Revolution in genomics and microbiology: the impact of technology

The Genomic & Microbiology Revolution: In Technology we Trust? was the title of the 37th Annual Microbiology Conference of the British Society for Microbial Technology, held at the RAF Museum at Hendon on 19 July. A fascinating programme looked at a range of different aspects of technology applied to microbiology, here summarised by Mark Wilks on behalf of the BSMT committee.

Technology is often understood in a very narrow sense such as the introduction of automation to what was a manual process. When this happens, the benefits are normally obvious immediately and very often cost-effective. For example, who would want to go back to manually inspecting blood culture bottles for signs of bacterial growth? Although we should note that even applying technology to this simple process has its own pitfalls as it requires the development of sophisticated algorithms to monitor bacterial growth. These can be confused by delays in transporting blood culture bottles to the laboratory prior to incubation, which upsets the expected increases in turbidity that are expected to occur when the culture is being incubated at 37°C.

The Royal Air Force Museum, Hendon, venue and backdrop to the BSMT Annual Microbiology Conference.

The Health Technology Assessment (HTA) arm of the National Institute of Health Research (NIHR) takes a much broader view of the term technology. For the NIHR, technology assessment could mean not just studying the effectiveness of a particular technology such as MALDI-TOF but the assessment of a particular product, such as an antibiotic or probiotic. Here, a yoghurt containing a probiotic is a technology.

Adapting to effectiveness

Our first speaker was Professor Paul Dark, an Intensivist from Manchester, and the National Deputy Medical Director of the NIHR Clinical Research Network, who has led a number of very large investigations of the effectiveness of different technologies, many supported by the HTA. He concentrated on reviewing efforts made to develop the best evidence for effective molecular diagnostic technologies in the diagnosis and management of sepsis as well as fungal infections by describing several different trials that are currently running. The common thread of these trials is what is often termed 'of very high quality'. For example, the research question is very carefully defined and the study is sufficiently powered to provide a useful answer. These studies also always involve a substantial health economics aspect,

Dr Katie Hopkins illustrated how to provide timely sequencing for local diagnosis.

rigorous study design, large statistical input and, importantly, a real degree of patient involvement.

Professor Dark described in great detail the ADAPT trial. A somewhat tortuous acronym standing for biomArker-guided Duration of Antibiotic treatment in hospitalised PaTients with sepsis. This large trial is designed to provide a definitive answer to the question does a treatment protocol based on serial monitoring of C-reactive protein (CRP) or procalcitonin (PCT) safely allow reduction in duration of antibiotic therapy in hospitalised patients with sepsis? It is worth pointing out that the drive to reduce excessive antibiotic usage is not solely due to justifiable concerns about the development of antibiotic resistance, but the actual harm that antibiotic use can cause. This is particularly so in neonates where concern over neonatal sepsis leads to what all neonatologists agree is a huge over-prescribing of antibiotics, leading to very common and sometimes very serious deleterious effects on the patient.

It is sobering to reflect that the use of the CRP test to guide the diagnosis of sepsis has been in widespread use for approximately 40 years, but there is still no good evidence to show its benefit, principally owing to the small size of the trials that have taken place. In the ADAPT study the total sample size of 2760 patients is being enrolled, and it's a tribute both to those who are running the trial and to all the ICU staff and patients who are working on the trial that it managed to keep ticking over during the COVID pandemic, and is now back on track to its predicted recruitment rate, and should finally provide a definitive answer on the value of these two tests.

Direct identification and sequencing

In the next talk Adela Alcolea-Medina, Clinical Scientist with ViaPath at St Thomas' Hospital, London described her work using quite a different technology – Oxford Nanopore (ONT) sequencing for the direct detection and identification of viruses in respiratory clinical samples. Next-generation sequencing (NGS) allows thousands to billions of DNA fragments to be simultaneously and independently sequenced. One great advantage of NGS in clinical microbiology is that it is 'agnostic' and should allow for an unbiased approach to the detection of pathogens.

