MASTmatters

Celebrating 60 years of Innovation

1957–2017

60 years of Microbiology, Antibiotics and Vaccines

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In 2007, I wrote "Fifty years of microbiology, antibiotics and vaccines" to commemorate the 50th birthday of MAST® and already it is time to consider the events of a further decade. In my mind, there have been many transformations but, in particular, four that stand out as "game changers".

Today's microbiology laboratory is facing the "perfect storm"; an aging workforce with a shortage of skilled scientists to replace them, the need to increase productivity and turnaround times and the necessity to decrease errors. Compared to 10 years ago, today's laboratories are much more unified, standardised and accountable. Chemistry, Immunology and Haematology departments are often merged into Blood Sciences although Microbiology and Histology have, by the nature of their specimens, resisted this merger. There has also been a tendency, over the past 10 years, to centralize clinical laboratories so that they serve several hospitals. Many laboratories now offer seven day services with a 24/7 on-site shift system and adopt LEAN approaches. Centralisation may distance patients and clinicians from the laboratory and cause delays in specimen transport but use of approved Point of Care (POC) tests may negate some of these problems, a development I would consider the first "game changer". Today, there are several syndrome or disease based POC tests for pneumonia, sexually transmitted diseases (STD), pharyngitis, influenza, MRSA, meningitis, gastroenteritis and fever in returning travellers.

Although Microbiology methodology is still largely culture based, there have been significant innovations in this field. Chromogenic culture media now permits specific detection of additional pathogens and pathogens with acquired antimicrobial resistance, including vancomycin-resistant enterococci (VRE), carbapenem-resistant-Acinetobacter spp. (CRA), and Enterobacteriaceae producing extended-spectrum B-lactamases (ESBL) and carbapenemases (CPE). Recent genomic and metagenomic studies have demonstrated that approximately 80% of bacterial species detected in the human gut are uncultured so far, uncultivable or overlooked because they are only present in low numbers. This has driven an interest in "Culturomics" and, in 2015, microbiologists discovered 35 new groups of bacteria in groundwater in Colorado using ultrafiltration which had evaded over 130 years of conventional cultivation studies[1]. Microscopy has also evolved, as has the use of acute-phase proteins. In the last decade, microbiology laboratories have gradually increased their use of automation which has been held back by the large number of diverse human activities and by the variety of specimen types and containers in bacteriology. Developments in laboratory automation such as automated methods for inoculation of culture plates and methods for automated digital analysis that enable detection and enumeration of coloured colonies, e.g. WASPLab detection of MRSA or VRE, strengthen the continued role of culture media and chromogenic media in particular. Full laboratory automation should be the norm within the next 10 years.

My second "game changer" of the last decade has been the introduction of Matrix-Assisted Laser Desorption Ionization–Time Of Flight Mass Spectrometry (MALDI-TOF MS) into routine laboratories. This proteomic approach has truly revolutionised identification both from culture and from clinical specimens as well as the detection of certain AMR mechanisms.

Microbiologists now make greater use of molecular techniques for the detection of pathogens directly from clinical material. Modern PCR panels can detect a range of Gram-positive and Gram-negative bacteria and *Candida* spp. along with a few resistance genes (for example *mecA*, *vanA/B*, NDM, KPC, OXA, VIM and CTX-M). In contrast to blood culture panels, enteric panels are standalone replacements for conventional methodology and can detect a range of bacteria, parasites and viruses. Modern and specialised panels are capable of detecting pathogens such as *Escherichia coli* K1, *Haemophilus influenzae*, *Listeria monocytogenes*, *Neisseria meningitidis*, *Streptococcus agalactiae*, *S. pneumoniae*, Cytomegalovirus (CMV), enterovirus, herpes simplex 1/2, human herpesvirus,

human parechovirus, *Varicella-Zoster* virus (VZV), and *Cryptococcus neoformans/ gattii* in cerebrospinal fluid (CSF). Although phenotypic Antibiotic Susceptibility Testing (AST), based on Minimum Inhibitory Concentrations (MICs) and clinical breakpoints, remains the gold standard for *in vitro* prediction of AMR, Real-Time PCR (RT-PCR) has been applied to the rapid screening and detection of common antibiotic resistant genes. Molecular techniques undoubtedly have a role for detection of *mecA*, *vanA/B* and known carbapenemases but cannot cover all resistance determinants (e.g. > 1500 β-lactamases), porin mutations, efflux pumps or binding site modifications. Further, the presence of a particular gene/mutation does not always confer phenotypic resistance. RT-PCR of universal genes has become a powerful technique. The sequence of some genes in 16S rRNA (e.g. *rpo*B) differ between bacterial species and can therefore be used for bacterial identification; these are held in databases along with increasing numbers of 18S rRNA sequences.

Whole Genome Sequencing (WGS) has become fast, relatively cheap and compatible with the routine clinical microbiology workflow. In one single step, WGS has the potential to provide, nearly all the information required to detect and characterise bacteria, to carry out AMR testing, to identify virulence determinants, to ultimately implement public health measures. A laptop-based handheld sequencer may soon be cost-effective enough for routine use. Online databases (e.g. GenBank) and bioinformatic pipelines have developed considerably. WGS can dissect out virulence determinants, AMR markers and genotypes of unusual or difficult to grow bacterial strains.

The introduction of Next-Generation Sequencing (NGS) technology is my third "game changer" of the last decade.

