The magazine of the
Society for Applied Microbiology

# microbiologist

## INSIDE

## COSMETIC MICROBIOLOGY

*Clostridium botulinum*:
From botulism to Botox

Dangers of cosmetic contact lenses

The microbiological hazards of tattooing

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**Paul Sainsbury** reviews the content of this issue

microbiologist

## Cosmetic microbiology encompasses a much more diverse field than the analysis of dirty old mascara

Historical evidence suggests that we humans have probably been using cosmetic procedures to change our appearance since…well…forever. Archaeological evidence of cosmetics usage and application certainly dates from ancient Egypt and Greece – at least 6,000 years ago. Some historians even argue that a form of cosmetic body art may have been the earliest form of ritual in human culture, dating back over 100,000 years and that this was associated with the emergence of *Homo sapiens* in Africa.

One thing is certain though, for those involved in the cosmetics industry, microorganisms represent both a curse and an opportunity. Contaminating microorganisms in consumable cosmetics represent a serious and expensive problem for the stability and safety of the final product. The *European Cosmetic Products Regulation*, outlined in this issue by Heather Moore, shows us exactly what manufacturers must do to ensure the safety of their products just to sell them within the European Union. On the other side, microorganisms can produce vital ingredients for the industry in a cheap and efficient way, and Nicola Stanley-Wall and Chih-Yu Hsu detail just a few of these found in everyday products including toothpaste! In addition to this, Clare Taylor shows us that biotechnology and genetic engineering are potentially paving the road toward even more versatile uses for microorganisms, such as sunscreen and anti-ageing medication.

Now, as someone who has frequented a tattoo studio more than once, I was particularly unsettled to read Nicolas Kluger's article. It is an eye-opener and describes in detail what can go wrong under the tattooist's needle. The cosmetic contact lens industry has exploded in recent years as more and more people choose to temporarily change their brown eyes to blue or give the appearance of cat- or zombie-like eyes for fancy-dress parties. However, wearing any kind of contact lenses, including decorative ones, can cause serious damage if the lenses are obtained without a prescription or not used correctly. Christin Henein and Francisco Figueiredo write about the hidden dangers of cosmetic contact lenses and why you should never buy them from a Halloween shop!

We certainly couldn't let this issue go without mentioning botulinum toxin. A couple of kilos of this would kill every human on Earth, yet many people pay large amounts of money to have it injected into their foreheads. Brendan Gilmore describes the history and mechanism of action of Botox and shows us that modern medicine has also found other less vain applications for one of the most poisonous substances known to man.

In other articles W H Pierce Prize winner, Jack Gilbert, discusses our constant misunderstanding of the microbial world and answers the question "*how clean is too clean?*" and Martin Adams gives us another fascinating insight into London's Microbiota. We also announce in this issue details of the Summer Conference 2017 on Food Safety, the Winter Meeting on Synthetic Biology and Vaccines, our Early Career Scientists event on Bioethics, and a special one-day AMR conference headlined by Dame Sally Davies. All these events can now be booked online via our website **www.sfam.org.uk**.

## NEWS IN BRIEF

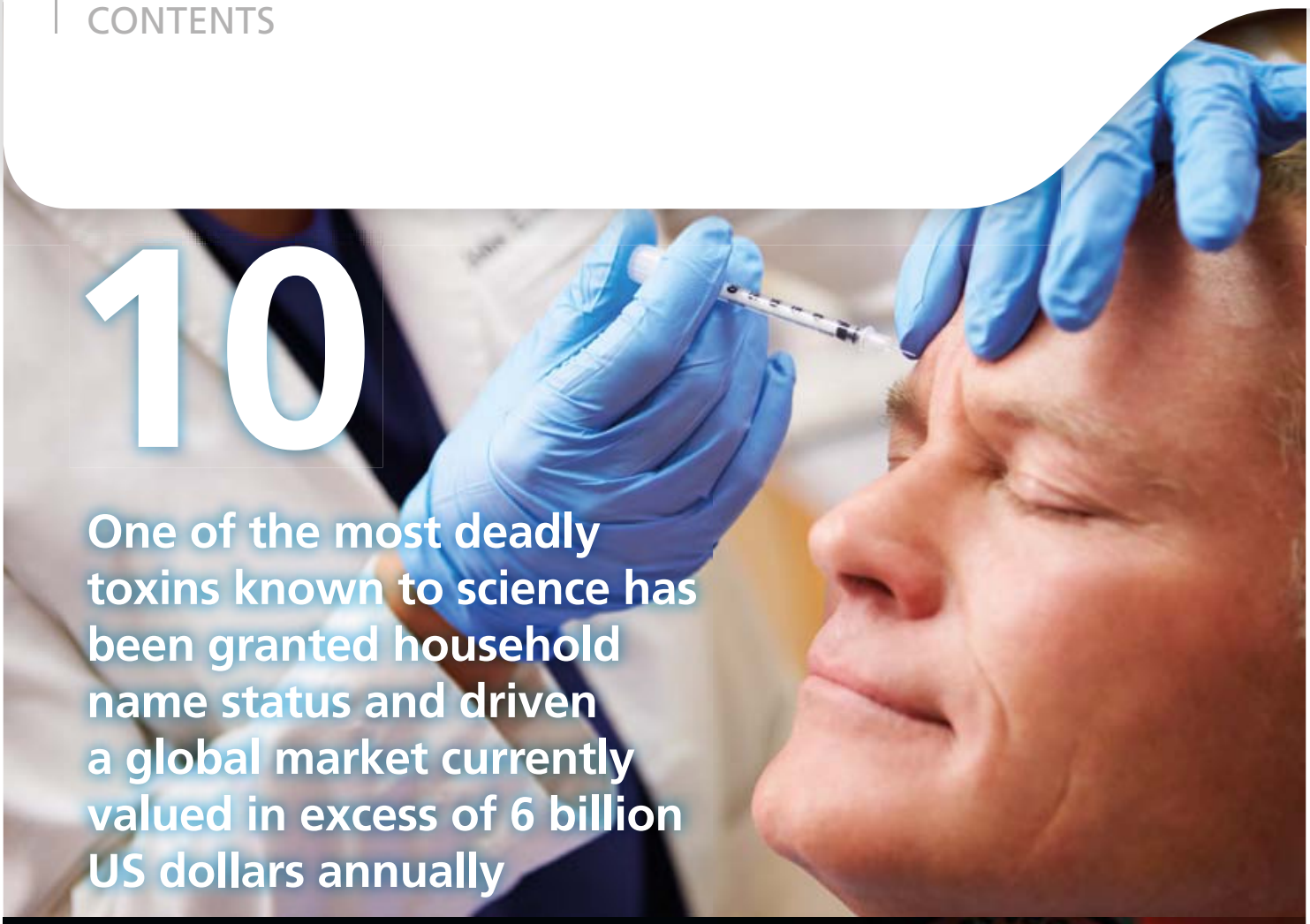
### Promising new antibiotic has been found in the human nose

Scientists say they have discovered a bacterium, *Staphylococcus lugdunensis*, lurking in the nose that fights methicillin-resistant *Staphylococcus aureus* MRSA by producing its own antibiotic named lugdunin. **http://bit.ly/2aqM1PV**

### Zika alert

The Centers for Disease Control (CDC) issued an unprecedented travel warning in August advising pregnant women and their partners not to travel to a small community just north of downtown Miami. The first time the CDC has warned people not to travel to an American neighbourhood.

**Paul Sainsbury**, *Editor*



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One of the most deadly toxins known to science has been granted household name status and driven a global market currently valued in excess of 6 billion US dollars annually

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## COMMERCIAL



# President's column

The Society for Applied Microbiology is proud to be an international Society with 36% of our Members coming from outside of the UK. This is apt as microbiology issues are not restricted to a single region of the world, with many of the microbiological problems we encounter either global issues or ones which have the potential to become widespread. Recent examples of outbreaks of diseases that raised concerns at an international level are evidenced by the Ebola and Zika virus outbreaks. These examples also further highlight that there may be a need for international expertise and cooperation to help counter them and contain the spread. The higher levels of foreign travel and tourism to more exotic destinations are often cited as one of the potential risks which might cause such diseases to spread and our Spring Meeting in 2017 will be focusing on some of these issues.

Of course such problems are not new: plague caused by *Yersinia pestis* (the Black Death) is believed to have been spread through trade routes from Asia into Europe and beyond and is estimated to have killed 5–200 million people over an approximately eight-year period during the 14th century. Such uncontrolled levels of disease and death seem unthinkable today which is why the estimate suggested by the O'Neill Report* in 2014 that the number of deaths attributable to antimicrobial resistance (AMR) globally could rise from the current estimated levels of 700,000 lives per year to 10 million lives annually by 2050 seemed so shocking. The final report published in May this year** also stresses that this is not a problem which can be addressed by a single country acting individually but needs coordinated international action on a number of levels. Antimicrobial resistance presents new challenges on a number of levels: it is not just the spread of one microorganism that needs to be controlled but many different species which individually present a disease problem; perhaps more importantly the genes producing resistance can themselves also spread independently of the microbial host species, thereby creating new resistant species more rapidly. The role that commensal microorganisms may play in this by acting as reservoirs for resistance genes has only recently received much recognition but contributes to the overall complexity of the situation that faces us. The need for interdisciplinary approaches to address the problem is also an important aspect that we as applied microbiologists need to recognize and be prepared to embrace as this is an issue which needs new thinking to address if the predicted figures for deaths from AMR microorganisms are to be avoided. The Society will continue to spotlight this issue in our conference calendar with our next one-day meeting focusing on AMR scheduled for 24 November 2016.

* *Antimicrobial Resistance: Tackling a crisis for the health and wealth of nations. The Review on Antimicrobial Resistance chaired by Jim O'Neill December 2014. http://amr-review.org/Publications*

** *Tackling Drug-Resistant Infections Globally: Final Report and Recommendations May 2016. http://amr-review.org/Publications*

**Plague caused by *Yersinia pestis* is believed to have been spread through trade routes from Asia into Europe and estimated to have killed 5–200 million people**

**Christine Dodd**
*President of the Society*

# Harper's Postulates

## Notes from the Chief Executive

# Evidence

For years I've wondered why so many headlines which relate to stories about bacteria have focused on the **number** of bacterial cells on a toilet seat/cash point/ your dining-room table.

These articles are invariably written to infer that because there are a lot of microbes on a surface, that surface is somehow dangerous to health because ALL bacteria, no matter what they are, will cause disease if there are enough of them. Bacteria are bad. Full stop.

However, in a recent* Washington Post article**, this year's **W H Pierce Prize winner**, **Jack Gilbert**, an environmental microbiologist at Argonne National Laboratory (see page 38 for his article), says: "*In the 150 years since we identified that bacteria were the cause of disease, we've become obsessed with the abundance of cells*". The thinking is, "*bacteria equals disease therefore more bacteria equals more disease. But that just isn't the case.*"

We are surrounded by bacteria: "*Scientists estimate that Earth is home to about 1 trillion species of bacteria. They dwell in the soil, the ocean, the air, your skin, your innards – they're unavoidable. Basically, it's the microbes' world and we're just living in it.*"

What many people may not appreciate, is that the **number** of bacteria on a surface means nothing in terms of how dangerous that surface is. We need to think critically and look beneath the surface (pardon the pun) of these headlines. Not everyone knows that the important thing to consider is the **type** of bacteria, rather than the number of cells. To use information appropriately we need **evidence** to draw a conclusion about the impact of the science behind these headlines.

So, to remain informed, we need **evidence**. As delegates heard at this year's Summer Conference Workshop entitled *Engaging with science policy*, many policymakers are not experts in a particular field, and so they rely on **evidence** from experts to inform policy decisions. In many cases it falls to scientists to provide that evidence. Some scientists are reluctant to do so because they perhaps don't think their knowledge or expertise is **relevant**, or sufficient to enable their voice to be heard. This is where learned societies, including SfAM can play a vital role: we work singularly, and in partnership with other organizations and learned societies, to provide expertise on a wide variety of subjects and policy issues. We respond to Government consultations, inquiries and provide expert witnesses on subjects such as STIs and 'Leaving the EU: implications' and 'opportunities for science and research'.

To ensure the Government is hearing evidence from experts, we rely on you, our Members, to provide that evidence and we encourage you to get involved. If you think you can help us to contribute to providing sound, good quality **evidence** to help inform policy decisions, get in touch. We're waiting to hear from you to ensure that we remain the voice of applied microbiology.

* *at time of writing.*

** *https://www.washingtonpost.com/news/speaking-of-science/wp/2016/07/05/dear-science-how-many-germs-are-actually-on-a-toilet-seat-and-should-i-care/?postshare=3711467810293139&tid=ss_tw*

**Lucy Harper**
SfAM *Chief Executive*

# Academic publishing


I have just returned from the Society's Summer Conference in Edinburgh where I helped chair part of the student session on academic writing. Now, many of you reading this will be PhD students and so will not have a lot of experience of writing for publication but, as the unofficial mantra of academia is 'publish or perish', it is a vital skill. I thought I would spend this column discussing a couple of important changes taking place in academic publishing at the moment which you may not be aware of but will become important to you down the line.

The first is the debate surrounding impact factors. The impact factor of a journal has been the metric by which its quality has been measured for more than 50 years. Impact factors are a measure of the average number of times articles in a journal have been cited over a given period. In recent years there has been a backlash against the use of this metric due to a number of well-documented failings. Recently, a paper has been submitted, authored by editors and policy advisors of some of the top international journals (Nature, Science, EMBO etc.), encouraging all journals to move away from impact factors as the measure of quality. So what are the alternatives? A number of other measures including Altmetric and Eigenfactor have been put forward but no one system has been universally adopted. So, for the moment, we continue to use impact factors but it is worth knowing there are other methods out there.

