Topics in microbiology and infection: a review of the BSMT conference

The British Society for Microbial Technology held its 38th Annual Microbiology Conference at the RAF Museum in Hendon, London, on 11 May, focusing on current infection issues facing laboratories and clinicians. Here, Dr Mark Wilks, Chair of the BSMT, and others on the committee, offer a review of the day's proceedings.

Professor Alasdair MacGowan, Professor of Antimicrobial Therapeutics, University of Bristol, set the scene with an authoritative overview of the development of antimicrobial resistance (AMR) and approaches to controlling it.

As described in a seminal report *The global burden of bacterial antimicrobial resistance in 2019: a systematic analysis*, published in *The Lancet* in 2022, death rates associated and attributable to AMR are highest in sub-Saharan Africa, closely followed by South Asia and Eastern Europe. Globally, *Escherichia coli* is the leading pathogen for deaths associated with resistance, followed (in order) by *Staphylococcus aureus, Klebsiella pneumoniae, Streptococcus pneumoniae, Acinetobacter baumannii* and *Pseudomonas aeruginosa*. Lower respiratory tract infections are most associated with death rates associated and attributable to antimicrobial resistance.

The local AMR situation was described using the latest English surveillance programme for antimicrobial utilisation and resistance (ESPAUR) report, published in November 2022 (other reports are available for Northern Ireland, Scotland and Wales). In England, the incidence of bloodstream infection (BSI) caused by *E. coli* and *S. pneumoniae* decreased noticeably during 2020–2021 in comparison to other key pathogens, such as *K. pneumoniae* and *Enterococcus*, which could be described as having an increasing trend. In comparison to Gram-negative BSI, there is little change reported in the incidence of resistance in *S. aureus* (MSSA and MRSA) and *S. pneumoniae* BSI.

Across England, the AMR burden has notable geographical variation and association with certain ethnic groups, and carbapenemase-producing Gram-negative bacteria have particular geographical distribution according to enzyme. The incidence of AMR in *Neisseria gonorrhoeae* is being closely monitored by the UKHSA GRASP surveillance. Over a 20+ year period, rates of resistance to ciprofloxacin have increased from <10% to almost 50%.

It is well-documented that antimicrobial resistance is driven by antimicrobial use. Between 2017 and 2021, antibiotic consumption of certain antibiotic classes has decreased (penicillins and macrolides) but consumption of other classes has remained the same. Other factors that drive AMR include inappropriate prescription of anti-infectives by human and animal health professionals, antibiotic courses that are not taken

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The RAF Museum in Hendon, North London, has become the regular venue for the BSMT's Annual Microbiology Conference.

Dr Nathaniel Storey from the Department of Bioinformatics, Great Ormond Street Hospital, London.

Dr Mandy Wooton, Scientific Lead at The Specialist Antimicrobial Chemotherapy Unit, Public Health Wales Cardiff.

as directed, poor hygiene and lack of infection prevention and control, and the movement of people (and possibly goods) around the world.

Preventing infection in the first instance is key to the management of AMR, emphasising the need for clean water, sanitation, access to immunisation, and good hand hygiene. Other approaches include improving anti-infective use to slow the development of resistance and stopping or limiting the spread of resistance when it occurs. Underpinning these key AMR management strategies are market interventions; legislation, including device and drug regulation; infection prevention, education and motivation, and innovation in areas of digital health, non-traditional therapies, and new diagnostics.

There is no shortage of development activity in the field of new therapeutics, with a recent review describing over 400 projects from 300 different institutions currently ongoing. In brief, the majority (46%) of new therapeutic approaches investigated direct-acting small molecules, 70% of which were completely novel and 50% were targeting Gram-negative bacteria. Other major areas of development include projects in antibodies and vaccines (14%), bacteriophages and microbiota modulators (13%); anti-virulence approaches (8%), and potentiators (8%, mainly β-lactamase inhibitors). Development in the pre-clinical antibacterial pipeline is healthy and needs to be coupled alongside the development and deployment of sustainable rapid diagnostics.

Surveillance support programmes

Low- and middle-income countries (LMICs) suffer disproportionately from antimicrobial resistance due to limited healthcare provision, antimicrobials used in lieu of access to healthcare, and lack of access to second/third-line drugs. In these countries, multi-drug resistance may not necessarily be the main problem, rather resistance to the most widely used/available drugs. Dr Claire Gordon, Consultant in Infection, Rare and Imported Pathogens Laboratory, UKHSA, described one initiative supporting laboratory development for AMR surveillance in LMICs.