Published rapid methods for clinical metagenomics-only sequence DNA or RNA have long turnaround times to provide clinical reports. Although Library preparations for combined RNA and DNA sequencing have been published, these take days to provide results, mainly owing to the huge quantity of human DNA present in clinical samples, which outnumber pathogen DNA by a factor of thousands if not more. She described a method for depleting human DNA while limiting centrifugation or the use of chemicals and a library preparation that can sequence RNA and DNA organisms at the same time. The benefits of a rapid unified, unbiased method for detecting and characterising DNA and RNA organisms are obvious and has become something of a 'holy grail' in recent years. It should remove the need for multiple samples or sample splitting the different tests and hopefully to exclude the need for targeted PCR. In addition, it has the potential to identify unexpected novel and emerging pathogens.

The workflow she described was simple and the first results were obtained seven hours after receipt of the specimen, and a 24-hour workflow generated full genome sequences of pathogens. The technique had been successfully applied to respiratory specimens, and several different viruses have been identified with results correlating well with conventional tests. In the last couple of months, the focus has switched, for obvious reasons, to testing skin swabs from patients presenting with a blistering rash, and a number of cases of monkeypox had been detected. Interestingly, in a patient with suspected monkeypox, varicellar-zoster virus (VZV) and not monkeypox was detected. The gene coverage obtained was a 100%, which gave a high degree of confidence in the result and obviously made a huge difference to patient management. Although the attractions of the technology are obvious, it is likely that the need to establish rigid quality control (QC) criteria rather than cost will determine how quickly it is introduced into the diagnostic laboratory.

Phenotypic versus genotypic sensitivity testing

After a break, the next talk was given by Dr Katie Hopkins, Lead Clinical Scientist, Antimicrobial Resistance & Mechanisms Service, UK Health Security Agency at Colindale, who delivered a comprehensive overview of the different technologies used for antimicrobial resistance and also the challenge of selecting which ones to use within a diagnostic laboratory.

Phenotypic methods are commonly used and while slower, with inconsistencies in reproducibility, they do provide a quick method of detecting resistance in organisms, even if the

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Natasha Weston provided an overview of WGS of *M. tuberculosis* and other mycobacterial species.

mechanism is not clear and requires further investigation. These agar-based methods, along with the gold-standard of broth-microdilution, have the additional difficulty of interpreting and reporting the susceptibility results using increasingly complex breakpoint tables. Ensuring the results tested within the microbiology laboratory translate to understandable results for users is critical for it to impact patient care.

Molecular methods offer a more rapid, gene specific mechanism that can be particularly useful in organisms with infection, prevention and control implications such as methicillin-resistant *Staphylococcus aureus* (MRSA) or carbapenemase-producing organisms (CPOs). Detection of the gene in a diagnostic setting can be used to rule-in patients as having a resistant organism and there are multiple commercial assays available for doing so, albeit this is more complex for Gram-negative than Gram-positive bacteria.

This captivating session showed the advantages of the amount of data gained when applying whole-genome sequencing (WGS) in antimicrobial resistance and how this can be used for epidemiological purposes, outbreak investigations and to examine biomarkers in organisms. While the genotypic methods are unable to allow understanding of the interactions of more than one resistance mechanism within one organism, and do not always predict therapeutic failure, they have significant public health and outbreak management implications.

Dr Hopkins discussed the literature and evidence body surrounding phenotypic and genotypic antimicrobial susceptibility testing, and while the volume of evidence is there, the drawback is the tendency for studies to focus on individual organisms, reducing its applicability into a diagnostic laboratory.

The need for most laboratories to use a combination of methods requires a sound knowledge of the methods, their limitations and the most appropriate application. This makes antimicrobial resistance (AMR) a key area of microbiology where multidisciplinary working of biomedical scientists, clinical scientists, pharmacists, medical microbiologists and infection control is crucial, and brings together the science with the clinical applications, for the patient.

Tuberculosis and whole-genome sequencing

Dr Natasha Weston, who was previously a Senior Clinical Fellow at the National Mycobacterial Reference Service in Birmingham, concluded the morning session with a look at how WGS is being applied to the diagnosis of TB and other mycobacterial infections. While PCR is playing an increasing diagnostic role, it can be costly and provides quite limited antimicrobial susceptibility information. Dr Weston demonstrated that WGS has advantages in diagnosis, treatment and epidemiologically. By applying WGS techniques, a reliable rapid identification can be obtained with an indication of multiple resistance markers potentially weeks before that possible by traditional techniques. Whole-genome sequencing is now the method of choice within the reference setting, replacing phenotypic methods as the first line for antimicrobial susceptibility determination.