The second major area I wanted to highlight is the issue of open access. In April 2013, Research Councils UK (made up of BBSRC, MRC etc.) who fund many PhD or postdoctoral positions made the decision to require all peer-reviewed work generated via their funding to be made freely accessible via a scheme known as open access. The NIH and NSF in the US have similar policies. There are two main types of open access: green and gold. Gold open access is paid for by the authors and means that the final version of the article is freely available via the publisher's website from day one of publication. There are many journals which only allow publishing via this model; examples include the PloS journals and MicrobiologyOpen from Wiley. These journals charge a publication fee which is normally more than £1400 per article. The alternative is green open access where the final formatted publication becomes freely available on the publisher's website after a set period (normally 12 months) but the unformatted accepted version of the paper can be uploaded to an institutional repository. Green open access is normally free and many journals now offer both options, e.g., the Biochemical Journal. For more information, there is an edition of Micropod (*Sf*AM's podcast) on open access from 2013, available via the website **www.sfam.org.uk**.

## FURTHER READING

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# WHAT'S YOUR POISON? *Clostridium botulinum:* from botulism to Botox

*"Poison is in everything, and no thing is without poison. The dosage makes it either a poison or a remedy."*

**Paracelsus** (1493–1541), the father of toxicology

According to 'Bergey's Manual of Determinative Bacteriology' *Clostridium botulinum* is a Gram-positive, toxin-producing, anaerobic spore-forming rod with straight or slightly curved cellular morphology. However, this matter-of-fact description betrays nothing of the infamy of this most feared of foodborne pathogens, and source of one of the most poisonous biological toxins known to man. The pathogen *Cl. botulinum* (from the Latin *botulus* and adj. *botulinum*, pertaining to sausages) is so-called because of its original association with sausages as the causative source of fatal paralytic food poisoning, and not the morphology of bacteria themselves as sometimes suggested. Today, the toxins produced by *Cl. botulinum*, whilst still posing a threat to humans and animals alike, are both therapeutically and cosmetically useful in a wide range of applications.

Botulism, or food poisoning associated with the bacterium *Cl. botulinum*, has probably been a threat to human health from the earliest attempts of man to store or preserve food. There are few ancient historical references linking food with paralytic disease but a number of references allude to an association between the consumption of certain types of foodstuffs and death by paralytic disease. Some early dietary laws and edicts are inferred to reflect some knowledge of food poisoning. The most prominent of these was an order by Emperor Leo VI of Byzantium (886–911), which outlawed the manufacture of blood sausages, and outlined the penalties for flouting this command. However, it was not until the 18th and 19th centuries that outbreaks of fatal food poisoning in Germany's Württemberg region led to the first systematic studies of *Cl. botulinum* food poisoning. This increased rate of food poisoning has been linked to reduced standards of hygiene as a result of the widespread crippling poverty, a hefty toll exacted on the region by the devastation of the Napoleonic Wars.

During this period, as outbreaks of paralytic disease associated with consumption of meat were documented, the finger of blame pointed steadily at blood sausages. So much so, that in 1802 the Royal Government of Stuttgart warned its citizens of the 'harmful consumption of smoked blood sausages'. Fatal paralytic disease associated with food consumption soon became known as 'sausage poisoning' and the search was on to find the reason for this scourge. Around 1811–1812, the medical faculty at the University of Tübingen was enlisted to help find the culprit. Two faculty members, after studying the case reports collected in the region suggested that it was likely to be a 'zoonic, possible organic poison' and concluded that sausage poisoning came about because the sausages were not boiled for long enough, in an attempt to prevent them from bursting. In the following years the first systematic collection of case reports were gathered and published by two medical officers, J. G. Steinbuch and Justinus Kerner (a physician and romantic poet) in 1817. Kerner continued to study sausage poisoning and published his first monograph in 1820. He continued his studies using experimental animals (birds, mammals, amphibians and insects), which he exposed to botulinum toxin contained in crude extracts of 'sour sausages'. Kerner even conducted self-experimentation with the toxin, until his old university teacher (wisely) advised him to desist. Remarkably, Kerner's painstaking experiments led to an accurate description of the symptoms of botulism, a description of the toxin's mode of action, advice on the prevention and treatment of botulism (including the first successful application of the gastric tube to provide nutrition to paralyzed patients) and, critically, the suggestion that the unknown toxin could be therapeutically useful. The therapeutic applications of botulinum toxin would not be realized for over 150 years. For all of this endeavour, Kerner earned the nickname 'Wurst Kerner' (sausage Kerner) and sausage poisoning was referred to as 'Kerner's disease' until 1870 when the term 'botulism' was coined.

The next major step in the elucidation of the aetiology of botulism followed an 1895 outbreak linked to pickled and smoked ham, which resulted in three deaths and 10 critically ill patients, in the Belgian village of Ellezelles. Microbiological examination of the ham by University of Ghent Professor of Microbiology, Emile Pierre Marie Van Ermengem (who had worked in the laboratory of Robert Koch in Berlin in 1883), led to the isolation of an anaerobic organism which Ermengem named *Bacillus botulinus*, later renamed *Cl. botulinum*.

### Botulinum toxins

*Clostridium botulinum* produces seven structurally homologous but antigenically and serologically distinct exotoxins (A, B, C [$C_1$, $C_2$], D, E, F and G). All botulinum toxins (BTX or BoNT) are neurotoxins of varying potency, with botulinum toxin A the most potent, followed by B and F. Toxins A, B and E are most commonly associated with human botulism, with some rare reports of BTX F botulism. Types C and D are neurotoxins in animals only. All toxins interfere with neural transmission through blockade of acetylcholine release at the neuromuscular junction. Thus, intramuscular administration of the toxin leads to muscle paralysis through inhibition of acetylcholine release at presynaptic motor neurons. Botulinum toxins are expressed as an inactive single polypeptide with a molecular weight of approximately 150 kDa which is subsequently cleaved to form a dichain molecule comprising both a heavy (H, 100 kDa) and light (L, 50 kDa) chain, linked through a disulfide bridge. The heavy chain binds the toxin to the presynaptic receptor, whereupon the toxin complex enters the cell via endocytosis and the disulfide bond linking H and L chains is cleaved to liberate the light chain into the cytoplasm and the endosomal compartment. The light chain acts as a zinc endopeptidase, interacting with proteins in the nerve terminal (synaptosomal-associated protein (SNAP), vesicle-associated membrane protein (VAMP) and syntaxin) preventing fusion of acetylcholine vesicles at the cell membrane. This neuromuscular blockade occurs at four sites in the body; the neuromuscular junction, autonomic ganglia, postganglionic parasympathetic nerve endings and acetylcholine releasing postganglionic nerve termini, leading to weakness in striated muscles or flaccid paralysis.

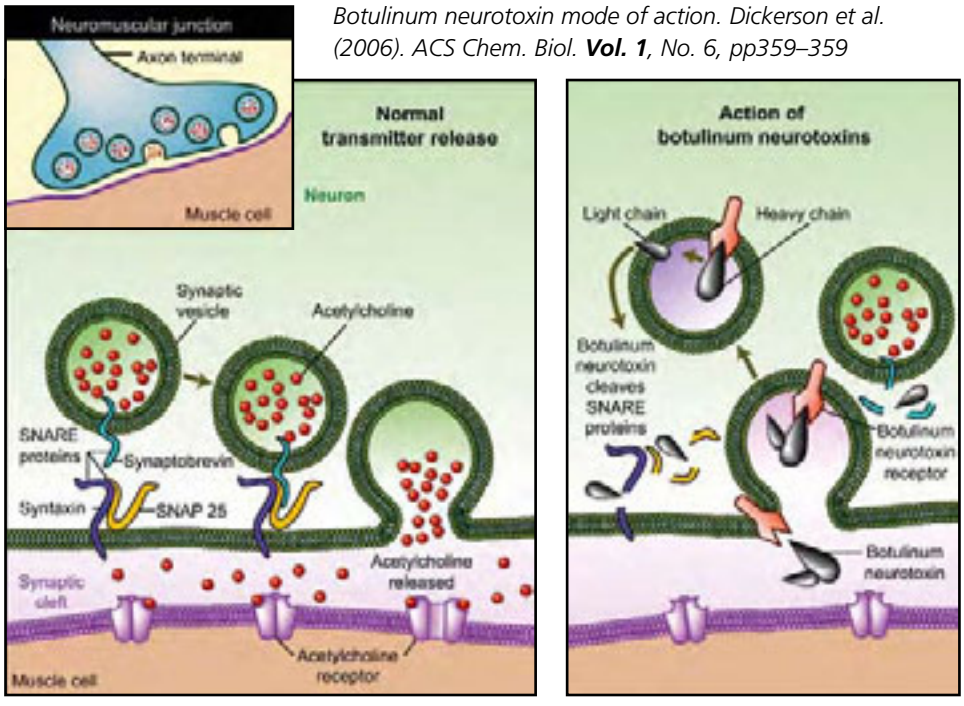
Since the toxins may take 24–72 hours to take effect, symptoms of botulism may initially include nausea, vomiting, diarrhoea, cramps or constipation. This can then progress to muscle paralysis starting from the head and spreading down the body to the legs. Advanced symptoms may include drooping eyelids, blurred or double vision, weakness of facial muscles, dry mouth, difficulty swallowing (dysphagia), slurred speech and breathing difficulties. Babies may experience flaccid paralysis and have a weak cry and difficulty feeding.

Nowadays, food poisoning with *Cl. botulinum* is rare in humans and can be effectively treated with botulinum antitoxins, neutralizing antibodies raised against the various toxins. Botulism can occur as a result of the ingestion of contaminated food, inhalation, colonization of the infant gut or from wound infection. Although *Cl. botulinum* is present in soil, river and sea sediments, the chance of contracting botulism is low. The US Centers for Disease Control and Prevention and USDA issue guidance for home canning of vegetables and other preserves for the avoidance of botulism and the NHS advise that children under one year not be given honey, known to be a source of *Cl. botulinum* spores.

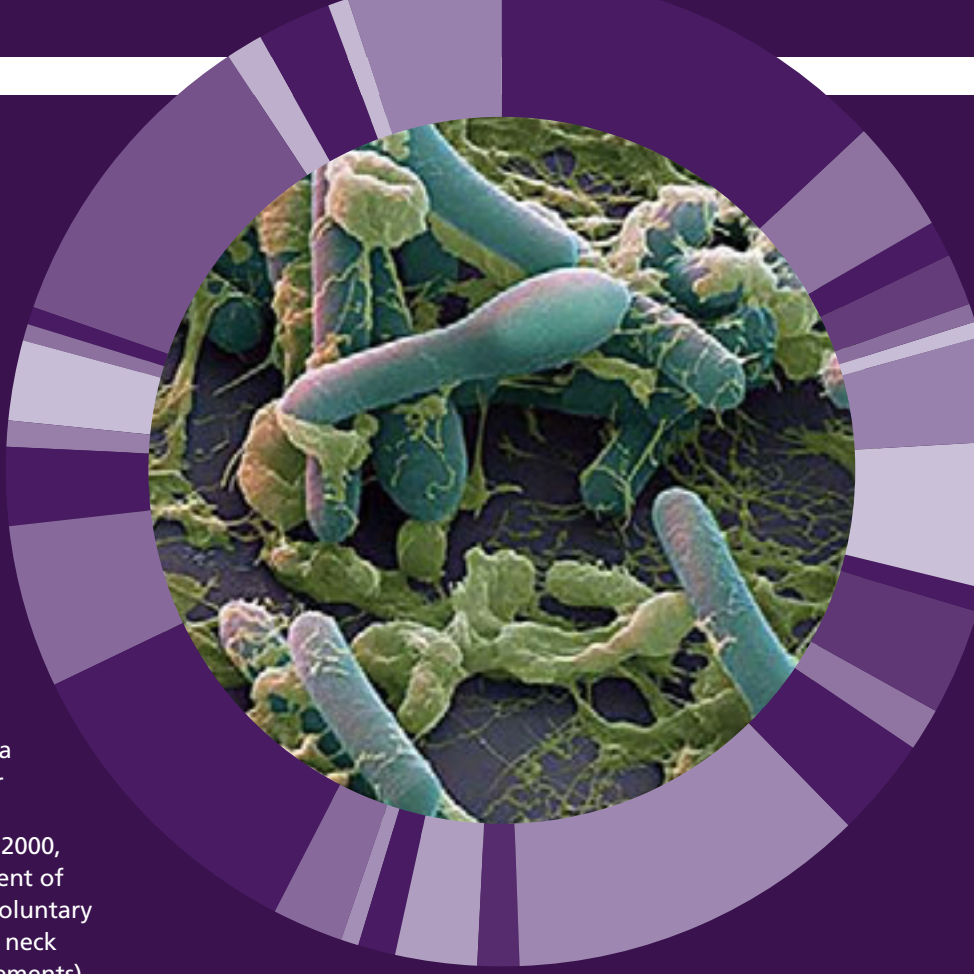
In 2013, the FDA approved the first botulism antitoxin for use in neutralizing all seven known botulinum nerve toxin serotypes. This antitoxin (a mixture of antibody fragments against all seven toxins) is indicated for botulism outbreaks, infant botulism and is to be stored in the US Strategic National Stockpile for emergency preparedness and responses, should botulinum nerve toxins be used in the event of a terrorist attack (post-exposure prophylaxis).

### Therapeutic and cosmetic use of botulinum toxin

In 1822, Justinus Kerner noted that the toxin "*administered in such doses, that its action could be restricted to the sphere of the sympathetic nervous system only, could be of benefit in the many diseases which originate from hyperexcitation of this system*" and that "*it can be expected that in outbreaks of sweat, perhaps also in mucous hypersecretion, the {toxin} will be of therapeutic value*". Although research into botulinum toxins would continue from the time of Kerner's predictions to the present day, the therapeutic applications which he prophesied would not be realized




*Botulinum neurotoxin mode of action. Dickerson et al. (2006). ACS Chem. Biol.* ***Vol. 1****, No. 6, pp359–359*



for over 150 years. Thanks to the work of Edward J. Schantz, (Department of Defense Laboratory, Fort Detrick, Md.) who succeeded in purifying BoNT A in 1946 and supplied it for use in scientific research, and the pioneering work of Alan B. Scott, (Smith-Kettlewell Eye Research Institute, San Francisco) who, in the 1980s used the toxin first in monkeys then in humans to treat strabismus (a condition which prevents both eyes aligning in the same fixation point, also known as cross-eye or squint). This led to the FDA approval of BTX A (Botox®, Allergan, now Actavis) for the treatment of strabismus, blepharospasm/eye dystonia (sustained, forced, involuntary blinking or closing of the eye, contraction of muscles around the eye) and hemifacial spasm. In 2000, the FDA approved BoNT A for the treatment of cervical dystonia/spasmodic torticollis (involuntary and sometimes painful contraction of the neck muscles leading to involuntary head movements).

