data requiring complex informatics pipelines to identify the presence of resistance mutations. What is not currently supported are mechanisms to ensure the quality of a given test pipeline. To overcome this the error associated with the technique and the bioinformatic approach used is needed.

Solution

The EMPIR project [Novel materials and methods for the detection, traceable monitoring and evaluation of antimicrobial resistance](#) developed the metrology to reliably evaluate new and existing clinical test methods for detecting and monitoring AMR. A WGS comparison study was performed between ten laboratories using sequences from clinical isolates of Carbapenem-resistant Enterobacterales (CRE) specifically chosen for varying quality and complexity. The UK's Great Ormond Street Hospital for Children (GOSH) analysed the results and found a disparity between laboratories in terms of the number of AMR associated genes reported. For the first time it was demonstrated that the bioinformatic analysis step, and in particular the database of resistance genes used, is a crucial area to focus on in order to standardise this technique. In a second study, the bioinformatics process was interrogated between thirteen laboratories using two mixed microbial community samples which were previously characterised using a reference value provided by digital PCR (dPCR) as a reference measurement procedure. Results described the reproducibility of the analysis step and indicated that bioinformatic tools struggled to identify organisms at low abundance and stressed the importance in using test data alongside sequencing experiments to determine any potential biases introduced.

Impact

GOSH, applied the WGS bioinformatic method developed during the project to identify the organism present and any associated AMR as part of routine management of patients suffering chronic infections – such as those found in cystic fibrosis patients or children with immunosuppression due to bone marrow transplants.

GOSH now has plans in place to perform WGS for CRE. For these patients the timeframe to obtain the data required to inform treatment needs to be rapid – only one to two days – thus

GOSH intends to implement the use of 3rd generation WGS instruments such as the MinION portable sequencer made by Oxford Nanopore, which was also evaluated in the project to identify antimicrobial resistance in *Mycobacterium tuberculosis*. The findings from this work presented a suitable bioinformatic approach for predicting resistance to anti-TB drugs which can therefore be applied in a clinical setting.

The new methodologies developed as part of AntiMicroResist will help to determine the presence and type or resistance in a patient, allowing better antibiotic targeting as well as improved control and prevention strategies to combat future threats due to AMR.

Combating bacterial resistance to antibiotics

The EMPIR project AntiMicroResist developed measurement procedures to predict antimicrobial resistance (AMR) and assess viral drug resistance.

Methodology for quantifying Methicillin-resistant *Staphylococcus aureus* (MRSA) using digital PCR (dPCR) were developed and multi-drug-resistant tuberculosis was used as a diagnostic model to produce materials to support point of care analysis.

Carbapenem resistant bacteria were used to assess bacterial strain analysis using matrix-assisted laser desorption ionisation–time of flight mass spectrometry and Next Generation Sequencing to detect resistance genes. Reference methods for the quantification of anti-viral resistant sequences in viruses were established for HIV and human cytomegalovirus (hCMV). Prototype external quality assessment schemes for MRSA, Tuberculosis and HIV were organised and materials assigned values using dPCR which can be used to evaluate different methods and laboratory performance.



© jfzsy



The EMPIR initiative is co-funded by the European Union's Horizon 2020 research and innovation programme and the EMPIR Participating States

www.euramet.org/project-15HLT07

Jim Huggett

LGC, UK

+44 208 943 7655 | Jim.Huggett@lgcgroup.com

11326/0721 - 15HLT07