Respiratory microbiology: BSMT meeting report on a day of inspiration

The 34th Annual Scientific Symposium of the British Society for Microbial Technology took place in May at the Royal Air Force Museum, Hendon. On behalf of the BSMT committee, Mark Wilks reports on a day of inspiration and excellence.

The RAF Museum in Hendon was a new venue for the British Society for Microbial Technology (BSMT), whose meetings traditionally have been held at the Public Health England laboratories in nearby Colindale. The theme of the meeting was respiratory microbiology, and full use was made of the increased space available, which permitted a record attendance of 150 delegates and a greatly increased trade exhibition.

Invasive fungal infections and the need for improved diagnostic testing

The morning session was chaired by BSMT President Professor Eric Bolton, who introduced the first speaker, Dr Samir Agrawal (Blizard Institute, Queen Mary University of London and Barts Health NHS Trust), who began with a discussion on invasive fungal disease (IFD) and the need for improved diagnostic testing. Dr Agrawal started by discussing the global burden of fungal disease, which is truly astonishing. There are around one billion superficial infections, around 1.5 million mucosal candidiasis infections, and more than 1.5 million deaths per annum due to fungi, yet they are still a neglected topic by public health authorities, even though most of the deaths are avoidable. Many of the serious fungal infections are a consequence of other health problems, including asthma, AIDS, cancers and organ transplantation. Early and accurate diagnosis permits prompt antifungal therapy, but this is often delayed or unavailable.

Looking at the most serious fungal infections by causative organism, recent global estimates have found 3,000,000 cases of chronic pulmonary aspergillosis, ~223,100 cases of cryptococcal meningitis complicating HIV/AIDS, ~700,000 cases of invasive candidiasis, ~500,000 cases of *Pneumocystis jirovecii* pneumonia, ~250,000 cases of invasive aspergillosis, ~100,000 cases of disseminated histoplasmosis, over 10 million cases of fungal asthma and ~1,000,000 cases of fungal keratitis occur annually. Patient management is key in IFD, as mortality can result in 40–90% of cases in some high-risk patient groups, with delayed treatment increasing mortality further.

Diagnosis is challenging and often clinicians treat on suspicion rather than the results of diagnostic tests. This means patient are exposed to unnecessary antifungal therapies, increasing the possibility of antifungal resistance; a state which Dr Agrawal characterised as "diagnostic nihilism". It is thought that 'optimal' management could reduce costs by £0.13 million a month and ultimately save the NHS England

Dr Mark Wilks and Dr Riccardo Alagna enjoying Dr Templeton's talk.

Dr Samir Agrawal discusses aspects of invasive fungal disease with a delegate.

Professor Chris van der Gast discussing cystic fibrosis with a delegate.

The Hendon event was supported by commercial colleagues from 20 companies.

budget ~£150 million per annum. In addition, many of the drugs are toxic.

Dr Agrawal went on to discuss his experience as a haematology oncology consultant stating quite often patients are treated for IFD with no evidence to suggest they actually have an IFD; whereas if laboratories had better diagnostic tools, this would benefit patient treatment and outcomes. Dr Agrawal showed a flow chart of IFD management which demonstrated screening strategies, diagnostic-driven strategies and empirical strategy, all of which never allow for a proven diagnosis. He went on to say it is important to know what tests are in your centre; currently β-D-glucan, galactomannan and *Aspergillus* polymerase chain reaction (PCR) are the most common tests and have been for several years, but the UK lags behind Europe and the USA in making these tests available. In addition, access to other newer tests is either not available or results are not available in a timely manner.

So, can laboratories do better? Yes! There are two rapid antigen tests (lateral-flow devices), OLM and IMMY, that are single-use tests for *Aspergillus*-specific antigens that will give results in one hour that could provide so much more information and guidance to clinicians. The results from these two lateral-flow tests were presented at the European Congress of Clinical Microbiology and Infectious Diseases (ECCMID) earlier this year, and have so far produced very impressive results, especially on bronchoalveolar lavage (BAL) samples.