Clustered Regularly Interspaced Short (CRISPR) Palindromic Repeats gene editing tools allow experts to edit genes with never-before seen precision, using a chemical process to cut and paste the DNA of any living thing. This opens the door to all kinds of potential medical and biological breakthroughs, from treating cancer and HIV to editing animal genes to make them more disease-resistant. In 2008, Craig Venter announced that his laboratories had synthesized the genome of *Mycoplasma mycoides* from a computer record and transplanted it into the existing cells of *Mycoplasma capricolum* devoid of DNA; the semi-synthetic bacterium remained viable[2]. This work cost $40 million (USD) and took 20 people more than a decade to complete, however it does show great promise for a future where organisms can be developed to meet very specific needs. Molecular and cellular biology are no longer discrete subject areas but vital tools and an integrated part of current microbiological research. The development of this variety of molecular tools has led to major changes in microbial taxonomy with the introduction of new genera, species and subspecies and the reclassification of established pathogens. As part of this revolution in molecular biology, the genomes of a growing number of pathogenic and model bacteria have been fully sequenced, with immense implications for our future understanding of microorganisms at the molecular level.

NEW & EMERGING PATHOGENS

The World Health Organization (WHO) has identified the top 8 emerging diseases that are likely to cause severe outbreaks in the near future. The list excludes diseases that already have sufficient Research and Development programs and funding, including HIV/AIDS, tuberculosis, and malaria. (CCHF) Crimean-Congo haemorrhagic fever is a tick-borne virus primarily found in Africa, the Middle East and Asia. It causes death in up to 40% of cases and has no vaccine[3]. Filovirus diseases include Ebola; there were severe outbreaks in West Africa in 2014-2015, the disease is often fatal if untreated and there are currently no vaccines. MERS-CoV and SARS are both respiratory syndromes caused by coronaviruses. MERS-CoV virus does not pass between humans (camels are the suspected transmitter) but SARS was the first pandemic of the 21st century. The first SARS-CoV infections were reported in late 2002, the pandemic escalated in February 2003 and, over the subsequent 100 days, more than 8000 cases were reported in 29 countries resulting in 775 deaths[4]. By contrast, Lassa fever has a death rate of only 1%, with only 1 in 5 infections resulting in severe disease where the virus affects several organs. Around 80% of people who become infected with the virus have no symptoms[5]. Nipah virus was first identified in 1998, and affects both humans and animals; there is no vaccine and treatment is in the form of intensive supportive care. Rift Valley fever primarily affects animals, but the disease has also been found in humans where a small number develop a more severe form which can lead to death. This virus is spread by a variety of biting insects but does not transmit from person to person and Man is infected by mosquitoes that have previously bitten livestock; the death rate is only 1% but rises to 50% if bleeding occurs[6]. A further three diseases were included and listed as serious, requiring action as soon as possible: Chikungunya (a viral disease transmitted by mosquitoes and identified in over 60 countries across the world); severe fever with thrombocytopenia syndrome (first identified in rural China, with 2500 reported cases by 2014 and a death rate of 7%)[7]; Zika (declared a public health emergency in Brazil in 2016 with more than 4300 cases of microcephaly recorded[8]; the disease has been found in 61

countries). Other infections to watch out for in 2017 are: Leishmaniasis (a problem among internationally displaced people, spread by sandflies); Oropouche (spread by widely distributed *Culex* mosquitoes) and Mayaro (spread by biting *Aedes* mosquitoes). In 2007, *Waddlia chondrophila* (causing miscarriages) and *Alloscardovia omnicolens* (causing UTI in the elderly and those with predisposing factors) emerged and 2010 saw the arrival of *Neoehrlichia mikurensis* (a zoonotic disease associated with the immunocompromised).

VACCINES

The Decade of Vaccines Collaboration (DoVC) was formed in 2011. The Global Vaccine Action Plan (GVAP) was endorsed by the 194 Member States of the World Health Assembly in May 2012 to achieve the Decade of Vaccines vision by delivering universal access to immunisation. The DoVC set out to save millions of lives, enhance economic activity and deliver on Millennium Development Goal #4 (reducing under-five mortality by two-thirds by 2015). In addition, the aim is to create vaccines for new, emerging and unknown diseases before they cause global health emergencies. It is estimated that improved vaccine coverage for *S. pneumoniae* could avert 11.4 million antibiotic days per year in children < 5 years worldwide. New vaccine delivery systems are anticipated and a number of heat-stable vaccines have become available. By 2020, developing countries may have the capacity to make their own bespoke vaccines. Considerable progress has been made in identifying the signalling pathways and receptors of the innate immune system.

