OCTOBER 2016 # 23

the Pathologist

The Slow Tsunami is Coming

Can we do enough to combat antibiotic resistance before it hits?

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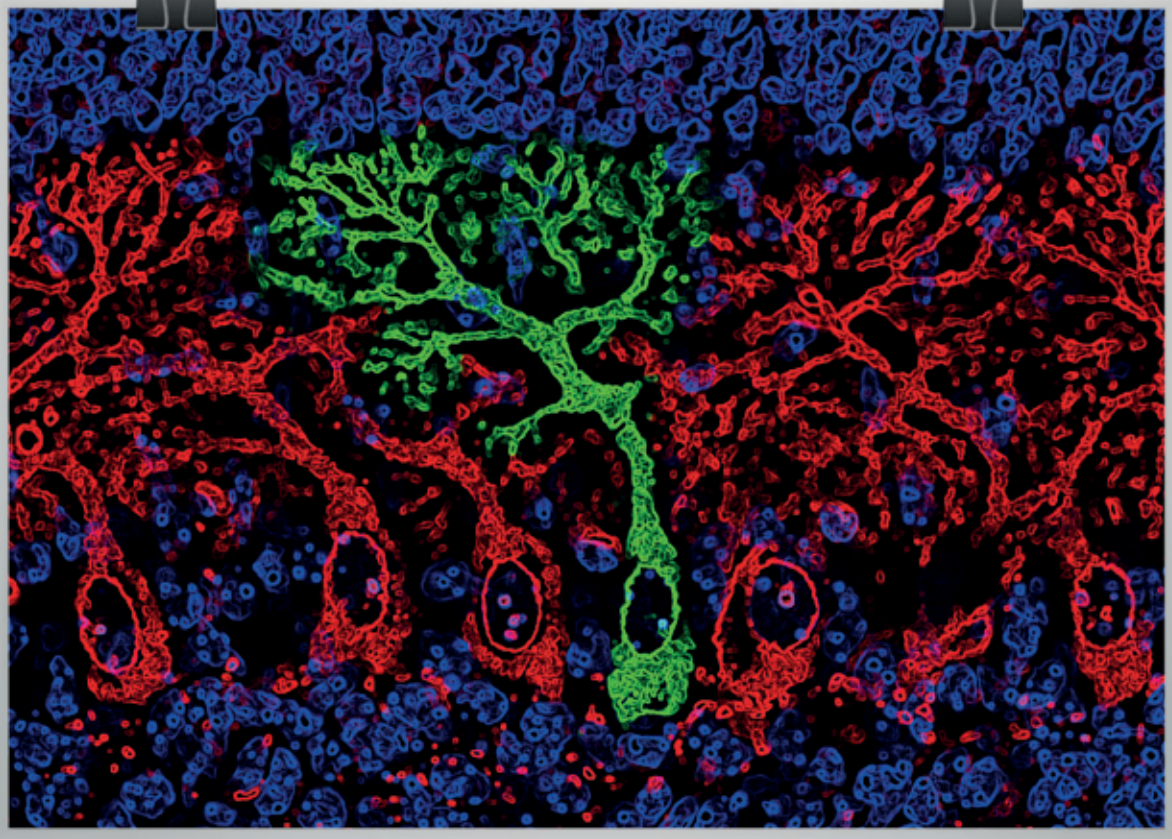

Mouse cerebellum stained to reveal a Purkinje neuron (green), by researchers studying the expression of ALS-associated genes in the brain. For the first time, the scientists discovered that the *C9orf72* gene is strongly expressed in the hippocampus of the mouse brain – a region where adult stem cells reside and which is known to be important for memory.

Image credit: Andrew L Bashford and Vasanta Subramanian University of Bath.

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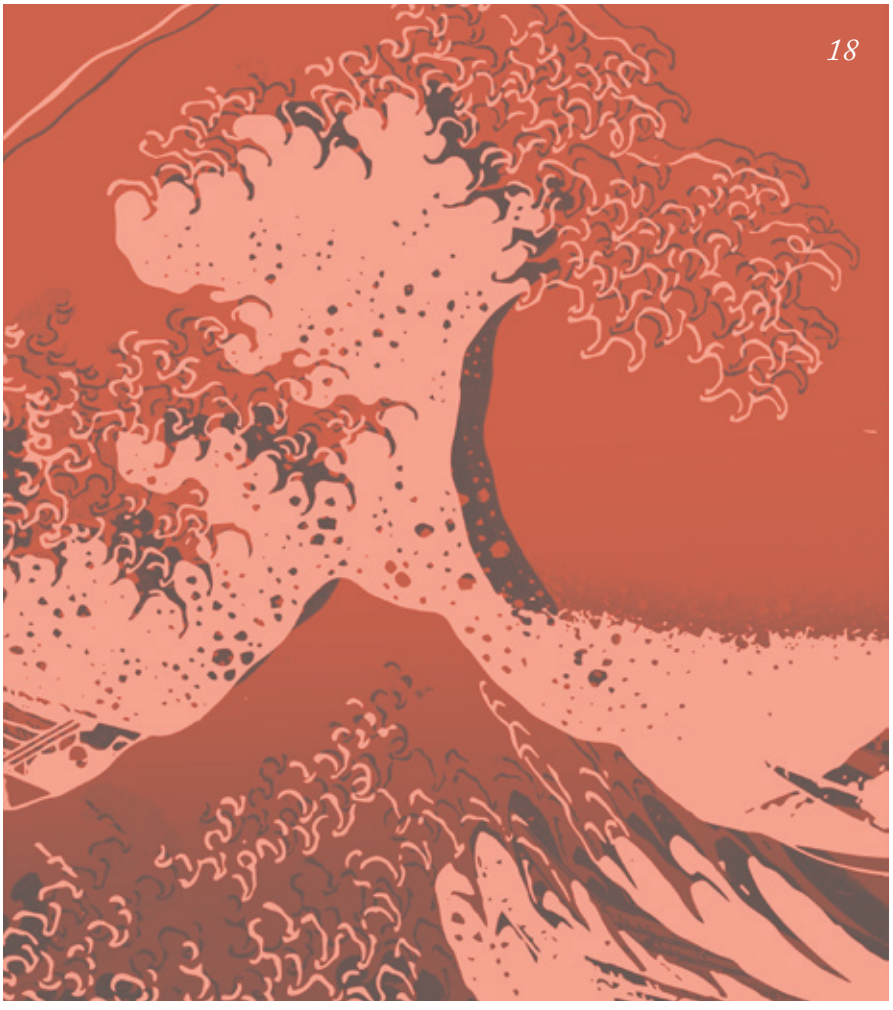
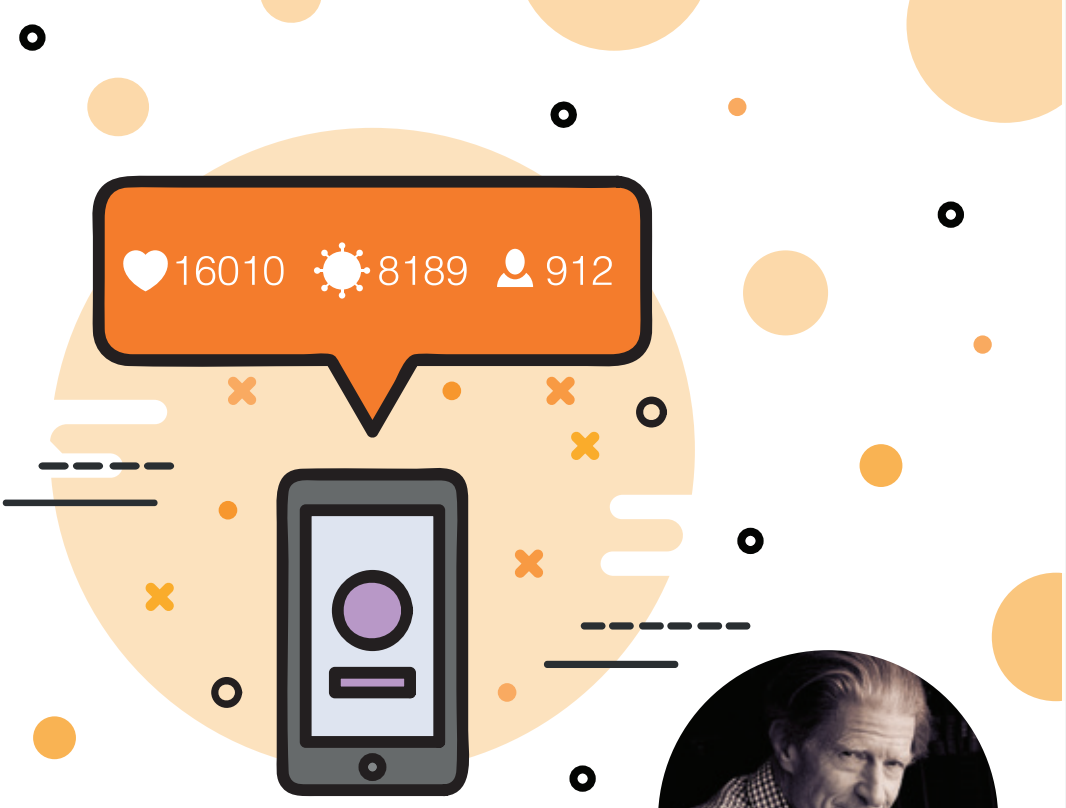
Contents



On The Cover

An illustration of the antibiotic resistance tsunami gaining momentum and threatening health on a global scale.



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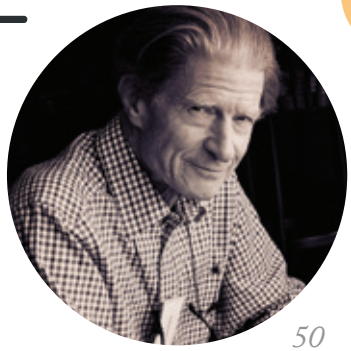

50

In Practice

Next Gen

Profession

Sitting Down With

the Pathologist

ISSUE 23 - OCTOBER 2016

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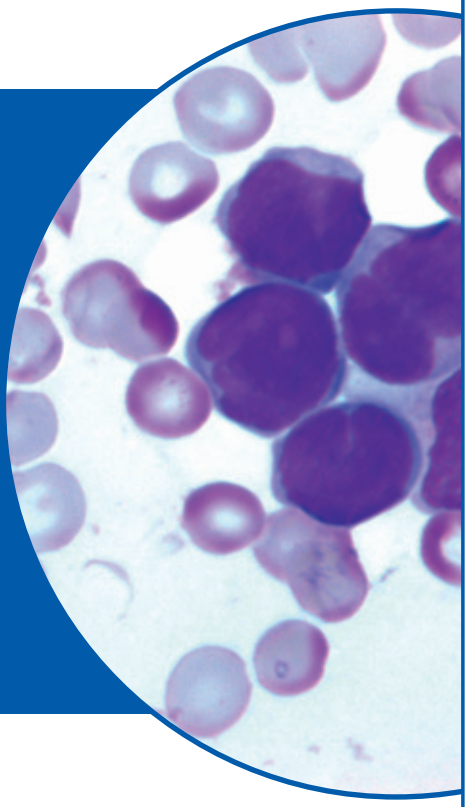

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The Times They Are A-Changin'

Or at least I hope they are, but I need your help…

This month saw the Nobel Prize in literature surprisingly (to most) awarded to singer-songwriter Bob Dylan. He becomes the first musician to win the award, and is probably the most radical choice in the accolade's history. The news was greeted with congratulations and praise by some, but criticism (some pretty extreme) from others. As you'd expect, social media was alight with comments: "I'm happy for Bob Dylan, #ButDoesThisMeanICanWinAGrammy?" quipped a best-selling novelist, Jodi Picoult. "Bob Dylan winning a Nobel in Literature is like Mrs Fields being awarded 3 Michelin stars," the novelist Rabih Alameddine wrote – Mrs Fields being a huge retail cookie brand in the US, so certainly not meant as a compliment.

What really struck me about the news (and this may be owing to my ignorance so please try not to judge me too harshly) was this: as well as the unusual choice, it was the high-profile nature of the recipient. More often than not, Nobel Prize winners tend to be fairly low-profile (at least that's my understanding). And isn't that one of the best things about the award – that it recognizes widely unrecognized brilliance?

As you know, pathology is sadly a profession whose value is not widely perceived, and the people within it don't tend to receive public accolades for their hard work. In fact, the reality is quite the opposite. Remember The Pathologist's cover feature a couple of years back on pathology stereotypes (1)? And the image of the eyes lurking from behind a basement door? Guess what? A lot of people think that comical depiction is true – that pathologists are basement-dwelling, corpse-handling non-doctors! You and I know that's not true. And it's at this time of year that we like to recognize the amazing work you do. With that, I'm pleased to announce nominations for The Pathologist's 2016 Power List are now open.

Last year, we asked you to put forward your most inspirational role models (2). This time, we're taking a different approach. We want you to nominate people in training or the early stages of their careers: the "rising stars" of pathology and lab medicine. I am personally inviting you to help give your profession and its amazing people the recognition they deserve. Are you with me?

The process is simple: go to our short online form (http://tp.txp.to/powerlist_form) and tell us whom you would like to nominate and why. The nominees will be judged by an independent panel of experts and the results announced in our December issue.

Let's crush those stereotypes together and show the world just how great pathology is. As Dylan beautifully wrote, "The times they are a-changin'." Let's change them together.

Fedra Pavlou
Editor

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Upfront

Reporting on research, innovations, policies and personalities that are shaping pathology today.

Do you want to share some interesting research or an issue that will impact pathology?

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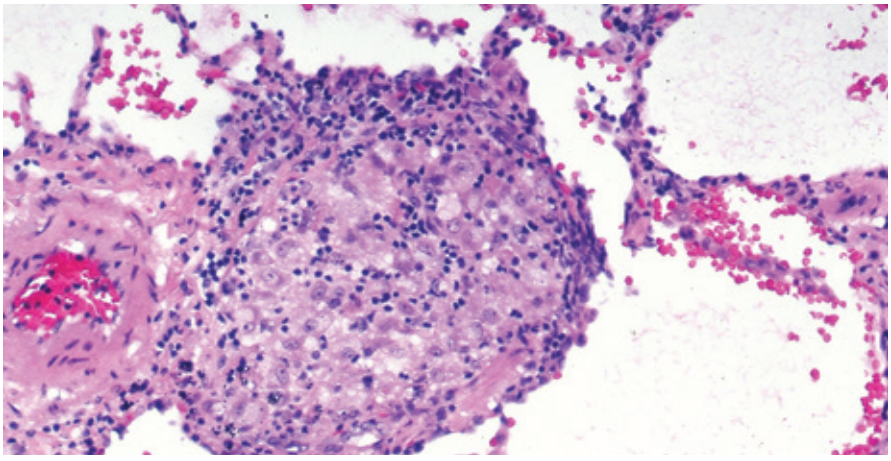

An Enduring Epidemic

The aim is to end the global tuberculosis epidemic by 2030 – but if that's to happen, we need to close the resource gap

"Global actions and investments fall far short of those needed to end the global [tuberculosis] epidemic."

World news has been full of bacteria lately, most notably in the wake of the United Nations General Assembly's resolution on antimicrobial resistance. Given the high-profile nature of that meeting and its outcomes, it would be easy to overlook some of the other recent data – such as the World Health Organization's 2016 Global Tuberculosis Report (1), which highlights a significant gap between the UN's goal to end the worldwide TB epidemic and the actions currently being taken to achieve it.

The targets involve an 80 percent reduction in the disease's incidence rate and a 90 percent reduction in deaths by 2030 – but since 2015, when those targets were established, the epidemic has proven to be larger than expected. Multi-drug-resistant TB (MDR-TB), in particular, is causing crisis: only one-fifth of eligible patients are enrolled for treatment, and the treatment success rate is just over 50 percent. The numbers for extensively drug-resistant TB (XDR-TB) are even worse, with only 28 percent of patients successfully treated.

That's not to say there's been no improvement – quite the opposite. More countries than ever are testing newly diagnosed TB patients for rifampicin resistance, at least 23 have introduced new, shorter treatment regimens for drug-resistant disease (with success rates up to 90 percent), and at least 70 countries have begun to use new antibiotics like bedaquiline and delamanid against MDR- and XDR-TB. It's a good start – but it's not enough. The current funding gap for TB care and prevention is nearly US$2 billion and expected to increase, and an additional $1 billion is needed to develop new vaccines, diagnostics and treatments. And the barriers aren't just monetary; patients need better education and improved access to care. Will the WHO's report prompt the necessary actions? That remains to be seen… *MS*

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Playing Chicken With Chicken

Why is antibiotic resistance in *Campylobacter* species on the rise – and how can we combat it?

Credited with nearly one million cases of food poisoning each year in the United States alone (1), it's no surprise that *Campylobacter* species are the major causes of foodborne gastroenteritis worldwide. Perhaps less well known is the fact that the species responsible for most human disease, *C. jejuni* and *C. coli*, are rapidly increasing their ability to resist antibiotics. This is a major problem: with *Campylobacter* contamination present on an estimated half of commercially available chickens (2), and over half of those bacteria resistant to common drugs like ciprofloxacin and nalidixic acid, it's becoming more and more difficult to treat cases of campylobacteriosis.

The increase in resistance is at least partly due to the routine use of fluoroquinolone antibiotics in poultry farming. The drugs are used to treat or even prevent disease in chickens raised in crowded environments, where a single outbreak of infection can result in significant cost to the farm. But they're also used to treat food poisoning in human patients – and if farming practices render the bacteria resistant to fluoroquinolones, doctors will lose one of their best options for eliminating human intestinal infections.