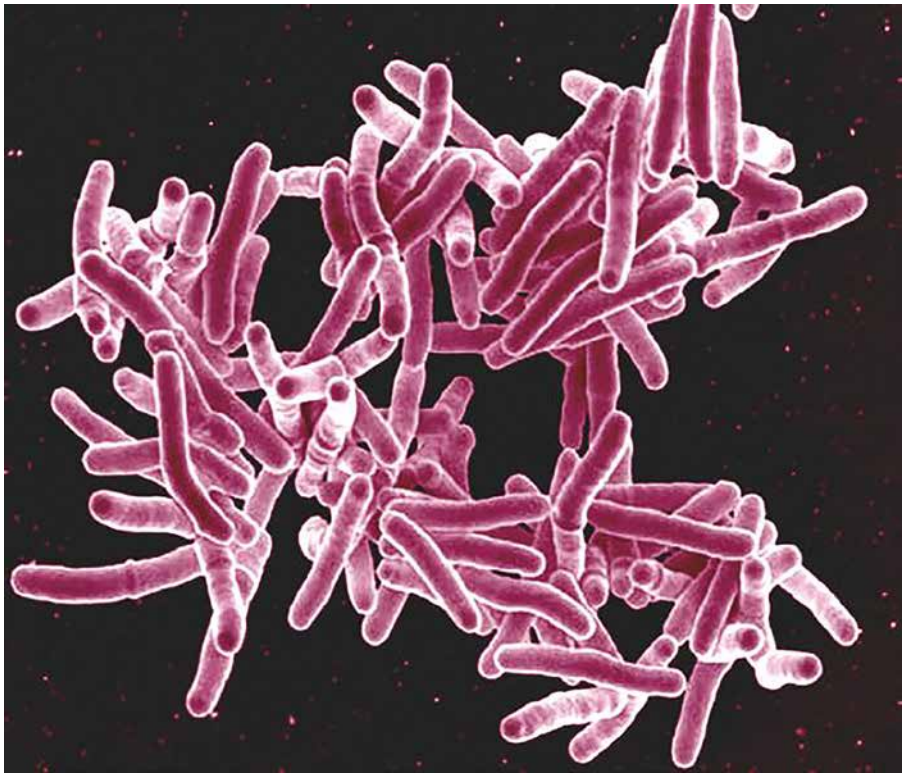
Whole-genome sequencing can simultaneously give information regarding the genetic relatedness of different clinical isolates. Characterisation of mycobacterial isolates using WGS works particularly well for *Mycobacterium tuberculosis* strains but is also effective for other well-described non-tuberculosis mycobacteria. Given the slow genomic evolution of *M. tuberculosis*, varying only around one single nucleotide polymorphism (SNP) every two years, the data derived from WGS can be used as an effective indicator to identify clusters of related cases.

The advantages of WGS as an outbreak investigation tool were vividly illustrated with a series of case studies showing how socially distinct cases can be linked using WGS. Data can now be available in less than a week from initial positive cultures, which allows a much more rapid and accurate picture of TB outbreaks and transmission events to be determined. This means that public health interventions, contact tracing and treatment can be targeted much more precisely and effectively than previously possible.

Transmission events that are transient or short lived can be difficult to connect but WGS has allowed the recognition of clusters with little or no social overlap. Dr Weston clearly demonstrated within these case studies the real benefits that more rapid availability of typing data can provide, where it is even possible to identify laboratory cross-contamination events.

External quality assessment: the challenges

The increasing complexity of the technologies that have been introduced has meant a greatly increased requirement for QC in the context of wider quality assurance. During this continual period of progression, the complexity of the analysers has increased markedly from detection of single gene targets to multiplex assays capable of detecting multiple targets from a range of different pathogens simultaneously. Conversely, the time taken to generate a result has markedly decreased, with some platforms providing an actionable result within 30 minutes. Nowadays, over 80% of microbiology laboratories in the UK report that they use molecular assays for virology diagnostic testing. It was with this background in mind that Dr Elaine McCulloch, Technical Project Manager, QCMD, Glasgow discussed the need for quality assurance in molecular diagnostics



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Whole-genome sequencing is being applied to *M. tuberculosis* (pictured) and other mycobacterial infections (scanning electron micrograph)

and next-generation sequencing (NGS).