ANTIBIOTIC RESISTANCE

The turn of the century saw the development, and spread, of AMR due to CTX-M ESBLs, VRSA and extensively drug-resistant *Acinetobacter* spp. Carbapenem resistance is associated with poor antibiotic stewardship and continues to be a growing problem worldwide. KPC producers were first reported in 1996 in the United States and then spread globally, particularly in Puerto Rico, Colombia, Greece, Israel, and China. Within a decade, they spread worldwide with a mortality of 40-80%[9]. The New Delhi Metallo-β-lactamase (NDM-1) was first described in *E. coli* in New Delhi in 2009[10]; it rapidly became endemic throughout South Asia and the Balkan states and spread to 40 countries within 5 years amongst Enterobacteriaceae, *Acinetobacter* spp. and pseudomonads. OXA-48 first appeared in 2001[11] and has spread worldwide over the last 15 years. *A. baumannii* causes severe infections that primarily affect ICU patients. In Chile, carbapenem resistance in the ICU is over 70%, leaving few therapeutic options (polymyxins and tigecycline) for treatment of severe cases[12]. Both clonal transmission within hospitals and inter-hospital transmission have been reported. *A. baumannii* has a high prevalence of multidrug resistance, including carbapenems (MDR) and can be extensively drug-resistant (XDR). In 2015, scientists in China identified a plasmid-carried gene conferring resistance to polymyxins[13].The gene, *mcr-1*, represents the first known plasmid-mediated resistance to polymyxins, which are considered antibiotics of last resort for Gram-negative bacteria. Since its discovery, *mcr-1* has been identified in Enterobacteriaceae from humans, animals and meat in at least 5 continents and, in at least one case, has passed into a highly resistant carbapenemase-producing organism[13].

Neisseria gonorrhoeae has developed increasing resistance to oral antibiotics (e.g. azithromycin, fluoroquinolones, and cefixime) previously used to treat this infection and has become one of the leading microbial threats to public health. Treatment failure has been confirmed in at least 10 countries and increasing rates of resistance to oral agents have left ceftriaxone as the last remaining reliable treatment for gonorrhoea, although resistance has been reported[14]. Treatment guidelines are moving towards the use of drug combinations, e.g. ceftriaxone plus azithromycin, and recent clinical studies have identified drug combinations that could be used for salvage treatment of non-responders, such as azithromycin in combination with either gentamicin or gemifloxacin.

Although there is a year-on-year decline in MRSA cases recorded by English NHS Acute Trusts, the annual decline in MRSA cases now is a third of what it was 6 years ago[15]. The incidence of Community-Associated MRSA (CA-MRSA) is <1% of all MRSA infections, but is rising. Daptomycin, linezolid, and oritavancin have become available for treatment of serious resistant Gram-positive infections, offering alternatives to vancomycin. Numerous isolates of MRSA with reduced susceptibility to vancomycin (hVISA) have been identified in clinical infections. Vancomycin resistance is prevalent in other bacteria, such as Vancomycin-Resistant Enterococcus (VRE), and several cases of Vancomycin-Resistant *Staphylococcus aureus* (VRSA) have been reported in the literature since 2002[16]. Hypervirulent strain BI/NAP1/027 of *Clostridium difficile* has increased resistance to fluoroquinolones and has a selective advantage in patients treated with this antibiotic. Recurrent *C. difficile* disease occurs in approximately 20% of patients[17], underscoring the importance of prevention. Newer antimicrobials, such as fidaxomicin, have some efficacy both in treatment of infection and prevention of relapse. Although global mortality from tuberculosis (TB) has declined over the past 20 years, this reduction may not be enough to meet the target set out in the Millennium Development Goals and, in 2011, there were more than 8 million infections and 1.4 million deaths from TB. WHO estimates that, in 2014, there were about 480,000 new cases of multidrug-resistant tuberculosis (MDR-TB) defined as being resistant to two first-line anti-TB drugs[18]. Globally, only half of MDR-TB patients were successfully treated in 2014; among new TB cases in 2014, an estimated 3.3% were multidrug-resistant. Extensively drug-resistant tuberculosis (XDR-TB) is resistant to at least 4 of the core anti-TB drugs and has been identified in 105 countries; an estimated 9.7% of people with MDR-TB have XDR-TB. In July 2016, resistance to the first-line treatment for *Plasmodium falciparum* malaria (artemisinin-based combination therapy) was confirmed in 5 countries of the Greater Mekong sub-region[19]. The spread of resistant strains to other parts of the world would pose a major public health challenge and jeopardise important recent gains in malaria control. In

2010, an estimated 7% of people starting antiretroviral therapy in developing countries had drug-resistant HIV; in developed countries, this figure was 10–20%. Some countries have recently reported levels at, or above, 15% amongst those starting HIV treatment, and up to 40% among people re-starting treatment. Second and third-line HIV regimens are 3 times and 18 times more expensive, respectively, than first-line drugs. Virtually all influenza A viruses circulating in humans are resistant to M2 inhibitors (amantadine and rimantadine) although the frequency of resistance to the neuraminidase inhibitor oseltamivir remains low (1-2%).

NEW ANTIBIOTICS

The pace at which new antibiotics have been introduced has slowed considerably; 16 antibiotics were approved by FDA between 1983 and 2012 whereas only 2 were approved between 2008 and 2012 and a total of 6 new antibiotics have been approved since the end of 2012. Scientific barriers to drug discovery (especially for Gram-negative bacteria), regulatory challenges (the licensing process has become more complex, expensive and discouraging), diminishing returns on investment (antibiotics are less commercially attractive than long-term treatments for chronic disease), the potential for rapid emergence of resistance, antimicrobial stewardship limiting access to new antibiotics and difficulty with relevant clinical trials have led major drug companies to scale back or abandon their antibiotic research. Of greater concern is the fact that nearly all antibiotics brought to market over the past 30 years have been variations on existing drugs and the emergence of antibiotic-resistant pathogens has accelerated.