What can be done? Some countries, including the United States and Australia, ban the drugs completely in the poultry industry. In those that don't already have a ban in place, experts recommend that fluoroquinolone use be phased out. Organizations like the British Poultry Council have declared a commitment to reducing antibiotic use, and state that it's already decreasing (3) – so hopefully, *C. jejuni* and *C. coli* will escape their fate as an object lesson in the effect that agricultural overprescription can have on human disease… *MS*

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A Pig In a Poke

When importing livestock for food or breeding, European countries may inadvertently open their borders to superbugs as well

Cast your mind back a few hours to the beginning of your day. How did you start it off? For many of you, the answer will include a bacon sandwich – ham and eggs – breakfast sausage. These kinds of hearty foods can make for a comforting breakfast – but perhaps somewhat less comforting once you consider the high rates of livestock-associated methicillin-resistant *Staphylococcus aureus* (LA-MRSA) in much of Europe's pork supply.

Where does this superbug come from? In large part, Denmark, where as much as two-thirds of the pig herd may be infected (1). Because the animals are not tested for LA-MRSA before export and there's no mandatory reporting of the disease, Danish pigs – which account for well over half of European pig exports for both food and breeding – are able to travel the continent unchecked, and with an unwelcome travel companion. Worse yet, the bacterium doesn't just cause disease in pigs; it's capable of infecting humans as well, and when it does, it's extremely difficult to treat.

So what's being done to combat LA-MRSA in Denmark? At the moment, not much. There's little in the way of regulation and many Danish exporters are adamantly opposed to introducing more. Meanwhile, infected pigs continue to be distributed across Europe at the rate of millions every year. It's clear that better screening and stewardship are needed to prevent the spread of this serious superbug – and that if the livestock industry won't stand up against it, others who understand the danger must. *MS*

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Sneaky Superbugs

Norway's strict LA-MRSA transmission measures prevent the import of almost all live pigs – but the bacteria have found a new way in

Denmark may not strictly curate its pigs' health, but not all countries follow suit. Nearby Norway, for instance, carefully maintains its herds' disease-free status through extensive screening and a tight lock on live imports. Nevertheless, several outbreaks of LA-MRSA overtook Norwegian pigs even after the introduction of import bans – so the authorities began asking: how?

The answer, it seems, lies in a novel transmission route. Humans – namely, laborers who had previously worked on Danish pig farms – likely brought the CC398 strain of LA-MRSA with them to Norway, where they passed it on to the livestock they handled (1). Although those workers, like most humans, were able to carry the bacterium without ill effects, some are not so lucky. The same strain has caused numerous illnesses and even six deaths in Denmark, where it is endemic. Patients need not have had direct contact with livestock to contract the disease; it can be transmitted through contaminated meat as well.

Although Norway's strict protocols have thus far prevented CC398 from becoming widespread, this new mode of transmission may mean that the country needs to crack down even more – and in fact, mandatory screening of farm workers has already been suggested. One thing is certain: the ability to transfer infections back and forth between species reinforces the need for both human and veterinary medical specialists to speak out for better stewardship wherever possible. *MS*

Reference

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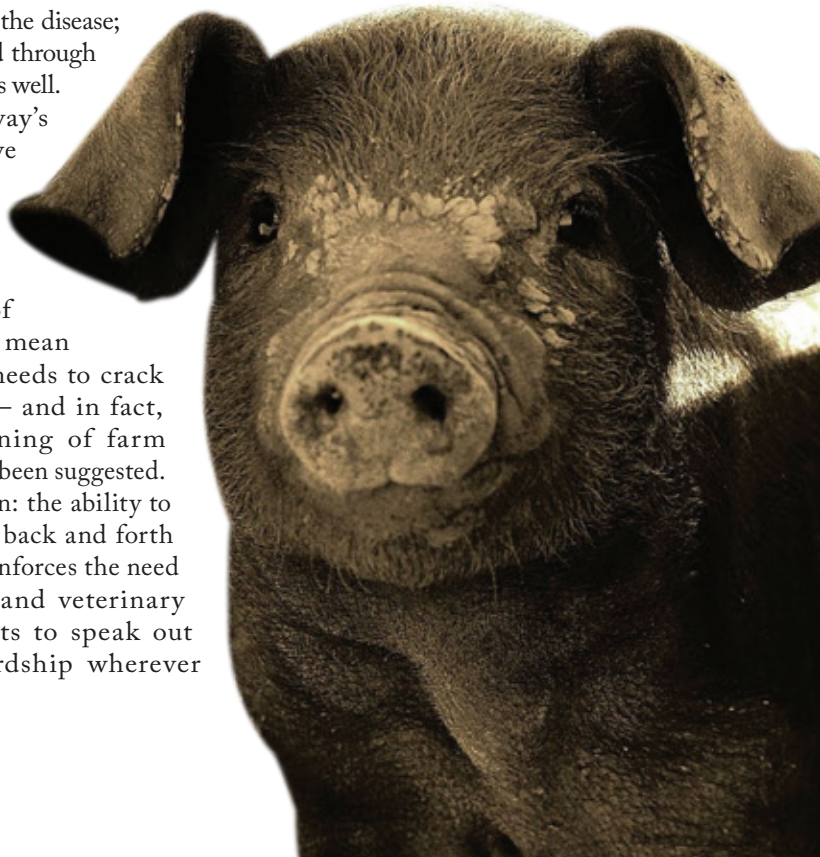

Resisting Resistance

Rapid, affordable tests to spot bacterial infections could reduce antibiotic overprescription in resource-limited settings

In countries with unregulated antibiotic access, stewardship for the prevention of drug resistance development can be a challenge. But how can obstacles like physicians' lack of time to conduct a full diagnostic workup, or the absence of resources that would allow the verification of bacterial infections before prescribing antibiotics, be surmounted? Researchers from the Oxford University Clinical Research Unit in Vietnam suggest introducing an affordable, five-minute C-reactive protein (CRP) test that could distinguish between viral and bacterial infections and prevent antibiotic overprescription (1).

"There's no current standard of practice for diagnosing bacterial infections in the Vietnamese primary healthcare setting," explains Nga Do Thi Thuy, first author of the study. "Doctors commonly prescribe antibiotics solely based on clinical examination, regardless of existing treatment guidelines." This is partly because Vietnamese primary care providers often have only minutes in which to see each patient, so they can't conduct thorough examinations. The problem of overprescription is compounded by the fact that most antibiotics are available over the counter, so many patients skip the visit to the doctor and simply buy the medications they think they need (2). Standard CRP testing, recommended in many western countries, is not available in Vietnamese primary healthcare – but the researchers hope that their new test will fill the gap.

Will rapid CRP testing be enough to solve the problem? "The key challenge is low compliance with the therapeutic algorithm," says Do. "Improved education, and the associated increase in adherence to the algorithm, could be a solution." He adds that a CRP test to be delivered in pharmacies selling over-the-counter antibiotics might also reduce the issue of patients' purchasing drugs they don't need. The setting still brings unique challenges to bear – like health centers prescribing antibiotics to get rid of old stock, rather than out of need – but as rapid diagnostics continue to be developed and improved, Do and his colleagues are optimistic that the fight against unnecessary antibiotic use can be won. *MS*

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Diving Into Diagnostics

Hashem Etayash explains how his team's "microfluidic cantilever" device works to trap bacteria and test resistance

When fighting a superbug, there's no victory without effective treatment. But all too often, researchers' attention is focused on the end of the patient pathway – on the antimicrobial agent itself, rather than on the journey to its administration. But how can we appropriately treat multi-drug-resistant infections unless we first have the ability to identify the causative pathogen and then determine which antibiotics might have a positive impact? That's the problem my colleagues and I chose to tackle in the hopes that we could shave hours, or even days, off the time taken to treat. Right now, there's a significant lack of rapid diagnosis because of the amount of time it takes to culture bacteria and confirm their identity – and during that time, an infection may spread, develop resistance, or even kill a patient. To address this vital gap, we developed a "microfluidic cantilever" – a small device that can trap bacteria and enable resistance testing.

The cantilever is a rigid structure, like a tiny diving board made of silicon, with a thin layer of gold on top. It has an embedded microfluidic channel whose inner surface is coated with biomolecular receptors. Those can be antibodies or antimicrobial peptides that specifically bind a harmful bacterium like *Escherichia coli* or *Listeria monocytogenes*. As a sample is introduced into the device, the bacteria are captured by the receptors and the device then sends three different signals to confirm the selectivity and sensitivity of the detection:

1. When the bacteria are captured, the cantilever's mass changes, generating a change in the resonance frequency of the cantilever.
2. Adsorption of the bacteria forces the cantilever to deflect, due to the way its bimetallic material responds to bacteria-induced surface stress.
3. By shining infrared light on the microfluidic channel of the cantilever, the trapped pathogens absorb light, vibrate and generate another confirmation signal in the form of a nanomechanical infrared spectrum.

The uniqueness of our device lies in its ability to integrate multiple signal generation techniques simultaneously into a single device to enhance sensitivity and selectivity. This synchronized detection of three orthogonal modes provides solid bacterial detection with no ambiguity. And once the bacteria are trapped, we can add antimicrobial agents to the channel and measure the cantilever's oscillations. That way, we're able to tell whether or not the antimicrobials have killed the captured bacteria – and thus, whether or not they'll be effective as a treatment.

Other techniques for detecting bacteria and drug resistance, like agar plates or broth dilution assays, require a minimum of 24 hours to complete. Not only is that inconvenient, but it doesn't meet our increasing demands for rapid detection. It's my hope that our microfluidic device can address those needs. Of course, this is the first time we've reported its use for biological applications, so it will still need extensive adjustment and verification – but ultimately, with a time requirement of only 30 to 60 minutes for a test, it could serve as a rapid diagnostic alternative to save precious hours in patient care and public health.

I fear that, with the rise of drug resistance, we're on the edge of a return to the decades when we didn't have antibiotics for many pathogens. The only difference I see is that, instead of having nothing in our hands, we'll have many things – but all of them unusable. Bacteria spread fast; they multiply rapidly and in large numbers; they quickly come up with new mechanisms to resist antibiotics. That means our responses must be equally quick – so tools for rapid diagnosis are urgently needed to combat infections worldwide. I hope that our device will not only help to save the lives of patients with multi-drug-resistant infections, but also reduce the development of new resistance in the future. Why? Because despite the amount of work focused on exploring new antimicrobials, we've added very few new antibiotics to our arsenal over the last decade or so – and with the speed at which resistance develops, we could easily reach a stage where even the simplest infections are deadly.

To combat this, we must remember that "one hand does not clap." We need to work collaboratively – from researchers to physicians to patients – to prevent the development of drug resistance. No creature is as uniquely outfitted to survive by any means possible as the humble bacterium, and if we diagnose inaccurately, prescribe unnecessarily, or fail to comply with treatment regimens, we're assisting their continued survival. We need researchers to provide tools for identifying bacteria and testing their antibiotic susceptibility; we need physicians to provide appropriate prescriptions at appropriate times; and we need patients to adhere to what their doctors tell them. If each of us does our share, we may be able to fight back against antimicrobial resistance and save millions of lives. *HE*

Hashem Etayash is a PhD candidate in Pharmacy and Medicinal Chemistry at the University of Alberta, Edmonton, Canada.

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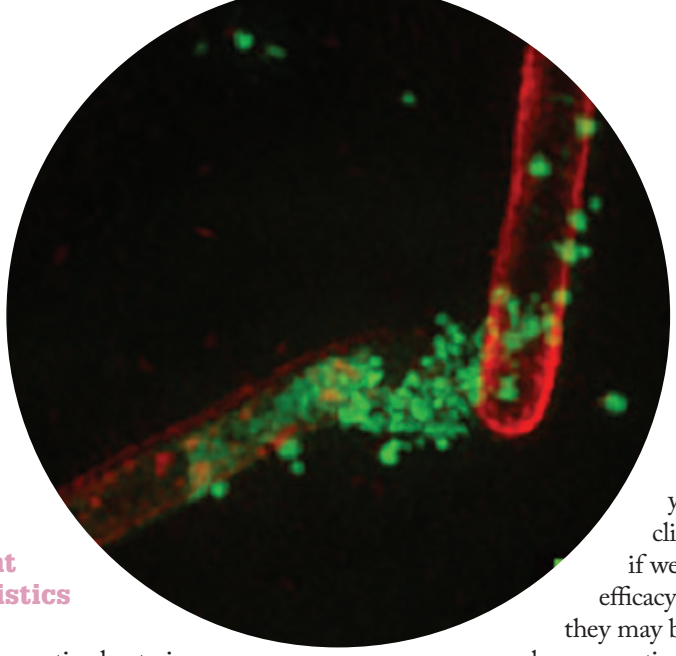

A SNAPPy Solution

Eric Reynolds describes a unique particle structure that has demonstrated potent antimicrobial characteristics

As multi-drug-resistant Gram negative bacteria become an increasingly urgent global threat, the need for effective treatments grows accordingly. Gram negative "superbugs" are particularly nefarious, because their lack of a thick peptidoglycan layer means there's one less thing for antibiotics to target. But rather than continuing to explore the well-trodden avenues of drug development, our group at the University of Melbourne took a different tack: developing structurally nano-engineered antimicrobial peptide polymers, or SNAPPs – star-shaped nanoparticles used to combat bacterial infections (1). SNAPPs contain peptide sequences based on the standard linear antimicrobial peptides, but their three-dimensional shape allows them to better disrupt the outer membrane of the bacteria. The actual "killing mechanism" is multimodal (including membrane destabilization, unregulated ion movement, and induction of the apoptotic-like death pathway), but it's initiated when a SNAPP interacts with – and destroys – the integrity of a Gram negative bacterium's outer membrane.

We had been studying linear antimicrobial peptides for many years, but realized that the 3D structure of the molecules was important. So when chemical engineer Greg Qiao developed a synthesis strategy for preparing star-shaped polymers, we were very keen to test them for antimicrobial activity. So far, we've seen great success; our SNAPPs have been shown to have potent (sub-micromolar) efficacy against a range of Gram negative bacteria – including those that are resistant to conventional antibiotics. We've even shown that they can protect mice from infection with multi-drug-resistant *Acinetobacter baumannii*, a bacterium where even colistin (the "last line of defense") fails. Best of all, and a surprise even to us, was our discovery that the molecules' toxicity to mouse (or human) cells is relatively low!

It's a promising beginning, but there's still a lot to be done. At the moment, we're continuing to improve efficacy by modifying the structure of the SNAPPs, and also working on further reducing toxicity so that our molecules have an acceptable safety margin for human trials. It's possible that the human immune system might "see" the SNAPPs and develop an immune response to them, although we haven't seen that happen in our mouse models. It's also possible that, when used intravenously, the activity of the SNAPPs could be negated by amphipathic molecules in the blood. Those are the types of potential problems we're attempting to address right now, so I expect that it will be at least another five years before we're ready for Phase I clinical trials. At that point, though, if we can demonstrate the safety and efficacy of SNAPPs in human patients, they may become the antimicrobial to use when conventional antibiotics fail. *ER*

Eric Reynolds is a Melbourne Laureate Professor at the University of Melbourne, head of the Melbourne Dental School, and a board member and CEO of the Oral Health CRC, Melbourne, Australia.

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In My View

In this opinion section, experts from across the world share a single strongly held view or key idea.

Submissions are welcome. Articles should be short, focused, personal and passionate, and may deal with any aspect of laboratory medicine. They can be up to 600 words in length and written in the first person.

Contact the editor at fedra.pavlou@texerepublishing.com

Questions of Quality

The importance of quality is broadly accepted – witness the plethora of standards and guidelines – but do they lead to effective implementation?

By James Westgard, Emeritus Professor in Pathology and Laboratory Medicine, University of Wisconsin Medical School, USA, and President of Westgard QC, Inc.

Quality continues to be an issue for many laboratories. In some countries, laboratories are only just developing quality management systems, processes, and procedures, not to mention practical implementation tools and techniques; in other countries, laboratories are so busy, and analysts have such heavy workloads, that finding time for quality has become more and more difficult. Often, the business strategy in laboratories is to "do more with less" – which means less time for activities such as performance evaluation, quality control, and quality improvement.

Improvements in QC practices won't lead to better quality management if laboratory analysts lack the time to learn new techniques and properly implement them, though. An example is the new US CLIA option to use Individualized QC Plans (IQCP) that use risk assessment to identify potential failure modes and then select appropriate control mechanisms to detect those failures. The approach sounds good in principle, but in practice it lacks the basics: standardized risk assessment methodology, documentation of the performance of control mechanisms (other than statistical QC), objective assessment of the acceptability of residual risk, and mechanisms for review by inspectors in accreditation programs. These shortcomings exist in spite of a new CLSI standard: EP23A "Laboratory Quality Control Based on Risk Management" and a new CDC/CMS "Step-by-Step Guide" for developing an IQCP.

"The fact remains that laboratories generally have no formal training and little experience in risk assessment."

So, although the existence of guidelines has influenced manufacturers to implement risk management, and despite manufacturers then persuading organizations like CMS and CLSI to adopt a risk management approach for quality control, the fact remains that laboratories generally have no formal training and little experience in risk assessment. They have to learn risk management on the job – which is

time-consuming and often problematic.

Not all laboratories are the same, however. For example, those situated in developing countries usually show more interest in improving the quality of their testing services and often invest more time and effort in it. By contrast, laboratories in developed countries are sometimes less interested in QC and quality improvement because of the increased pressures for productivity and efficiency that they face.