In 2002, BoNT A (Botox® Cosmetic and Xeomin®) received FDA approval for what is perhaps the most widely recognized application of the toxin, the cosmetic, temporary removal of moderate to severe glabellar lines, or frown lines (also known as hyperkinetic facial lines). This application of one of the most deadly toxins known to science has led to botulinum toxin, in the guise of Allergan's Botox® being granted household name status and has partly driven a global market which is currently valued in excess of $6 billion (US) dollars annually. This market is split roughly 50:50 between cosmetic and therapeutic use. Interestingly, the toxins themselves are not patentable but the major pharmaceutical companies Allergan (Botox®) and Ipsen (Dysport®) developed European and US markets for numerous indications, and have acquired intellectual property for a broad spectrum of indications.

Today, the therapeutic use of BoNT extends from the treatment of hyperhidrosis (as predicted by Kerner in 1822), overproduction of saliva (in cerebral palsy and other neurological disorders), limb dystonia (writer's cramp) and other focal dystonias (spasmodic, patterned involuntary muscle activity), spasticity indications (stroke, brain injury, cerebral palsy, MS), non-dystonic disorders of involuntary muscle activity (including nocturnal bruxism (teeth grinding), tremor, tics, trismus and anismus), chronic pain indications, smooth muscle hyperactive disorders including haemorrhoids, anal fissures, neurogenic bladder and bladder dysfunctions, and the treatment of chronic migraine. The term 'miracle poison' has been coined to describe this remarkable toxin which, at first deadly, has been domesticated to treated a staggeringly diverse array of serious clinical applications. In many cases, botulinum toxin has fewer side effects than pharmacological interventions and often with superior outcomes.

Despite gaining notoriety and public awareness as a remedy for wrinkles, crows feet and frown lines, botulinum toxins, still amongst the deadliest poisons known to man, the human lethal dose is approximately 1 ng/kg of body weight, are proving truly transformative therapeutic agents, miracle drugs, some 150 years since their first description.

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# CAN BACTERIAL PRODUCTS turn back time?

Are you concerned about skin wrinkles and lines? Have you ever dreamt of drinking from the fountain of youth? Pick up any health and lifestyle magazine and you will be sure to find an article, or adverts for products that claim we can reverse the ageing process. Anti-ageing is big business in the health and cosmetic industry, but is it all bunkum, or could bacteria play a part in helping us to 'turn back time'?

As you will have just read in the previous article, one of the best known bacterial products used in the cosmetics industry is the toxin produced by *Clostridium botulinum*, used in Botox. Approved by the FDA, Botox has been available since 2002, but there are other areas of interest in the cosmetics market related to anti-ageing.

We are constantly reminded by advertisers and the popular media that exposure to UV radiation from the sun accelerates the ageing of skin. Indeed, in a study that focused on Caucasian women, researchers from L'Oreal suggested that UV exposure was ~ 80% responsible for the effects of photoageing, which could also be termed 'premature ageing', of facial skin (Flament *et al*., 2013). Thus the advice is to protect skin from the sun, presumably by wearing products that contain sunscreen. In 2010, Balkus & Walsh reported the identification of 'sunscreen' molecules in the cyanobacterium *Anabaena variabilis*. Cyanobacteria produce small molecules called mycosporines and mycosporine-like amino acids (MAAs) that can absorb UV radiation, although the biosynthetic route was not previously known. In their study, Balkus & Walsh demonstrated that *A. variabilis* contains genes that produce MAAs and that when these genes were cloned and expressed in *E. coli*, the same 'sunscreen' molecules were produced. These cyanobacterial MAAs are similar to those produced by eukaryotic algae; MAAs harvested from red algae are already used in some cosmetic products such as 'Helioguard 365' which is marketed as 'a natural UV-screening active to protect against photoageing' (**https://mibellebiochemistry.com/products/helioguard-365/**). Hence it is possible that future sunscreen products could contain bacterial MAAs produced by recombinant *E. coli*.

**Who wants to live forever?**

Rapamycin, a macrolide produced by *Streptomyces hygroscopicus*, has been shown to extend the life of mice (Arriola Apelo *et al*., 2016), and a new project is examining its use in dogs

**Rapamycin is being tested in canines with a view to extending the lives of healthy dogs; if successful, the treatment could also be applied to age-related diseases in humans**

(**http://dogagingproject.com/**). Known as an immunosuppressant, and normally used to prevent the rejection of organs following transplant, rapamycin is being tested in canines with a view to extending the lives of healthy dogs. If successful, the treatment could also be applied to age-related diseases in humans.

## FURTHER READING


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*Bacillus cereus* is commonly found in soil and is associated with the foodborne illness 'fried rice syndrome'. However, a strain isolated from Siberian permafrost in 2009 – *B. cereus* F – has been used in a bizarre 'experiment' by one of the scientists that isolated it. Anatoli Brouchkov is reported to have injected himself with a culture of the bacterium and is reportedly "*stronger and healthier than he has ever been before*" (**http://www.telegraph.co.uk/news/health/11901105/Russian-scientist-says-he-is-stronger-and-healthier-after-injecting-himself-with-eternal-life-bacteria.html**). This experiment followed earlier research on mice that suggested the bacterium had slowed down ageing in mice (**http://phys.org/news/2012-01-permafrost-bacteria-ageing-scientists.html**). The team hypothesized that the bacterium had survived for 3.5 million years thus it must possess some anti-ageing mechanism. Perhaps Anatoli Brouchkov can tell us in a hundred years from now.


**Clare Taylor**
*Edinburgh Napier University*

# The microbiological hazards of tattooing

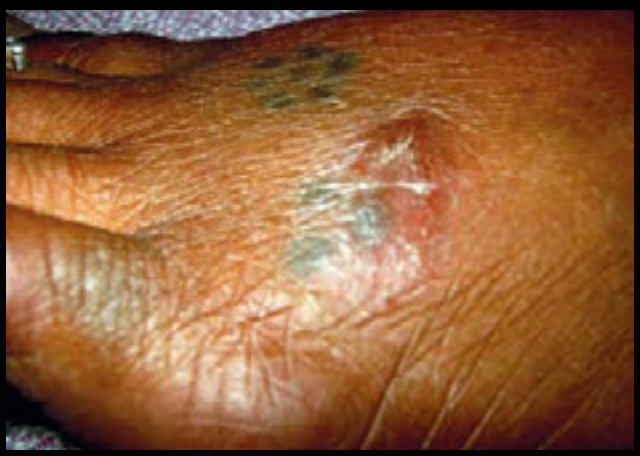
Tattooing has become increasingly popular nowadays and a definite mark of the generation born after 1978. It is estimated that 10 to 15% of the general population is currently tattooed, with a higher prevalence among young adults aged between 18 and 39 years. Unfortunately, tattooing is not completely harmless. With the breaking of the skin barrier during the procedure, there is a risk of inoculation of various germs that can lead to local and sometimes systemic infections.

Inoculation may occur at different steps of the procedure through the use of contaminated inks or instruments (electric tattoo machines and needles), the lack of hygiene during the procedure or, secondarily, a lack of proper aftercare during the healing process. Tattooing should be performed in a professional tattoo parlour. Any situations that cannot guarantee asepsis and sterility of the procedures may lead to an increased risk of infection and should be avoided (home-tattooing, jailhouse tattooing, traditional tattooing in villages etc.).

The spectrum of possible agents of cutaneous infections is quite varied including viruses, bacteria, environmental rapidly growing mycobacteria and fungi.

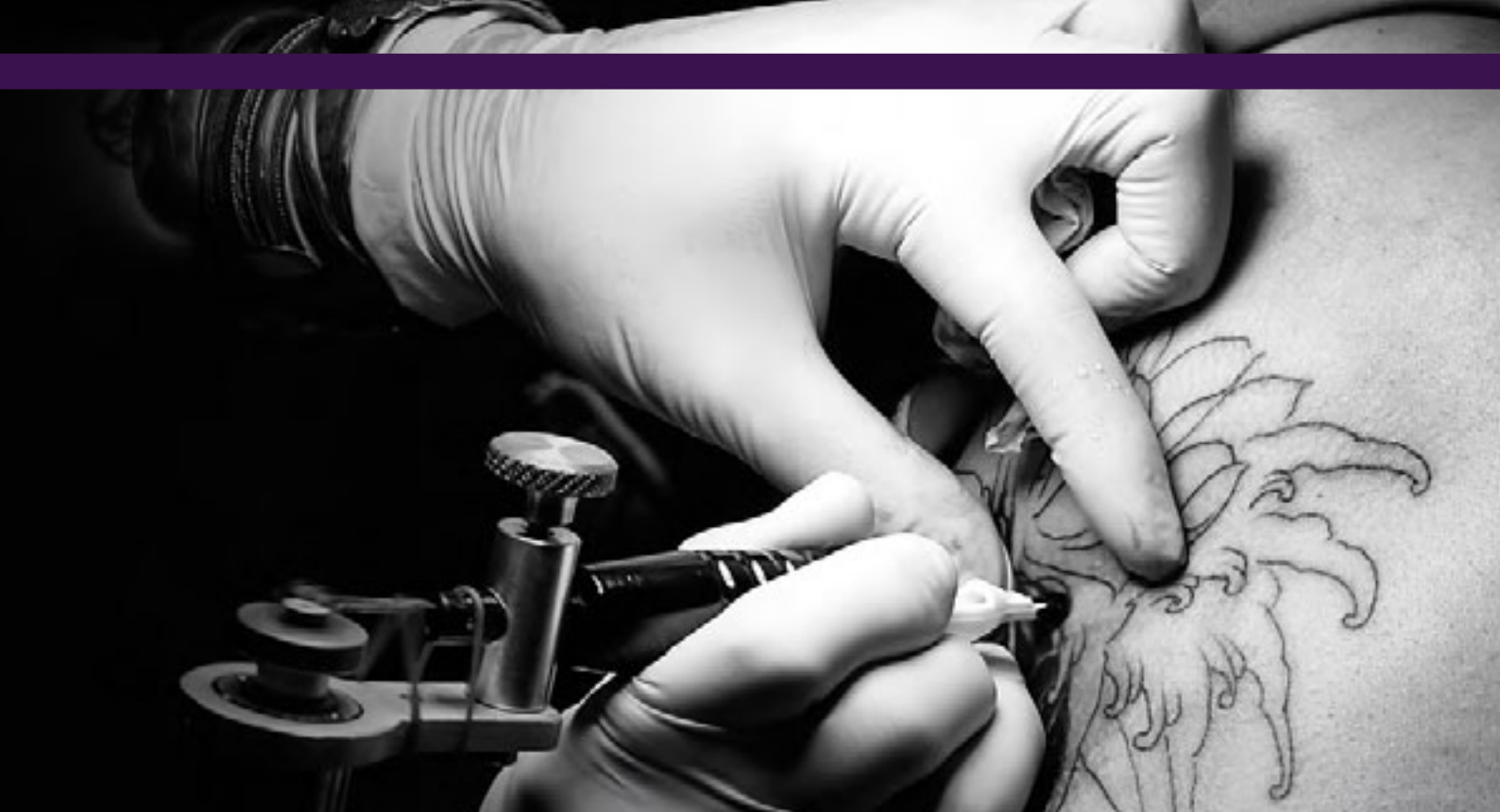
Acute superficial pyogenic infections such as folliculitis, impetigo, ecthyma as well as deep pyogenic infections such as furunculosis, erysipelas and cellulitis remain exceedingly rare. We cannot rule out a potential underestimation of superficial bacterial infections because customers may not seek medical advice in cases of minor infection that can be successfully treated with soap and disinfectant. Gangrene, amputations and deaths were reported among the French sailors at the end of the 19th century. However, the antibiotic era, rules of hygiene and asepsis and improvement in tattooing techniques made such complications rather exceptional. Such cases occur only in the case of lack of sterilization and cleanliness. In 2004, a cutaneous epidemic infection with MRSA was reported in three different states of the USA. More recently, polymicrobial necrotizing fasciitis has been reported in New Zealand


*Sterile and single use material ready before tattooing in a professional tattoo shop*



*Tuberculoid leprosy*

COURTESY OF DR A GHORPADE, BHILAI (CHHATTISGARH STATE), INDIA



# A definite mark of the generation born after 1978

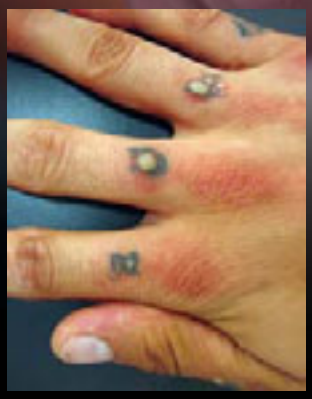
and Australia among pacific islanders who got traditional Samoan tattoos (Pe'a). In all those cases, unlicensed tattoo activity and asepsis were responsible for such dramatic adverse events.

Inoculation syphilis used to be the major complication during the 19th century in Europe until the beginning of the 20th century and was mostly related to the use of the tattooist's saliva during the procedure. Such cases have totally disappeared and belong to history now, thanks to the discovery of penicillin, sterile needles and electric tattoo machines.