The Fleming Fund provides £265 m UK aid to support LMICs to generate, share and use AMR data as part of global efforts to understand and reduce the impact of drug resistant infections. Managed by the UK Department of Health and Social Care, in partnership with Mott MacDonald, the programme works in 24 priority countries across Africa and Asia. The vision of the programme is for clinical laboratories to complete basic bacteriology, and data that are generated are reported to centralised AMR coordinating committees that report to relevant ministries.

In recent decades, matrix-assisted laser desorption/ionisation-time of flight (MALDI-ToF) spectrometry has revolutionised microbiological identification in high-income countries. By applying the concept of 'leapfrog' technology, defined as bypassing the introduction of traditional methodologies to jump directly to the latest technologies, the current programme aimed to introduce MALDI-ToF into LMIC laboratories that have had minimal acess to culture methods because of limited resources. Aside from rapid and accurate identification, compared with traditional biochemical identification methods, MALDI-ToF requires little additional consumables and reagents, solving supply chain issues. Nor does it produce much waste, with little environmental impact, and doesn't require specialist skills or training.

In terms of cost, based on current estimated instrument lifetime of seven years, capital outlay will be recouped if sample throughput is maintained at 5000 isolates / year, with a cost similar to that of standard processing using traditional identification methods. Additional samples cost $3.81 for identification,

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compared with $12.23 for standard testing.

The first installation was completed in the National Reference Laboratory in Uganda in February 2020. Then the COVID-19 pandemic struck causing logistical problems and a shift in priorities. Dr Gordon described other non-COVID-associated logistical challenges associated with transporting MALDI-ToF instruments, which do not tolerate tilting into airports such as that in Bhutan that has a steep approach or needing to plan flood resilience by installing a benchtop MALDI-ToF rather than a floor-standing equivalent.

An important consideration for such a programme is ensuring the sustainability of the solution. Applying MALDI-ToF methods for use in other investigations, such as mosquito identification (Kenya), research and public health applications (Timor Leste) and investigating deaths in fish (Ghana), could lead to additional funding and support for long-term service provision.

While this ambitious project has been additionally challenging due to the COVID-19 pandemic, lessons have been learned for rollout of future programmes. In addition to the logistical learning points, although local doctors were supportive of laboratory development, early engagement with clinicians in the grant application and implementation stages is also key if programmes in LMICs are to be successful.

Detecting resistance mechanisms

Dr Mandy Wooton, Scientific Lead at The Specialist Antimicrobial Chemotherapy Unit, Public Health Wales Cardiff, discussed how the routine diagnostic laboratory detects AMR focused on Gram-negative resistance mechanisms including extended-spectrum β-lactamases (ESBLs), AmpC cephalosporinases and carbapenemases. Detection of these enzymes remains a challenge because of the diversity of emerging resistance, which is continuously evolving with newer targets being increasingly identified, with currently 5522 core AMR genes in the reference gene catalogue.

Dr Wooton discussed the complexity of each of the molecular classes of β-lactamases and their different resistance profiles and mechanisms, and how these can be manipulated in phenotypic tests to aid their detection. The β-lactamases are the most significant group of enzymes involved in conferring resistance to β-lactam antibiotics in Gram-negative bacteria, which work by hydrolysing the β-lactam bond of substrates thus rendering the antibiotic ineffective.

While standard antibiotic susceptibility testing detects most resistances, Dr Wooton described the current methods and phenotypic tests as the preferred methods in routine diagnostic laboratories for the 'difficult to test' resistances.

In general, isolates are screened by either chromogenic/differential agar, disc diffusion or a variety of automated methods. Phenotypic confirmatory tests include combination disc testing and gradient strips, while MALDI-ToF colorimetric tests and automated methods have so far proved less popular. Genotypic confirmatory tests include PCR/real-time PCR, rapid testing direct from sample, whole-genome sequencing (WGS) and microarrays.

Dr Wooton discussed how inhibitor-based tests with an indicator antibiotic, including combination disc testing, are relatively cheap, easy to perform and extensively validated for definitive identification and differentiation of enzyme types. Although there are some limitations due to the complex nature of resistance, for example, the detection of OXA-48 resistance, and difficulty for detection in non-fermenters, they have a good overall sensitivity and specificity.

While rapid technologies have a clinical benefit, in that they are rapid and easy to use, and are particularly useful in outbreak situations, they are expensive and the probes and primers may not be updated regularly, leading to limitations in the detection of novel or rare targets and an increase in false negatives. Similarly, WGS is currently used for special circumstances (eg tuberculosis detection), and in the future could be implemented in a routine setting; howeve, challenges in cost and timelines could be a problem.

Delegates to the conference contributed to the success of the conference, and it is planned to make the presentations available to all who attended.