There are also differences in practices depending on how laboratories are inspected and accredited, and this too can vary geographically. For example, the US follows the "CLIA Rules" for inspection and accreditation, whereas many other countries are adopting the ISO 15189 standard for quality and competence in medical laboratories. That ISO standard adheres to certain metrological concepts, which in turn requires adoption of new quality practices. In particular, laboratories have traditionally embraced the TAE (Total Analytic Error) model for understanding, measuring, and managing quality, but ISO 15189 instead recommends the use of the metrology "uncertainty model" (Measurement Uncertainty, MU). MU has been advocated for many years now, especially in Europe, but has yet to find broad acceptance and practical use in service laboratories, and is mainly a requirement for accreditation by ISO 15189. So the field is still not adequately harmonized.

This complexity means that many organizations find that they need outside support to develop, understand or meet quality requirements; but the different organizations may themselves bring different challenges. In the US, I have found the government agencies particularly taxing, as it seems that they mostly listen to trade organizations, then to large manufacturers and then professional organizations. Laboratory practitioners are the last to get heard, and this can lead to problems. The change of QC practices from EQC (Equivalent QC) to IQCP is a good example. In the original proposal from industry, manufacturers were to provide laboratories with risk information pertaining to their analytic systems, so that the laboratory could develop risk-based QC plans accordingly. Two CLSI documents were developed – one for guidance to manufacturers on how to present their risk assessment information to laboratory users, and another for guidance to laboratories on how to use that risk assessment information to develop QC plans. CLSI submitted both of these documents for review, but only the one intended for laboratory guidance was finally approved; the document for manufacturers was canceled, because manufacturers dissolved the committee! Thus, it remains the case that laboratories lack sufficient information from manufacturers about the performance and expected failure modes of manufacturers' controls. Nonetheless, CMS adopted IQCPs even though laboratories lack the risk assessment information to properly plan and develop an IQCP. So we're getting there, but slowly; perhaps the quality of patience is an essential attribute in QC!

"There are also differences in practices depending on how laboratories are inspected and accredited, and this too can vary geographically."

Improving Cancer Survival

Malignant effusions are increasingly relevant specimens in the era of targeted therapy

By Ben Davidson, Senior Pathologist, Department of Pathology, Oslo University Hospital, Norwegian Radium Hospital; Professor, University of Oslo, Faculty of Medicine, Institute of Clinical Medicine

Serosal cavities – peritoneal, pleural, and pericardial – are frequently affected by cancer. The majority of tumors are carcinomas, particularly adenocarcinomas, originating from the lung, breast, female genital tract, and gastrointestinal tract. However, carcinomas of other origin – hematological cancers, germ cell tumors, melanomas, sarcomas, and childhood cancers originating from primitive tissues – may all be associated

with malignant effusions. Additionally, malignant mesothelioma is a primary cancer of serosal cavities.

Serous effusions are not infrequently the first anatomic site sampled in a newly found or clinically suspected cancer, or the only site from which material is available in the event of disease recurrence (1). Indeed, many surgical pathologists have been reluctant to accept that serous effusion specimens constitute adequate material for diagnosing cancer, one of the most contentious areas of dispute being malignant mesothelioma. This ignores the fact that effusion specimens contain large numbers of viable tumor cells, which are often far better preserved than those in biopsy specimens. Moreover, educating clinicians to send non-fixed, large-volume specimens immediately to the laboratory provides pathologists with ideal material for diagnosing both new cancer and recurrent disease. Recently, an international group of experts published a consensus document detailing guidelines and ancillary methods for the diagnosis of malignant mesothelioma in effusions, supporting the relevance of these specimens in tumor diagnosis (2).

Beyond the issue of tumor diagnosis, effusions are becoming relevant in targeted therapy. Indeed, any molecular test that can be applied to biopsy material or surgical specimens may be applied to effusions. The use of paraffin-embedded cell blocks allows for the application of identical immunohistochemistry and in situ hybridization protocols as in biopsies, and the availability of high-quality fresh-frozen material makes molecular analyses feasible (3). This is likely to have greater relevance in the near future, when next generation sequencing will become part of the normal repertoire of pathology labs.

While many pathologists use primary and metastatic tumor interchangeably in molecular analyses, there is extensive literature documenting that these specimens have different molecular profiles (1,3), emphasizing the need to analyze the metastatic lesions that are to be targeted by therapy rather than the primary tumor. Metastases are not all equal either. Cancer cells in recurrent disease differ from those in metastases sampled at diagnosis and demonstrate complex patterns of clonal evolution that are evidence of the dynamic changes undergone by cancer cells during tumor progression, as exemplified by ovarian carcinoma effusions (4,5). The prognostic relevance of cancer-associated molecules similarly differs among chemo-naïve effusions obtained at diagnosis and post-chemotherapy specimens (6).

"Many surgical pathologists have been reluctant to accept that serous effusion specimens constitute adequate material for diagnosing cancer."

Finally, I believe it is worth taking note of the rapidly increasing body of literature that implicates effusion-located cancer cells in the development of chemoresistance, most evidently in the case of ovarian carcinoma (7–14). This appears to occur via diverse cellular mechanisms, including expression of postulated stem cell markers, partial epithelial-to-mesenchymal transition, modulation of cytokine levels, and inhibition of apoptosis. This should draw our attention to focusing on strategies that would sensitize these cells to chemotherapy and improve treatment of metastatic cancer.

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Breaking with Tradition

Can immunophenotyping challenge classical approaches to cytology?

By Areej Khatib, Medical Director, Center of Advanced Pathology Labs, Head of IAH pathology and clinical lab department and Assistant Professor of Pathology, Bethlehem University, Palestine

In my view, a profession is an accumulation of knowledge and experience, affected by one's personality and background. Many describe me as being "different" and this quality has accompanied me ever since I began my independent pathology practice. And perhaps it's the reason why I challenge classical approaches to cytology.

For example, take the investigation of malignancy versus benignancy by means of the cytology of body effusions. There have been few innovations in this field since Muller laid the foundations of clinical cytology as we know it today in 1838 (1). Even with Papanicolaou's great discovery of his famous "Pap test" in 1928, much remained the same, and those pathologists that followed him added little to classical cytology practice (2).

Later, immunocytochemistry as an ancillary diagnosis method became widely accepted as an important improvement in effusion cytology (3,4). Then came fluorescence in situ hybridization (FISH), comparative genomic hybridization (CGH), and other molecular biology techniques such as PCR, all of which have been used successfully to characterize malignant neoplastic cells and improve diagnostic accuracy (5).

Most of the above methodologies "highlight" the cell of concern. However, the sensitivity and specificity of these "fancy" techniques are decreased by a number of obstacles, such as variable mixtures of normal and tumor-derived cells, presence of rare atypical cells, and low cell numbers.

One technique worth considering is flow cytometry. This was first used in the late 1980s with aneuploid cells, on the basis that their presence indicated malignancy, but remained a secondary technique in the detection of malignant cells in peritoneal fluids (6,7). However, immunophenotyping of effusions with flow cytometry has become more popular over time (8,9). In my opinion, flow cytometry can be very useful in detecting malignant cells in effusions of cancer patients but may damage cells, rendering them useless for further morphologic characterization. Also, I don't accept that detection of a population of cells carrying a specific marker necessarily indicates that those cells are malignant; benign epithelial cells will also carry an epithelial marker that is usually looked for, and in blind flow cytometry, you can never know.

I'd be more confident about drawing conclusions from the examination of separated cells under a microscope.

In our institute, we looked for a technique that allows separation of metastatic cells by immunophenotyping, thereby keeping the morphology intact for further verification of the separated population with microscopy. We did not need to look too far because magnetic cell separation (MACS) provides a tried and tested gold standard (10). This cell isolation method has been in use for about 25 years and is cited in more than 20,000 publications. As far as we are aware, the technique has been used for separating labeled epithelial cells from body effusions, but it has not been compared with traditional cytology methods for positive effusion.

The method requires metastatic cells to be labeled with an epithelial marker, separated and then examined with a light microscope. For the marker, we chose CD326 – also known as human epithelial antigen – which is present in the majority of carcinomas (11). The basis of our work was to split the sample into two, and then to examine one half traditionally and the other half after CD326-based separation. The results were amazing; we reduced false negatives in the pre-label and separation step by 15 percent!

So, how should we proceed? New ideas are often viewed with skepticism, and many will argue that the cost of the technique outweighs its benefit. However, the cost of immunophenotyping is falling, and we may soon see antibody costs fall to about US $7–10 per sample. Additionally, costs could be minimized by limiting use of the technique to verification of initial negative results. Nevertheless, the method needs to be tested on a large scale, and perhaps I can tempt other pathologists and cytologists to get involved so that we may design and perform an extensive study to translate our knowledge into practice. Such a study would no doubt help with the evolution of medicine in general and pathology in particular.

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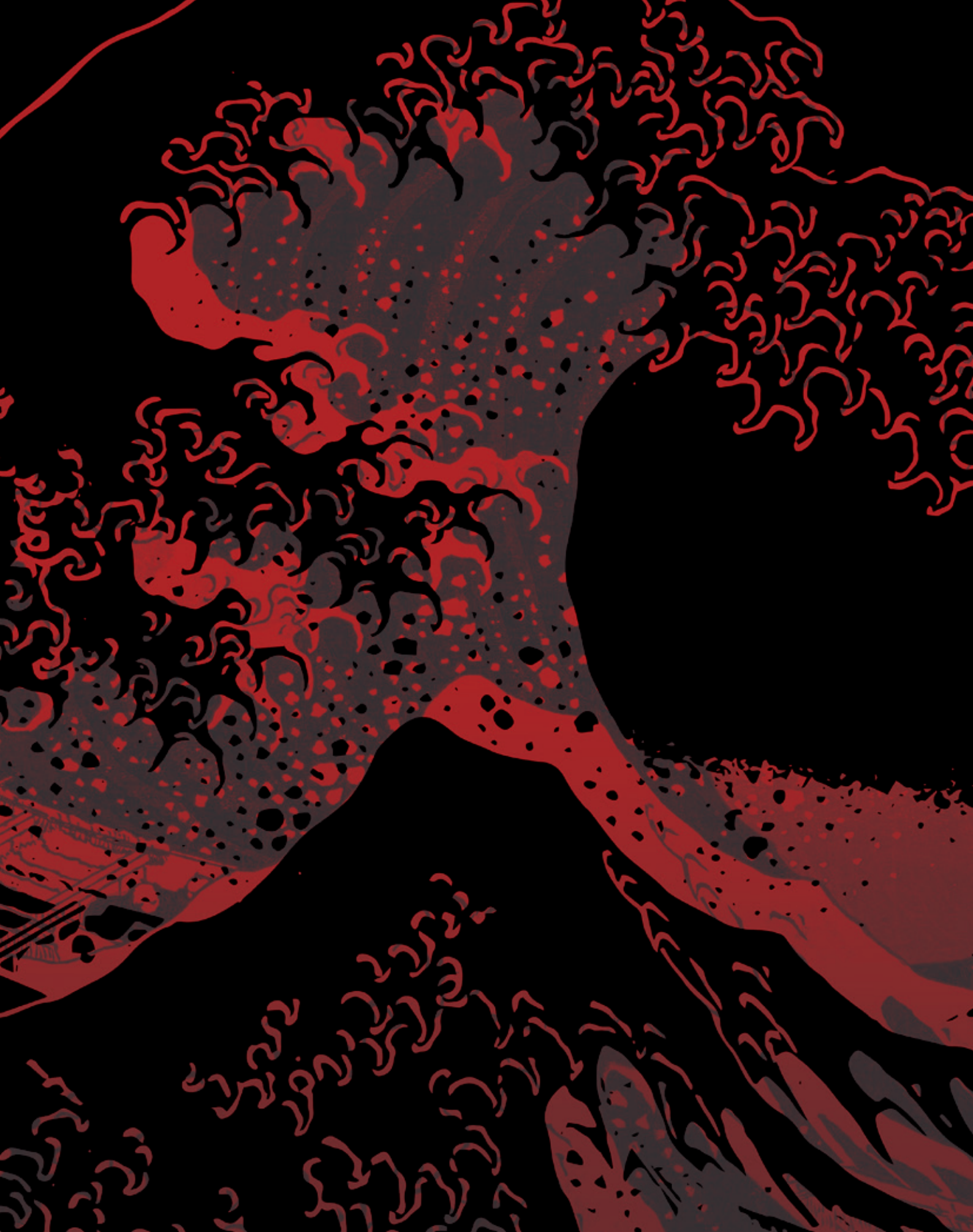

The Slow Tsunami Is Coming

And in its wake, a flood of drug-resistant superbugs… unless we do something to stem the tide

By Michael Schubert

Imagine that you're about to go on a holiday. You're packed and prepared, wavering between excitement for your upcoming trip and the familiar, slightly panicked feeling that you've definitely forgotten something. You're a little nervous about the flight, but as you take your seat, the pilot's reassuring voice comes over the intercom. "Good afternoon," he says. "This is a two-hour flight from London to Málaga. You have an 80 percent chance of safely reaching your destination." A one-in-five chance of disaster? Surely those odds are far too high for a simple vacation trip. But if that's the case, then why are such statistics acceptable in a healthcare setting? Dilip Nathwani, President of the British Society for Antimicrobial Chemotherapy, says, "We have a 50 to 70 percent chance of getting the right antibiotic for a patient's infection – and we sit and congratulate ourselves on those odds." To him, that's unacceptable – and that's only the surface of the problems with antimicrobial stewardship.

Antibiotic resistance is a critical issue in today's medical care, so much so that even the United Nations General Assembly felt the need to tackle it head-on. It's only the fourth time the UN has given such serious attention to a health issue, and they've taken a hard line, warning that antimicrobial resistance threatens worldwide development and requires a global response. All 193 member countries agreed – and all 193 of them have made a commitment to develop "superbug-fighting" action plans within the next two years.

What might these plans look like? There are three key aspects to a successful approach:

- Stewardship: a commitment to establishing and improving regulation and surveillance of antimicrobial use, sales and prescription;
- Research and development: not only of new types of antibiotics, but also of rapid diagnostics that can spot bacterial infections and identify effective treatments; and
- Education: both for healthcare professionals and for the general public.

With drug-resistant infections estimated to claim 700,000 lives per year globally – and that number expected to grow to 10 million by 2050 (1) – it's not hard to understand why there's a need for immediate intervention. But the $64,000 (or, in this case, $100 trillion) question is: what can we do?

The slow tsunami

Antimicrobial resistance has been referred to as a "slow tsunami." There's a steady increase in the number of drug-resistant pathogen strains – and not just to hard-hitting drugs like the carbapenems, but also to the common, low-cost drugs that are often prescribed for minor ailments. Nathwani says, "I think that we're at a pivotal stage in the fight against antimicrobial resistance. One of the UN recommendations was for each country to have a plan in place within two years – but to me, the challenge is much more than that. Not only do we need action plans, but we need to actually implement them and achieve some measurable goals." That, he says, is where the UN declaration falls short. "It doesn't really come up with targets. I think those are critical, because you need that kind of leverage within political and healthcare systems to bring about the change we all desire," (see Sidebar "Steps to Success").

A lever and a place to stand

The problem – according to Elizabeth Tayler, Senior Technical Officer of Antimicrobial Resistance for the WHO – is that we as humans tend to engage in short-term risk avoidance, rather than taking a long-term view. "If there's a 5 percent chance that an infection might be bacterial, we'll treat just in case," she says. "If I take antibiotics, I might feel better slightly earlier, or be marginally less likely to get a secondary infection." It's a prevalent behavior, and one that can only be defeated through education. "The fundamental problem we have is making people more and more aware of the long-term risks."

To that end, the WHO teamed up in 2015 with the UN's Food and Agriculture Organization and the World Organization for Animal Health to develop a global action plan with five key objectives:

- **Education**
  "The first objective is to raise awareness among healthcare professionals, agricultural workers, and the public."
- **Surveillance**
  "The second is about strengthening our knowledge base around resistance patterns and consumption."
- **Infection prevention**
  "In low-income countries, a lot of that is about improving vaccination, water, and sanitation – community prevention. It's also important to improve infection control in health facilities and in the animal sector."
- **Stewardship**
  "We need to improve responsible use of antibiotics in both the human and animal sectors."
- **Resources**
  "We need adequate resources to do all of this, and we need to improve the models for drug and diagnostic development to ensure sustainable investment."

It's a plan that was endorsed by the UN General Assembly – "and so now," Tayler says, "what we have to do is action." The first step is well on its way. "I'm sitting in Trinidad at the moment, working with the Caribbean countries to develop their national action plans. To date, 32 countries have plans in place and 59 more are working on them. That includes the big countries – India, China, Brazil, Mexico and so on – who arguably have the most significant impact on antimicrobial resistance. Our biggest challenge now is to translate those plans into action, but we're seeing exciting signs that countries are beginning to take this seriously."

A global balancing act

Both Tayler and Nathwani are clear about one thing – that if we are going to succeed in defeating antimicrobial resistance, we need to take a broader view. This means considering not just the long term, but also the long distance. "If I take a global view," says Nathwani, "although we need to preserve the effectiveness of current antimicrobials, we also need to ensure that, in the parts of the world with little access to antibiotics, these treatments become available. There's a rather staggering statistic – that more people die from lack of access to antibiotics than die of drug-resistant infections. We need to reach a balance between stewarding our antibiotics and ensuring that everyone has sustainable access to them." It's a balance he hopes can be achieved by emphasizing infection prevention through methods like vaccination, hygiene, sanitation, and clean water – and by ensuring that, when antibiotics are made available, they're prescribed by professionals who understand their use.