Linezolid (discovered in 1995 and approved in 2000) was the first representative of a major new structural class of antibiotics (the oxazolidinones) to be approved in 35 years. This innovation gap still exists, with representatives of only four new classes of antibiotics (lipopeptides, glycylcyclines, pleuromutilins and diarylquinolines) reaching the market. Other antibiotics introduced around this time included telavancin (lipoglycopeptide discovered 2000, approved 2009), ceftaroline (novel cephalosporin, approved 2010), fidaxomicin (macrocyclic for *C. difficile* associated diarrhoea, approved 2011) and bedaquiline (diarylquinolone anti-mycobacterial, approved 2012). It can take inordinate lengths of time to progress antibiotics even as far as clinical trials (60 years from discovery in the case of the pleuromutilins)[20]. Registration of antibiotics goes through three phases:

Phase I; to see what dose can safely be given to the patient
Phase II; to see if it cures infections
Phase III; to compare its efficacy to that of "standard of care treatment".

This process takes at least five years and costs around £500 million. A proposed new registration scheme would allow provisional registration at phase II (solving the unmet medical need). In 2012, Congress passed the Generating Antibiotic Incentives Now (GAIN) Act - my fourth "game changer", which provides five extra years of patent protection and a fast-track approval process through the FDA for new antibiotics; as of February 2014, 17 of the 45 new antibiotics in development qualified for benefits under the GAIN Act. To temper this, all these antibiotics are modifications of known classes and only 1/3 of them showed activity against Gram-negative ESKAPE pathogens (*Klebsiella pneumoniae*, *Acinetobacter baumannii*, *Pseudomonas aeruginosa* and *Enterobacter* spp.).

Three new antibiotics were approved for the treatment of acute bacterial skin and soft tissue infections usually caused by *S. aureus* or *Streptococcus pyogenes*: dalbavancin (lipoglycopeptide discovered 1999, approved FDA 2014); oritavancin (lipoglycopeptide discovered 1996, approved FDA 2014) and tedizolid (oxazolidinone discovered 2005, approved FDA 2014). Two new cephalosporins with or without an old beta-lactamase inhibitor and an old cephalosporin in combination with a relatively new beta-lactamase inhibitor have been recently approved: ceftobiprole (a 5th generation cephalosporin); avibactam (discovered 2004 and approved as ceftazidime/avibactam by FDA in 2015); ceftolozane (discovered 2004 and approved as ceftolozane/tazobactam by FDA in 2014).

Scientists are now examining underexplored environments for antibiotic-producing microorganisms e.g. cave bacteria. Deep sea sediment samples have given rise to abyssomicins, polycyclic antibiotics from the new marine actinomycete taxon *Verrucosispora*. Recently, nasal *Staphylococcus lugdunensis* has been shown to produce lugdunin, a novel thiazolidine-containing cyclic peptide antibiotic that prohibits colonisation by *S. aureus* giving hope that human microbiota could be considered as a source for new antibiotics. Formicamycin-producing *Streptomyces formicae* have been isolated from the African plant ant *Tetraponera penzigi*. The Community for Open Antimicrobial Drug Discovery is a nonprofit initiative that aims to pool together the many thousands of chemicals made by academic chemists and screen them for potential antibiotic use. The majority of the antibiotics we have today were discovered as growth by-products of cultivable bacteria, many from soil. However, we've cultured less than 1% of the bacteria on the planet[21]. Technology such as iChip (NovoBiotic Pharmaceuticals) permits the "dark matter" to be mined as it allows microbes to be cultured *in situ*. Using this technology, researchers identified teixobactin, an antibiotic with a novel mechanism of action, together with a further 24 new compounds. Teixobactin is produced by the soil organism *Eleftheria terrae* and has activity against Gram-positive (but not Gram-negative) organisms and mycobacteria and a novel mode of action

inhibiting peptidoglycan biosynthesis. It is also difficult to select teixobactin resistance. However, the exciting prospect lies with the technique rather than the antibiotic *per se*. In 2014, Professors Boger & Okano at Scripps Research Institute, San Diego modified vancomycin chemically to give increased binding and, in 2016, scientists at Rockefeller University identified which genes in a microbe's genome ought to produce antibiotic compounds and then synthesised those compounds to discover two promising new antibiotics (humimycin A and humimycin B from *Rhodococcus* sp.).

Regardless of the introduction of new antibiotics, it is imperative that we preserve the antibiotics we already have. This decade has seen the introduction of robust antibiotic stewardship programmes. In December 2013, the FDA issued voluntary guidance regarding antibiotic use in animals, asking antibiotic manufacturers to remove their drugs from over-the-counter status and place them under veterinary supervision[22]. In December 2016, the FDA stated that antibiotics used in animal feed (other than ionophores, bambermycins, bacitracin and tiamulin) should be classified as "medically important", requiring a veterinary feed directive (VFD). Novel approaches to addressing AMR include the use of bacteriocins; bacteriophages; passive infusion of monoclonal antibodies; innate immune modulators; faecal microbiota transplant; CRISPR *vs*. genes encoding AMR; use of toxins, iron acquisition systems, secretion systems, quorum-sensing pathways, adhesins, and biofilm formation as novel therapeutic targets and "resistance breakers" that damage membranes and permit antibiotic access.

CONCLUSION

I have noticed many changes over the last 10 years but I've been particularly impressed by developments in Point of Care testing; MALDITOF MS; Next-Generation Sequencing (NGS) and the introduction of the GAIN Act as an incentive to antibiotic development. We have been given the Rosetta Stone to help us unlock the remaining secrets of Microbiology. Although many techniques are still in their relative infancy, their impact has already been immense and the next 10 years will witness a paradigm shift.

