Publishable Summary for 15HLT07 AntiMicroResist
Novel materials and methods for the detection, traceable monitoring and evaluation of antimicrobial resistance

Overview

In 2014 a World Health Organisation (WHO) report stated that antimicrobial resistance (AMR) is so serious, that it threatens the achievements of modern medicine, and while new therapies to treat resistant pathogens are needed, the diagnostic tools required to guide their application are equally lacking. This clinically focussed project applied innovative metrological concepts for developing quantitative higher order methodologies and materials to support the development and application of diagnostic testing for the detection and management of AMR. The project developed the materials and methods to support the implementation of standardisation of a protein biomarker for bacterial infection, screening of resistance in bacteria using genetic methods and for screening of membrane targeting antibiotic action. A reference measurement procedure was developed to support higher order methods for quantitative monitoring in virology and guidelines were published to assist the implementation of new tools, such as next generation sequencing (NGS), in detection of unknown antibiotic resistance.

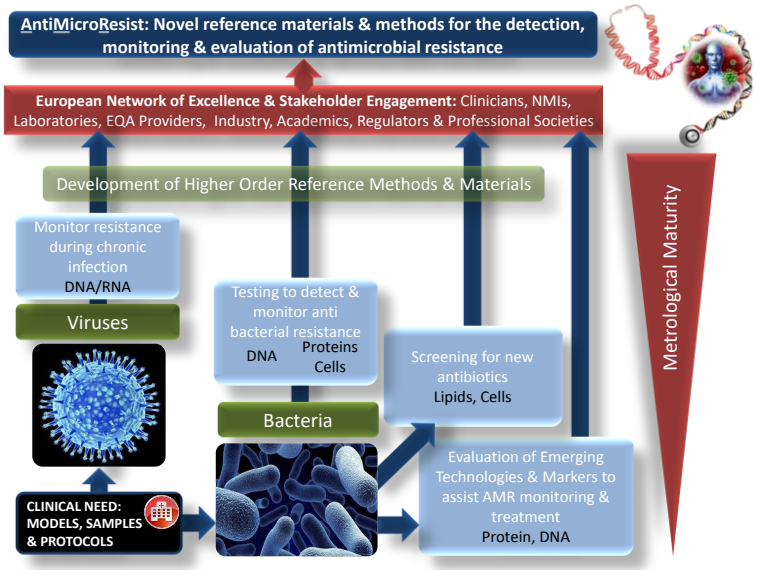
Need

Pathogens resistant to antimicrobial treatment threaten effective prevention of a range of infections. A recent review estimated AMR will account for a staggering 45 % of global deaths by 2050 (http://amr-review.org/). In recognition of this rapidly growing problem, several European activities to monitor detection and treatment of AMR have been initiated, including the European Centre for Disease Control (ECDC) interactive database for clinical detection of AMR antimicrobial resistance (EARS-Net).

Despite such initiatives, there is continued stakeholder need for methods to:

- More rapidly diagnose patients with infections that do need antimicrobials
- Detect infections that are already resistant
- Guide clinical practitioners with respect to correct and effective therapies, to reduce over prescription of antimicrobials
- Support testing of innovative antimicrobials

A recent report in association with The World Alliance Against Antibiotic Resistance (WAAAR) stated that "*Today, we do not have the diagnostic tools to effectively address AMR*" a fact that was further confounded by the lack of mechanisms to standardise the methods that do exist. At the start of the project the most advanced application of traceable measurement methods to manage infectious diseases was for viruses. While some internationally recognised reference materials do exist, AntiMicroResist developed reference measurement procedures that could improve the accuracy and reproducibility of reference material production. Standardisation for clinical testing



associated with bacterial resistance was even less mature. It was acknowledged by the Joint Committee for Traceability in Laboratory Medicine (JCTLM) that the development of reference measurement systems for infectious disease diagnostics and AMR was a key requirement to support both comparable clinical measurement and the industry compliance with the IVD regulation. The objectives of AntiMicroResist addressed these issues by progressing the development of reference methods and materials to underpin the development and application of diagnostic methods to identify and manage AMR, and to support the measurements required for the testing of new antimicrobials.