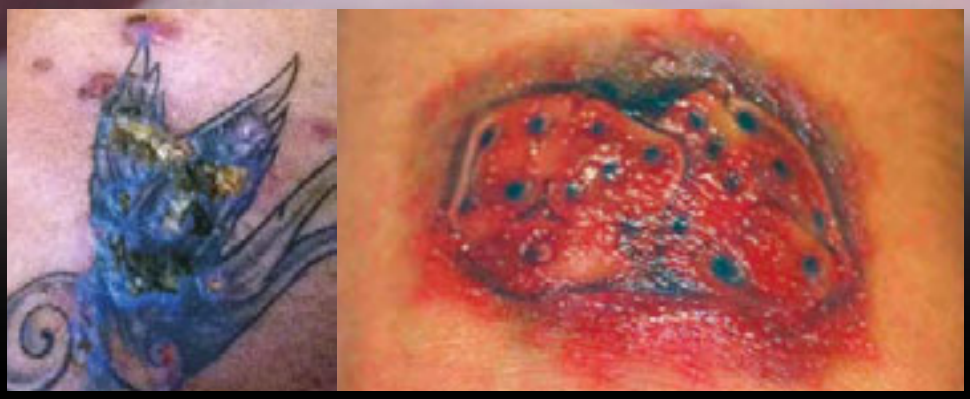
Inoculation leprosy remains limited to India, where leprosy is endemic, because of the tradition of roadside tattooists using an unsterile needle to tattoo a whole village at once. Delay of onset is highly variable ranging from 10 to 20 years after tattooing. Similarly, inoculation of tuberculosis in the skin by tattooing is rather rare and was contemporary of syphilis outbreaks at the end of the 19th century. In a more rapid scenario, outbreaks of rapidly growing mycobacteria have been reported since 2003 in western countries, especially with *Mycobacterium chelonae* and more rarely with *Myco. abscessus* or *Myco. immunogenum*. The story is usually always the same; an outbreak situation and several cases before the tattooist refers the customers to a clinician. The customer develops papules and pustules within the drawing of their recent tattoos within one to several weeks after tattooing, usually on the grey shadings. Diagnosis is usually delayed with application of local corticosteroids and/or of topical and oral antibiotics against *Staphylococcus aureus*, before the diagnosis is correctly made by biopsies and skin cultures. The use of tap water by the tattooist to dilute the ink to obtain a grey colour is usually the cause of inoculation. In some rare cases, mycobacteria were in the ink bottle, without dilution or even in sealed bottles.

Viral warts and molluscum contagiosum have also been reported in association with tattoos. Lesions develop in variable numbers and size, sometimes only to one colour. The delay of onset ranges from one month to 10 years after tattooing. Inoculation remains a mystery, but, the most reasonable hypothesis remains the pre-existence of lesions prior to the tattooing process.

In the literature, there are limited cases of systemic complications after tattooing. A few cases of bacterial endocarditis have been reported in patients with pre-existing valvulopathies that ended in replacing the valves. We usually suggest that any customer with a known history of cardiac murmur, even if only in childhood, should delay the procedure and seek medical advice, followed if necessary by further



*Viral warts on a tattoo*



*Above L to R: Pyogenic infections after tattooing*

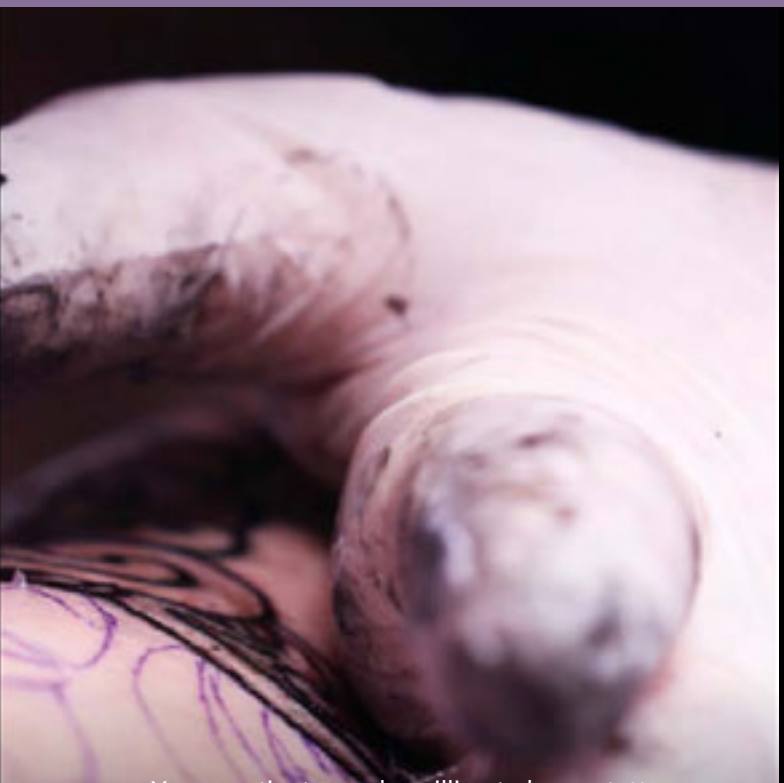
COURTESY OF DR E EHRSAM, LE CATEAU CAMBRÉSIS, FRANCE

specialized explorations and that any patient with a known congenital heart disease should warn the general practitioner/cardiologist about the procedure to discuss the opportunity of antibiotic prophylaxis.

Unhygienic procedures and needle sharing expose those being tattooed to the risk of bloodborne virus transmission; mainly hepatitis C, but also hepatitis B and HIV. Recent studies pointed out that there was no definitive evidence for an increased risk of HCV infection nowadays, for tattoos received in professional parlours. However, the risk of HCV infection remains significant, especially among high-risk groups, when tattoos are applied in prison settings or by friends.

Prevention interventions are needed to avoid the transmission of hepatitis C from tattooing in prisons, homes and other potentially non-sterile settings. The risk of inoculation of HIV is rather theoretical. There are very few debatable cases in the literature, usually from Third World countries (Vietnam, Jamaica) with unprofessional practices and poor infection control methodologies. Of note, tattoo complications in HIV customers are the same as any tattooed patients. It is suggested to avoid tattooing when the CD4 count is low and viral load is high. Skin rashes on tattoos associated with HAART and immune restoration syndrome have also been described.

**Gangrene, amputations and deaths were reported among the French sailors at the end of the 19th century**



Young patients may be willing to have a tattoo, while being under immunosuppressive therapy. They do not always perceive the risks and get tattooed without any medical advice, especially when their treatment is fully effective and their disease is controlled. No cases of (severe) infection after tattooing in patients undergoing immunosuppressive therapies has been reported to our knowledge. Unfortunately, a lethal case of infection after tattooing in a young man with acute leukaemia after tattooing was reported. Therefore it is suggested that:

i) When initiating an immunosuppressive treatment, enquire regarding potential wishes for a tattoo.

ii) The clinician administering immunosuppressive therapy should be non-judgmental in order to increase the likelihood of the patient being receptive to advice against tattoos.

iii) The level of immunosuppression should be evaluated (therapy, disease itself and potential co-morbidities, such as diabetes).

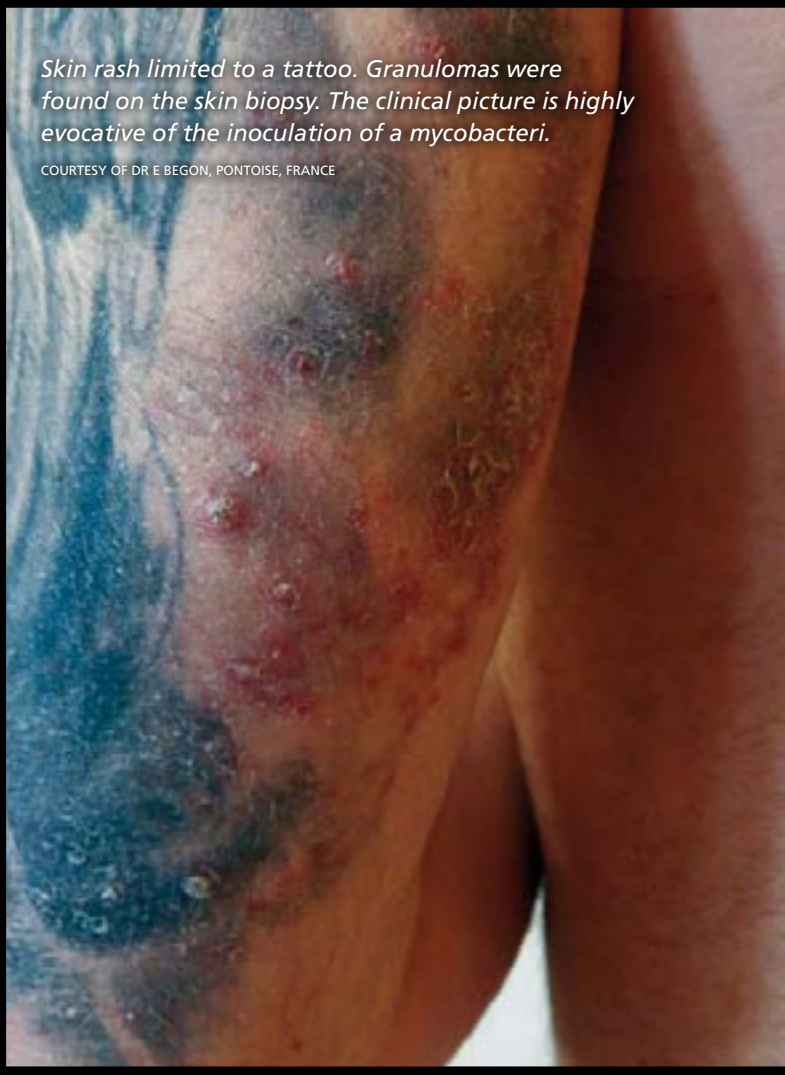
iv) The reasons for contraindications should be explained, stressing that they are only temporary and in relation to the risk of severe infections.

v) Tattooing can always be reconsidered, when the treatment is withdrawn or at a maintenance level.

vi) Therefore, any body-art procedure has to be avoided when the disease is active and the treatments are at high dosage.

According to the Council of Europe Resolution ResAP(2008)1 on requirements and criteria for the safety of tattoos and permanent make-up, sterility should be declared on the ink packaging. However, several studies in Norway (2004), in Switzerland and Liechtenstein (2010), and in Denmark (2013) pointed out that some inks may be contaminated by various bacteria (Gram-positive rods, Gram-positive cocci or Gram-negative rods), even in unopened bottles. For instance, Høgsberg *et al*., found *Streptococcus sanguinis*, *Staphylococcus* sp., *Pseudomonas* sp., *Enterococcus faecium* and *Acinetobacter* sp. in seven out of 58 bottles purchased via the Internet.

Pyogenic infection remains quite rare if the tattoo has been performed in a professional tattoo shop. Backyard tattooists and unclean studios should be avoided. Proper education and training of the tattooists and regulation of tattooing activity is mandatory. There is a necessity for collaborative work between the customer, the tattooist and the clinician to prevent infectious disease transmission.



*Skin rash limited to a tattoo. Granulomas were found on the skin biopsy. The clinical picture is highly evocative of the inoculation of a mycobacteri.*

COURTESY OF DR E BEGON, PONTOISE, FRANCE

**Nicolas Kluger** MD PhD
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# The hidden dangers of

Contact lenses (CLs) for medical conditions are composed of soft (hydrogel or silicone hydrogels) or rigid (typically gas permeable) materials. Cosmetic contact lenses (CCL) or zero-powered CLs are mainly designed to change the colour of the eyes or their appearance, but can also be used therapeutically to obscure disfigured eyes. The majority of CCL wearers use them for aesthetic purposes where there is no medical indication. Side effects/complications of CCL on the eye range from mild discomfort to sight-threatening disease, such as secondary corneal infection. Unfortunately, CCL-related microbial keratitis has been reported and is a significant health concern for millions of CL wearers.

An estimated worldwide CL market size currently stands at roughly $7.1 billion with the US as the largest global market share. Although the US and UK have regulated the sale of CCL as a medical device, this level of regulation has not been adopted by the rest of Europe and elsewhere. However, US and UK consumers are still able to easily obtain CCL from unregulated online vendors. Not only has there been a growth in online sales of CCL over the last few years but greater over-the-counter availability in supermarkets, nail salons, beauty parlours and costume stores particularly at certain times of the year, such as Halloween. Unfortunately, online sales often result in increases in unsupervised CL wear. Despite legal requirements for a valid CL prescription some people obtain CCL for extended periods of time without appropriate ophthalmic examination. Internet purchasers have fewer follow-up examinations with eye care professionals (ECP) and no one to consult in case of an emergency, resulting in significant delay between the onset of symptoms/signs and medical care. Consequently, changes in the CCL marketplace has had an impact on the overall safety record of CL wear.