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Sudan – Impressions in a Medical Health Care Environment

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Africa is a very diverse multi-ethnical continent. The African culture is highly diverse and knowledge of one or two countries should not lead to a conclusion about the African mentality and living conditions in other areas. The various climate zones from North to South and East to West have influenced the tradition of each population. Information on country, economy and culture cannot be transferred from one country to the other. Nations with political tensions, poor export / import business, or almost no tourist industry are mainly blind spots on our inner map. Beside daily news we know little to nothing about the living conditions in such areas.

Looking from a European perspective on Sudan, this country is one of these blind spots. Business contacts are marginal and the most information we receive is about the civil war, hunger, drought and more recently about the armed conflicts between tribes in Southern Sudan. UNHCR organizations try to control the social tragedy for the individual but there are also health care and epidemiological issues creating enormous risk potentials in terms of uncontrolled epidemics.

International and European research centers have established co-operations with universities and local reference laboratories in Khartoum to control outbreaks of Dengue virus, Zika virus, Chikungunya virus, Rift Valley Fever, West Nile virus, Malaria, Brucella, and many other infectious diseases. Since a large majority of local people who live in rural areas have contacts with animals, a wide range of zoonotic diseases are spread which becomes a daily problem for medical doctors and veterinarians.

Through contacts made by former local colleagues and European infectious disease reference centers, MAST Diagnostica GmbH joined an international team to teach local scientists on modern molecular techniques, thus enabling them to perform clinical and epidemiological trials in Sudan. The first contacts were made in 2015 and continued in a second visit at the end of 2016. The cultural and climatic changes are always a welcome experience for one or two weeks, and should not be missed! Owing to a cold spell, with the lowest temperatures between 33 °C and 37 °C, followed by a dry period in December 2016, the malaria pathogens were not present in Khartoum. However, from the point of view of an infectious disease epidemiologist, the sample pools in the reference institutes can be considered as an "Eldorado" of tropical disease pathogens. Having such a wide array of sample pools available, it is no wonder that local virologists and bacteriologists have an extremely high level of expertise in understanding the very diseases symptoms and the appropriate diagnostic solutions required. Looking at the Sudanese GDP as an indicator of economy performance no one can expect a high standard health care system. Among the 69 hospitals in Khartoum, 52 are operating as private hospitals, often supported by foreign organizations.

The leading hospitals (national and the private ones) offer a western standard health care with the lab equipment being of an excellent standard whilst the basic structure of the laboratory buildings may not be in the most pristine of conditions. Modern instrumentation allowing automated assay procedures methods e.g. molecular DNA and RNA diagnostics are available, which allowed the MAST® team to perform testing on Malaria, *Salmonella typhi* and *Salmonella paratyphi*, Chikungunya and Dengue viruses. All tests were run on the latest models of real time PCR machines and results were compared to

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"However, from the point of view of an infectious disease epidemiologist, the sample pools in the reference institutes can be considered as an "Eldorado" of tropical disease pathogens. Having such a wide array of sample pools available it is no wonder that local virologists and bacteriologists have an extremely high level of expertise in understanding the very diseases symptoms and the appropriate diagnostic solutions required"

the molecular MAST® platform which is under development. Laboratory work in Sudan is a wonderful experience because samples are available in large numbers, both the bio-banked samples and the indigenous ones. Freshly collected samples are also available, many associated with eating improperly prepared food or by drinking unboiled and non-bottled water. As a result, gastrointestinal infections are frequent and non-locals face a higher risk of becoming a potential host for these infectious agents.

The local cuisine is excellent and the lab team together enjoyed a pot of "ful"(cooked beans) and Sudanese Pizza. Traditional food is eaten observing the cultural norms which eliminates the use of knives, forks and spoons, with every bite taken using the right hand from a shared plate. Lunch time meals and dinners were an entertaining event spent talking about infections and the epidemiology of bacteria, viruses and parasites.

Life in Khartoum is generally very safe with the main risks associated with crossing busy roads on the streets. Traffic follows its own incoherent pattern similar to a picture of Brownian molecular motion whereby vehicles must find their own paths to avoid a road accident. The major risk in these regions will always be tropical diseases, thus rapid and reliable modern diagnostics are invaluable tools in the fight against these serious life threatening conditions.

Malawi

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In a modern age of microbial disease and morbidity in less developed countries, the cooperation of IVD companies with medical professionals is crucial for improving the outcomes of global health. One form of cooperation is mediated through nonprofit partnerships, which can potentially address the problems faced and generate effective solutions for treatment. MAST® has recently been involved in such a collaboration with Shirley Westwood, who is a BMS Microbiologist at James Cook University Hospital. Shirley has undertaken extensive work in the Eastern African nation of Malawi and here she describes her amazing journey and the complications she faced.

I first went to Kamuzu Central Hospital (KCH) in Lilongwe in November 2012 with the African Partnership Programme for patient safety, as part of the Infection Prevention and Control team. My role was to give a Quality Management System presentation for the pathology department, who were preparing for accreditation, and surveillance of Hospital Acquired Infection. My presentation was well received and the department was one of only two in the hospital which achieved accreditation. Unfortunately, my surveillance wasn't as successful as I quickly realised that the laboratory wasn't equipped for such a task. It was seriously underfunded, had no technicians, reagents or consumables.