Objectives

This clinically focussed project applied innovative metrological concepts for the development of higher order quantitation methodologies, and it applied them to provide a step change in evaluating and treating microbial resistance.

The specific objectives of this project were:

1. *Identification of approaches for the management of bacterial antimicrobial resistance.*
   Metrological frameworks were developed for the management of bacterial antimicrobial resistance with a focus on protein biomarker monitoring, routine screening and cellular bioassays (Minimum Inhibitory Concentration (MIC) analysis). This included developing External Quality Assurance (EQA) materials and investigating candidate reference methods to improve reproducibility and better support of established measurements.
2. *Establishing higher order methods and candidate reference materials for quantitative monitoring of viral antimicrobial resistance.*
   A metrology framework based on digital PCR (dPCR) as an SI traceable reference measurement procedure was developed to support the use of higher order reference materials and methods for the quantitative monitoring of viral antimicrobial resistance. This approach brought the potential for SI traceability to the quantitative molecular approaches that are routinely used for the management of patients.
3. *Evaluation of reference materials and methods for the functional validation and screening of antibiotics.*
   Candidate reference materials and methods were evaluated for the functional validation and screening of last-resort and emerging antibiotics. Quantitative measurements of microbial cell walls and artificial membranes were assessed when challenged by antibiotics where intact membranes served as an indicator of resistance. These studies included the kinetics of antimicrobial action against resistant bacteria.
4. *Evaluation of future reference measurement needs.*
   The standardisation of innovative next generation approaches for the detection of emerging and more challenging antimicrobial resistance mechanisms was investigated. Preanalytical, analytical and informatics stages of the process were investigated to determine sources of error and recommendations derived from these findings.
5. *Uptake by reference testing laboratories, EQA scheme providers, health care professionals and diagnostic industry.*
   Uptake of the technology and reference measurement systems developed in the project by EU reference testing laboratories, EQA scheme providers, healthcare professionals (hospitals and health centres) and industry (diagnostic companies) opens up access to SI traceability to support the standardisation of comparable and traceable measurements in the field of antimicrobial resistance management.

Progress beyond the state of the art

At the start of the project, current methods employed to reduce antimicrobial drug resistance included approaches to determine whether the patient had an infection and what type, what treatment to administer and whether the infection in question was resistant to a certain treatment. Crucially, while there was a critical need for the development of new therapies, the fight against antimicrobial resistance also needed to consider the tests available to clinical practitioners to assist in their selection of the most appropriate treatment, having in mind the globally recognised over prescription of antimicrobials leading to the growth in AMR.

AntiMicroResist progressed beyond the state of the art by contributing to:

1) Improving the calibration of tests to determine if bacterial infection is present
2) Standardisation of molecular detection of genetic identifiers for AMR screening
3) Improved reproducibility of the physiological assessment of bacterial drug sensitivity
4) Development of reference measurement procedures for the assessment of viral drug resistance
5) Developing methods to assess and predict antimicrobial membrane resistance and targeting
6) Application of the innovative novel methods required to tackle the latest challenges in AMR

Specific innovations included:

- The evaluation of dPCR as a reference measurement procedure for the value assignment of reference materials for pathogen load determination and antimicrobial resistance diagnostics
- Evaluation of AFM nanocantilevers as a method for the rapid assessment of MIC
- Novel, quantitative biophysical measurements of microbial cell walls/membranes and microorganisms challenged by antibiotics, and the kinetics of antimicrobial action against resistant bacteria
- An assessment of the application of next generation sequencing and advanced mass spectrometry to identify and measure diagnostic markers for emerging and more challenging resistance mechanisms
- Development of accurate and robust reference measurement procedures for individual microbes as well as microbiomes that enable the detection of unknown resistance mechanisms