Tayler points out that the rise in resistance to affordable antibiotics disproportionately affects resource-poor countries. "Those drugs have been the backbone of medicine in developing countries," she says, "but those countries are going to be very vulnerable if the drugs no longer work – so we're in a difficult position at the moment." And working within weak health systems to improve standards isn't easy. "Part of the problem is simple inertia. Even when people and organizations are enthusiastic for change, actually making it happen is another matter. "I think the impact is greatest in the poorest countries," says Tayler. "Those are the ones buffeted by Zika, by yellow fever, by political instability. It's interesting talking to people there, because there's a lot of enthusiasm – but when people change, or political systems change, inertia takes over."

Perhaps even more significant is the challenge of enforcement. "When you have poor or non-existent clinical governance, improving stewardship or enforcing regulations becomes much more difficult," says Tayler. "Although there can be quite good legislation, for instance as regards over-the-counter sales or restrictions on agricultural use, actually having the capacity to enforce that is very difficult – and there are plenty of people with a vested interest in maintaining the status quo. It's a challenging environment in which to try to make progress." Nathwani chimes in with an example: "In India, where they have significant problems with unregulated antibiotic use, they've introduced a 'red line' concept. Antibiotic packages feature a dark red line, which signals that they should not be taken unless prescribed by a qualified professional. But that's very difficult to enforce, and its impact is as yet unmeasurable. So there's a lot of ambition, but actually enforcing the regulations is a huge challenge."

A question of resources

It's clear that developing countries will need special attention as we work to steward our antibiotics and stem the rise of resistance. But what about healthcare professionals in countries with more resources? The availability of funding and infrastructure doesn't guarantee that those things will be appropriately allocated – and there are already concerns that the approximately US$790 million pledged by the UN won't be enough (3).

"I think the resource question is critical. It's important that antimicrobial resistance is not seen as a specific project. It should be built in when we strengthen agricultural, health or laboratory systems – not treated as an add-on. Why? Because if we do it that way, it's much more likely to be sustained." Although we still have to make the case for additional resources, Tayler has some powerful arguments to suggest. "The O'Neill report (1) talked about a potential 2–3.5 percent fall in GDP by 2050. That's like something the size of the UK economy dropping out of the world

Steps to Success

According to Dilip Nathwani, certain key measures must be taken to improve the chances of success of a program to tackle antibiotic resistance.

1. **Increase public understanding of the potential risks of antibiotics.**
   "They need to understand that the massive desire for antibiotics is counterproductive and harmful. These great therapeutic agents are also a threat, and abusing them can actually challenge and even negate many of the advances we've undertaken in medicine. That education needs to begin very early – from a school level."

2. **Increase healthcare professionals' understanding.**
   "We now have healthcare professionals outside medicine – nurses, pharmacists, dentists – who can prescribe, so we need them to engage with the principles of good prescribing. That's both an educational and a behavioral change.

3. **Adopt organizational empowerment.**
   "We need to make sure this happens in our communities, our nursing homes, and our hospitals. Here's a depressing fact: the United States has had extensive campaigns about antibiotic stewardship, but a recent study (2) showed that 55 percent of all hospital patients receive at least one antibiotic – and over the last decade, there has been no change in antibiotic consumption. So the question you have to ask is: what have all these attempted interventions actually achieved?"

4. **Take action – and track results.**
   "We need to see the measurable impact of all the good things we've been talking about for the last decade. Our focus must be on implementation, evaluation, and then further implementation – that is the proof in the pudding. Unless we do that, and unless we have systems to measure appropriateness and consumption and feed back to prescribers and the public, we won't bring about sustainable change."

market. The World Bank has done similar studies and says that the financial impact of antimicrobial resistance will be similar to the 2008 financial crisis, but much more protracted (4). These are the kinds of data that have a serious impact on policymakers."

That isn't to say that we haven't already made strides. "In the United Kingdom, we've seen fantastic results in primary care," says Nathwani. "We are beginning to reduce both overall antibiotic use and the misuse of broad-spectrum antibiotics." Nonetheless, he says, this impact – especially in hospital practice – needs to be greater still. In Scotland, where Nathwani practises, he says they've significantly reduced the use of cephalosporins and quinolones, but not total prescribing. "We must not be complacent," he warns. "We need to focus our efforts on the hospital and long-term care facility setting – but without underestimating the importance of community prescribing, because 80 percent of all human prescribing occurs outside specialized care facilities."

Maintaining momentum

So what's the biggest factor in all of this? Is it the amount of funding available? The pace of research into new drugs? According to Tayler and Nathwani, it's communication.

"It's quite interesting how you communicate things," observes Tayler. "Sometimes, people are much more captivated by the scary numbers; sometimes, it's the personal stories, or the fact that procedures like Caesarean sections, joint replacements or cancer treatment will become much riskier without the protective cover of antibiotics." She emphasizes, though, that the role of diagnostic professionals is critical. "You have the credibility, and also the local data. We are very short of good data in many contexts. At the WHO, we can talk about the global picture – but people are inherently worried about what's going on locally. Showing them that this problem exists in their own backyards focuses attention very well.

"People still trust doctors, and we need to leverage that to change the way we think about infection and antibiotics. We have a window of opportunity at the moment, while politicians are engaged and interested, but we know they'll move on. So it's vital that we put in a concerted effort to sustain that interest – or at least to revive it periodically."

"I think the clinical community needs to understand what the laboratory can offer," Nathwani adds, meaning not just the capabilities of medical science, but also its limitations. "Sometimes, I think the community expects too much of the laboratory. But if we understand each other and work together, then the outcome of each consultation will be more effective. I think pharmacists, nurses and infection specialists need to come together with primary care physicians to monitor antibiotic prescribing. Pharmacists can identify prescriptions that don't comply with policy; nurses can identify where intravenous drugs aren't required or question why a broad-spectrum antibiotic is in use where a narrower one would suffice. I think you need a culture of effective teamwork across the disciplines, working very closely with the laboratory, to ensure that the quality of prescribing is good."

Nathwani's own hospital sets the standard. "We're quite protective of broad-spectrum antibiotics like the carbapenems. If one is prescribed on a surgical ward, the prescription will be reviewed by a pharmacist the next day – and if there isn't a good reason for it, that person will have a conversation with the attending team. If there's still no reasonable response, then the pharmacist will email the stewardship and infectious disease teams, and we'll review the patient that same day and make an analysis based on the clinical situation. And if we feel that the antibiotic is not appropriately prescribed, we'll have a conversation of our own with the attending team at a clinician-to-clinician level and recommend changing or stopping the drug. We also train and empower our nurses to get involved, asking questions like, 'Why can't we administer this treatment orally?' or 'Have you taken the blood level for this drug?' All of us – nurses, pharmacists, junior doctors, specialists – work together to optimize each prescription."

Damaging diagnostic discrepancies

"One major challenge to prescription reduction is diagnostic uncertainty. If you're a clinician and you're not sure of your diagnosis, then you're likely to prescribe or continue antibiotic treatment. I think that if we're really going to personalize medicine, we need a diagnostic test to determine whether infections are viral or bacterial – and, if bacterial, what antibiotics might be effective. We need these results in a timeframe rapid enough to inform clinical decision-making. And that's something

we don't currently have." Nathwani believes that such tests hold the key to the future of antimicrobial stewardship – and he's not thinking only of those that are under development. Many biomarkers and point-of-care tests, like procalcitonin or C-reactive protein, already exist, but simply aren't available in many areas. "Although we rely on new solutions," he says, "we already have effective tests that will reduce diagnostic uncertainty; they just need to be brought to the bedside more quickly. I think the laboratory community needs to embrace them and lobby for them to be made available – because these kinds of tests are cost-effective and will ultimately bring about a reduction in antibiotic consumption."

He does caution that new tests need to be combined with good stewardship, though. "Clinicians love sexy new diagnostics because they're 'better.' But their benefits are often overblown – and what they really do is make people over-investigate and overtreat. If you can't combine rapid diagnostics with specialists who are able to interpret them and provide advice, then the tests alone are useless."

Tayler's expectations for the future are similar. "I think that research on diagnostics is really important, because if we can get cheap, rapid tests that help people to prescribe more appropriately and manage the risk of missing something, that's a way to change behavior." She also believes that emerging evidence of the microbiome's positive effects will change people's perceptions of antibiotic risk. "If it's possible that I could be harming myself by taking antibiotics – not to mention the long-term harm to society – then I think that changes the inherent risk calculation, and I think that's important." She hopes that a desire to preserve the helpful microbiome may make patients think twice about demanding antibiotics when there's no real need.

Think big

Eric Reynolds, Melbourne Laureate Professor at the Melbourne Dental School, points out a major concern healthcare professionals may not always consider – the widespread use of not just antibiotics, but antimicrobials. "We have recently shown that long-term use of chlorhexidine (used as a disinfectant) is associated with the presence of multi-drug-resistant bacteria," he says. "The bacteria exchange genes for 'efflux pumps' to pump out small antimicrobial molecules like chlorhexidine and triclosan – but these efflux pumps can also be used to pump out conventional antibiotics. Therefore, these bacteria develop resistance not only to the antimicrobials, but also to antibiotics. They can then survive on skin or in mouths without causing any problems until we become otherwise immunocompromised." It's not just antibiotics that must be carefully stewarded, he warns – disinfectants and other antimicrobial agents need to be considered with the same level of caution.

But how can we encourage such significant changes?

"Clinicians love sexy new diagnostics because they're 'better.' But their benefits are often overblown – and what they really do is make people over-investigate and overtreat."

Nathwani thinks that targets are the key. Measuring and feeding back data on consumption and quality is fundamental, because if that information is available to the clinicians and managers of healthcare systems, it can be used to inform metrics and future targets – and those can be powerful in influencing them to prescribe more effectively. "The other bit," he adds, "is to ensure that antibiotic prescription becomes a patient safety issue. I think that if you're prescribing poorly, then people coming into your healthcare facility should be told that they are not in a safe environment – and I think that kind of language will help people recognize that it's important to get this right."

Tayler has one more reminder for pathologists and laboratory medicine professionals involved in antimicrobial resistance. "I think this is one of the most exciting and important challenges that we face, so the work you're doing is massively important, and it's underpinning a global movement. Don't forget that – and instead of just focusing on the challenges that face your area, think about what you can do that will have the greatest global impact."

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Open-Access Education

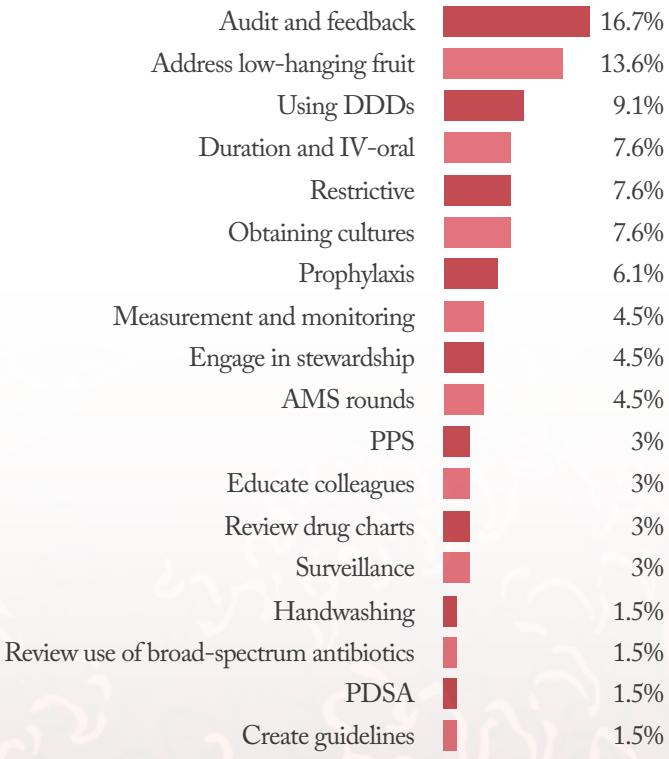
By Dilip Nathwani, President of the British Society forAntimicrobial Chemotherapy

High-impact, low-cost educational solutions with a global overview are the way forward for antimicrobial resistance and stewardship. My colleagues at the British Society for Antimicrobial Chemotherapy and I have set up the world's first massively open online course (MOOC) on antibiotic stewardship in hospitals – although of course we include many generic methods applicable to other healthcare settings and even the community at large. The course is free at the point of access, and since we started running it, we've had nearly 32,000 students enrolled. Their enthusiasm has been quite staggering!

Learn more about the course at:
http://tp.txp.to/MOOC/course/futurelearn

What one thing will you now implement?

According to survey respondents, they will now...

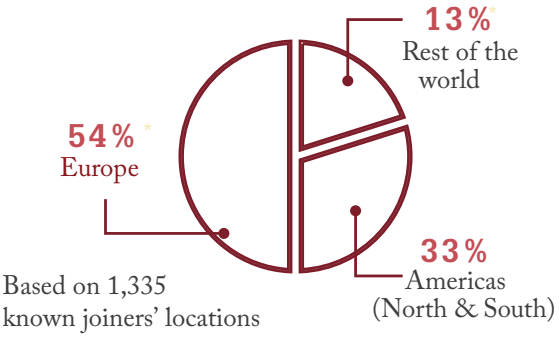


Data correct as of August 5th, 2016

Antimicrobial Stewardship Program

27565

Total MOOC Enrollments



Based on 1,335 known joiners' locations

178

participating countries

"I have learnt a great deal about the importance of reducing prescribing and consumption of antibiotics as the way of combating resistance. I now understand clearly what I must do to contribute to AS at my clinic."

"A big thank you to all the facilitators and participants for all the discussions and feedback. I particularly enjoyed the sections on behavioural change. Finally, our new formed AMS group has been given a wealth of information, strategies and tools to help make it a success in my country."

Post-course survey results combined for all three runs

80% liked or strongly liked subject expert discussions

90% found the course engaging or very engaging

86% would recommend the course to others

78% found the course the right length

87% did the course or part of the course at home

91% course exceeded expectations about learning and/or adding fresh perspective to their role

68% were healthcare workers

89% met or exceeded their overall expectations for an online course

Of those healthcare workers who responded...

21% Medics

23% Pharmacists

11% Microbiologists

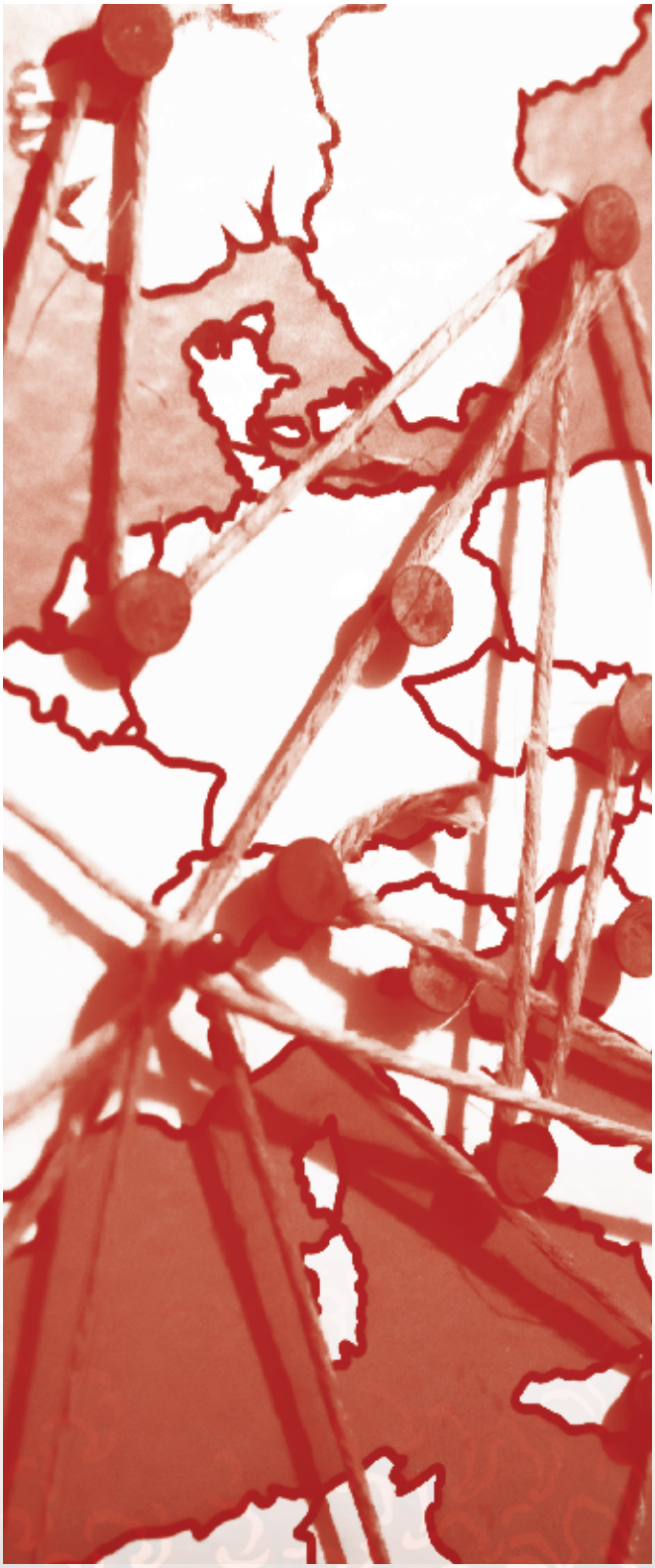
18% Nurses

1% Dentists

26% Others

From 41 countries

The above results are based on Run 1 post-course implementation survey respondents.