I found a few swabs and antibiotic discs which were past their expiration date and with the reluctant help of the Tidziwe Centre, which is a clinical research and training centre for the University of North Carolina's AIDS's Research in Malawi, I did manage to show there was a significant problem with AMR in the hospital. As there is a high percentage of HIV positive patients in the hospital and their infections are often related to their status, I encouraged the laboratory supervisor to seek funding. This year they have finally been given some funding from the Lighthouse Trust, a HIV testing, treating and care facility supported by the global fund.

I decided that if I was ever to return I would need to take my own supplies. When asked to be part of a project funded by the Tropical Health and Education Trust (THET) I wrote myself a comprehensive list of what I would require. I didn't return until December 2015 due to family commitments and the Ebola outbreak in 2014 which took me to Sierra Leone with Public Health England.

Based on the findings of my previous trip to Malawi. I decided to screen for MRSA, ESBL and CPE. I chose three areas. Firstly, as

women and children were my main priority, I chose The Ethel Mutharika Maternity Unit. Secondly I chose the burns unit which treats a lot of children, particularly in winter when open fires are the only way to stay warm and cook food. Finally, I chose the male surgical unit, where the conditions were appalling and some patients were found on old mattresses in the corridor outside of the ward.

I arrived in Malawi with the full 46kg luggage allowance containing plates, swabs, urine bottles and kits. Despite my concerns over the safe transportation of my cargo, and thirty six hours of travel, I stocked the laboratory fridges ready to start work the next day. I then spent two weeks taking samples and persuading medical staff that the microbiology laboratory was now up and running. Once again I found evidence of substantial antimicrobial resistance, however not in the maternity unit which was a bit surprising. After a lot of questions and detective work I discovered they had been using Imipenem as a first-line antibiotic, which had been supplied from a donor.

"I aim to focus most of my efforts on the women and young children, this is where my heart truly lies in Africa, those babies are dying when they really shouldn't be and this is heartbreaking"

> *"On our two most recent trips, we have gone out to the district hospitals where they have no access to Microbiology and admit that they treat blind. Unfortunately, most of the patients with severe infection die as a result of this."*

I brought as many clinicians from the wards into the lab as possible and presented them with their patients results, indicating that the antibiotics used were either ineffective or not required. We met with hospital directors, ministry of health representatives, pharmacists and other hospital staff, to generate support for our project. We also agreed on an antibiotic policy for KCH.

The next visit in July 2016 presented us with new challenges. The hospital staff were demoralised as their budget had further been cut due to international donors pulling out.

We presented the antibiotic policy which was well received and I conducted more surveillance, this time screening staff on the burns unit for MRSA. I also discovered that several staff members were positive for MRSA from their nasal swabs.

The problem of ESBL on the Maternity unit had returned and their donor supply of Imipenem had run out. We advised on MRSA eradication therapy and how pharmacy can monitor the use of antibiotics. In-between visits we have been supporting the hospital staff by encouraging the laboratory, infection control and pharmacy groups. It's been very slow to change and some staff, particularly the older ones, are still not using the microbiology laboratory, however, there has been a large increase in the number of specimens being processed and an interest in susceptibility testing results. I've been working very closely over the last 18 months with the microbiology lab manager, who is very encouraged by our help, we just need to sustain it.

I took ESBL ID Disc Set (D52C), AMPC & ESBL Detection Set (D68C), AMP C Detection Set (D69C), **MAST**DISCS® combi range *Carba plus* (D73C), a simple and efficient assay for the confirmation of carbapenemase-producing-Enterobacteriaceae (CPE) along with antibiotic discs (Tobramycin 10, Cefotaxime 5 and Metronidazole 5) all provided by MAST®.

Once again I collected samples from my target wards, which were then processed and the results shared with the relevant staff. There was one tragic case where a young girl had presented at a district hospital and she was over 30 weeks pregnant. Her stillborn baby was delivered by Caesarean section, and the mother was septic. She was on Ceftriaxone and transferred to the central hospital after 10 days. Her condition worsened and she had a hysterectomy for a necrotic uterus and developed peritonitis. They put her on Imipenem but sadly she died two days after I saw her. She had *Acinetobacter baumanii* in a wound on her ankle where she had been tied to the bed. There were so many things that tragically went wrong here and this is not an uncommon occurrence.

On our two most recent trips we have gone out to the district hospital in Dowa where they have no access to Microbiology and admit that they treat blind. We visited the hospital during the first week and gave presentations on hand hygiene and control of infection practices. I also did a short presentation on antimicrobial resistance and the importance of culturing samples to identify the pathogen and obtain sensitivity tests.

I gave each staff member attending the course a swab and urine sample bottle. They were asked to take their samples and return them to us, while we prepared lunch! I took these samples back to the central hospital where I cultured them, obtained the results, which I then photographed and made into a presentation. We went back to Dowa the following week and I was able to present the results and discuss each individual patient with the relevant clinician. Sadly, most of the patients had not improved and several had died as a result of their infection, which had not been treated with appropriate antibiotics. These findings were visibly shocking to the staff, the majority who had no previous understanding of antimicrobial resistance.