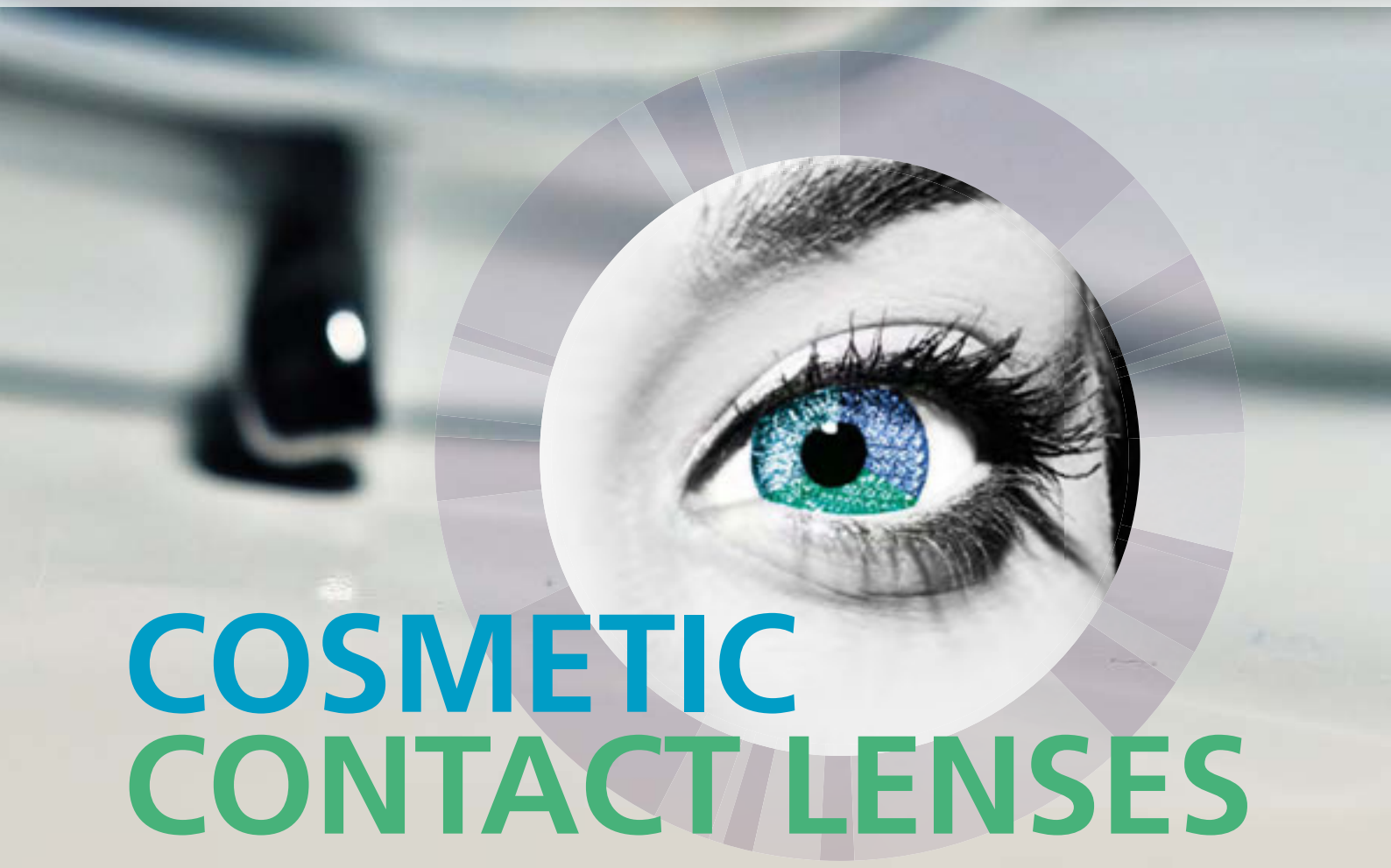
Several different types of CCLs are available. Zero-powered coloured CLs are offered with a diverse colour palate, shades, tints and intensifiers. Opaque coloured CLs are designed to cover the area overlying the iris and have a fixed pupillary aperture which restricts the visual field and blurs peripheral vision. Extra-wide cosmetic lenses have an extended area of coverage to make the

eyes appear larger providing a 'doll-like' appearance. These extra-wide lenses have recently gained popularity in Asia and North America particularly with younger customers. Newer CCLs on the market are silicone hydrogel, high dielectric constant (Dk) material, allowing for more oxygen permeability and with cosmetic capabilities. These lenses utilize technology which encapsulates the colour pigments within the lens – addressing many of the safety concerns of CCLs, including high oxygen permeability and a smooth lens surface. Cosmetic lenses with pigment on the surface resulted in significantly higher bacterial adherence, which is overcome when the pigment is encapsulated.

CCL wearers tend to be younger with a female preponderance. The lenses are less likely to be dispensed by an ECP (Odds Ratio, OR 12.3) and more likely to be bought online. Education about lens care and handling tends to be deficient (OR, 26.5). They have a high relative risk for microbial keratitis, which is the leading cause of visual loss in a young population with no refractive error. Culture-positive *Pseudomonas* and *Acanthamoeba* cases are more common amongst CCL users and are associated with poor prognosis. Suboptimal outcomes include vision below 20/200 (60% CCL versus 13% CL), corneal perforation and the need for surgical intervention such as corneal transplantation. Overnight wear and poor CL care are modifiable risk factors for the development of CL-related problems, which tend to be higher in CCL wearers.

The US Food and Drug Administration (FDA) has made recommendations on appropriate wearing schedules for CLs. It advises against the substitution of sterile saline solutions for multipurpose solutions, topping up solutions, reusing lens solution and avoidance of contamination from tap, bottled and lake water. In addition, the FDA promotes rub and rinse of CLs and clean, rinse and air-dry procedures for lens cases each time lenses are removed. Some multipurpose lens solutions can increase the binding of *Pseudomonas* to epithelial cells and decrease the rate of epithelial cell exfoliation. Infrequent lens wearers have a higher contamination rate of lens solution. Bypassing optometric assessment and fitting means many of the dangers of CCLs are attributable to poor education on CL care and hygiene. CCL overuse, overnight wear and poor CL hygiene are particularly associated with microbial keratitis.

CCLs pose the same risks as any other CLs and if improperly fitted, cleaned or handled can cause serious damage to the eye. Evidence suggests a growing incidence of serious microbial keratitis related to CLs that may be associated with more sight-threatening pathology. Complications include CL-related papillary conjunctivitis, superior limbic keratoconjunctivitis,



# COSMETIC CONTACT LENSES

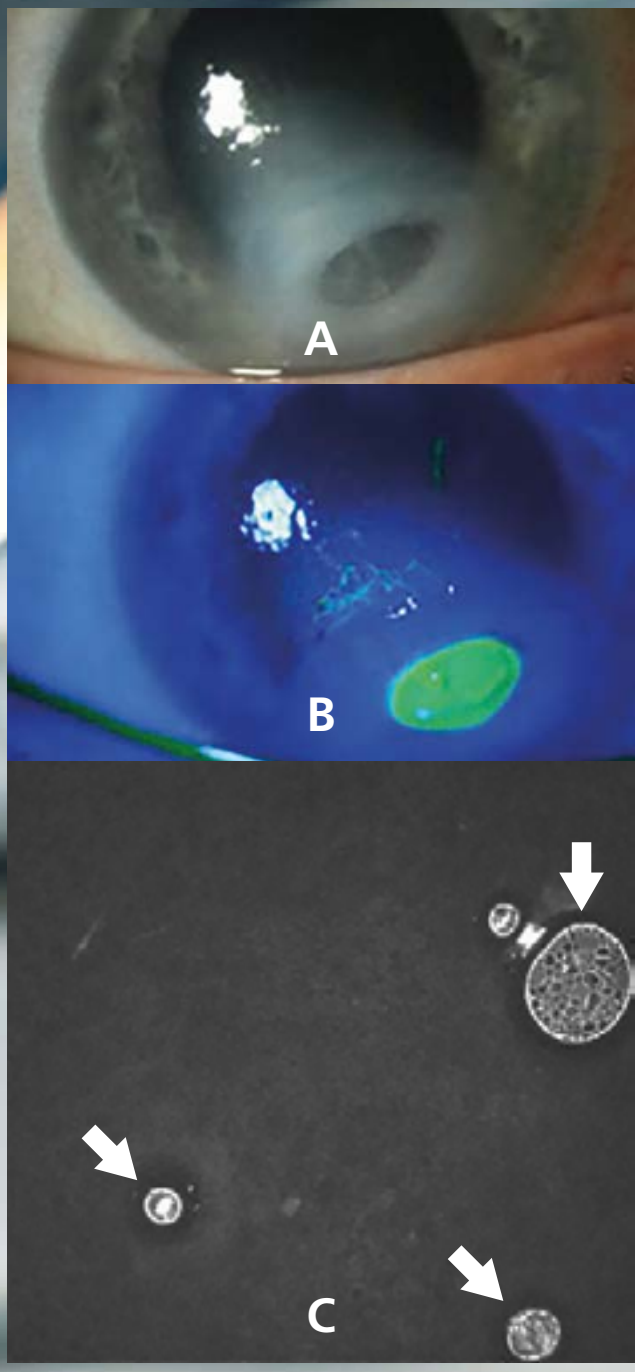



***Figure 2*** *Acanthamoeba keratitis. Colour photograph of Acanthamoeba-related corneal ulcer (***A***) and with fluorescein staining (***B***). In vivo confocal microscopy showing double-walled Acanthamoeba cysts (arrows), which can persist for many months (***C***).*

keratopathy, corneal oedema, corneal neovascularization, infiltrative keratitis, microbial keratitis and corneal perforation. It has been reported that 65% of all new corneal ulcers can be attributed to CL wear (Figure 2A). The most common isolates from CLs are *Pseudomonas aeruginosa*, *Staphylococcus* spp., fungi and *Acanthamoeba*. Higher rates of *Pseudomonas* and *Acanthamoeba* have been reported in CCL wearers.

CCL wear markedly increased the relative risk (RR) of microbial keratitis (RR, 16.5) compared with wear of conventional CL. A gap of greater than one year since a wearer's last visit to an eye care provider (RR, 3.4) also increased the risk of microbial keratitis, which can be bacterial, fungal or protozoan. Fungal infections are a rare cause of CL-related microbial keratitis – immunosuppression is considered a more important risk factor. *Acanthamoeba* keratitis is a highly symptomatic and sight-threatening infection due to free-living amoebae found in swimming pools, tap water, soil and fresh water. Exposure to contaminated water when wearing CLs puts individuals at increased risk of infection. CCL wearers are often at an increased risk of *Acanthamoeba* keratitis due to poor CL hygiene. It is important to highlight that early diagnosis of *Acanthamoeba* keratitis is crucial for a better visual prognosis.

Clinically, *Acanthamoeba* keratitis can be sometimes difficult to diagnose as it may present similarly to other conditions, particularly herpetic keratitis, which can often cause delays in diagnosis and consequent treatment. Features of *Acanthamoeba* keratitis include disproportionate pain to clinical findings. Clinical findings may include radial keratoneuritis with infiltrates along corneal nerves (which is a pathognomonic sign) and ring infiltrates. Stromal infection tends to occur centrally and appears as a diffuse stromal haze with an overlying epithelial defect (Figures 2A & B), which can progress to stromal melt and corneal perforation.

To diagnose microbial keratitis, cultures and Gram stains from corneal scrapes should be taken. Culture of CLs, CL case and solution are also recommended. Unfortunately, delayed diagnosis of *Acanthamoeba* and fungal keratitis is often not unusual, and may result in significant vision loss. This is partially due to the low sensitivity and time delay of corneal cultures. *In vivo* confocal microscopy is a novel technique for the study of the cornea, in particular its cellular structure (Figure 3). In cases of *Acanthamoeba* keratitis, *in vivo* confocal microscopy can detect trophozoites and cysts and is useful if available (Figure 2C). Studies using *in vivo* confocal microscopy report higher rates of *Acanthamoeba* keratitis.

The aim of treatment is to sterilize the corneal ulcer and promote healing with minimal damage, including scar formation. Progression of the infectious process may

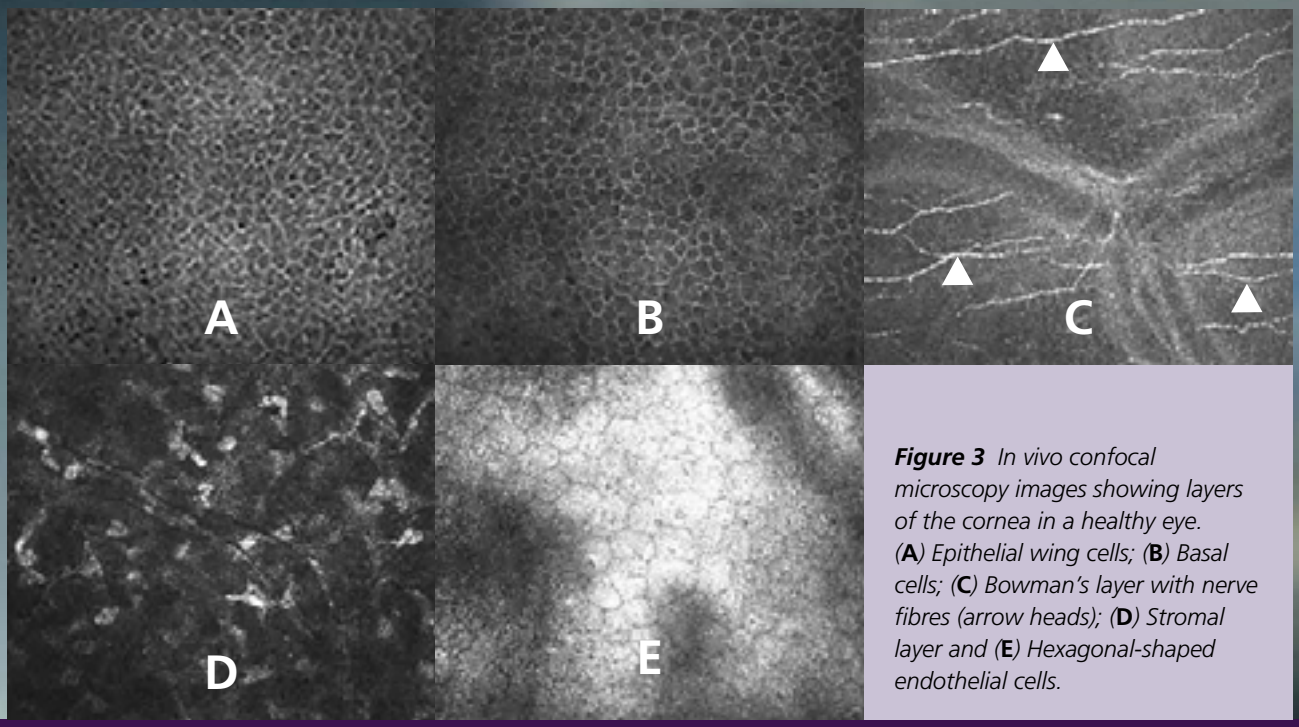



***Figure 3*** *In vivo confocal microscopy images showing layers of the cornea in a healthy eye. (**A**) Epithelial wing cells; (**B**) Basal cells; (**C**) Bowman's layer with nerve fibres (arrow heads); (**D**) Stromal layer and (**E**) Hexagonal-shaped endothelial cells.*

result in corneal perforation and secondary endophthalmitis. First-line treatment is monotherapy using a broad-spectrum antibiotic administered at a high frequency for the first three days then reducing the frequency for 7–14 days depending on clinical response. Positive cultures, high index of clinical suspicion, *in vivo* confocal microscopy and/or corneal biopsy should be obtained in challenging cases such as fungal and *Acanthamoeba* keratitis.