Dr Agrawal concluded by saying laboratories need 'rapid' assays that can be run daily or with turnaround times <48 hours to give better diagnosis and therapy options for patients with IFD. Currently, there are no laboratories in the UK that provide these diagnostic tests. Although it is not easy for laboratories to introduce new tests, we need to. Laboratories, clinicians need you!

Cystic fibrosis lung microbiome

The next speaker was Professor Chris van der Gast (Manchester Metropolitan University), who spoke about the cystic fibrosis lung microbiome. He gave a comprehensive review of the conventional microbiology culture-based approach and the new molecular ways of studying the condition, which are leading to new insights and may revolutionise the treatment of cystic fibrosis (CF) in the near future. Cystic fibrosis is a common autosomal recessive genetic disorder, affecting approximately 10,000 and 30,000 people in the UK and USA, respectively.

A mutation is present in the CF transmembrane conductance regulator (*CFTR*) gene, which encodes for the CFTR epithelial cell membrane protein and chloride channel. The disorder is multi-systemic, affecting the lungs, gastrointestinal tract, pancreas, reproductive organs, liver and kidneys. However, lung disease, as a result of chronic microbial infection and concomitant airway inflammation, is the leading cause of morbidity and mortality in the majority of CF patients.

Absent or impaired mucociliary clearance leads to a viscous cycle of infection and inflammation, giving successive rounds of inflammation, increased mucus production and lung damage, leading to mucus retention.

Overgrowth of cultures, particularly with *Pseudomonas aeruginosa*, means that selective media are essential. This in turn means that by definition only a limited range of potential pathogens

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can be grown, typically, *P. aeruginosa*, *Staphylococcus aureus*, *Burkholderia cepacia* complex, *Haemophilus influenzae*, *Stenotrophomonas maltophilia*, and *Acromobacter xylosoxidans*.

However, it is increasingly clear that the traditional 'one microbe, one disease' concepts of infection pathogenesis and infection control are not optimal in CF management. Absent or impaired mucociliary clearance means eradication is rarely possible. Chronic lung infection in CF is polymicrobial – an infection microbiota of interacting microbes (interacting with each other and the host), ranging from strict aerobes through to anaerobic bacteria and fungal species.

How then can we look at the whole lung microbiota in CF? And what might the gains be from this comprehensive approach? Professor van der Gast described the workflow through all the different stages that he has developed, and guided delegates through the numerous pitfalls on the way, which have led to numerous studies on the microbiota of different body sites and different conditions giving questionable results.

Although his talk necessarily focused on the lung, the general principles apply to the study of all sites that have a relatively low microbial load. In fact, anyone wishing to embark on a study like this in whichever body site would be well advised to look at Professor van der Gast's presentation on the BSMT website (www.bsmt.org.uk).

Starting with sample handling and preparation, he described how respiratory samples, whether BAL or sputum, must be stabilised at –80˚C or significant changes in microbiota composition will occur. Repeated freezing and thawing samples also leads to significant changes in composition.

One important question is to decide whether extracellular DNA – which may be derived from dead cells or damaged cells – needs to be included in the analysis. He described a method using propidium monoazide to remove DNA from dead

bacteria. Propidium monoazide (PMA) is impermeable to the cell membrane so cannot enter living cells; rather it binds to the extracellular DNA of bacterial cells and when exposed to light modifies the DNA template, making it impossible to amplify. Therefore, any DNA which is amplified is derived from living cells and therefore likely to be more relevant.

The next question is which DNA extraction protocols should be followed, In addition, the cell walls of some bacterial species, particularly high GC containing Gram-positive bacteria and especially mycobacteria, are very hard to disrupt.

An important caveat here is to bear in mind that commercial kits are often contaminated with low levels of bacterial DNA. A large number of negative controls and possibly mock communities as positive controls need to be run. This is in contrast to the normal use of water as an adequate negative control in PCR.