Results

1. *Identification of approaches for the management of bacterial antimicrobial resistance.*
    - Development of reference materials and methods for the measurement of proteins and DNA associated with the diagnosis of bacterial infections and identification of strains that carry resistance like MRSA.
        - A candidate reference method using mass Isotope Dilution Mass Spectrometry (IDMS) was developed that was able to quantify procalcitonin in serum samples. This included the successful characterisation of a protein primary calibrator, as well as the evaluation and characterisation of suitable peptide primary calibrators.
        - A candidate reference measurement procedure using dPCR for the measurement of MRSA was developed, which can also be used in the EQA of routine laboratories. Interlaboratory comparisons between partners have been completed and have shown variability in the reproducibility of the results which was in contrast to good repeatability within laboratories. Improvements in the reproducibility of the extraction step would be required in order to apply these methods for the quantification of the analyte, however they are currently fit for analyte detection.
        - Materials to support point of care analysis have been developed using multi-drug-resistant tuberculosis as a diagnostic model. The characterisation of this material was completed, and it is now available for inter-laboratory evaluation which could be used beyond the scope of this project.
    - Evaluate AFM nanocantilevers as a candidate reference method for phenotypic analysis of bacteria to offer a complementary method to evaluate the minimum inhibitory concentration.
        - Atomic-force microscopy (AFM) nanocantilevers have been evaluated as a candidate reference method for phenotypic analysis of bacteria via mechanical signal. High variability was observed in some of the core procedural steps. However, an alternative signal, observed during this evaluation is now being investigated as a novel and potentially simpler alternative method for phenotypic analysis of bacteria. This work will extend beyond the end of the project.
2. *Establishing higher order methods and candidate reference materials for quantitative monitoring of viral antimicrobial resistance.*
    - Development of a higher order reference method for the quantification of anti-viral resistant sequences in viruses such as HIV and human cytomegalovirus (hCMV) to monitor and reduce the development of antimicrobial resistance.
        - A reference method for the quantification of hCMV has been developed and submitted to the Joint Committee for Traceability in Laboratory Medicine (JCTLM) database for review.

- This method was successfully tested in several hCMV ring trials organised as EQA for routine virological laboratories (approximately 100 participants each). A similar method was developed for HIV.
- The CCQM Working Group on Nucleic Acid Analysis (CCQM-NAWG) initiated the pilot study P199 (Copy number concentration of HIV-1 RNA genomic sequences) with measurements completed by September 2019. Materials were prepared by the consortium and were distributed to study partners in June 2019.

3. *Evaluation of reference materials and methods for functional validation and screening of antibiotics.*
   - Development of reference materials to model resistance and susceptible bacterial membranes.
     - An inter-comparison study to characterise candidate reference materials consisting of different lipid compositions mimicking resistant and susceptible bacterial membranes to a common specification is now underway under VAMAS TWA40.
4. *Evaluation of future reference measurement needs.*
   - Determine the reference needs for advanced next generation diagnostic tests by measuring proteins and nucleic acids, individual microbes, as well as microbiomes in order to enable the detection of unknown resistance mechanisms.
     - Carbapenem resistant bacteria were used as a clinical model system to evaluate the accuracy, robustness and reproducibility of strain analysis using matrix-assisted laser desorption ionisation–time of flight mass spectrometry (MALDI- TOF-MS) and detection of resistance genes using Next Generation Sequencing (NGS). Factors effecting performance of the selected pipelines were evaluated at a single site and reproducibility between laboratories was tested. The findings from this study formed a good practice guide and will be submitted for peer review publication.
5. *Uptake by reference testing laboratories, EQA scheme providers, health care professionals and diagnostic industry.*
   - Maximum impact was ensured via knowledge transfer, training and uptake through interaction with the established and growing project stakeholder network and contribution to the drafting of guidelines.
     - The project organised prototype EQA schemes for MRSA, Tuberculosis and HIV. These materials were value assigned by the partners using dPCR. These values can be used to evaluate different methods and laboratory performance.
     - The project contributed to EQA schemes organised for routine virological laboratories for the quantification of CMV and HIV which were organised by the collaborator GBD.
     - The project inputted into the draft ISO standard ISO/CD 17822-2 – 'In vitro diagnostic test systems -- Qualitative nucleic acid-based in vitro examination procedures for detection and identification of microbial pathogens – Part 2: Quality practices for nucleic acid amplification.