Threatened corneal perforation can be treated with systemic antibiotics such as oral ciprofloxacin 750 mg twice daily. Oral ciprofloxacin can be particularly useful in cases where there are peripheral ulcers close to the limbus. Once the ulcer is sterilized corneal glue can be applied to restore globe integrity in cases of corneal perforations measuring up to 1 mm in diameter, otherwise endophthalmitis can be induced. Corneal transplantation may be indicated in impending corneal perforation not responding to treatment or significant central corneal scarring accompanied by significant visual loss after the infection has been eradicated.

In summary, CCLs from unlicensed vendors and no involvement of an ECP are associated with more sight-threatening complications. However, advances in CL technology have improved the safety profile when properly prescribed by an ECP and used in a compliant manner. For this reason, it is important to highlight that complications with CCL are often associated with poor compliance and hygiene while handling the lenses.

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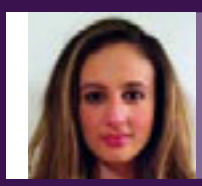


**Christin Henein** left
**Professor Francisco C Figueiredo** right
*Royal Victoria Infirmary & Newcastle University, Newcastle upon Tyne, UK*

# The European Cosmetic Products Regulation

The European Cosmetic Products Regulation (EC) No. 1223/2009 was implemented in 2013. One of the main purposes of this regulation is to improve the safety of cosmetic products made available to the public on the European market.

Currently, there are over 4,500 cosmetic companies in the EU, with exports representing one-third of the global market. European cosmetics are a €72.5 billion business; it is vital that these products are safe under normal and reasonably foreseeable uses by consumers and in line with the requirements of the regulation.

**Product recall**

RAPEX (Rapid Alert System for non-food dangerous products) is the European Commission's rapid information exchange system between Member States. RAPEX is publicly available and aims to prevent or restrict the marketing or use of products posing a risk to consumer/professional health and safety. Notifications are published weekly and contain useful information on product origin, countries the product is available in, type and level of risk, product details (type, brand and batch) and measures taken by the public authorities or the EU RP to control the risk. During the period of 2005–2016, 97 cosmetic products were recalled from the European market due to microbial contamination; 10–20% of the total annual recalled products. The majority of risks in cosmetic products originate from prohibited preservatives or whitening ingredients/ preservatives exceeding the maximum permitted level. Together with microbial contamination, these make up nearly all product recalls.

From a microbial perspective, the main offenders are *Pseudomonas aeruginosa*, over-abundant aerobic mesophilic microbes, *Staphylococcus aureus*, *Candida albicans*, *Enterococcus* spp., Enterobacteriaceae, *Burkholderia cepacia*, *Klebsiella* spp. and moulds. Since the regulation was implemented, the number of product recalls has not markedly changed. Numbers appear to be reducing, particularly for chemical hazards, but more data over time is needed before reliable comparisons can be made. Figure 1 shows the non-compliant cosmetic products reported between 2013 and 2015.

**Product manufacture**

Cosmetic products are hospitable survival grounds for microorganisms. They often contain a variety of nutrients such as water, lipids, proteins, glucosides, polysaccharides and vitamins to sustain survival. Additional factors such as balanced pH, oxygenation, essential oils, raw plant or animal materials and suitable osmotic concentrations also favour growth. To control the prevalence of microorganisms during consumer use, approved chemical antimicrobials can be used in the product composition. The EU Regulation (Annex V)

provides a positive list of these preservatives with maximum formulation limits to ensure product safety and compliance is met (Regulation *Article* 14). Independently from the use of preservatives, good manufacturing practice (GMP) should always be used to restrict the potential for microbial contamination at the source.

GMP and microbial quality assurance must be standard practice in the production of cosmetic products (Regulation *Article* 8); CEN and ISO Standards are available to govern compliance. These practices define procedures for the cleaning and sanitization of materials, production rooms, personnel and equipment in the raw material stages, bulk/finished products and packaging stages to control microbial interference. Use of closed systems, filtered air systems, minimal residue build-up, flushing systems and use of high-quality stainless steel for product contact are all recommended. During production, the chemical and microbial quality of water should also be monitored and materials delivered should be checked before storage. Physical contaminants such as cardboard or wooden pallet fibres should not enter the clean manufacturing facility as these can have a high bioburden. Finished products should be packaged immediately or within a maximum specified storage time period. In common with any industry striving to control microbial contamination, the essential procedures are the prerequisite protocols, good processing practices, hazard and risk analyses, routine sampling, quality and safety control and assurance.

**Microbial limits**

The microbiology of cosmetics should be assessed from the raw material ingredients analysis step through to the manufacturing and consumer use stages. Different product types have different associated risks depending on such factors as their origin, formulation and predicted consumer exposure.

The physical barrier of skin and the body's natural defence mechanisms protect the body from microbial attack. Microbiologically contaminated products may cause trauma to the skin such as irritation or allergy and could possibly invoke microbial infection. However, a risk of infection is also possible where products are intended for use on mucosal membranes, damaged skin and/or young children. An increased risk is also possible in immunocompromised persons or the elderly. The European Scientific Committee on Consumer Safety (SCCS) therefore defines two categories of cosmetic products in terms of the assessment of microbial safety:

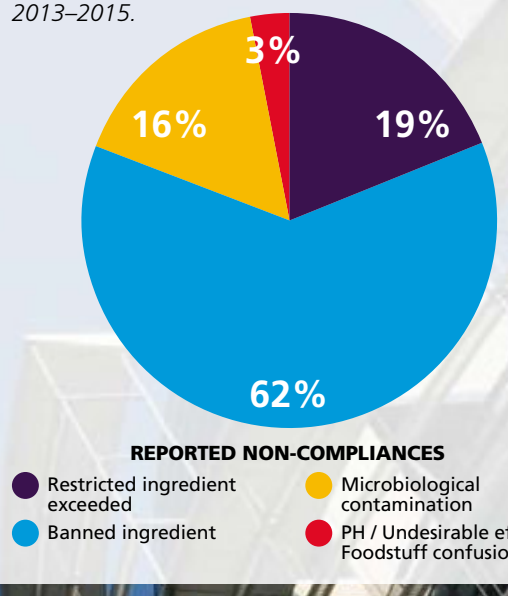
*Category 1: products specifically intended for children under 3 years, to be used in the eye area and on mucous membranes.*

*Category 2: other products.*

Routine batch testing is standard procedure for cosmetics product manufacture and microbial analysis determines the safety of the products entering the market. In some cases, testing may not be deemed necessary (e.g., > 20% alcohol content, low water activity, low/high pH) by use of a risk assessment carried out to a known standard, e.g., ISO 29621: 2010.

For all other products, total viable counts and potential pathogen analysis are required. Microbial limits are generally defined by product category; a product in Category 1 should not exceed 1 x $10^2$ aerobic mesophilic microorganisms (CFU/g or CFU/ml) per product and Category 2 products should not exceed 1 x $10^3$ aerobic mesophilic microorganisms (CFU/g or CFU/ml) per

***Figure 1*** *RAPEX reported non-compliance statistics 2013–2015.*



product. Due to the inherent variability of the plate count method, results are considered out of limit if > 1 x $10^2$ CFU/g or ml for Category 1 products, or > 1 x $10^3$ CFU/g or ml for Category 2 products. For pathogen analysis in cosmetic products, quantification of *E. coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Candida albicans* is required. These species must not be detectable in 1 ml or 1 g of either Category 1 or 2 cosmetic products.

### The preservation system

Microbial contamination of products can occur at the raw material stages, during product manufacture or as a result of consumer (mis)use. It is therefore essential that the product and consumer are protected against potentially harmful microorganisms throughout the product's life. Microbial proliferation can cause not only a variance in stability and quality of the product such as unacceptable changes to colour, viscosity, odour and pH, but may well cause harm to the user. Chemical preservatives are used to continually reduce microbial contamination in products during their life but different types of packaging and use instructions should also be considered. Pump dispensers that prevent the product coming into contact with the air, aerosols or single use products (e.g., creams or face masks) may contain fewer/no preservatives as the product is physically protected from microbial contamination or washed off quickly; only microbiological quality tests are necessary for these finished products with a scientific justification. However, cosmetics with exposed product, such as screw tops or open pots, will most likely need chemical preservation systems to ensure the user is protected to the end of the product's stated life. These products require both microbiological quality tests and a preservation challenge test to be performed on the finished product.

### The preservation challenge test

A product's preservative system protects it from microbiological contamination during manufacturing and normal and foreseeable consumer use. The Challenge Test (or Preservative Efficacy Test (PET)) identifies the type and minimal effective concentration of preservatives needed to reduce inoculated levels of potential pathogens over time. This test is also recommended after a period of storage (end of shelf life) to check that the preservation system is efficacious over time. *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Candida albicans* and *Aspergillus brasiliensis* are the standard test species and a test 'PASS' ensures that products are microbially stable and preserved during storage and use. This test is a mandatory requirement of the EU Regulation for products that are likely to deteriorate under normal conditions of use, storage or present a risk of infection to the user.

### Conclusions

Although microbial contamination in cosmetic products does not account for a high proportion of the reported non-compliant products that may pose risk to a user, harm is potentially significant in those with weakened immune responses or if used in eye/mucosal areas. The EU Cosmetic Products Regulation has been implemented to ensure greater product safety and products must be manufactured and tested to maintain microbial limits to a reasonable shelf life during use and storage. Consideration therefore must be given to the manufacturing environment, the raw materials/water used, manufacturing processes, packaging and choice and efficacy of preservation system if used.

Microbial tests must be performed as part of a routine quality assurance system. This analysis is a standard requirement of GMP. Only through this can companies demonstrate compliance of products to the required product category limits and be confident of their product safety.

## FURTHER READING

Regulation (EC) No. 1223/2009 of the European Parliament and of the Council of 30 November 2009 on Cosmetic Products.

The SCCS's notes of guidance for the testing of cosmetic substances and their safety evaluation. 9th Revision (2016); SCCS/1564/15.

International Standard ISO 17516:2014 Cosmetics – Microbiology – Microbiological limits.

International Standard ISO 29621 Cosmetics – Microbiology – Guidelines for the risk assessment and identification of microbiologically low-risk products.



**Dr Heather Moore**
*TSGE Consulting*

# Shining a light on the **unseen heroes** of cosmetic products

Cosmetics, and other personal care items, are complex multiphase formulations, meaning that they comprise multiple individual ingredients that are combined to generate the final product with distinct properties.

Here we briefly shine a light on the unseen heroes that are used for the production of fast-moving consumer goods including toothpaste and face cream.

| | | |
|---|---|---|
| **LEVAN** | **Bacterial Source:** | Production requires an enzyme extracted from *Erwinia*, *Streptococcus*, *Pseudomonas*, *Zymomonas* spp. and *Bacillus subtilis* |
| | **Properties:** | Skin protecting, film forming, increase viscosity |
| | **Usages:** | Skin-moisturizing, reduction of skin irritation and skin-whitening effects |
| **GELLAN GUM** | **Bacterial Source:** | *Sphingomonas paucimobilis* |
| | **Properties:** | Increase viscosity |
| | **Usages:** | Emulsion stabilizer, viscosity-increasing agent |
| **HYALURONIC ACID** | **Bacterial Source:** | *Streptococcus equi* subsp. *zooepidemicus* |
| | **Properties:** | Skin conditioning, increases viscosity |
| | **Usages:** | Dermal filler via injection, anti-ageing creams |
| **XANTHAN GUM** | **Bacterial Source:** | *Xanthomonas campestris* |
| | **Properties:** | Increases viscosity |
| | **Usages:** | Viscosity controller, oral hygiene products such as toothpastes |

The utility of bacteria in producing materials that are used in everyday cosmetic products is diverse, and it is likely that more materials will come onto the market. One polymer that is at more advanced stages of development is bacterial cellulose, which can be extracted from *Gluconacetobacter xylinus* and is being considered as a replacement to plant-derived material (cellulose gum). Cellulose gum has reported uses in wound-healing, skin repair and in the construction of artificial nails. Increasing consumer demand for innovative products, balanced by growing concerns about the sustainability of source materials, drive the development of components derived from microbial systems.

**Watch this space (and your cosmetics) to see what new materials will enter into commercial use!**

***Acknowledgements:***
*C-YH doctoral studies are funded by BeautyHsiao Biotechnology Inc. (registered in Taiwan, R.O.C.) NS-W acknowledges the Biotechnology and Biological Sciences Research Council [BB/L006804/1] for funding.*

**Chih-Yu Hsu** left **Nicola Stanley-Wall** right
*Division of Molecular Microbiology*
*School of Life Sciences*
*University of Dundee*

# Celebrating biology for the fifth year running

Biology Week is an annual celebration of the biosciences, with events around the UK and beyond. Now in its fifth year, the week aims to showcase the important and amazing world of the biosciences, getting everyone from children to professional biologists involved in fun and interesting life science activities. We're busy getting ready for Biology Week 2016: Saturday 8 – Sunday 16 October.

Biology Week aims to bring the biology community together in a hive of activity, making more noise than anyone could individually in order to share a passion for the subject. The message travels beyond boundaries – bringing together those across life science disciplines and engaging new audiences.

Biology Week 2015 was a huge success with over 130 events taking place from London to Land's End, Malta to Mauritius. It was an incredibly busy and inspiring week, and each year we try to make Biology Week bigger and better than ever before!