Dr McCulloch described how an external quality assessment (EQA) scheme needs to be fit for purpose, meeting regulatory requirements (ISO 17043:2010 as an EQA / PT provider) and the requirements of a clinical laboratory (ISO 15189:2012 or equivalent). A scheme needs to be practical and cost-effective, and be driven from a regulatory perspective, but also include educational elements. External quality assessment samples that are as close to a clinical sample as possible ensure that the scheme is as clinically relevant as practicable. An EQA scheme for molecular diagnostics needs to keep up with current clinical and technological developments that are occurring in 'real-life' clinical and laboratory settings. In terms of QC requirements for accreditation, QC materials should be different from calibration materials, and the use of independent third party controls should be considered.

Dr McCulloch stressed the importance of internal QC (IQC), in particular for monitoring variation in test results. Variation is expected and may indicate different issues according to its nature. For instance, random variation is represented by individual spikes observed in the data. These are easy to detect and are likely to be caused by unpredictable fluctuations. Conversely, systematic variation is represented by trends or drift in the data. This type of variation is harder to detect in a single dataset and may lead to hidden, persistent and/or growing errors.

While the majority (86%) of molecular assays remain qualitative, there is a need for viral quantitation in certain infectious diseases (eg cytomegalovirus [CMV], Epstein-Barr virus [EBV] and bloodborne viruses) and certain patient populations (eg transplant recipients). Currently, 'Official Standards' are only available for less than 8% of all the QCMD EQA schemes and pathogen/strain level coverage is less than 0.5%. In the absence of an international standard, an EQA provider needs to find suitable reference material, and QCMD chose to use an alternative approach and developed International Reference Materials (IRMs). Once an international standard becomes available, it will be possible and necessary to back calibrate IRMs.

With over 60% of laboratories reporting the use of commercial molecular assays, the diverse nature of molecular assays available can complicate the quality assessment (QA) process.

In particular, multiplex testing presents certain challenges to molecular QC and QA, particularly when balancing the need for a clinically relevant and cost-effective EQA service in line with the relevant regulatory requirements (ISO 17043 and ISO 15189). Supporting diagnosis based on clinical presentation, multiplex assays often comprise a combination of bacterial, viral and fungal genes (± antibiotic resistance genes). To support a decreased turnaround time, multiplex assays may report preliminary findings at an early stage with a final identification report later.

In the scenario where more than one pathogen has been identified from a single sample, identifying the causative agent of infection rather than colonisation can be challenging as it is possible that the pathogens present may represent clinically relevant dual infections. From an EQA sample perspective, combinations of multiple pathogens in a single tube are not considered appropriate, although such 'mixtures' can be used as control material. QCMD supports the rotation of different pathogens through the annual EQA challenges to be more relevant than providing all pathogens in a single tube/ challenge.

The diagnostic landscape is changing and there is an increased interest in the provision of near-patient testing in community settings, which presents another set of challenges to molecular QC and QA. In the near patient setting, the location and personnel completing the testing can be varied, ranging from testing being completed in a different laboratory by personnel not employed by your laboratory (eg by satellite laboratories), in a primary care facility (eg GP surgery), in an accident and emergency department, or in an ambulance or other 'in the field' setting. In addition, there are many different point-of-care (POC) devices/platforms on the market with different specimen requirements and testing algorithms, all of which need to be covered by an EQA scheme. Whatever the setting, personnel and device used, according to ISO 15189:2012 near-patient testing comes under the responsibility of an accredited laboratory.