Impact

More than thirty-six presentations/posters were made at conferences, including the 4th Joint EFLM – UEMS Congress, Poland, the 'qPCR dPCR & NGS 2017' Symposium, Germany and the European Congress of Clinical Microbiology. During the first half of the project four workshops were run including the LESPAR Early Career Researcher workshop on diagnostics for Antimicrobial Resistance in 2017 and the ESCMID (European Society of Clinical Microbiology and Infectious Diseases) 3rd Course on the Principles of Molecular Microbiological Diagnostics in 2018. Early project findings describing the first molecular reference measurement procedure for infectious diseases was also presented at the JCTLM Member's and Stakeholders' Workshop in December 2017. Three open access papers were published in international peer-reviewed journals, including Nature Communications and an additional seven papers drafted for peer review submission and planned open access publication. A project symposium was held at the British Society for Antimicrobial Chemotherapy (BSAC) Spring Conference in March 2019 to showcase project findings and associated research and to encourage uptake to members of industry, healthcare scientists and academia.

Impact on relevant standards

Impact on standardisation for AMR measurements was achieved through contribution to international activities including:

- A new work item proposal for ISO TC212 (Clinical laboratory testing and in vitro diagnostic test systems) WG4 (Microbiology and Molecular Diagnostics) on AMR measurement. Contribution to standard development in ISO TC276 (Biotechnology) WG3 (Analytical methods).
- A project proposal for a VAMAS inter-laboratory study for predicting an appropriate antibiotic treatment.
- A good practice guide on methods for the standardisation of next generation molecular methods for monitoring AMR was developed in association with the IFCC Committee for Molecular Diagnostics and this was submitted to the CCQM working group for nucleic acid measurements.
- A reference method for the quantification of microbial nucleic acids was submitted to JCTLM.

Impact on industrial and other user communities
AntiMicroResist generated early impact through the application of metrological concepts in a clinical context and the development of new measurement capabilities that includes novel, precise (and potentially SI traceable) reference methods for the measurement of surrogate biomarkers, resistant microbes and resistance levels. These concepts are applied by our clinical laboratory partners (Great Ormond Street Hospital (GOSH), The Royal Free Hospital (UCL), Helios Kliniken (UWH) and Klinika Golnik (UCG)). GOSH has already implemented a whole-genome sequencing method for AMR detection in the clinical laboratory that is based on the good practice guide delivered by the project.

Techniques for the development of reference materials to support a wide range of measurements associated with AMR management including methods to monitor nucleic acids, proteins and cell phenotypes were developed and incorporated into appropriate guidelines and disseminated (alongside the reference methods developed to characterise and quantify them) for use by the IVD industry and to support EQA provision. Early inclusion in relevant EQA schemes for both antiviral resistance and antimicrobial resistance testing was facilitated through our partnership with EQA scheme providers (both as partners, Helios Kliniken (UWH) and named collaborators Gesellschaft für Biotechnologische Diagnostik mbH (GBD)).

Close liaison with our clinical and industrial stakeholders and partners on AntiMicroResist is allowing the validated next generation diagnostic methods to be applied in preclinical and translational research in order to perform accurate measurements, which enable a better understanding of antimicrobial resistance. Furthermore, close discussion is progressing based on the materials and methods developed in the project to support near patient point of care AMR testing with a number of industrial collaborators including Oxford Nanopore, a leading sequencing technology provider, regarding the development of reference materials.