This year we are organizing a debate at the Royal Institution with the Biochemical Society and Cancer Research UK on using DNA to predict people's chances of getting cancer. We will celebrate the best in biology books and photography at our annual RSB awards ceremony and host a parliamentary reception for MPs, Ministers and our membership organizations in the Houses of Commons.

Our branches, MOs, members and the public are also organizing events all over the UK and beyond. Events get more diverse every year; science festivals, Big Biology Days, dino digs, competitions, lectures, fungal forays, music and storytelling. We would love you to get involved with the biggest week in the biology calendar. If you are interested in running an event yourself then please let us know via **media@rsb.org.uk**.

There are lots of ways people can get involved in their schools, colleges or universities too. *Sf*AM contribute each year to our Biology Week quizzes to put students' knowledge to the test. You can compete in a Bio-bake off, hold an Insect Poll, get creative with a BioArtAttack, go on a Bioblitz, try out some kitchen science, attend a local event or have a STEM speaker come to visit. You can find lots of resources and ideas, and download the poster for schools on our website.

The great thing about Biology Week is that absolutely anyone can get involved and there are no limits to the type of activity or area of life sciences you want to explore. It really is great to be at the heart of it – seeing hundreds of Biology Week events popping up all over the world – being enjoyed by children and professional scientists alike. Our vision is that more people understand the role biology will play in addressing some of the biggest challenges of the century, such as climate change, food security, antimicrobial resistance, biodiversity loss, and age and lifestyle-related disease.

Find out about events and activities at **www.rsb.org.uk/biologyweek** and let us know what you're planning for Biology Week via Facebook or Twitter using **#BiologyWeek**, or via email. Biology Week 2016 is fast approaching and we hope you enjoy it as much as we do.

**Dr Mark Downs** CSci FRSB
*Chief Executive of the Royal Society of Biology*

# What is the LONGITUDE PRIZE?

The Longitude Prize is a five-year challenge with a £10 million prize fund. It commemorates the 300th anniversary of the Longitude Act of 1714, the first British challenge prize, to determine longitude at sea. In 2014, with the support of the BBC and Amazon, the public decided the focus of the new Longitude Prize to be antibiotic resistance.

The Prize aims to conserve antibiotics for future generations, revolutionizing global healthcare. It is looking to award one prize of £8 million to a team that can develop a transformative, accurate, affordable, rapid point-of-care diagnostic test that is easy to use, anywhere in the world. This global Prize is being developed by Nesta, supported by Innovate UK.

## How to win

To win the Prize, the team must prove to our Prize Advisory Panel (judges) that their diagnostic meets the overall objective of transforming treatment decisions to significantly reduce the misuse and overuse of antibiotics. We have not defined the test we are looking for, as we are looking for something truly novel and unforeseen. Tests that could theoretically win the Prize are those that accurately distinguish between bacterial and other infections – ruling antibiotics in or out. At the opposite end of the spectrum tests could go further and look at the resistance and susceptibility profile of the infection to various antibiotic classes.

Deadlines for submitting the information needed to win occur every four months. The next deadline is on 30 September 2016, then 31 January 2017. Applications will remain open until the Panel and Committee agree there is a winner, or until the last submission date of 30 September 2019.

## Entering the Prize

### Registration

Teams sign up on the website by providing information on who and where they are, and a short description of their idea. They then work on their idea until they decide if and when they are ready to make a full submission to win the Prize. Registered teams are at different stages of development with their ideas and are under no obligation to submit a winning solution.

### Submission

When teams are ready to submit a full entry to win the Prize, they need to provide an equivalent of a target product profile. This includes full details about their diagnostic and its performance and a business plan showing how they would scale up the manufacture and distribution of their test. They also need to have developed a design locked prototype.

We expect the full assessment process, which includes clinical trials, to take at least 18 months. However, as we do not define the test, nor sample type, it is impossible for us to predict this with any accuracy. If a team makes it through this assessment process, the Prize will be awarded by the Longitude Committee, chaired by Lord Martin Rees and including Dame Sally Davies, Baron Peter Piot and Professor Jeremy Farrar.

# Spring Meeting 2016

This year's S*f*AM Spring Meeting was entitled "**What can whole genome sequencing do for clinical and public health microbiology?**" Held on 19 April at the Bloomsbury Hotel in London, a diverse group of delegates arrived on one of the warmest and sunniest days of the year, eager to discuss one of the exciting new techniques in microbiology: whole genome sequencing.

Once we'd had our fill of hot drinks at registration, S*f*AM's Meetings Secretary Dr Andrew Sails welcomed us and began his chair of the morning's talks.

The first presentation – "**Laboratory and bioinformatics challenges before whole genome sequencing can be applied routinely in clinical and public health microbiology**" – was given by Dr Anthony Underwood from Public Health England. An engaging talk about a realistic way to get whole genome sequencing into the clinic set the tone for the day. As a microbiologist who has moved towards bioinformatics, Anthony Underwood highlighted some of the difficulties in bringing a technique that generates so much data into routine use. We need to get biologists and software developers talking together in order to develop transparent software that is easy to use – that way we can have centralized bioinformatics with one assay.

In a change to the scheduled line-up, Professor Noel McCarthy from the University of Warwick followed, asking "**What does a public health epidemiologist need from whole genome sequencing?**" This talk really showed the audience a glimpse of what could be achieved with whole genome sequencing, as we were presented with some of the previous problems in epidemiology – and how whole genome sequencing can solve them. A particularly exciting example showed that whole genome sequencing of bacteria from a number of individuals in an infectious outbreak could identify where transmission was person-to-person, or from zoonotic source to person. It was also shown that for us to be sure we're undergoing an outbreak we need to be 99.9% sure of the geno- and phenotyping of the bacteria involved, and with whole genome sequencing, we can do that.

Changing tack was Professor Edward Feil from the University of Bath, with a talk on "**Nomenclature for bacterial whole genome sequence genotyping: can we all speak the same language and who decides what it is?**" Edward began by apologizing for the meaty title of his talk, he then went on to urge us that, as we move into the third millennium, it's time to move away from using phenotypes as our first call for identifying bacterial species. The talk drew in examples from all walks of life, pointing out that our current use of binomial species assignments, much like how most cars use their make and model as a binomial name, is "*intuitive and works well in most cases*". It may not always be pragmatic to have a defined unique species, and small changes in genotype can have major changes in the phenotype, so we have to throw all the information we have at the problem of nomenclature. The talk ended with an idea drawn from astronomy to give us a glimpse of what the future of nomenclature may be like – using the addresses of SNPs much like star coordinates to position an organism on the phylogenetic tree.

The final session of the morning asked "**What can bacterial whole genome sequencing actually do for the clinical microbiologist and infection specialist?**" presented by Dr Grace Smith of the Heart of England NHS Foundation Trust. As an expert in tuberculosis, Grace understands the need for rapid and discriminating tests to best help a patient at the bedside. It takes a long time (4–8 weeks) to grow *Mycobacterium tuberculosis* in culture and analyse it for clinical phenotypic information, and whole genome sequencing can greatly cut this down. Estimating that it will be 12–18 months before whole genome sequencing is used routinely in the clinic, the talk concluded with examples of a pilot study on using whole genome sequencing within the NHS.

After a break for lunch and a chance to talk with other delegates and exhibitors, we began the afternoon session, chaired by S*f*AM's Corporate and Parliamentary Liaison officer Professor Mark Fielder.

This session was opened by Professor Judith Breuer of University College London, introducing a new point of view with "**What can whole genome sequencing do**

**for the clinical virologist?**" An expert in cytomegalovirus, Judith looks at the genomics of antiviral drug resistance. Unlike bacterial microbiology, the virology field is dominated by genotyping rather than phenotyping. The issue with this is that identifying resistance genes in a virus can take a lot of rounds of PCR, which can be time-consuming and prone to errors. Whole genome sequencing circumvents this, and by using probes to isolate the viral genetic material, it is possible to gain large amounts of information on resistance genes in a short amount of time. Using deep sequencing, we are able to identify non-consensus mutations in resistance genes that give rise to problems further down the line in patient treatment. "*We are using information from whole genome sequencing to be more intelligent about treating patients*" – a sentiment shared by many of the speakers.

Our final talk of the day was somewhat more specialized – "**Whole genome sequencing studies on *Salmonella* Typhimurium DT104**", given by Dr Alison Mather from the University of Cambridge. Despite the focused view on a particular organism, the talk provided much interest in the wider implications of the findings. By looking at sequences from many different populations of DT104 and running statistical analysis, it was shown that most transfers of this organism are human-to-human or animal-to-animal, with infrequent transfers between the two. The genotypic and phenotypic data lined up well with each other as well as with the epidemiology, showing that by using a combination of these techniques we are able to find new levels of depth of information on the spread of bacteria that were previously unobtainable.

We wrapped up the day with a panel discussion on whole genome sequencing, which was opened up to questions from the audience. The panel highlighted some of the problematic areas we need to overcome before seeing whole genome sequencing in the clinic, many of which harked back to Anthony's first talk on bioinformatics, and the need for transparent and simple data interpretation. Other questions posed asked if we would be saying goodbye to the Petri dish and microscope anytime soon, yielding a mixed response from the panel. With improvements in technology, whole genome sequencing could well be done in under an hour and progressively cheaper, meaning bacterial culture becomes the time-consuming and expensive option – "*Would you wait for an overnight culture if you could get results in 10 minutes?*" asked Grace Smith. The panel wrapped up with a simple yet difficult question from the audience: how should we teach the microbiologists of the future? The answers came one by one from the panel to reach a resounding consensus: teach about where whole genome sequencing and genomics can fit into the course – but don't take the Gram stain out of your lectures just yet.

**Robert Millar**
*University of Warwick*

# *An Audience with* Margaret McFall-Ngai

**6pm | 11 October | 2016**
**Royal Society of Medicine, One Wimpole Street, London**

*The lecture will be followed with a drinks reception and canapés.*

**Margaret McFall-Ngai (University of Hawaii, Manoa) is a pioneer and international leader in the field of animal–microbe interactions and her research into the relationship between a host and its microbiome continues to challenge traditional microbiological notions.**

Her major research interests focus on the associations between animals and their specific symbiotic prokaryote partners.

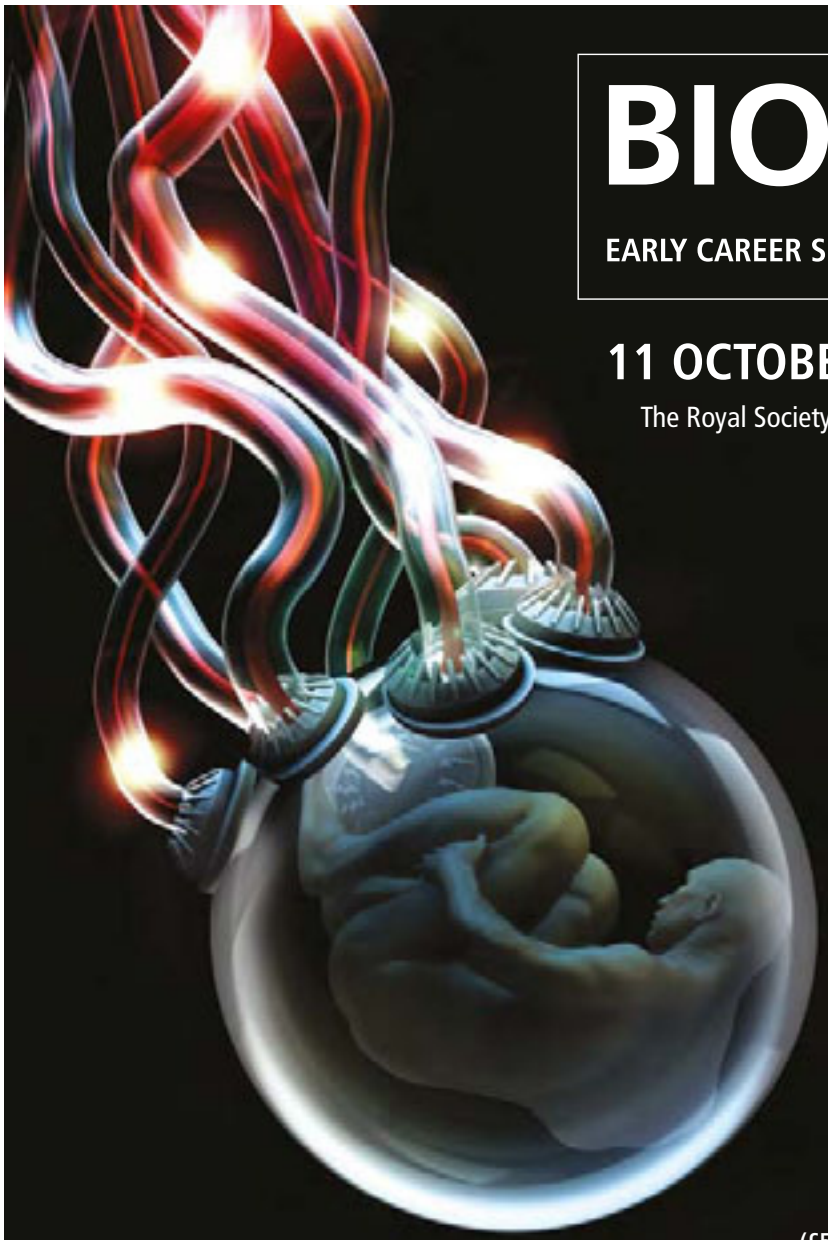
In particular:

- How environmentally rare bacteria are harvested from the host's habitat during the onset of a horizontally transmitted symbiosis.
- The establishment and maintenance of the specific symbiotic microbiome.
- The influence of bacteria on the development of the host tissues with which they associate.
- The similarities and differences between pathogenic and beneficial animal–bacterial interactions.
- How the symbiont population is maintained over the host's lifetime.