A Call for Collaboration

By Elizabeth Tayler, Senior Technical Officer of Antimicrobial Resistance, WHO

The Global Antimicrobial Resistance Surveillance System (GLASS) aims to encourage global standardization of antimicrobial resistance surveillance. It's vital for us to begin developing standardized approaches, so that we have systems and data that can be compared between countries. GLASS, which is currently under development, focuses very much on the importance of linking laboratory data to patients. One of the challenges we have in many countries is that labs have an awful lot of data, but if it's not actually linked to patients, it's very difficult to interpret.

If possible, diagnostic professionals should consider getting engaged with GLASS, no matter where in the world they are or how they work. The system tries to build from where countries are right now, acknowledging that some countries have much more capacity than others; it's trying to get everybody involved and starting to work to the same standards.

Who?
The World Health Organization.

What?
A system that collects and reports data on antimicrobial resistance rates. Ultimately, it will allow data to be shared and compared between countries, as well as expanding its data collection activities to include information on the implementation status of national surveillance systems.

Where?
http://tp.txp.to/GLASS/world/health

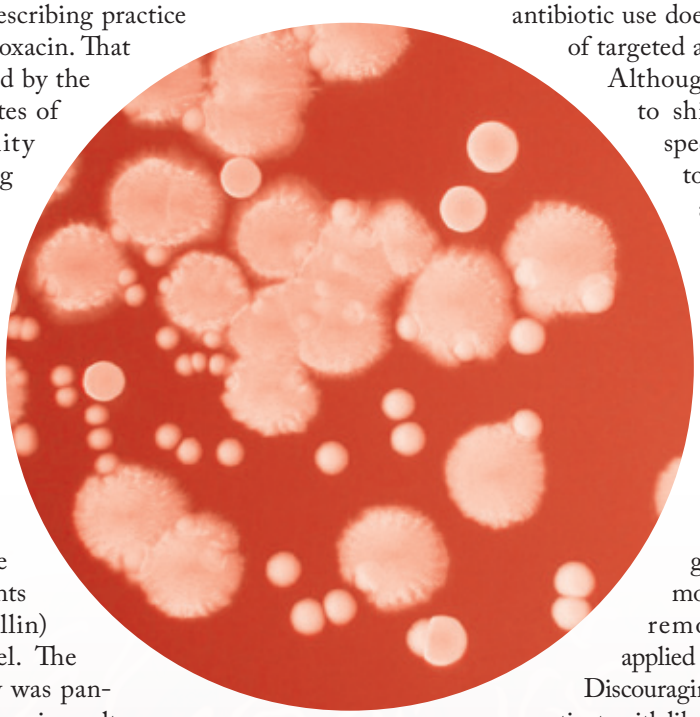
When?
The first formal call for 2016 data will be open from April to July of 2017.

How?
Those wishing to enroll should contact the GLASS Secretariat. Once enrolled, they will register in the GLASS IT platform, submit data on the current and retrospective status of national antimicrobial resistance surveillance, and participate in technical discussions.

A Nudge in the Right Direction

Brad Langford and Larissa Matukas describe how their work aims to influence prescribing patterns, and remind of the criticality of collaboration

Several years ago, our hospital faced a number of *Clostridium difficile* infection (CDI) outbreaks. Our antimicrobial stewardship program (ASP) decided to look for ways to reduce patient susceptibility to this burdensome disease. Knowing that fluoroquinolone use is a major risk factor for CDI, and that ciprofloxacin – a fluoroquinolone drug – was one of our most commonly used oral antimicrobial agents, we thought we might be able to influence prescribing practice by selectively reporting ciprofloxacin. That decision was further supported by the fact that, at the time, our rates of Gram negative susceptibility to the drug were dropping steadily, making it less useful for infections where it was truly needed. We hoped that selective reporting might be able to reduce the development of resistance by reducing overall use of fluoroquinolones.



The general rule of thumb we established was not to report ciprofloxacin for Enterobacteriaceae that were fully susceptible to all the agents (or only resistant to ampicillin) on the Gram negative panel. The most typical example we saw was pan-susceptible *Escherichia coli* from urine culture. Unfortunately, because our reporting system isn't automated, compliance with the policy was not perfect – and many clinicians requested reporting ciprofloxacin for blood cultures, due to its good bioavailability and tissue penetration for deep-seated infections. There were some confounding factors, too, like in any before-and-after study – we were in the process of implementing our ASP during the intervention; new Infectious Diseases Society of America guidelines had just come out urging clinicians not to prescribe first-line fluoroquinolones for uncomplicated cystitis; and clinicians may have been discouraged from ciprofloxacin use because of Gram negative organisms' low susceptibility to it. Nonetheless, we think the rapid and sustained drop in usage – and, indeed, in *E. coli* susceptibility – after selective reporting definitely indicates that our intervention was a contributing factor. As a result, we plan to look at other selective reporting practices, now in the context of a more robust and stable antimicrobial stewardship program, to see if they also have an impact on utilization and susceptibility.

But selective reporting comes with a warning label. We found that, after our intervention, the use of amoxicillin-clavulanate increased significantly. There were a number of reasons for this, but the problem was likely exacerbated by selective reporting of ciprofloxacin. Selective susceptibility reporting and other types of restrictive approaches are likely to result in "squeezing the balloon," a phenomenon where overall antibiotic use does not change, but prescribing of targeted agents shifts to another agent. Although we can use this method to shift prescribing from broad-spectrum or powerful antibiotics to more suitable ones, it's still a concern because our main goal is an overall reduction in unnecessary antibiotic use. That's something we can achieve by performing selective reporting in combination with other, more active, approaches like prospective audit and face-to-face feedback.

"Nudging" – the concept of guiding the prescriber to make more rational decisions without removing autonomy – can be applied to many other scenarios as well. Discouraging the initiation of antibiotics in patients with likely colonization or contamination by carefully worded comments or interpretations can be useful, as can adding more detail about each therapeutic option (like cost or breadth of spectrum) on a susceptibility report. Even something as simple as placing a more desirable option first on a susceptibility list may increase the likelihood that a prescriber will choose that option. All of these kinds of communication are important, and so is ensuring that prescribers have easy access to laboratory staff if they have additional questions. We need to keep the lines of communication open if we want our interventions to be effective.

There's a big "know-do" gap between what we know about appropriate use of antibiotics and what is actually implemented in everyday practice. Education is a key component to help change practice, but it won't be effective on its own; we also have to recognize the central role that behavior plays in prescribing practices. Fear, often related to uncertainty of diagnosis, or complacency, can lead to unnecessary antimicrobial use. In our opinion, the biggest challenge will be implementing effective antimicrobial stewardship interventions that address these behavioral aspects of prescribing in a variety of settings to a variety of audiences – and "nudging" will likely continue to play an important role.

Our study reflects the need for a truly collaborative, multidisciplinary approach to intervention. There are too many complexities in modern healthcare delivery, and in the emergence of resistance, for laboratories to make assumptions about how clinicians will best interpret and apply our results. As laboratory physicians, we need to actively seek end-user input to formulate individual antibiograms that are clear and useful. Part of that, too, is ensuring that laboratories receive the right specimens at the right times – because as we develop increasingly diverse and sensitive tests, the old adage of "garbage in, garbage out" is truer than ever. Selective antibiotic reporting can target and improve the post-analytical phase of the total testing process, but if we want to make a real difference to stewardship, we need to combine it with education, communication, and interventions at every stage of the process.

Brad Langford is a consultant pharmacist for Public Health Ontario's Antimicrobial Stewardship Program and lead pharmacist in antimicrobial stewardship at St. Joseph's Health Centre Toronto, Canada.

Larissa Matukas is Head of the Division of Microbiology and an Infectious Disease Consultant at St. Michael's Hospital and Assistant Professor in the Department of Laboratory Medicine and Pathology at the University of Toronto, Canada.

The Hollywood Approach

Roy Kishony and Michael Baym walk us through the MEGA project, which delivers a powerful, visually impactful message: bacteria spread and develop resistance… fast

It's easy to see that education is a vital component of antimicrobial stewardship. The better professionals, patients and the public understand how resistance evolves and how their own behaviors can contribute, the more likely they are to bring about change. But what's the best way to educate? Researchers from the Kishony laboratory at Harvard Medical School and the Technion – Israel Institute of Technology think it's through visual aids – and they've undertaken a big project to help.

What exactly is a "big project?" In this case, it's MEGA (the Microbial Evolution and Growth Arena) – a 2'x4' Petri dish that took 14 liters of agar to fill. The dish was divided into nine sections with increasing doses of antibiotic, then seeded with *Escherichia coli* and filmed continuously in time-lapse for two weeks. The result was a fascinating visualization of the way bacteria move through space – and the speed at which they can evolve resistance to even the highest concentrations of antibiotics.

Roy Kishony: Much of the credit for the idea isn't ours – it's all thanks to Hollywood! I was inspired by a video of a gigantic Petri dish billboard made as an ad for a movie, and thought it would be cool if we could use an experiment like that to demonstrate evolution. Tami Lieberman, a doctoral student in my lab, came up with the idea of patterning the plate with increasing concentrations of antibiotic to challenge the bacteria to evolve. And Michael made it all happen!

Michael Baym: It was really a joy to work with such a talented team. The idea of making movies of evolution actually happening was really compelling, and when we saw them, we realized that we could not only use this as a teaching tool, but could learn new things about the process itself.

One thing we learned is that small difficulties that aren't a big deal in a small experiment can become hugely important in a large one. We had enormous difficulties with condensation, contamination, temperature control, and even being able to see the bacteria. Once, yeast managed to get into our plate – and since it was unaffected by the antibiotics, it took over the whole plate shockingly fast.

RK: We also found that it's not always the "fittest," or most resistant, mutations that expand fastest into higher antibiotic concentrations. Just like we know from our own lives, bacteria too can have trade-offs – doing one thing well can mean doing other things less perfectly.

Watch the video at: http://tp.txp.to/MEGA/petri/dish

MB: They can also just be unlucky. Bacteria don't just need the right mutation; they have to have it in the right place at the right time. Evolution doesn't always select the very best. Sometimes, it's just those that are "good enough" and get there first.

RK: Eventually, we hope that by systematically mapping the mutations that lead to resistance to a range of antibiotics, we will be able to develop a new paradigm of "anticipatory diagnostics." Based on a pathogen's genome, we'll be able to tell doctors which antibiotics to use and even which specific combinations to use to best limit the chance of evolution.

It's no secret that antibiotic resistance is growing as a major public health concern. If we don't do anything about it, we may be facing a "post-antibiotic era" in which common infections and minor injuries can kill. So we need to make changes – like reducing antibiotic use and misuse. We also need to rethink the paradigm of antibiotic treatment and come up with new ways of combining drugs in order to slow down, and perhaps even reverse, the acquisition of resistance.

It makes us very happy that our videos can be used to help explain and visualize otherwise vague concepts of evolution, like mutations, selection and gradual adaptation – and we were humbled to learn that our video was chosen to be shown in the United Nations event on antibiotics. Both Michael and I were invited to the event, and seeing our work used to make a difference in international public policy-making was a highly rewarding experience!

Roy Kishony is Marilyn and Henry Taub Professor of Life Sciences at Technion – Israel Institute of Technology and visiting professor at the Department of Systems Biology at Harvard Medical School.

Michael Baym is a postdoctoral fellow in the Kishony laboratory at Harvard Medical School, Boston, USA.

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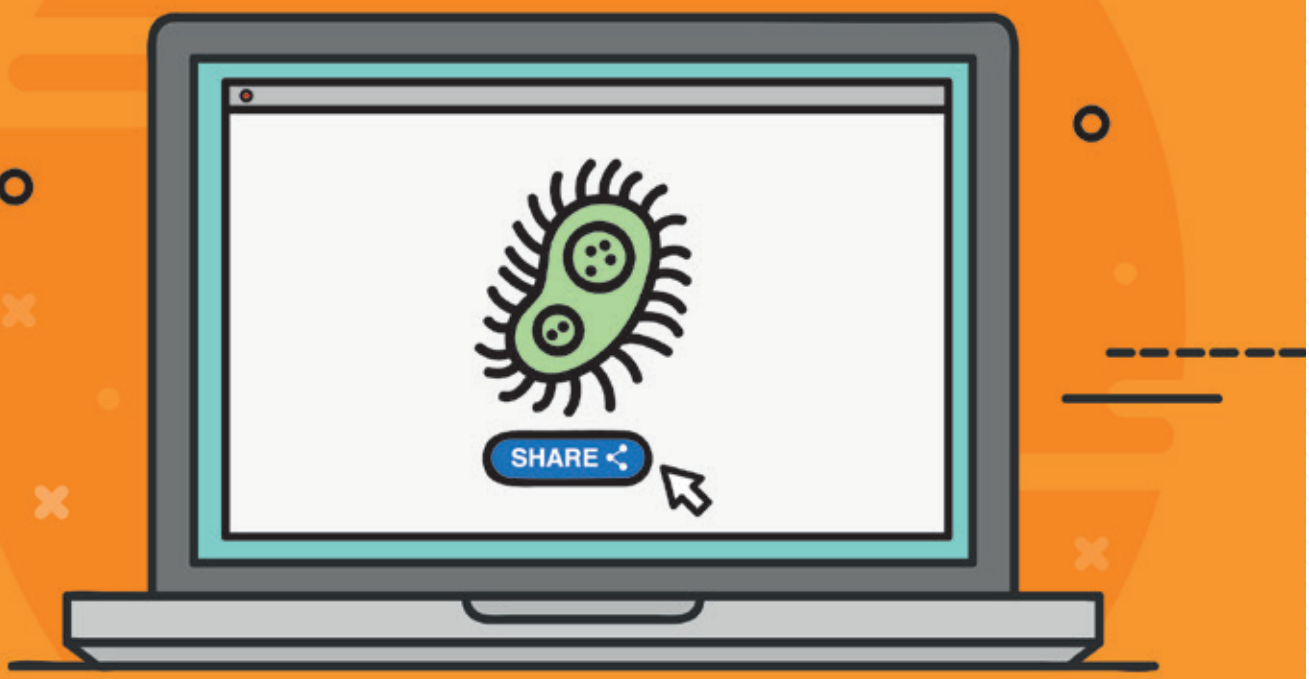

Going Viral

How social media posts are providing doctors with a new diagnostic and epidemiological tool

By Santhosh Nadipuram

With the digital revolution firmly underway, pathologists hear a lot about new microscopes, automation devices and long-distance reporting software designed to make their jobs easier. But now, a different kind of diagnostic tool is catching physicians' attention: social media. It's a tool I recently had cause to experience for myself, as my colleagues and I dealt with a cluster of cutaneous leishmaniasis cases that had arisen in a youth group on a trip to Israel – noteworthy because we were able to detect this cluster thanks to the teens' social media posts (1).

The original pictures of the leishmaniasis lesions were taken by the kids themselves. One of them shared the very first photograph with friends after diagnosis by traditional means – and once the first picture was distributed, other kids replied over social media, sharing their own lesions along with the likely diagnosis. By the time the fourth or fifth child had made a doctor's appointment, they were actually suggesting the diagnosis themselves (often along with showing the photographs they'd taken, or sending them to offices at the doctors' request). My co-authors and I alerted one another to the presence of an outbreak fairly early on, after gathering histories from our patients and using the Emerging Infections Network (EIN; ein.idsociety.org), an online resource for infectious disease monitoring. After that, we were able to track leishmaniasis cases and provide information as needed – something that might never have happened without the teens' initial ability to share their diagnoses and the doctors' subsequent ability to connect with one another.

At a Glance

- *Social media use is rising exponentially – with surprising benefits for disease diagnosis and monitoring*
- *An outbreak of leishmaniasis was detected when teenagers shared photographs of their lesions on social media and alerted their doctors*
- *For healthcare providers on social media, the value is immense – but it's important to be careful about maintaining patient privacy*
- *This is only the beginning of a social media revolution in healthcare, and as guidelines develop, I anticipate that its utility will only increase*

Public posting about public health

The utility of social media for disease diagnosis and monitoring is actually an emerging area of study, and we've heard of a number of other experiences similar to our own. For instance, in 2012, Facebook was used to help diagnose a group A streptococcal outbreak (2), and reports on other platforms like the review site Yelp have allowed us to not only spot outbreaks, but identify the foods responsible (3). The information doesn't just flow one way, though – doctors have also used social media to alert patients and communities of outbreaks in places ranging from college campuses to war zones (4–6). Even some public health

systems have set up successful systems for citizens to report outbreaks; Chicago, for instance, has an online food poisoning alert form (foodbornechicago.org).

Social media is here to stay, and people are going to use it to share stories about their health. If we as providers and epidemiologists can find a way to harness this ubiquitous form of communication, we not only become better informed ourselves, but we can effectively partner with the community at large to track, diagnose and hopefully stop outbreaks. Learning more about how to best leverage social media will require traditional epidemiologists to partner with scientists conversant with large-data analytics, so that they can sift through the massive amount of traffic that social media sites experience. Picking out relevant data will be a challenge, but it's one I believe is worthwhile for the knowledge we can gain.

"Social media is here to stay, and people are going to use it to share stories about their health."

Social media safety

There isn't much guidance available to healthcare professionals who want to use social media for epidemiology – or any other innovative purpose. Current guidelines (set by individual institutions, medical boards, or organizations) focus on protecting patients' privacy and ensuring that providers maintain a professional relationship with

An Outbreak of Misinformation

In the age of social media, stories of disease epidemics spread across the Internet at an impressive rate. From Ebola to measles, rumors and anecdotes can travel fast. At the same time, news stories abound on so-called "anti-vaxxers" – members of the public who believe that vaccines are unsafe and refuse to have their children inoculated against common diseases.