Most of Professor van der Gast's work has been done on the Illumina MiSeq, which is reasonably easy to use and it is possible to multiplex up to 384 samples in one run to keep the costs down and throughput up. Bioinformatics pipelines are needed to convert raw sequence data into something usable. The raw data consist of millions of sequences and they have to be organised and poor sequences removed to get a reliable result. He described the use of two programs, QIIME and Mothur, to analyse data and the move away from operational taxonomic units to exact sequence variants. All this means that is possible to study comprehensively the microbiota of the CF lung and obtain reliable results.

Professor van de Gast's talk focused on methodology rather than results. A previous presentation given by the professor at the BSMT meeting in Liverpool in October 2018 is also on the BSMT website. This shows how careful attention to detail described above can yield reliable results and shows the quantitative and qualitative leaps in our understanding of the microbiology fibrosis can be made using this approach. These methods are being applied to a long-term study of the Manchester paediatric and adult CF patients.

Non-tuberculous mycobacterial infections

The next speaker was Dr Katherina Kranzer (recently Clinical Director of the Supranational and National Tuberculosis Reference Laboratory in Germany, and currently based at the London School of Hygiene and Tropical Medicine). Dr Kranzer has worked in South Africa and other sub-Saharan countries with a focus on TB and HIV. In her presentation, she gave delegates a great insight into non-tuberculosis mycobacteria (NTM), highlighting the challenges of collating national data and susceptibility testing.

Dr Kranzer's experience working in different countries allowed her to present some of the challenges with collating national data on isolates and sensitivities. She highlighted the advantage of having robust national reference laboratories, with guidance on isolates to be sent for confirmation, compared to countries with private laboratories or fragmented services where not all isolates are sent routinely. The former allows for clear conclusions on NTM and *Mycobacterium tuberculosis* (TB) infections to be made, or changes in resistance patterns to be monitored.

The low numbers of NTM in the UK, some of Europe and the USA (range 1–6/100,000) certainly fulfil the definition of a rare disease (<5/10,000 people) based on numbers affected, but there is ambiguity when assessing whether NTM disease is life-threatening or chronically debilitating.

Non-tuberculosis mycobacteria are certainly reported to have high mortality, but patients often have multiple co-morbidities, which contribute to this rather than the organism itself. Furthermore, the difficulty in establishing whether a patient is colonised or has true disease makes reaching a clear conclusion about whether or not the NTM caused death unattainable.

Dr Kranzer discussed the number of challenges faced for drug susceptibility testing for TB and NTM, as follows:

- EUCAST breakpoints unavailable therefore micro-broth dilution required
- the effect media used can have on MIC values (eg clarithromycin)
- use of TB gene targets to assess resistance, not specific NTM targets
- clarithromycin is the only drug with clinically meaningful breakpoints where resistance has been correlated with clinical outcomes.

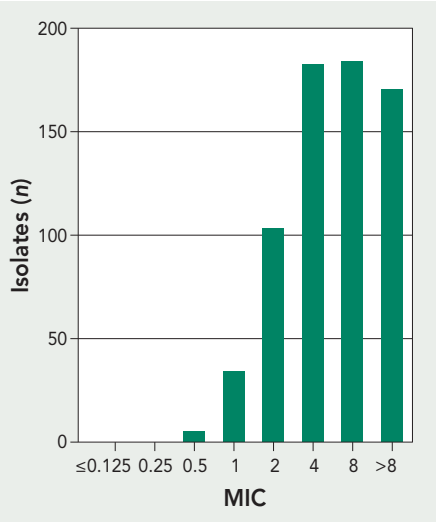

Fig 1. Aggregated MIC distributions for all of the tested *Mycobacterium avium-intracellulare* complex (MAIC) isolates for rifampicin.