Next-generation sequencing (NGS) is an emerging area of diagnostic microbiology as we heard in the previous

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talk. Currently, QCMD is focused on two areas: drug resistance and viral metagenomics. It has seen an increase in the number of NGS datasets submitted for assessment of drug resistance. This has led to workflow information being updated, additional files collected, and improvements to analysis processes to identify quality parameters. Next-generation sequencing for viral metagenomics results in vast volumes of output data. In order to support this molecular diagnostic method, QCMD launched a new pilot in 2020 (ongoing), which aims to assess performance of existing metagenomics protocols as currently implemented by participants. Plans include additional EQA schemes for bacterial metagenomics and genotyping.

After this comprehensive overview, delegates were left with a new appreciation of the continued efforts of QCMD to provide a high-quality EQA scheme covering the range and complexity of molecular assays now available.

Antimicrobial stewardship

The principles of antimicrobial stewardship could be regarded as well-established within the UK, documented in policy and protocol, and enshrined within the framework of clinical decision-making. Dr Esmita Charani, Research Lead (Practice, Design and Engineering), NIHR Health Protection Research Unit, at Imperial College London, provided a different perspective and delivered a thought-provoking talk which considered antimicrobial prescribing as a complex social process and from an international angle.

Research funding in this area is focused on developing and evaluating technological solutions, and technology will now doubt have a significant role to play. However, when these solutions fail to consider factors, such as end-user requirements or resource availability they will struggle to realise their potential. Dr Charani highlighted the limited research in areas such as implementation and the influence of social contexts in which antimicrobial prescribing occurs. The focus on technological solutions, more likely to be initially employed in high- and middle-income countries, perhaps indicates a disconnect between those areas that carry the burden of antimicrobial resistance and those with the resources to pursue and direct research funding.

Even in regions where there are clear policies in place, decision-making can be influenced by a wide range of factors that are difficult to quantify and assess. Power dynamics in strictly hierarchical institutions can lead to a lack of engagement with wider healthcare professionals such as pharmacists who can contribute key knowledge. Incredibly, there is clear evidence that pharmacist gender still has a quantifiable influence on the acceptance of prescribing advice and stewardship recommendations. The references presented to support these conclusions were drawn from a high-income, technologically developed country suggesting this remains a concern despite the perception of social progress in these areas.

Access to a high-quality diagnostic service was recognised as crucial to guide decision-making. However, the capabilities of laboratories and crucially the confidence in the accuracy and clinical relevance of results produced is critical to these tests positively influencing antimicrobial prescribing. This is an area in which there remains significant variation across different global settings.

Managing AMR on the international stage requires coordination and collaboration between many different groups. Overarching policies are important but consideration must also be given to local interactions, and educating both prescribers and patients/carers is as essential. Dr Charani demonstrated that behind every simple subject lies a more complex reality. More research into the individual social dynamics and socioeconomic inequalities both between and within different regions must be considered to allow effective interventions to have the desired effect.

Looking to the future

We hope to return to some of the other topics of the meeting, in particular the use of NGS in microbiology, in a future article. The BSMT is grateful to all the speakers and to Professor Brian Duerden CBE who chaired the afternoon session in what proved to be a stimulating day of excellent presentations. We were sorry that Dr Kate Templeton, BSMT President, who was due to chair the morning session, was prevented from attending by a cancelled flight from Edinburgh. Cancellation of a different flight from Edinburgh also thwarted Michael Croughan, the BSMT treasurer, who could not get to London for the conference, the first one he has missed in 37 years of the BSMT conferences.

It can't have been easy to cope in the afternoon session when the air conditioning failed in the extreme heat, despite the best efforts of the museum staff to look after the welfare of everyone who attended. Thanks also to the 20 commercial companies who sponsored the event – more details about these companies and their products relevant to the conference were provided in a recent issue of *Pathology in Practice* (June, page 42), and details are also on the BSMT website (https://bsmt.org.uk).

Watch *Pathology in Practice* and the BSMT website for more details about the next Annual Microbiology Conference to be held on Thursday 11 May 2023, again at the RAF Museum, Hendon. Alternatively, email Valerie Bevan (vbevan@bsmt.org.uk), who will add your name to the list of people kept informed about the conference by email. Topic to be decided – ideas welcome!

Dr Mark Wilks would like to thank David Westrip, Carolyne Horner, Samantha Manyanga and Valerie Bevan from the BSMT Committee, all of whom contributed to this article.

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Sequencing using the Oxford Nanopore system.