Impact on the metrology and scientific communities
AntiMicroResist has increased the focus of the metrology community on this medical priority by ensuring that AMR is part of the discussions amongst the CCQM and JCTLM and of the research portfolio of many European and global NMIs conducting bioanalysis. The impact on the wider scientific community was achieved by demonstrating how antimicrobial resistant measurement comparability can be improved. This was ensured through peer reviewed papers in high impact journals, conference presentations, workshops and other dissemination activities, including best practice/required information guidelines for clinical research which are needed to assist in its translation to the patient.

Longer-term economic, social and environmental impacts
AntiMicroResist inherently responds to the needs of healthcare sectors, where its long term impact will be strongest. Although different sectors can benefit from the project results, the diagnosis and treatment of AMR remains the main impact domain where this coordinated metrological programme will have major long-term contributions.

Longer term social impact
In response to specific criteria, which WHO set in 2009-2011 and which retain their urgency today, in the longer term, the successful uptake of the project's outputs will improve the measurements that are required to understand the underlying reasons behind a high percentage of hospital acquired infections, monitor infections resistant to first line medicines and support the use of effective antimicrobials for the treatment and prevention of infections.

Longer term economic impact

The scale of the challenge is compounded by the fact that the most severe gaps in European biotechnology and pharmaceutical sectors remain at the early, high risk stages, making it difficult for small and medium enterprises to survive. The outputs of this project will aid in reducing the associated risks by improving the quality of the materials required to support accurate measurements. Given the growing market size in the diagnostics, and therapy of AMR, this will assist even small contributions in bringing significant benefits.

Longer term environmental impact

The emergence of virulent bacterial strains has adverse ecotoxicological consequences on environmental organisms, which is one of the reasons for the spread of antimicrobial resistance. While drugs and their breakdown products can be harmful or mutagenic to microorganisms, plants and animals, new medicines must be as specific as possible to provide the desired effects on human health without undesired consequences on other organisms. Consequently, many of the measurement findings will directly impact on the study and management of antimicrobial resistance in the environment by supporting both better application of antimicrobials, but also by demonstrating the accuracy of some of the technologies that are also used in environmental microbiology such as metagenomic analysis. More confident measurement and monitoring of AMR is also directly applicable to animal husbandry in both the agricultural and veterinary industry.

List of publications

Phelan, J, O'Sullivan, DM, Machado, D, Ramos, J, Campino, S, O'Grady, J, McNerney, R, Hibberd, M, Viveiros, M, Huggett, JF, Clark, TG (2019) Integrating informatics tools and portable sequencing technology for rapid detection of resistance to anti-tuberculosis drugs. Genome Medicine, 11:41 https://doi.org/10.1186/s13073-019-0650-x

Shaw LP, Doyle RM, Kavaliunaite E, Spencer H, Balloux F, Dixon G and Harris KA (2019). Children with cystic fibrosis are infected with multiple subpopulations of Mycobacterium abscessus with different antimicrobial resistance profiles. Clin Infect Dis, ciz069, https://doi.org/10.1093/cid/ciz069

De Santis, E., Alkassem, H., Lamarre, B., Faruqui, N., Bella, A., Noble, J. E., Micale, N., Ray, S., Burns, J., Yon, A. R., Hoogenboom, B. W. & Ryadnov, M. G. Antimicrobial peptide capsids of de novo design. Nature Commun, 8, 2263 (2017) https://doi.org/10.1038/s41467-017-02475-3

Project start date and duration:	1 June 2016, 36 months	
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Internal Funded Partners: 1 LGC, UK 2 LNE, France 3 NIB, Slovenia 4 NPL, UK 5 PTB, Germany 6 TUBITAK, Turkey	External Funded Partners: 7 BSAC, UK 8 GOSH, UK 9 UCG, Slovenia 10 UCL, UK 11 UWH, Germany	Unfunded Partners:
RMG1: TUBITAK, Turkey (Employing organisation); LGC, United Kingdom (Guestworking organisation)		