Dr Kranzer spoke of her personal experience examining minimum inhibitory concentration (MIC) distribution curves for multiple drugs tested against *M. chimaera*, *M. intracellulare*, *M. colombiense* and *M. avium* in order to ascertain an epidemiological cut-off (ECOFF). Although possible for certain drugs, many of the distribution curves do not have the 'tail-end' result that you would expect, making it impossible to be able to establish a true ECOFF value, with most of the MIC values that were tested condensed around the higher values (Fig 1). Cut-offs for rifampicin, ethambutol, ciprofloxacin, isoniazid, trimethroprim/sulfamethoxazole and doxycycline could not be determined as a result of this.

Her insightful talk on NTM certainly emphasised the clinical importance of these infections and also the significant difficulties and suboptimal nature of phenotypic drug susceptibility testing and correlation to outcomes for these infections.

New diagnostics for MDR/XDR-TB

The meeting resumed after lunch, and an opportunity to interact with the sponsors supporting the event. The session was chaired by Professor Brian Duerden CBE, who introduced the first speaker, Dr Riccardo Alagna (Emerging Bacterial Pathogens Unit, WHO Collaborating Centre and TB Supranational Reference Laboratory, Milan).

Dr Alagna opened the talk with an update on the global burden of drug-resistant TB and advised delegates that the problem is getting bigger. The estimated future burden of MDR-TB in 2040 obtained by mathematical modelling suggests significant increases of 12.4% in India, 8.9% in the Philippines, and a worrying 32.5% increase in Russia. He then went on to explain the differences between phenotypic and genotypic diagnostic methods for TB, and suggested that phenotypic diagnostics for susceptibility testing were becoming outdated and we should consider using genotypic diagnostics in the future.

Examples included the difference between wild-type and mutated isoniazid resistance related to MICs, where the mutation promoter or coding may affect the MIC distribution. He advised that mutations in different genes related to the same antibiotic (eg isoniazid) and could produce different MIC values, some of which may be considered sensitive and

some resistant. There was concordance between genotypic prediction and phenotypic resistance for rifampicin and isoniazid, but less for ethambutol and pyrazinamide. He advised that detection of mutation in injectable drugs for TB should overrule the phenotypic DST result.

Dr Alagna suggested that the Cepheid GeneXpert assay has revolutionised the detection of rifampicin resistance with a high sensitivity for MTB genomes and the detection of mutations found within the DNA fragment, which are responsible for 95% of rifampicin-resistant cases. The GeneXpert MTB/RIF assay is now a World Health Organization (WHO)-approved molecular diagnostic assay for a limited number of genes and a limited number of specific mutations. However, he pointed out that use of such an approach is not risk free and some gene mutations within DRS strains carrying the *rpoB* mutations may be missed.

He suggested that the use of whole-genome sequencing (WGS) may change the way we diagnose TB significantly and have a huge impact on studies of TB epidemiology. The time to result is significantly reduced and WGS replaces several molecular diagnostic assays and will screen for all mutations in any gene with a high specificity and sensitivity. Whole-genome sequencing is ready to be incorporated into European programmes for cross-border identification, management and prevention of TB outbreak scenarios.

A single method would allow for standardisation of diagnostics and grading of mutations, and the prediction of drug sensitivity. However, one important current disadvantage of this method is the need to start analysis from a positive culture. Although studies are underway to screen directly from sputum and other LRTI samples.

Finally, Dr Alagna went on to describe the multinational CRyPTIC studies using a microtitre sensitivity MIC plate to complement WGS when high-throughput antibiotic susceptibility testing is required to confirm WGS susceptibility prediction.

Respiratory tract infections: diagnostic tests which make a difference

The next speaker was Dr Kate Templeton (Consultant Clinical Scientist and Honorary Lecturer in Medical Microbiology, Edinburgh) on the subject of respiratory tract infections, and the diagnostic tests which make a difference. Dr Templeton explained that community-acquired pneumonia (CAP) is the most frequent cause of death due to infection. While TB deaths have decreased over the past 100 years, those due to pneumonia as the root cause from other pathogens have not.

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Mortality from a simple chest infection is less than 1%, but some cases can progress to respiratory failure or acute respiratory distress syndrome, requiring intensive care.