Next-generation sequencing

After the morning break, Dr Nathaniel Storey from the Department of Bioinformatics, Great Ormond Street Hospital (GOSH) London reviewed advances in next-generation sequencing (NGS) for the detection of antimicrobial resistance and how this could be used in diagnostics and infection prevention control (IPC). The majority of existing NGS services use short read Illumina sequencing technologies. These are highly accurate and use short DNA reads (typically 100 bp) but generate enormous amounts of data. However, library preparations can be slow, taking one to four days, and sequencing can be expensive. These problems are compounded by the need to batch process samples to keep down costs as far as possible.

In contrast, Dr Storey described the approach used by Oxford Nanopore for NGS. This is becoming increasingly popular and may supplant Illumina-based sequencing technology if sequencing is to be implemented in smaller laboratories. Nanopore sequencing offers a number of benefits including decreased library preparation time yields very long reads of up to several megabytes of sequence and reduced hardware and consumable costs.

Hardware costs for NGS are a fraction of Illumina-based technology costs with a starter kit costing less than £1000. The space required is also low and the cost of consumables reasonable. The accuracy of this method of sequencing was much lower than that of Illumina initially but has improved hugely over the last few years.

Dr Storey took us through the use of bacterial WGS at GOSH using software from Aries Genetics – an Austrian company – enabling cloud-based data analysis that facilitates the identification and typing of bacterial pathogens from

Twenty commercial partners sponsored and supported the event.

genomic data obtained in the laboratory. This in turn enables outbreak surveillance, infection control, and AMR prediction to be carried out. He described a proof-of-principle trial in which 50 Gram-negative isolates were sequenced as they were thought to potentially contain OXA-48 or KPC. The isolates were extracted and then sequenced simultaneously using the Nanopore minION system. Some 41% of the final data were available within 24 hours and a very high coverage of the whole genome was achieved. He showed that one staff member could sequence up to 96 bacterial isolates in a working day, and the turnaround for complete typing results was between 24 and 72 hours and the cost per sample was approximately £45 per isolate.

Importantly there was no requirement for highly trained staff or bioinformaticians and a huge amount of data was provided for IPC use and outbreak analysis. Clearly, this could be a way forward and should reduce or avoid altogether the need to send samples to external reference laboratories.

Enteric issues in Wales

Michael Perry, Clinical Scientist from the Anaerobe Reference Unit in Cardiff, presented his experience and the impact of introducing molecular techniques as a replacement for enteric culture. The arguments for nucleic acid amplification tests (NAATs) in this area have been well made previously. The resource demands and slow turnaround times of traditional methods contrast with the more streamlined and increased sensitivity offered by PCR or other molecular methodologies.

Michael Perry presented a comprehensive study conducted within the Welsh network encompassing over 960,000 data points. This highlighted a significant increase in the diagnosis of key infections specifically *Campylobacter* and *Giardia*. Given that *Giardia* frequently requires intervention and treatment, this represents a significant diagnostic improvement. Another advantage to molecular methods is the ability to target specific gene targets linked to pathogenicity and the molecular approach showed a significant increase in the detection of non-O157:H7 shiga toxin-producing *E. coli*.

When implemented across the network some initial reluctance was recognised with biomedical scientists concerned about the loss of traditional skills. However, as the advantages became clear, both to laboratory workflow and patient care, support was easily built. Culture is retained (for now) through the need to isolate pathogens for further investigation and typing but skilled biomedical scientist time can now be focused on those specimens with the greatest likelihood of positivity. This has removed the need for resource-heavy manipulations of large numbers of ultimately negative stools.

Clostridioides difficile testing has also often been an area of keen debate within diagnostic microbiology. Significant variation is seen in EIA-based assays and again comprehensive data gathered from the experience in Wales suggests that fears of a surge in positivity with PCR appear to be unfounded. In fact, by using NAAT as the primary test there was a better ability to identify mild and moderate disease *C. difficile* excretors, and there was an overall drop in CDI test positivity.

In a final assessment using that most scientific of measures (a show of hands!), it was apparent that around half of the audience was currently using some form of molecular technique within their enteric sections, with the other half seeking to in the future. It would be interesting to see how this would compare in future years.

Orthopaedic device infections

Next, Dr Kevin Cole, a senior biomedical scientist from North Tyneside General Hospital, Newcastle upon Tyne, gave a review of the advantages and disadvantages of molecular methods over culture in the detection of orthopaedic device infections, and described work he had undertaken at the Royal Sussex Hospital in Brighton for his PhD.

Although rates of orthopedic device-related infection (ODRI) may be fairly low at between 1% and 2%, they can be a disaster for the individual patient, and the increased number of procedures is leading to a large increase in the overall burden of infection. The device provides a nidus for bacterial growth allowing the formation of biofilms. The lack of a vascular system means that an effective immune response cannot be mounted, and antibiotics may not be able to penetrate the site. He took us through the familiar methods of culture and antibiogram determination using methods that are common, standardised, easy to perform and give results that are normally easy to interpret, and of course are relatively cheap. Plus, the causative organism is obtained, and further work can be done on it, such as determining relatedness of different isolates.