In recent years, these two issues created something of a social media storm, culminating in the December 2014 outbreak of measles that began in Disneyland, California and traveled to multiple states. Parents' failure to vaccinate their children was considered a crucial factor in the spread of the disease (1), and the outbreak brought the controversial topic into the limelight.

The decreased uptake of vaccination in some areas and populations has become a serious concern for healthcare providers (2) – but how do you convince people who are scared or mistrustful of vaccines to give them to their children? You could take a cue from social media…

"Misconceptions regarding vaccine use and safety are frequently shared and transmitted over social media," says David Broniatowski, co-author of a study that mined available data to gain insight into how the Internet, the public, and viruses interact (3). "Furthermore, social media allow these misconceptions to spread rapidly, with almost no regulation or factual verification. We wanted to understand what makes these misconceptions spread and what strategies public health communicators could use to combat their spread."

The team used 4,581 news articles concerning the outbreak and looked at their content, including statistics, stories, and "gists" – the bottom-line meaning of the information presented. They also looked at the number of Facebook shares each piece garnered. Ultimately, they found that articles containing a gist were shared most on social media, and articles containing statistics were also frequently shared. Personal stories did not greatly increase Facebook shares, which the team concluded may mean that stories are only effective when they also include a gist. "For example," says Broniatowski, "someone might tell a story about how their child was not vaccinated and then got the measles after going to Disneyland. Such a story is likely to be effective if it communicates a bottom-line gist of, 'If you don't vaccinate your child, they are at risk.' On the other hand, a story that does not communicate a gist is less likely to be effective. You're left asking, 'So what?'"

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Communicating With the Public

David Broniatowski's team suggest the following tips for effective communication with non-experts

1. Start with an evidence-based fact or statistic
   "Vaccines, like any medicine, can have side effects, but most children who get the MMR shot have no side effects. However, measles can lead to pneumonia, deafness, lifelong brain damage, or even death, and almost one-third of children with measles have to be hospitalized."

2. Use an explicit link to connect this fact to the bottom-line meaning
   "And the reason that's important is…"
   "What that means to you is…"
   "So what I tell patients is…"

3. Finish with the gist
   "Taking any risk that your child could get measles and suffer serious complications just isn't worth it. Vaccination is the best way to protect your child."

David Broniatowski is Assistant Professor at the Department of Engineering Management and Systems Engineering, School of Engineering and Applied Science, The George Washington University, Washington, USA.

them online. They tend to be focused on physician-patient relationships, assuming that doctors will use social media as a tool to communicate with individuals and occasionally post more broadly regarding public health (for instance, offering advice on using sunscreen, keeping up with vaccinations, water safety, and more). Outbreak reporting and monitoring is so new that most of these guidelines don't even acknowledge, much less address, the possibility.

My advice to healthcare providers on social media? As far as communicating directly with your patients, or with other providers about particular patients, make sure to look up your institution's guidelines on social media communication and sharing; those are usually in line with both good ethical practice and legal protections for patient privacy. Obtain a patient's direct consent before sharing, posting, messaging or tweeting any details about the case. From a laboratory standpoint, I advise against any sharing of patient samples (for instance, as photographs or videos). These are a minefield of protected health information. Labs that want to participate in online campaigns regarding public health should use the Centers for Disease Control and Prevention (CDC) social media toolkit (2) to maintain best practices and get their message out. As far as patients communicating with one another, it's up to them – but I suggest that physicians advise patients to look after their personal information; it can be disseminated quickly if they're not careful.

The main source of outbreak reporting by healthcare workers in the United States is the CDC. Although their toolkit for communicating outbreaks over social media is useful, the key thing to remember is that it's still just as important to be aware of your patients' privacy and their individual rights, especially when sharing photographs where patients can be identified. That can be an issue for pathologists who may need to share images of their patients for disease identification, consultation, or monitoring. Luckily, it wasn't a problem for us, because our patients mainly spoke to one another over social media and then brought the outbreak to our attention in person. We were able to avoid the potential pitfalls of social media use by not disseminating any patient information – they did that themselves.

The strength of sharing

It's a wonderful thing to be able to partner with the non-medical to help diagnose and treat disease. If people take the initiative to participate in their own care, everyone benefits. This is true for outbreak reporting (in our case), treatment and monitoring (as when parents and loved ones can take care of sick family members and report to their doctors), emergency response (when people can almost instantly communicate environmental and health conditions), and more – and information can flow from the provider to the public, or from the public back to the provider.

The greatest advantages of social media are rapid communication and an "amplification" effect, as certain topics start to trend on social media and are more frequently shared. In the case of our leishmaniasis outbreak, one teenager was diagnosed, and in a matter of days more than 10 others knew what infection they had and whom to talk to about it. Likewise, the treating physicians were able to quickly communicate their findings with one another using the EIN. Before social media became commonplace, this level of recognition might have taken weeks.

The biggest disadvantage, though, is that we're dealing with a massive amount of data. Everyone on social media has an equal voice – whether they're laymen or trained professionals. That means that

"It's a wonderful thing to be able to partner with the non-medical to help diagnose disease."

there is a potential for false reporting, of course – but more importantly, it poses the problem of separating fact from fiction. If a public health agency is monitoring social media in order to detect outbreaks or other health emergencies, they'll need a method by which to sift through the torrent of information and identify which reports need follow-up.

My dream for the future is that social media will allow anyone to connect to their medical teams and healthcare agencies, so that they can discuss their health and the health of their community without compromising privacy. My colleagues and I would also love to see the EIN used more broadly to inform fellow healthcare workers of outbreaks and emergencies, and crowdsourced consulting (medscape.com/consult) used to request assistance and second opinions from colleagues anywhere on the planet. These tools must be used with caution, though, because there are restrictions on the information that can be shared – and that can lead to faulty consultation. It's our job to ensure that we're using these systems as well as possible, but also to keep in mind their limitations.

The application of social media to healthcare is definitely a field of rapid growth – from simple posting all the way to using advanced analytics to track pandemics. Just as we had to create laws and ethics to communicate with patients in our offices and via telephone, we will have to address questions of privacy and public information on the Internet as well. The benefits are worth the teething troubles, though, as our world becomes increasingly connected and healthcare information is freely available with the click of a "Share" button.

Santhosh Nadipuram is a postdoctoral research fellow in Microbiology, Immunology and Molecular Genetics at the University of California, Los Angeles, USA.

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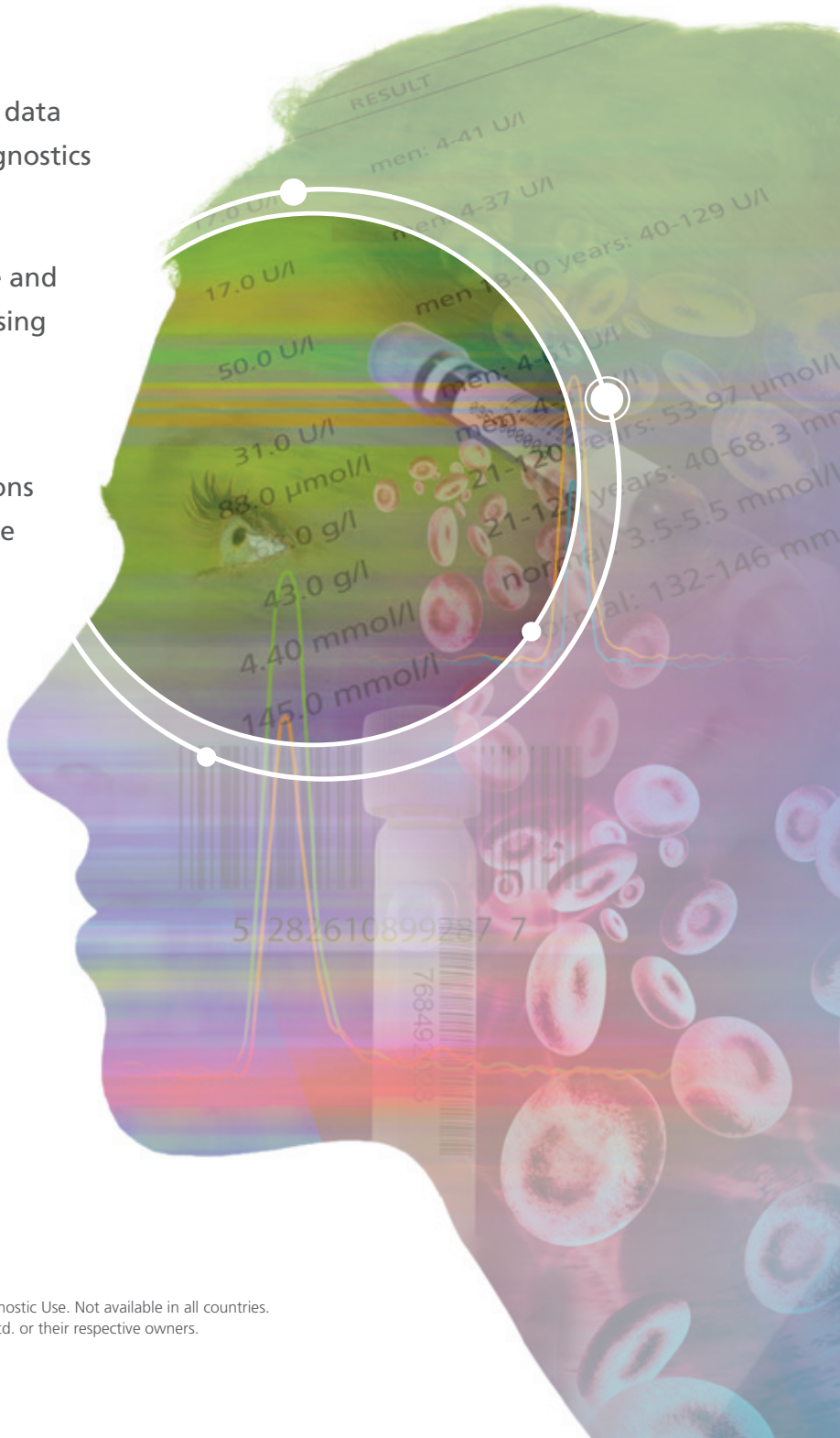

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(Virtual) Reality Check

How VR can enhance surgical pathology education

By Shyam Prajapati and Emilio Madrigal

Defining virtual reality (VR) is akin to defining art. With either one, it's controversial to confine a diverse range of human expression to just one concrete idea. The widespread perception of VR is one of simulated, lifelike artificial environments – but that doesn't reflect the spectrum of experiences available today, which range from passive encounters with three-dimensional videos to entirely digital environments manipulated by interactive devices. There's even augmented reality, which supplements our view of the real world with a layer of computer-generated content. Why the recent proliferation of variability and versatility in VR? We've built newer and more capable technologies (compact high-resolution displays, motion sensing, voice control, even gaze tracking) – and more importantly, processing power for mobile devices has become increasingly affordable. That means big-name manufacturers are racing to dominate the market with their own VR platforms. Google, Microsoft, Samsung, HTC, Sony and even Facebook have thrown their hats into the ring, and have flooded the market with low-cost headsets ultimately driving adoption. A recent report from Goldman Sachs presented a base case scenario estimating US$80 billion in revenue from VR/AR hardware and software by 2025, with healthcare contributing US$5.1 billion (1). VR is the next big computing platform – not just for entertainment, but for retail, education, and even medicine.

At a Glance

- *Virtual reality (VR) encompasses a broad spectrum of simulated experiences and is gaining popularity as technology advances*
- *It has the potential to make medical education safer, more affordable, more accessible, and more readily adaptable to change*
- *We propose a 2D and 3D VR project that supplements traditional surgical pathology teaching methods*
- *VR gross dissection libraries like the one we have initiated could become a cornerstone in standardizing surgical pathology practice*

Medicine's digital makeover

VR itself is not new to the medical field, but the explosion of affordable, capable equipment is. Recent articles have highlighted the technology's possibilities in patient care, including phantom limb pain management (2), post-traumatic stress disorder psychotherapy (3), 3D visualization for colonoscopy studies (4), and even as a "warmup" to improve performance in the surgical suite (5). The teaching and training benefits are easy to see; residents in multiple medical and surgical fields have used VR to acquire new skills at minimal cost and with no risk to patient safety (6–8). But despite its utility in other specialties, VR adoption in surgical pathology practice and education has been surprisingly slow.

Why does gross specimen processing need an update? Proper examination of surgical tissue is fundamental to patient care, especially in tumor identification and staging. Cancer diagnosis and treatment require a multidisciplinary approach involving surgery, radiology, oncology, and – last, but not least – pathology, which objectively supports clinical decisions. Although cancer identification has improved over the years, a highly skilled pathologist may still ultimately mean the difference between appropriate care and potentially dangerous treatment. Especially as we increase our comprehension of just how heterogeneous cancer is, it becomes more and more important to ensure efficient, standardized education for our residents and pathologists' assistants.

Enhancing education

Last July, we were faced with the month-long challenge of passing on as many of our "senior resident" skills as possible to our incoming first-year pathology residents. In doing so, we noticed a few shortcomings in the teaching process. First, trainees typically enter the hectic environment of the working laboratory with minimal prior exposure to surgical pathology – and yet they're expected to swiftly reach a high level of autonomy in performing tasks that require medical knowledge, fine motor skills, and keen attention to detail. Second, the level of responsibility should increase as trainees demonstrate proficiency, but that often doesn't translate into reality because of high workloads, limited instructor availability, or the push for decreased turnaround times. Third, the traditional resources for gross specimen processing include text-based instruction and two-dimensional diagrams – but our work itself is inherently visual and 3D. We noticed how time-consuming it was becoming for junior residents to diligently review and re-review manuals of surgical pathology procedures that simply weren't making the grade.

After contemplating these issues, we decided our trainees needed a modern solution. It needed to be innovative and progressive, while still capable of effectively teaching the fundamentals of gross specimen examination: orientation, description, dissection, and sampling. From our shared interest in informatics, we drew the inspiration to use VR – a logical choice because it's safe, inexpensive, enjoyable, and has proven benefits in motor skill learning (9). We had the added benefit of being able to tap into New York City's growing VR community, which offered a wealth of insight and collaboration from other creative minds like independent software developers and 360° video producers.

What we created was an instructional library viewable on both 2D and 3D platforms (see Sidebar "How to Build a Virtual Reality Environment for Pathology"). The content was designed as a passive, immersive environment with audio voice-overs that provide instruction and highlight clinically relevant aspects of different specimen types. Off-the-shelf products, including a stereo camera system, metal mounts, and a smartphone, were used to strategically capture video from the prosector's point of view. During filming, specimens were placed on a white surface alongside a fixed ruler that provides the viewer with an approximate scale in every frame. We chose our cases with consideration for the most commonly encountered specimen types at our institution, so that we could offer our trainees an experience as close as possible to "real life."

When filming was complete, we asked nine junior residents to watch the content in both 2D and 3D viewing formats (see Figure 1) and provide feedback on the experience. We knew that some viewers of 3D media may develop simulator sickness – a type of motion sickness characterized by visual discomfort, drowsiness, disorientation, nausea and possible vomiting – but a questionnaire completed at regular intervals during the study told us that none of the participants developed significant symptoms. After the experiment, our residents provided positive opinions of the tested videos. All of them

How to Build a Virtual Reality Environment for Pathology

Footage from each camcorder was shot in high definition (1080p) at 60 frames per second.

Raw files were converted to head-tracking-capable stereoscopic 3D, with adjustments to horizontal and vertical convergence to obtain the desired depth of field.

Post-production editing included contrast and color level adjustments, speed modifications, voice-over audio, and complementary multilingual subtitles.

Videos were exported at a 16:9 aspect ratio.

Study participants watched the 2D format on a desktop monitor, and the 3D format using a smartphone-based, head-mounted display.

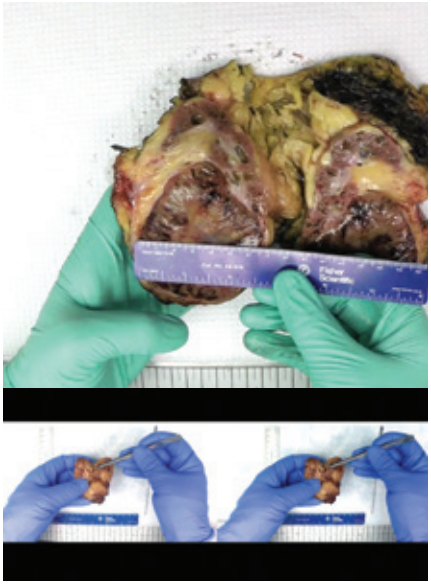

Figure 1. Examples of virtual specimen examination seen in 2D (top) and stereoscopic 3D (bottom) viewing formats.

said that they would use a gross processing video library to prepare for examining a new specimen type, and most reported that they were more confident in gross specimen processing after trying our simulation. A majority also said that the 3D aspect improved the viewing experience. Overall, although this was a proof-of-concept experiment with preliminary content, it's a promising beginning (10).

The future (of VR) is so bright, we gotta wear shades

Thanks to technological advances, increasing popularity, and ease of use, we anticipate that VR will be a key component in teaching the next generation of physicians. Ultimately, it will enable safer, cheaper, more accessible, and more adaptable medical education. And where better to begin than with surgical pathology? It's a highly visual discipline, and by building on the strengths of traditional teaching methods, our dynamic approach allows viewers to appreciate the procedural actions involved in specimen processing. To improve it further, our main focus is on continuing to develop the gross video library until it includes all of the specimen types a trainee might encounter in an anatomic pathology laboratory. Although it's an ambitious endeavor, it has the potential to educate not only pathology residents, but also medical students, pathologists' assistants, and surgical residents and fellows. Impressive claims, perhaps – but our goal, and the reason we developed our VR system, is to maximize efficient and meaningful education in surgical pathology so that our patients can receive better care.