Although successful in raising awareness it now poses the problem of what to do about it! The hospital relies on very limited antibiotics from donors and currently has no access to microbiology services. This situation may improve if staff from KCH transfer to Dowa and set up a microbiology service in the existing department or transport between sites can be funded. I'm very keen to try and get some access to Microbiology for them and to work on a way to get reliable transport for the samples.

The other aim of this trip was to set up antimicrobial stewardship, but as they hadn't implemented our policy, this wasn't possible. The policy has since been accepted and should be implemented soon. One of the problems with local staff in Malawi is that they rely very heavily on international support from volunteers, which cannot be maintained on a long term basis.

I aim to focus most of my efforts on the women and young children, this is where my heart truly lies in Africa. These babies are dying when they really shouldn't be and this is heartbreaking. In February of this year I returned to Malawi for the fourth time where I reinforced the teaching and surveillance that was previously introduced. I was encouraged to find more clinical staff using the laboratory services, however unfortunately they have now run out of supplies. I was to make this my final trip to Malawi, but now I am not so sure…

MAST® and AMR

Edited by **Asdaq Hussain**
Marketing and Technical Support
MAST(UK) Group Ltd

Antimicrobial resistance is regarded as one of the greatest issues facing 21st century healthcare, and antibiotics were awarded the Longitude Prize 2014. The development of resistant strains of microorganisms to antimicrobial agents is a natural phenomenon of adaption. This process has been enhanced by the overuse and misuse of antimicrobial agents. Since the discovery of penicillin 87 years ago, the years that passed have witnessed an increase in many common infections becoming potentially life threatening and in turn difficult to treat.

Clinicians and researchers are increasingly advocating strategies to maintain antibiotic potency, through the use of better diagnostic tools to guide and control dosage and therapy duration, this is a good example of antibiotic stewardship. Similarly, companies in the healthcare sector are being urged to accelerate the development of accessible new drugs together with more informative in vitro diagnostics.

As well as adding stress to the vast financial burden that most healthcare budgets already suffer, AMR poses many economic threats that go beyond the health sector such as damaging international trade and travel increasing the risk of spreading resistant infections. Particularly worrying is the prevalence of resistance in Gram-negative pathogens as isolates producing extended-spectrum β-lactamases (ESBLs), AmpC β-lactamases and Carbapenemase enzymes are rapidly disseminating. This results in limited treatment options which give rise to an increase in life threatening infections.

Extended-spectrum β-lactamases: Extended spectrum β-lactamases (ESBLs) are enzymes that are produced by Gram-negative bacteria, commonly the Enterobacteriaceae, which inactivate various β-lactam antibiotics against microorganisms. ESBLs hydrolyses 3rd and 4th generation cephalosporins, whilst many remain susceptible to cephamycins.

AmpC β-lactamases: AmpCs are mainly termed cephalosporinases as they hydrolyse 2nd and 3rd generation cephalosporins and cephamycins as well as most β-lactams. They also have a conferred resistance against some carbapenems due to diminished porin loss and porin expression.

Carbapenemases: For patients with ESBL/AmpC related infections, carbapenem antibiotics are most often the last line of defence. They are often the only surviving β-lactams that demonstrate activity against such enzymes, but with the development of carbapenemases their effectiveness is compromised. Carbapenemases are β-lactmases that confer resistance via hydrolysis to a number of β-lactams including penicillins, monobactams and carbapenems.

MAST® has been continually responding to this challenge in the last 10 years by updating its portfolio of **MAST**DISCS® combi range of AMR Detection Sets for definitive identification and differentiation of enzyme types. The disc sets rely on a combination disc method incorporating specific enzyme inhibitors to seek β-lactamases.

The **MAST**DISCS® combi range includes a comprehensive list of ESBL detection products to integrate into standard laboratory methodologies, either offering a full set of cephalosporin/indicator combinations or allowing the choice of selecting either cefpodoxime±clavulanate, with cefpodoxime being the best general substrate to detect all ESBLs, or cefotaxime±clavulanate and ceftazidime±clavulanate, which are good substrates for CTX-M and TEM/SHV-derived enzymes,. These third-generation cephalosporin/clavulanate synergy tests are compromised in organisms that have AmpCs, in which case synergy should be sought with a fourth-generation cephalosporin and clavulanate. The **MAST®** AmpC & ESBL Detection Set (D68C) can be used on all isolates of Enterobacteriaceae to identify and differentiate AmpC and/or ESBL production, which is important as some combinations used to treat ESBL infections can induce AmpC production. If an equivocal result is generated, then the use **MAST®'s** AmpC Detection Set (D69C) can be used as further work to determine AmpC production. Unlike many other commercially available products, **MAST®'s** AmpC Detection Set detects plasmid and intrinsic chromosomally encoded AmpC enzymes, encompassing inducible, derepressed and hyperproduced strains to provide a comprehensive, reliable and cost effective test.

Recent developments include the AmpC, ESBL & Carbapenemase Detection Set (D72C), Carba plus (D73C) which are further additions to the **MAST**DISCS combi range and the **MAST®**ICT which is the newest addition to the **MAST**DISCS ID range. All of these products fulfil a specialised niche within the laboratories for antimicrobial testing

AmpC,ESBL & Carbapenemase Detection Set (D72C) is a six disc system that has been developed for the identification and confirmation of AmpC and ESBL enzymes in Enterobacteriaceae, both singularly and in combination, while also providing an indication of carbapenemase activity. *Carba plus* (D73C) has been developed for the simple phenotypic confirmation of Carbapenemase activity amongst Enterobacteriaceae. It is a five-disc system that enables the differentiation of MBL, KPC and OXA-48-like Carbapenamases, including reliable discrimination of KPC from AmpC producing isolates. The addition of a Temocillin disc containing a MBL inhibitor, removes the ambiguity of MBLs being incorrectly identified as OXA-48.