Disadvantages include the slow turnaround times, the problems of negative culture and the fact that different strains can have identical or similar antibiograms. In addition, resistance genes may not always be expressed or indeed carried on all isolates of a particular strain if they are on mobile genetic elements (MGE).

Dr Cole then gave an overview of the various molecular methods that are available. Apart from the obvious in-house PCR methods for the detection of specific organisms and resistance genes, there are two readily available commercial systems: BioFire Film Array Joint Infection Panel and Unyvero Implant and Tissue Infection (ITI). These are expensive and have a limited number of targets, but are rapid and easy to perform and have obvious advantages over an in-house PCR method.

Going beyond this Dr Cole described the advantages of metagenomic NGS (mNGS) which is 'agnostic' as it can

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detect any organism present rather than being restricted to a limited number of predefined targets. In addition, it is more able to detect resistance and virulence markers and a lot more data about the organism are obtained, making typing for example using existing MLST schemes possible using the sequencing data obtained. Against this it has the disadvantages that is slower than other molecular methods, uses more specialised techniques and requires bioinformatic analyses.

As is always the case sequencing contaminating DNA will reduce sequencing of infecting organisms. Currently, mNGS is best placed to determine relatedness of isolates (by antibiogram, typing and sequence alignment). But there is still some way to go before molecular methods replace culture-based methods in the laboratory.

Post-COVID childhood infections

The last presentation of the day was the most topical! Dr Charlene Rodrigues, Consultant in Paediatric Infectious Diseases, St Mary's Hospital, London, and the London School of Hygiene & Tropical Medicine, updated us on group A streptococcal infections and other infections in children in the post-COVID pandemic period. The presentation gave an insight into the changes in paediatric infectious diseases that followed the COVID pandemic and restrictions in social interactions. Existing diseases re-emerged; there was an unusually high peak in group A streptococcal infections in children, which was accompanied by a corresponding increase in invasive streptococcal (iGAS) disease. Although the isolates were no more deadly and the case fatality rate was no higher, the media coverage made the public aware of the infection, and large numbers of parents were presenting in emergency departments, concerned that their children may have iGAS disease.

Some of the challenges were that the initial symptoms of iGAS are non-specific; children were swabbed but due to the relatively slow speed of culture, penicillin had to be given before culture results were available, which led to shortages. Culture did allow retrospective analysis of treatment pathways, but a rapid point-of-care test would have been more useful. Children seemed to be sick for longer and have a lot of inflammation, with different clinical phenotypes than previously seen, such as increased lung involvement, which posed the question as to whether this GAS could have other, additional virulence factors post-pandemic.

New conditions were identified in children in the post-pandemic period. Paediatric inflammatory multisystem syndrome (PIMS) occurred in some children about six weeks after becoming infected with COVID. The most serious complications of this syndrome were cardiac involvement, specifically myocarditis. Another new syndrome was acute hepatitis in young children, which was not caused by any of the hepatitis viruses (A–E). Affected children had liver failure and required transplantation. Here, metagenomics was used to sequence samples from clusters of severe cases, and was able to identify adeno-associated virus 2 in symptomatic children, although the pathogenic mechanism of this disease is still unknown.

The pandemic also had a negative effect on infection prevention, shown by the steady decline in measles vaccine uptake during this period. The UKHSA briefing in May 2023 reminded us that measles was eliminated in the UK in 2016, but vaccine hesitancy has been worsened by the COVID pandemic, resulting in an increase in cases. Measles is a highly contagious disease, easily tested by serology and PCR, but it is better to reduce the burden of infection by encouraging parents to have their children vaccinated.

There were also cases of more severe complications from enterovirus infections in neonates, with clusters of enterovirus myocarditis in South West England and South Wales – these were all disproportionately severe, but it is not known why. A possibility is that social distancing in mothers during the pandemic could have affected the severity of disease experienced by infants. There are no good management strategies for these cases, and there are currently no approved antiviral medications for enteroviral infections.

Postscript

The event did indeed cover 'Current Topics in Microbiology and Infection', with excellent speakers sharing insights into their specialist areas, from the development and control of antimicrobial resistance to infections in children in the post-COVID pandemic period.

The BSMT committee would like to extend warm thanks to all who attended and participated to make the conference a great success. The programme for the day is available on the BSMT website (https://bsmt.org.uk/), and it is planned to make the presentations available to all who attended the conference, plus to others for a small fee – please email Valerie Bevan (vbevan@bsmt.org.uk) if you are interested in receiving them.

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