Shyam Prajapati is born resident physician in Anatomic & Clinical Pathology at The Mount Sinai Health System in Manhattan, New York, USA, with an interest in hematopathology and clinical informatics.

Emilio Madrigal is a Cuban-born resident physician in Anatomic & Clinical Pathology at The Mount Sinai Health System in Manhattan, New York, USA, with an interest in surgical pathology and clinical informatics.

#pathologyVR

We have started to use the metadata tag #pathologyVR across social networks to make it easier for users to see who's discussing the subject and find relevant information. To learn more about our project, or to find 2D and stereoscopic 3D samples*, visit our pathology and VR interest website at pathologyvr.org

**To view the stereoscopic 3D content, you'll need a head-mounted display or stereoscopic glasses.*

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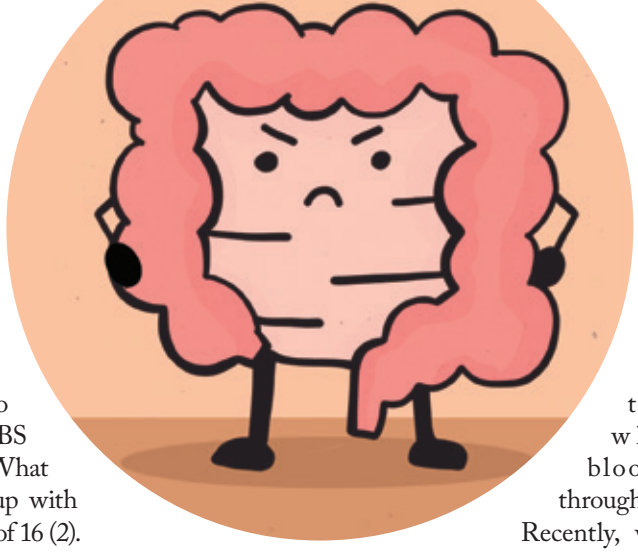
A Breath Test for the Bowel

A new panel of biomarkers present in exhaled breath may offer the first reliable, noninvasive way of diagnosing irritable bowel syndrome

By Frederik-Jan van Schooten and Zlatan Mujagic

Irritable bowel syndrome (IBS) is currently somewhat of a mystery to the medical community. There's no clear cause of the disorder, no reliable way of testing for it, and no cure. Patients are diagnosed based on tests to exclude other conditions, classified according to symptoms, and treated to alleviate the impact on their daily lives. But with a problem as common as IBS – believed to affect up to 15 percent of the population in developed countries (1) – the less guesswork involved in detecting and treating the condition, the better. That's why we decided to focus on finding a quantitative biomarker to assist with IBS diagnosis. What we ended up with was a panel of 16 (2).



At a Glance

- *Although it's a common and disruptive disorder, there's currently no reliable way of testing for irritable bowel syndrome (IBS)*
- *Diagnosis often relies on invasive testing to exclude other diseases – but a new breath biomarker panel might offer a better way*
- *The panel measures 16 volatile organic compounds that appear in different quantities in the exhalations of IBS patients*
- *Eventually, it's hoped that a point-of-care test for the condition could be developed that uses a small, simple electronic device to obtain and analyze the breath biomarker data*

Why focus on IBS?

About 10 years ago, we were looking for possibilities to describe noninvasively inflammatory processes in the body. Our initial research suggested to us that measurement of chemicals in breath were promising for that purpose. In recent years, analyses of breath metabolites have shown potential as a method of identifying biomarkers for a range of disorders. Why is breath so interesting? It contains a surprising amount of information. There are numerous anecdotes about nurses and doctors who diagnose their patients' problems by smell. Liver disease can show itself in bad breath due to sulfurous compounds; uncontrolled diabetics exhale acetone, giving their breath a sweet smell; even some dogs can spot cancer by smelling the patient's breath (3).

What actually causes the odors that can lead to a diagnosis? These scents are combinations of specific volatile organic chemicals (VOCs) that activate a unique set of olfactory receptors in the brain. Human beings can detect up to 10,000 different scents – but which chemicals are related to which diseases? That's the $64,000 question – and the reason we developed a biomarker discovery platform based on gas chromatography mass spectrometry (GC/MS). With that and a liberal application of multivariate statistics, we hope to find the chemicals associated with different diseases (see Figure 1).

IBS might not be the first condition that you would consider suitable for breath testing. Lungs are the obvious choice, but we soon realized that other internal organs release chemicals into the blood, and that those chemicals can enter the breath when the blood travels through the lungs. Recently, we showed that Crohn's disease activity could be monitored by breath testing (4) – so we thought we would turn our attention to another bowel disease with an equally urgent medical need. IBS is a common functional gastrointestinal disorder for which no reliable biomarkers currently exist. Its diagnosis is based on subjective symptoms at the moment, but objective biomarkers could improve the process and reduce the psychological stress and potential health risks of invasive testing.

The pathophysiology of IBS is poorly understood – and so far, none of the markers that relate to the potential pathophysiological processes have proven to be reliable. The great advantage of analyzing VOCs is that every person's breath contains a large number of these compounds. Many are products of metabolic or inflammatory processes in the human body – and some may result from the pathological processes of a specific disorder like IBS. Advanced statistical methods can identify small sets of compounds that are altered in patients relative to healthy control subjects. The beauty of this method is that we don't need to know what causes the production of these metabolites; we just need to be able to measure them. So even without fully understanding the pathophysiology of IBS, we can measure all of the metabolites to discover biomarkers that are characteristic for the disorder, or for gut health in general.

Selecting specific markers

We found 16 VOCs – including butane, n-hexane, methylcyclohexane and aziridine – which together form the biomarker panel for IBS. Most of these

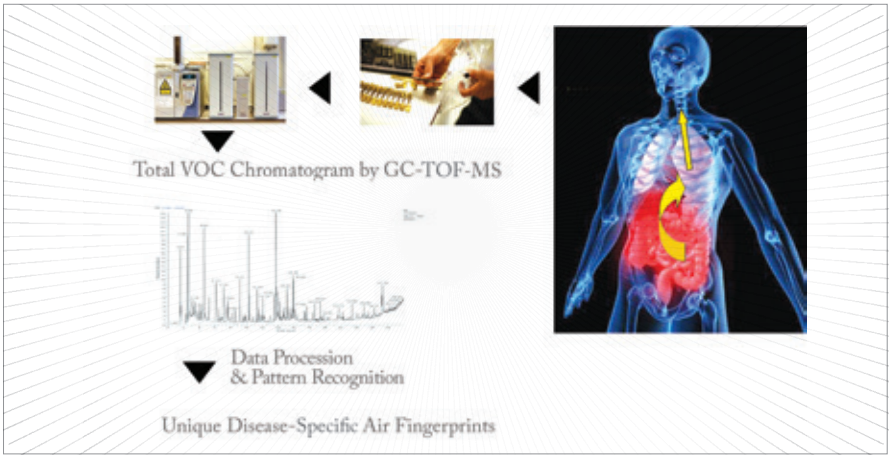


Figure 1. The process of using exhaled VOCs as biomarkers for IBS and other disorders. Breath samples are captured in tubes and subjected to GC/MS; the resulting spectrum is analyzed to yield information on disease-specific alterations to concentrations of the relevant compounds.

compounds can't be linked directly to a specific pathophysiological mechanism or metabolic process. The analysis of breath metabolites on this level is quite new, so we're still working to determine the origin of many of the chemicals. One of the most interesting ones is aziridine, which is increased in breath of IBS patients compared to controls. It's known to be produced by the Actinobacteria, one of the four predominant microbial phyla in the human body, so we can speculate that the aziridine we detect in breath may originate with these or similar gut bacteria. We also know from previous studies that the composition of the gut microbiota in IBS patients – including Actinobacteria – may be altered (5), so the changes to aziridine levels struck us as a finding worthy of further investigation.

To test our VOC biomarker panel as a whole, we asked subjects from a general population cohort to complete questionnaires on gastrointestinal symptoms. We selected 239 respondents with moderate to severe symptoms, regardless of whether or not they had an IBS diagnosis, and found it very interesting to see that the biomarker panel showed a moderate (r=0.54) – but highly significant – correlation with the severity of a range of gastrointestinal symptoms. This indicates that our biomarkers might be useful not only for patients with potential IBS or similar disorders, but even for studies that focus on gut health in the general population.

As in most scientific studies, every finding leads to new questions. From a scientific point of view, we're very interested in identifying the biological processes from which the breath markers originate. We are working on that with cells in culture, mimicking individual pathological processes to see which compounds are released. From a clinical point of view, though, the next important step is the external validation of our biomarker panel in a second cohort of IBS patients. In particular, we want to test its potential to discriminate not only between IBS patients from healthy controls, but also between IBS and other disorders with similar clinical presentations.

Turning theory into reality

Eventually, we'd like to see the panel used for IBS diagnosis in the clinic. Of course, we'll need more research before we reach that stage – but, if it continues to prove its potential throughout the next validation steps and in future studies, I can imagine an exciting future for it. For instance, at the moment, we trap the breath VOCs in tubes that are transported to the clinical chemistry department for GC/MS measurement and statistical analysis. That results in a diagnosis we can deliver to clinicians within hours. Our ultimate goal, though, is to develop a handheld device (like a miniaturized GC/MS or an electronic nose) that sits on the desk of the treating physician and exclusively detects the compounds included in our biomarker panel. The device would also have the ability to process the raw data, leading to an immediate diagnosis!

There's plenty of potential for methods like these to reduce our current invasive diagnostics. One day, we hope they might replace tests like endoscopies, intestinal biopsies, and even blood draws. Right now, we're still some way away from using our IBS breath test panel in day-to-day clinical work – but we're optimistic that we'll soon have that goal in our sights.

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Art From the Heart

How images created by pathologists and patients can forge a connection between the two – and raise awareness of pathology's role in patient care

An interview with Marilyn Bui and Katherine Galagan

There's no one better placed than a pathologist to understand the connection between art and medicine. From those who find the hidden humor in histopathology to those who see the beauty in bacteria, it's clear that the people behind the microscope have an eye for the unusual and the visually fascinating. But it's not just the professionals who see it – the patients do, too. And when Ray Paul, an artist and sarcoma patient at Moffitt Cancer Center, wanted to see what his tumor actually looked like – to "stare his devil in the eye" – it was his pathologist, Marilyn Bui, who viewed it with him and later sent him digital images to enlarge and use as a canvas for his art. This unusual collaboration led to a better understanding of his disease for Paul, a better understanding of her patient for Bui, and a deeper connection between pathologist and patient. Now, along with College of American Pathologists publications committee chair Katherine Galagan, Bui has turned that experience and others into a book. "The Healing Art of Pathology" features artwork and essays about pathology from the perspectives of both doctors and patients – something both editors hope will give each side insight into the other, igniting communication and collaboration.

Bui and Galagan discussed the project and its potential with The Pathologist...

At a Glance

- *Many patients are still unaware of what a pathologist is, much less the vital role they play in patient care*
- *Inspired by an artist and sarcoma patient, a book of art by pathologists and patients, hopes to bring the two groups together*
- *"The Healing Art of Pathology" features paintings, sculptures, drawings, essays and more by people on both sides of the microscope*
- *The editors hope the collection will contribute to pathology awareness, and give patients and pathologists some common ground for communication*

Are patients aware of pathology's involvement in their care?

Katherine Galagan: When I say that I am a pathologist, people ask me, "What's that?" They often don't even realize that we have medical degrees, and they're even less aware of the different roles within pathology. If you mention television shows like CSI: Crime Scene Investigation or Quincy, M.E., their eyes light up – but all they know is forensic pathology. I have to tell them that forensics is only a small branch of pathology, and that most pathologists work with hospitals or universities to diagnose diseases, run laboratories, and conduct research.

The more outreach pathologists do, the more we will be understood. Before I retired, I spent many years running a Career Day for high school students to help them understand pathology and the different career paths related to the clinical laboratory. That's one thing our book is intended to do – help patients and others understand the role of pathologists. Jerad Gardner has written a fascinating piece in it about his experience as a pathologist member of an angiosarcoma support group on Facebook. It's these types of outreach efforts that will help the public understand not only what we do, but also how we can be of help to them.

Marilyn Bui: For most patients and their families, pathologists are silent, invisible physicians. This book approaches patients and families from a unique angle that emphasizes letting pathologists be seen and heard. Once patients have read the book and know what we are capable of doing for them, we hope that they'll reach out to us and give us the opportunity to better help them. We also hope it will inspire an enthusiastic and talented next generation of pathologists. After all, it's pretty cool to be a pathologist!

How did the "Healing Art of Pathology" book come about?

KG: The book was Marilyn's brainchild; she was inspired by the artwork of one of her patients – Ray Paul – created in response to his tumor. After meeting with her to review his slides, he asked her for photomicrographs of his tumor, from which he created the beautiful artwork featured in "The Healing Art of Pathology."

We gained so much from assembling the book! First of all, I learned a lot about the depth and passion of the human spirit in the face of adversity, challenges, and reflection. Second, I learned about the incredible talents hidden within our patients, colleagues, family and friends. And last but not least, I acquired a deep appreciation for Marilyn's dynamic energy and vision. She saw the beauty of this book from the beginning and pushed it forward with incredible persistence and resolve.

MB: When I found out that the initial layout of the book had a prohibitive

price tag, Katherine uttered the most encouraging words to me. "Don't worry," she said, "we will get it done." By learning a new computer program and doing the entire initial layout of the book, she saved the project and also made it affordable to the readers. She became a partner, a mentor and a friend during the bookmaking process, and I still have so much to learn from her.

We'd both like to thank all of the contributors for letting us share their personal stories. It is a privilege and an honor to be the keeper of their voices. I have learned so much from this experience and everyone involved. I sincerely believe that everyone has the potential to be a pebble in the water, generating a positive "ripple effect" in the world if we choose to do so.

Credit: Mary Lachman

Mermaid's Tale. Mary Lachman, an anatomic pathologist and mixed-media artist, says that this image "symbolizes my personal transformation through art. It represents an ever-changing and ever-evolving approach to creativity."

Should pathologists interact more with patients?

KG: Definitely! Not only do we have a lot to offer patients – a unique perspective on their disease without the heavy overlays of treatment options, symptoms, and so on – but we also have a lot to learn from them. If we are insulated from their concerns and misunderstandings, we can't ensure that their questions are answered from the pathology point of view. Although in many cases the primary care physician serves as the intermediary, the depth of that doctor's understanding of the pathologic process is necessarily more limited than that of the pathologist who's actually studying the tumor or disease. For some patients, like Ray Paul (see Sidebar, "More Than a Cluster of Cells") visualizing their disease can be an important step in understanding and fighting it. In addition, their questions may create new insights and suggest new avenues of study for us.

MB: Absolutely yes! I'm a cytopathologist. Performing fine needle aspiration biopsies at my patients' bedsides has always been a rewarding experience. By directly communicating with patients and the clinicians while examining fresh tissue samples in real time, cytopathologists play a critical role in patient care. Many patients are wowed by the microscope, my portable "lab," which reminds them of their high school science classes; others are impressed by the immediate diagnoses we render. Most of us became physicians because we like to help people – and once you meet the patient, you're no longer just "signing out a case," but helping a real person. You're more willing to go the extra mile, so the patient gets our best efforts.

The CAP (College of American Pathologists) Foundation has a signature program called See, Test & Treat (http://foundation.cap.org/get-involved/see-test-treat-program/). Created and led by pathologists, the program directly benefits underserved patients by providing them with breast and cervical cancer screening, education, and a connection to health care. My home institution, the Moffitt Cancer Center, has a Mole Patrol program (https://moffitt.org/cancers/melanoma/screening/) that promotes sun safety and provides free skin cancer screening to the public. Its medical director for the past two decades has been a fellow pathologist, who has led the program to great success – screening over 15,000

More Than a Cluster of Cells

How my pathologist helped me regain my confidence and self-worth, and confront my cancer

By Ray Paul

I am a 53-year-old artist, musician and science enthusiast with a Bachelor's degree in biology and a Master's in painting. I am also a sarcoma survivor.

I first noticed a rapidly enlarging lump protruding from my left flank in the spring of 2011. A urologist friend of mine agreed to remove it in his office, believing it to be a lipoma, but he quickly realized it was something more sinister. Marilyn Bui at Moffitt Cancer Center confirmed a diagnosis of high-grade myxofibrosarcoma, for which my primary oncologist gave me a prognosis of "better than a coin flip." Since then, I've had metastases and recurrences, repeated resections, chemotherapy, and several rounds of radiation therapy including participation in a clinical trial for high-risk soft tissue sarcomas (1) – and follow-up is still ongoing.

Before my experience, I had been aware of the pathologist's vital role in diagnosis and was able to read pathology reports, but I'd had no direct interactions with my own pathologists. In 2013, when my cancer returned for the third time, I was at my lowest point – physically, emotionally and spiritually. I had abandoned my art and was simply going through the motions of life. A concerned friend suggested I find a way to combine my artwork and cancer. Together, he and I came up with the idea of having images of my cancer cells printed onto canvas, serving as a substrate for my painterly expression. Marilyn graciously invited me to her lab to view my pathology slides and provided me with digital images of the tumor histology. The resulting paintings were exhibited in The Healing Arts Gallery at Moffitt Cancer Center in 2014 and also appear in "The Healing Art of Pathology." Why did this matter so much to me? I envision my art to be a persistent, visual manifestation of the battle raging within, and a powerful testament to the beauty and healing powers of hope. It was cathartic and inspirational for me to be able to attack my cancer directly, through the act of frenetically and meticulously painting over the printed images of my cancer cells.