The **MAST®**ICT (D74) method utilises a cell permeabilising agent to release Carbapenemase enzyme from a Carbapenemase-Producing-Organism (CPO) to hydrolyse an indicator antibiotic in the test system. This allows a normally carbapenem susceptible reporter organism to grow where antibiotic has been hydrolysed, thus producing a distorted zone of inhibition. If the test organism produces no Carbapenemase, the reporter organism will form a regular, circular zone of inhibition around the indicator tip. The **MAST®**ICT (D74) is a reliable screening tool for OXA, KPC, NDM, IMP and VIM enzymes with the added and unique benefit of being suitable for use with Acinetobacter, Pseudomonas spp as well as Enterobacteriaceae.

mastpharma®

In a decade of innovation and utilising its 60 years' experience of development and manufacture, **MAST®** also offers **mast**pharma®. **mast**pharma® is a range of services (**mast**pharma® *development* and **mast**pharma® *stability*) tailored to the pharmaceutical industry for the design of diagnostic discs to determine microorganism susceptibility of novel antimicrobial compounds. Clinical trials of antimicrobials require such discs before the compound enters Phase 2 and 3 trials. The result of this is that antimicrobial susceptibility test discs are proven to be the most effective and validated method to determine bacterial susceptibility in both clinical and veterinary practice, and must be commercially available at the launch of the compound to assure full market penetration. **mast**pharma® is a bespoke service and **MAST**DISCS® pharma can be custom manufactured according to customer requirements to determine and compare the antimicrobial activity of novel compounds.

mastpharma® ***development***

During the disc development phase, an antimicrobial susceptibility test disc containing a novel compound is formulated and assayed to determine disc content is verified. Stress studies are undertaken to identify the most suitable formulation to produce stable, investigational use (IUO) only AST discs. Which are measured against defined quality control acceptance parameters.

mastpharma® ***stability***

mastpharma® *stability*: A minimum of three replicate batches of discs are manufactured and entered into real-time stability studies. Studies are carried out under both normal storage and additional stress conditions which allows trending to be monitored to ensure the final product retains effective performance for the duration of its allocated shelf life as a commercial product, meeting the International standards of both CLSI and EUCAST.

In conclusion

MAST® has a long history of working with the Pharmaceutical industry, developing new AST products and innovative solutions to aid antibiotic stewardship in an increasingly challenging environment. Further, **MAST®** remains committed to supporting the fight against the threats of microbial resistance by undertaking its own Research and Development programmes and providing an extensive range of high quality in-vitro diagnostic products to aid the microbiologist.

For more information please visit our web site **www.mastgrp.com**

MASTmatters

1957-2017

Decade of innovation (2007-2017)

2009
New Disc Dispenser MDD62

The new Mast DiscMaster2™ disc dispenser (MDD62) is launched which combines ergonomic design with practicality for routine, reliable dispensing with additional lock-off functionality. MDD64 is the latest upgraded model in use.

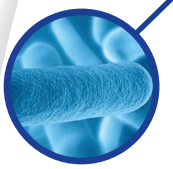
2011
MAST Launch Uri System in UK

A flagship product of **MAST®**
The Mast **Uri®** *System* streamlines the urine screening process, allowing greater than 95% of urines to be reported within 24 hours and offers savings in labour and consumables plus much reduced wastage. Currently installed in many locations across the United Kingdom and internationally.

2013
Carbapenemase Detection Set (D70) Launched

Providing a reliable phenotypic identification of metallo β-lactamases (MBL) and *Klebsiella pneumoniae* carbapenemases (KPC), with 100% sensitivity for NDM-1, whilst differentiating KPC positive isolates from those expressing ampC coupled with porin loss.

2014
CAT-ID™ (D71C) Launched



CAT-ID™ was developed for the simple screening of carbapenemase activity amongst Enterobacteriaceae with the ability to identify OXA-48-CPE.

2015
Medilink National Award - Outstanding Achievement

Mast Group Ltd succeeded in winning the coveted Medilink Outstanding Achievement Award in recognition of the MAST® **Uri®** *System.*

"The Mast Uri System represented an excellent mix of cutting edge technology, commercial success and importantly, a genuine contribution in helping with the global problem of increasing antibiotic resistance." Judges Quote

2016
Ceftolozane tazobactam (C/T40C)
Ceftazadime avibactam (CZA14C & CZA50C)

MAST® becomes first company to market both specialist antibiotics for multidrug gram-negative resistance in disc format meeting the parameters for EUCAST & CLSI.

2017
Infrastructure Investment

Commissioning of MAST®'s brand new state of the art facility to confirm a decade of excellence and innovation.

MAST® ICT - Indirect Carbapenemase Test (D74)

Reliable screening tool for OXA, KPC, NDM, IMP and VIM enzymes with the added and unique benefit of being suitable for use with Acinetobacter, Pseudomonas spp as well as Enterobacteriaceae.

MAST® ISOPLEX DNA LYO

Ready to use isothermal amplification kit in convenient lyophilised format.

Mast Group

IVD solutions through partnership

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