Dr Templeton reported on an innovative approach combining state-of-the-art diagnostics using PCR to identify possible pathogens and complementary critical care guidelines. Traditional methods only yield a putative pathogen in 50% of cases, and are particularly poor for atypicals and viruses.

A pre-study audit revealed that of 44 CAP patients, all had received antibiotics. The question was posed, can we do better? A flow diagram of testing and treatment algorithm produced in conjunction with clinicians was produced, aligned to testing protocols. In the study, all patients had requests for tests by electronic orders, and patient notes enforcing the treatment algorithm, essentially telling clinicians what to do in response to a particular result. Results were reported and linked to clinical care guidelines (eg 'stop antibiotics'). In particular if the results of *Legionella*, *Mycoplasma* and *Chlamydia* testing were negative, then clinicians were asked to stop prescribing clarithromycin.

A repeat audit of 50 CAP patients showed that although there was still some inappropriate specimen requesting, it was much improved; in particular, more BALs and urinary *Legionella* antigen tests had been requested. For example, there were only 12 out of 44 requests for a urinary *Legionella* antigen test before the study, and this had risen to 40 out of 50 cases in the repeat audit. Similarly, a treatment decision to stop clarithromycin was noted in the electronic patient record (EPR) in 78% of cases.

A similar approach was described for the paediatric ICU where ward rounds involved IPC nurses, virology, microbiology and infectious disease specialists. Regular reporting and increased help with the interpretation of tests led to significant changes in antibiotic prescribing; for example, if a pertussis PCR was negative then it was safe to stop clarithromycin. Similarly, an uncomplicated viral infection, diagnosed by PCR, does not need antibiotics.

Does point-of-care testing (POCT) have a role? Dr Templeton reported on a multicentre study attempting to answer this question using PCR platforms installed at several trusts. Before the study commenced a huge amount of preparation was needed, a business case was written, ward staff were trained and competence tested, and cascade training initiated, SOPs and algorithms on how to respond to the results agreed. During winter pressures, initial duplicate testing was stopped as 99% concordance had been established between POCT and repeat testing in the laboratory. Change of antibiotic use was recorded, treatment processes were updated and significant sums of money were saved on antibiotics, which funded the POCT.

Next, Dr Templeton described a recent study involving the comprehensive molecular testing and conventional testing to cover a very large number of respiratory pathogens in CAP. This study, probably the most comprehensive of its kind, showed that a putative pathogen could be isolated from up to 90% of cases – generally bacterial, but in a quarter of the cases bacterial and viral co-infections were detected. Antibiotic exposure prior to sputum sampling occurred in 84.8% of patients and not surprisingly this was associated with culture negativity.

Although it did not affect PCR positivity in the same way, the mean combined bacterial load was significantly higher. Encouragingly, the results showed that it would have been possible to de-escalate patient therapy in 77% of cases, with only 5.9% requiring an escalation.

The talk concluded with a look beyond the mere detection of possible pathogens by considering recent studies that have looked at the host response; for example, to differentiate between bacterial and viral pneumonia. Similarly, temporal dynamics of host molecular responses suggest that there are differences between symptomatic and asymptomatic influenza A infection. This is likely to be an increasingly important field in the future.

Severe respiratory infections in the ICU

The meeting concluded with an exhilarating presentation by Professor Mervyn Singer (Bloomsbury Institute, Professor of Intensive Care Medicine, University College London) on severe respiratory infections in the intensive care unit (ICU).

Before guiding delegates through the alphabet soup – CAP, hospital-acquired pneumonia (HAP) and ventilator-associated pneumonia (VAP) – of severe respiratory infections, he discussed the apparent severe increase in the rate of sepsis recorded on the NHS England sepsis dashboard. In some cases, however, the distinction between sepsis and a severe respiratory infection is not clear-cut. Since February 2017, the number of emergency sepsis admissions has more than doubled. It's hard to believe that this is due to a real increase in this condition and various reasons were discussed.