The importance of this interaction with my pathologist can't be overstated. Marilyn stepped out of the shadows of the lab, put a face to pathology's mysterious workings, and made me realize that pathologists are an integral part of the patient care team. My interaction with her helped me regain my confidence and self-worth by directly confronting my cancer through art and collaboration. It helped me, a devastated cancer patient, return to life as a productive artist. And it gave me a major boost of confidence to know that such brilliant and dedicated professionals cared about me as a person – that I was more than just a cluster of cells gone haywire.

Healthcare services should never discourage contact between pathologists and patients. That would deny the patient the emotional, psychological and spiritual benefits of direct interaction – and for me, at least, that relationship has been invaluable.

To other patients, I would say: educate yourselves on the vital role of pathology and pathologists. Know that they are an integral part of the team fighting for you. Go to the lab, view the histological slides, and get to know your own pathologists. You will find, as I have, that they are caring human beings, dedicated to helping the patient win the battle and hopefully, one day, finding a cure.

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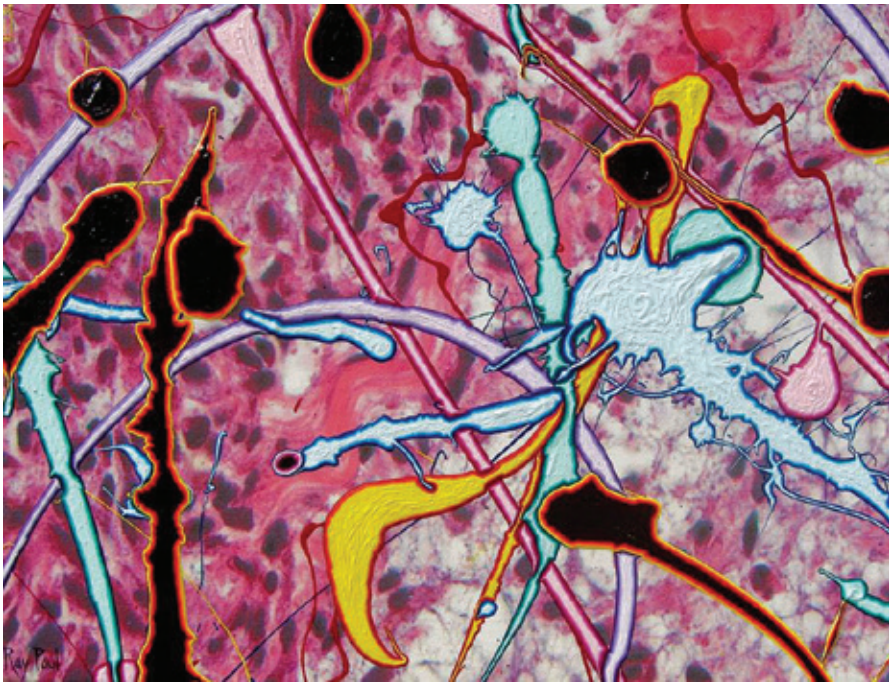


Credit: Ray Paul

Dendritic Swarm. Ray Paul, artist, biologist and sarcoma survivor, says, that this piece is a visual manifestation of the dendritic cell trial he underwent in an attempt to fight his cancer.

people. Quite a few pathologists I know volunteer at local and national patient education events on topics like cancer, heart health, or infectious disease. Most of us agree that interacting with patients reinforces why we became doctors and gives us the opportunity to learn from and be inspired by them.

How has open communication helped you and your patients?

KG: I am now retired – and during my career, my options for patient interaction were unfortunately very limited. However, I did review slides (and even gross specimens) with patients on several occasions, and I always found the interactions meaningful and considered them time well spent.

In addition, as a clinical pathologist, I occasionally had conversations with patients in the phlebotomy area, or as part of the transfusion service, especially early in my career. Later on, when I became involved with Lean process improvement, my medical center occasionally had patient team members who invariably provided a unique and useful perspective.

MB: I practice at Moffitt Cancer Center, which has an exceptional reputation in the US. At Moffitt, we put our patient first and work as a multidisciplinary team where pathologists are well respected. The sarcoma surgeons, medical oncologists and radiation oncologists feel comfortable sending people to me with pathology questions, knowing that I'll have the patient's best interests in mind and will be supportive of the group. I sometimes get phone calls from the clinic asking, "Could you please explain to our patient what this diagnosis means," or emails that say, "Can our patient email you about his pathology?" Because of the convenience of the Internet, patients also reach out to me directly. It's not uncommon for me to be the one helping patients get scheduled and seen by our sarcoma group at Moffitt.

What do you think the book will do for pathology advocacy?

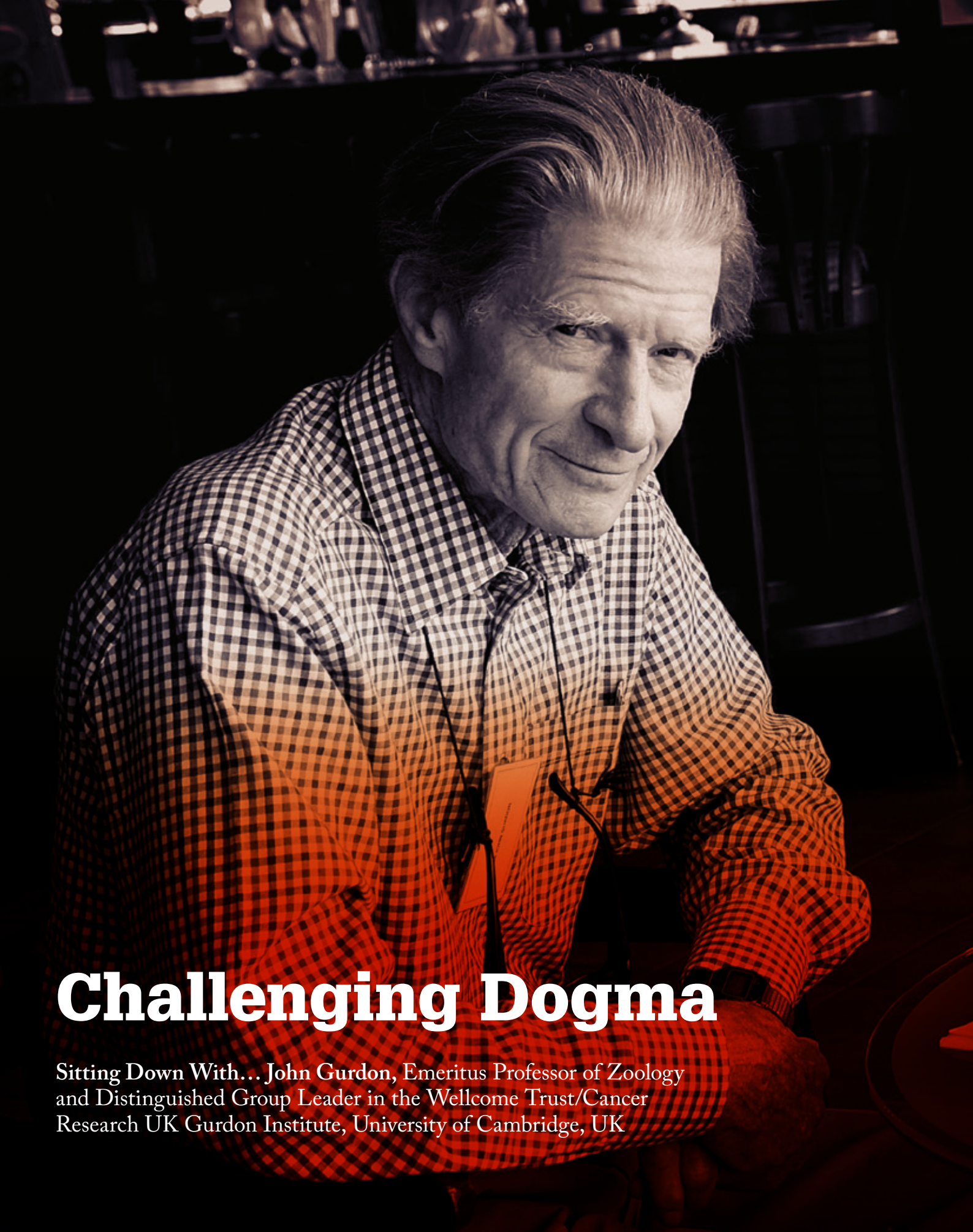
KG: We can only hope this book adds a few grains of sand to the mountain of advocacy efforts occurring worldwide. We hope to inspire pathologists to become even better leaders, team members, and patient advocates.

MB: In the circle of life, our individual roles as physicians and providers are transient. But as human beings, we are connected to each other and to the environment. We hope that "The Healing Art of Pathology" will show readers the beauty and the magnificent healing power of our discipline – so that together, we can make a better life for all of us.

Marilyn Bui is a Senior Member of the Department of Anatomic Pathology & Sarcoma, Section Head of Bone and Soft Tissue Pathology, and Scientific Director of the Analytic Microscopy Core at Moffitt Cancer Center. She is also a Professor and Director of the Cytopathology Fellowship Program at the University of South Florida Morsani College of Medicine Tampa, USA.

Katherine Galagan is an Anatomic and Clinical Pathologist, with subspecialty boards in Cytopathology. Prior to retirement, she was Chief of Pathology and Director of Clinical Laboratories at Virginia Mason Medical Center in Seattle, USA, and has volunteered in various roles for the College of American Pathologists.

"The Healing Art of Pathology" can be ordered at www.cap.org or by calling the College of American Pathologists at +1 800-323-4040 (option 1, request PUB315). Purchasing this book will support pathologists and royalties from the book will be donated to the CAP Foundation, to support their patient advocacy efforts, such as See, Test and Treat.



Challenging Dogma

Sitting Down With… John Gurdon, Emeritus Professor of Zoology and Distinguished Group Leader in the Wellcome Trust/Cancer Research UK Gurdon Institute, University of Cambridge, UK

What initially drew you to study science, and in particular cell development?

I always had an inherent interest in development from a very early age. I used to grow plants, make hybrids between different strains and became intensely interested in *Lepidoptera*. My removal from science education at school took me away from this career path – in fact, I ended up studying Classics. Although I was always interested in biological phenomena, I was put into Classics because they had spare teachers in the subject. Fortunately, I was able to get back into biology later on by pursuing a PhD in zoology. The work I undertook as a doctoral student wasn't standard zoology – it was nuclear biology, in fact, which is at least somewhat related to cancer.

Your career spans more than 50 years of biomedical research, much of which is highly lauded by the scientific community. What are your personal highlights from that time?

One of the pleasures of doing research is when some unexpected experiment proves successful – as most exploratory experiments are, of course, unsuccessful. An example of one that wasn't is the unexpected great success of injecting purified mRNA into eggs and oocytes of *Xenopus*, the African clawed frog commonly used as a model organism. All the predictions were that this could not possibly work because of the huge amount of RNA in such cells. But then again, I knew that every species is different – so experimentation is always worth a try!

How do you think the nature of biomedical research has changed since you began your career?

The range of experimental possibilities, especially at the biochemical level, has changed out of recognition during my time in science. When I started it was not possible to analyze any individual gene activity; all we could do was measure ribosomal and transfer RNA transcription. Now it is possible to determine the activity of each individual gene and even in individual cells. All this has happened during my time in science. I would say that technology has changed out of recognition in the last half-century. Now, we're able to answer questions in a way that could never have been done 50 years ago.

What was it like, as a young scientist, to challenge a scientific dogma as you did with your Nobel prize-winning discovery that mature cells can be reprogrammed to become pluripotent?

I was lucky that my experiments gave me an opportunity to reconsider the conventional dogma of the time. To be able to do this is itself an enormous incentive to continue work in a field.

Of course, my results were challenged by experts in the field – and this was entirely justified. In response, I extended my experiments and used genetic markers, which allowed me to prove beyond reasonable doubt that the results I had originally obtained were indeed valid.

Your research into epigenetics and pluripotency is still going strong. What are you currently finding most intriguing – and what do you think lies ahead for the field?

The question that most interests me at the moment is what causes the differentiation of nearly all cells in the body to be so stable. I want to understand the mechanisms involved in stabilizing the differentiated state of cells and that resist nuclear reprogramming. If we knew how those mechanisms worked, we could improve nuclear reprogramming for cell replacement therapy and hope to discourage cells from leaving their normal differentiated state to become aged or cancerous.

Even with the remarkable Yamanaka procedure (in which four transcription factors – *Myc, Oct3/4, Sox2* and *Klf4* – were introduced to mature cells to "reprogram" them into induced pluripotent stem cells), only a very minor proportion of cells make the change imposed by transcription factors. It would be of great interest, both scientifically and clinically, to understand what normally keeps cells firmly anchored to their intended destination so as to prevent disease and aging from causing cell change.

"Of course, my results were challenged by experts in the field – and this was entirely justified."

If you could go back and counsel a young John Gurdon just beginning his career, what would you say to him?

If all of the work you do fails to answer the question you've engaged, there comes a time when you have to acknowledge that and give up. A large proportion of my time in science has been devoted to experimental approaches that have not been successful, and have therefore been set aside.

However, an experiment that doesn't prove the hypothesis is not the same as a "failed" one. In my case, I was much attracted to working further on any result that seemed at the time not to be explicable. There is always something very rewarding in being able to understand what happens when an experiment either doesn't work or gives the unexpected conclusion. Therefore I recommend anyone now to pay special attention to any of their failed experiments.

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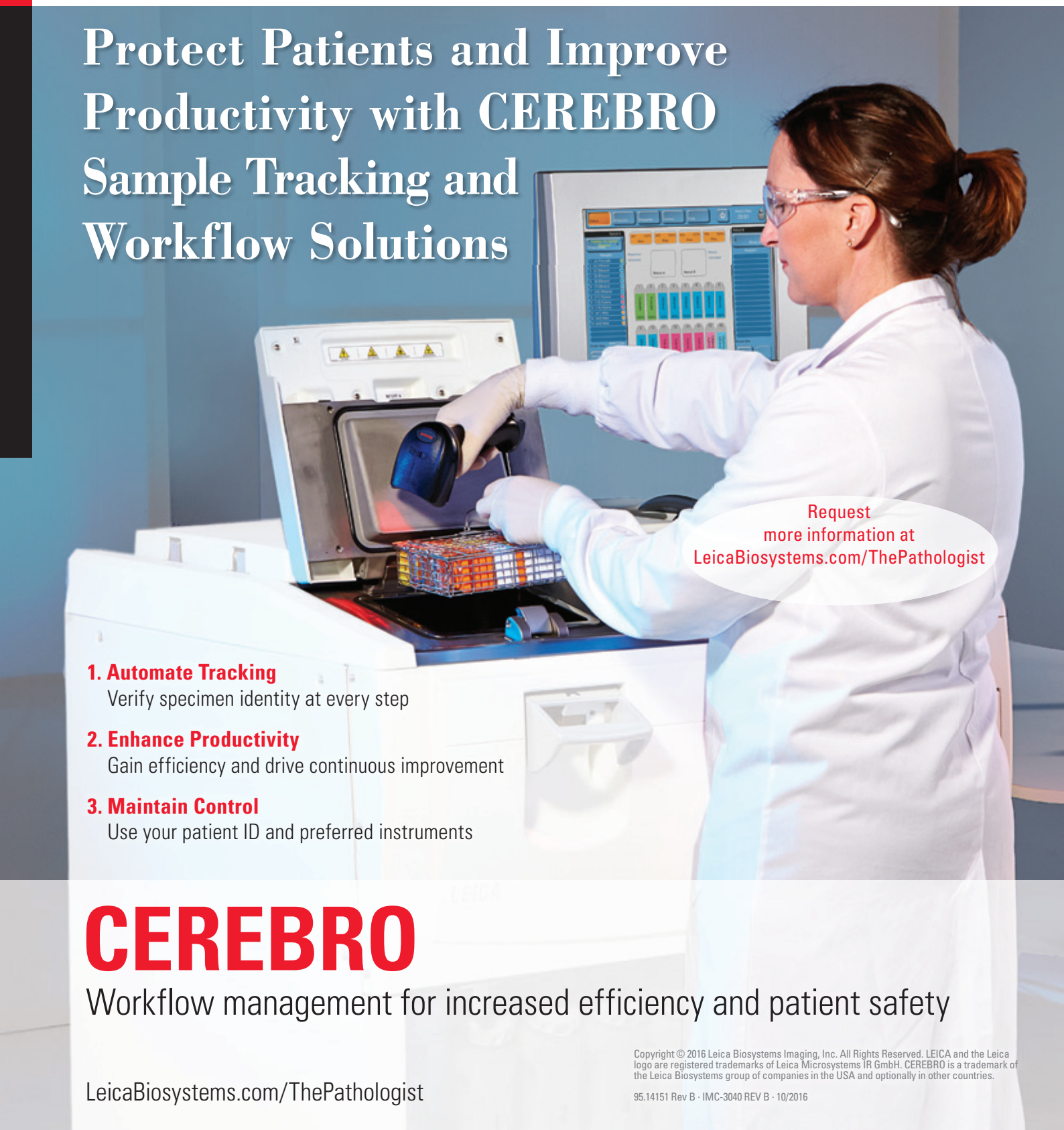

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