> An innovative approach has combined diagnostics using PCR to identify possible pathogens and complementary critical care guidelines

One factor that is rarely mentioned is the tariff received for a patient admission, so a patient with a primary diagnosis of pneumonia will attract a tariff of £7000 to £8000, whereas a patient with a primary diagnosis of sepsis may actually be not as severely ill but would nevertheless attract a tariff of >£10,000! There is therefore an incentive to report cases as sepsis rather than pneumonia

The mortality from a simple chest infection is less than 1%; however, some cases can progress to respiratory failure or acute respiratory distress syndrome (ARDS) where the host response to insult leads to an outpouring of fluid and white cells into the lungs. At this stage there will be a need for oxygen with or without non-invasive ventilation, and while mortality increases it is still less than about 5%. Full ventilatory support carries much higher mortality in the range of 10–60%, and in some cases requires ECMO where the patient's blood is removed, oxygenated and returned.

Professor Singer then guided delegates through numerous challenges and diagnostic conundrums of the different respiratory infections from the perspective of an ICU consultant. In CAP, patients are most likely to be infected with a Gram-positive organism, especially *Streptococcus pneumoniae*. Viral infections are usually seasonal and may be accompanied by secondary bacterial infection such as a *Staphylococcus* or *Streptococcus*. In patients with underlying pathology or co-morbidities, colonisation may lead to infection; for example, *Pseudomonas aeruginosa* in cystic fibrosis or *Haemophilus influenzae* in COPD

patients, and of course there is always a possibility of reactivation of disease (eg tuberculosis).

Although microbiology laboratories spend a lot of time worrying about the so-called 'atypicals' and whether they should introduce a PCR test for these, he suggested that in fact infections with *Legionella* and *Mycoplasma* are very rare in this group. A quick show of hands suggested this was also the experience for others in the audience. Probably more attention should be focused on unusual pathogens (eg *Pneumocystis jirovecii*, cytomegalovirus [CMV], fungal, TB) with endogenous (eg HIV) or therapeutic immunosuppression.

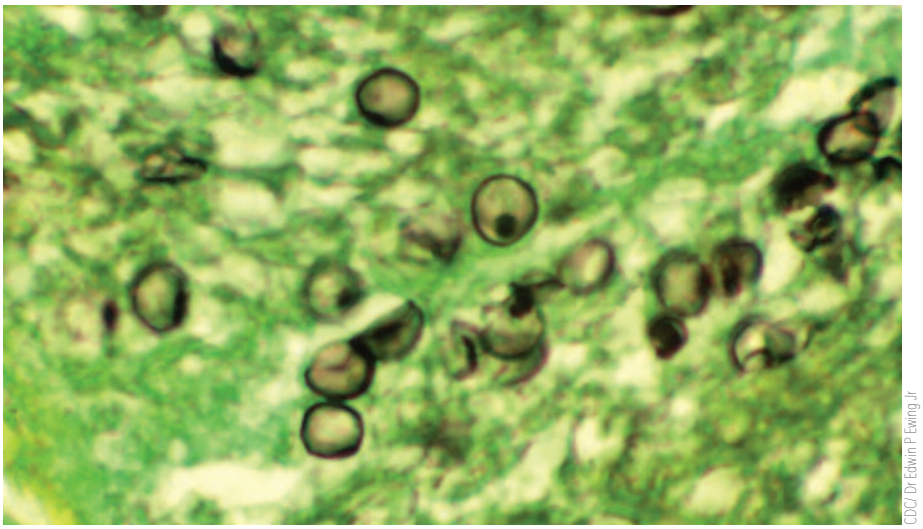
In HAP or VAP, this often relates to transmission of local pathogenic flora (mainly by cough/droplet spread) or endogenous (from oropharyngeal/intestine). Bacteria, when found, are predominantly Gram-negative and the particular organism and sensitivity pattern often follows the local hospital pattern.

In immunosuppressed patients, those on chemotherapy or post-transplant and those who are long-term ICU patients, you should not only think about Gram-negatives, but Gram-postives and more importantly other organisms such as fungi CMV, Epstein-Barr virus [EBV] and *Pneumocystis jirovecii*.

Whatever the condition, the diagnostic challenges are numerous and a causative organism is infrequently identified. An overall yield of 30–50% (blood, sputum, BAL) is typical. It is hard to know why this is so but three factors were mentioned. A putative bacterial pathogen may not grow, especially as the patient will almost certainly be on antibiotics, it might not be due to bacteria at all – a third of community infections are thought to be viral – or it might not be due to infection at all. False positives are also thought to be common, which could be due to overgrowth of commensals, low levels of multi-organism, or even high-level mono-organism, growth.

Professor Singer made the point that WBC activation and other markers of host response, which could help differentiate colonisation from infection, are rarely looked for.

These problems may be compounded by long delays before identification of organisms and sensitivity patterns are obtained, and molecular techniques underused compared to other European countries. These problems are compounded by the patient's underlying conditions, which may be nothing to do with infection. There may be other acute and/or chronic pathologies and co-morbidities that necessitate complex treatments and which make interoperation of microbiology results more difficult, including chest trauma, ARDS, lung fibrosis, and complications of chemotherapy.



CDC/ Dr Edwin P Ewing Jr

Lung tissue containing *Pneumocystis jirovecii* (methenamine silver staining).

Even when a plausible pathogen has been isolated, Professor Singer pointed out that there are still numerous treatment uncertainties – should we use mono- or combination treatments, for how long, and what about the role of adjuvant therapy (eg steroids)?

Intensive care unit patients with new signs or symptoms are often treated empirically and often on rather tenuous grounds. This may relate to clinician confidence or lack of confidence in withholding treatment, and probably leads to excessive and unnecessary antibiotic use. This in turn encourages resistance and overgrowth. It has also become more apparent in recent years that this has deleterious effects on the patient's microbiota, not to mention the direct toxicity of a lot of antibiotics.

There remain numerous uncertainties about the best treatment regimen; for example, in the treatment for CAP. Cephalosporins are common, but should you include a macrolide? Is mono- or combination therapy best for HAP or VAT? It is often not clear in outcome studies whether death was attributable to the HAP/VAT or not. Some studies use a clinical test of cure and others are microbiological. How long should therapy last? There is an increasing tendency towards short duration of treatment; is four to five days sufficient and if so could it even be shorter? There is particular paranoia around pneumococcal infection where long treatment regimens are common. Is this justified?

Professor Singer moved on to take a look at current novel diagnostic tests, describing first the results of the Abbott Iridica system in a multicentre trial for the rapid diagnosis of infection in the critically ill. This used a combination of PCR and mass spectrometry to obtain a result directly from blood in about six to eight hours. The results he described showed a much higher yield compared to culture, which makes sense as many of the patients had been on antibiotics. He then described work on the T2 system which gave pathogen identification, resistant detection and susceptibility even quicker – in one to two hours – from blood, urine or sputum. Whereas the Abbott Iridica system detected a huge range of bacteria, the T2 system is restricted to a limited number of organisms, essentially the so-called ESKAPE pathogens.

Professor Singer concluded his talk by describing some work by a colleague, Professor Kev Dhaliwal from Edinburgh University, using the novel technique of optical endomicroscopy, in which a fibreoptic catheter is passed down into the lung, making it possible to visualise what is actually happening in the alveoli. He showed fascinating photographs of the normal lung, contrasting with a *Staphylococcus aureus* infection where the developing bacterial colony can be seen. Cases of oedema and alveolar collapse were also shown, and examples can be seen in Professor Singer's presentation on the BSMT website (www.bsmt.org.uk). While this is still very much a research technique, in the future it might be possible to visualise bacteria and to observe directly the infection and the host response to that infection in real time. A fantastic prospect on which to finish!

Dr Mark Wilks is Clinical Scientist in Microbiology at Barts Health NHS Trust. Copies of all presentations given at the Annual Scientific Conference at the RAF Museum, Hendon, may be found on the BSMT website (www.bsmt.org.uk).