This is a **FREE** event by invite only and for delegates of the Early Career Scientists Research Conference, although a limited number of places at **£15** are available to other scientists who wish to attend.

Register at **www.emilecture2016.eventbrite.co.uk**

**+44(0)1933 382191** **www.sfam.org.uk** | **sally@sfam.org.uk**



# BIOETHICS

## EARLY CAREER SCIENTISTS RESEARCH CONFERENCE

## 11 OCTOBER 2016 | 11:00 –17:00

The Royal Society of Medicine, 1 Wimpole Street, London UK

### SPEAKERS INCLUDE:

Anne Glover, Professor and Vice-Principal External Affairs & Dean for Europe, Aberdeen

Bobbie Farsides, Professor of Clinical and Biomedical Ethics and Law, Brighton

David Jones , Director of the Anscombe Bioethics Centre, Oxford

John Bryant, Head of HEA Committee on Bioethics, Exeter

**£50 non Member £30 Member**

**(£5 early bird discount before 9 September 2016)**

**VISIT OUR WEBSITE TO BOOK OR CONTACT US FOR FURTHER DETAILS**

**S*f*AM and the ECS committee are proud to invite undergraduate, postgraduate and early career scientists to attend and participate in the fourth ECS Research Conference on 11 October 2016 at The Royal Society of Medicine, London.**

Bioethics is an emerging topic in the media and will be a familiar subject to those working with animal models and gene editing, however, in the context of microbiology it is rarely discussed.

This year's conference is specifically aimed at highlighting how bioethics is relevant in microbiology and will feature a panel discussion with leaders in the field. As always, the conference is a chance for early career scientists to present their data and network with other early career microbiologists.

**This year's event will be followed in the evening by the S*f*AM's annual *Environmental Microbiology* Lecture on animal–microbe interactions given by Professor Margaret McFall-Ngai.**

**www.sfam.org.uk | +44(0)1933 382191 | ecs@sfam.org.uk**

sfam ECS EarlyCareerScientists

# SUMMER CONFERENCE 2017

## NEW INSIGHTS INTO FOOD SAFETY

## 3–6 JULY 2017

BALTIC Centre for Contemporary Art
Gateshead, Tyne and Wear, UK

## SPEAKERS INCLUDE:

Sarah O'Brien – University of Liverpool, UK

Suresh Pillai – Texas A&M University, USA

Rob Kingsley – Institute of Food Research, UK

**Foodborne diseases are a chief concern of many microbiologists as they not only affect people's health and well-being, but also have major impacts for countries' economies too.**

**Although there have been some successes in the reduction of foodborne disease caused by particular pathogens, despite significant efforts the level of foodborne disease globally caused by microbial agents remains unacceptably high, and is a major cost and burden to society.**

This conference will look at our current understanding of the key pathogens that are causing the greatest burden on our health and our economy, focusing on new insights into these individual agents that aid our understanding of the problems they cause.

New issues such as the transmission of antibiotic resistance through the food chain will also be presented, as will new techniques for reducing levels of disease through approaches to control and food safety education.

**FEES BEFORE 2 JUNE 2017:**
**Full Member £250**
**Student Member £200**
**Student Non-Member £400**
**Non-Member £600**

**STUDENTSHIP GRANT AND ABSTRACT SUBMISSION DEADLINE: 10 March 2017**

society for applied sfam microbiology

**www.sfam.org.uk**

**sally@sfam.org.uk**
**+44 (0)1933 382191**

# ANTIMICROBIAL RESISTANCE MEETING

## FINDING SOLUTIONS TO A THREAT ON WORLDWIDE PUBLIC HEALTH

## 24 NOVEMBER 2016

One Great George Street, London

### KEYNOTE BY:

Dame Sally Davies
Chief Medical Officer

### OTHER SPEAKERS INCLUDE:

Susie Singleton
Public Health England

Paul Hoskisson
University of Strathclyde

Elaine Cloutman-Green
Great Ormond Street Hospital

**The final report issued by the Review on Antimicrobial Resistance, a project chartered in 2014 by UK Prime Minister David Cameron and backed by the Wellcome Trust, declared "We may be on the cusp of a post-antibiotic era" and must commit more resources to ensuring we do not return to a dark age of medicine, where even a papercut can kill.**

In this one-day conference, we aim to explore the latest in tackling AMR in terms of the key points of the review including infection control, improved diagnostics, and the development of new and novel compounds to address the evident "discovery void" when it comes to antimicrobials.

*Sf*AM warmly invites you to attend an event that promises to provide colleagues from across the academic, policy, public and private sectors with the opportunity to network and discuss cutting-edge research on antimicrobial resistance.

**FEES:**
**Full Member £35**
**Student Member £20**
**Student non-Member £30**
**Non-Member £50**

**Abstract submission deadline (abstracts must be on the topic of AMR): 7 October 2016**

society *for* applied sfam microbiology

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CAREERS

# Microbiology in glass

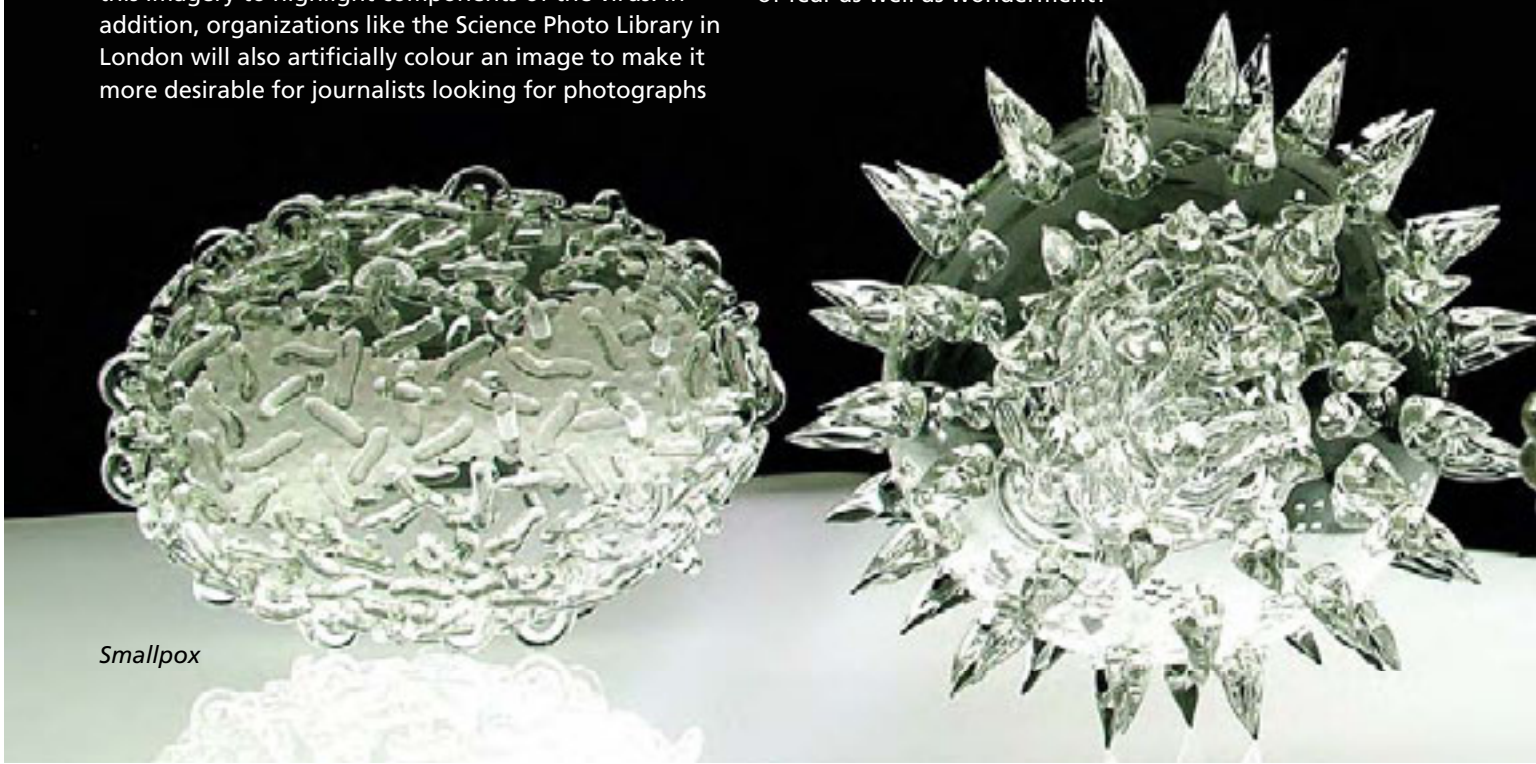
I am a multidisciplinary artist living in the UK, but have been working internationally for 18 years. My practice involves the creation of sculptures, installations and live arts projects, which have excited and inspired people around the globe. I have a set of different narratives that make up my practice which are developing in parallel with one another, one strand of which is the intersection of art and science.

My *Glass Microbiology* series of artworks were created to offer an alternative representation of viruses to the artificially coloured imagery received through the media. In fact, viruses have no colour as they are smaller than the wavelength of light. By extracting colour from the imagery and creating jewel-like sculptures in glass, a complex tension arises between the artworks' beauty and what they represent.

Since 2004, I have been investigating the implications of artificially coloured or doctored images of microbiology for communication purposes. Commonly, images of viruses are made using an electron microscope which produces black and white imagery. Scientists colour this imagery to highlight components of the virus. In addition, organizations like the Science Photo Library in London will also artificially colour an image to make it more desirable for journalists looking for photographs

*Swine flu and Luke Jerram*

to illustrate articles. I was interested in the effect which the colouring of these images has on how viruses are understood by people and how colour conventions are negotiated by those creating the imagery. If some images are coloured for scientific purposes, and others altered simply for aesthetic reasons, how can a viewer tell the difference? Because of their size and physical characteristics, viruses have no intrinsic colour, yet it's hard to find an image of a virus in a newspaper or scientific journal which isn't coloured. Presented with an air of scientific objective truth, could it be possible that some images are created to help promote a sense of fear as well as wonderment?



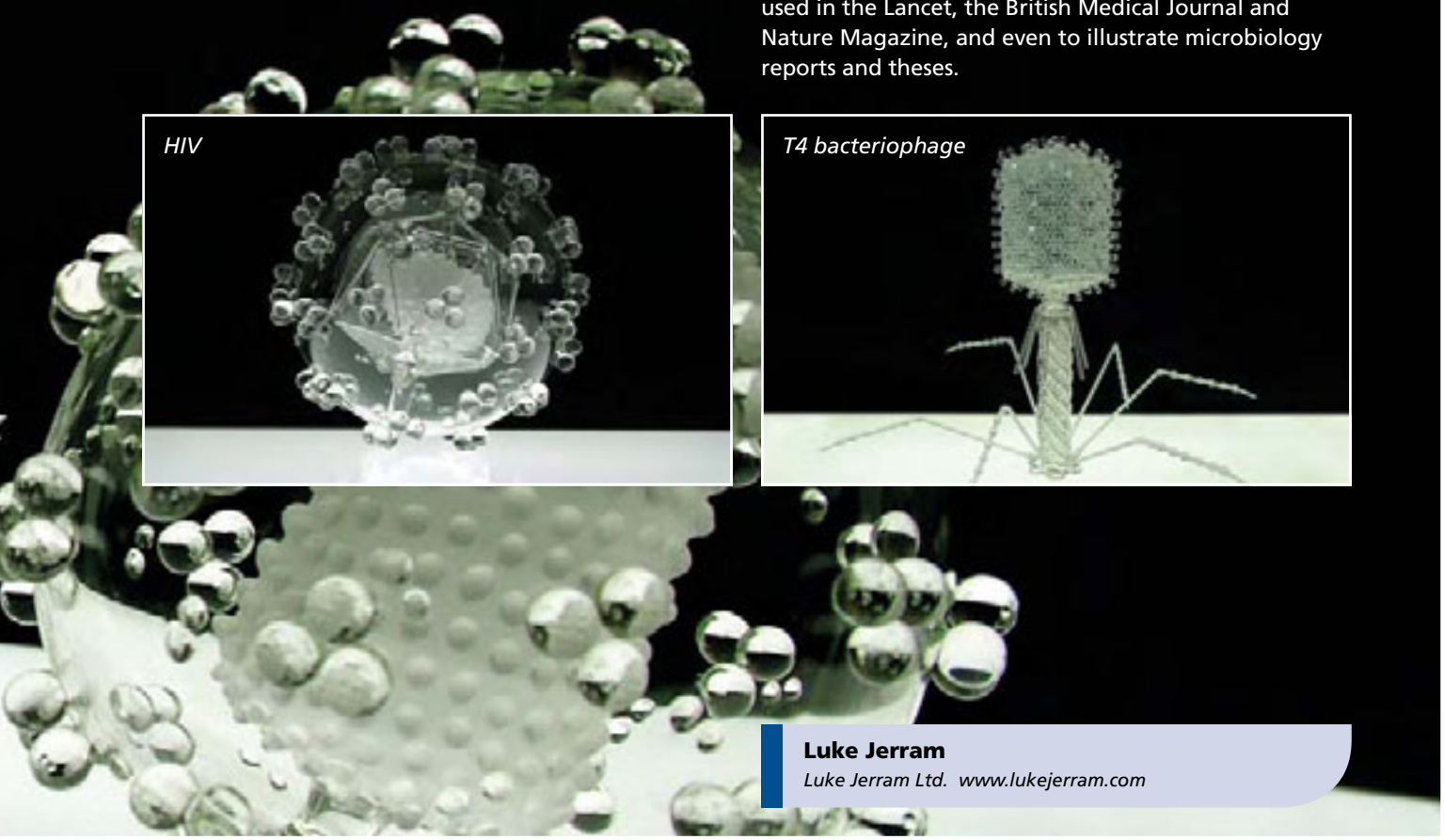
*Smallpox*

To develop the first artworks in the series, I worked in consultation with virologist Dr Andrew Davidson, from the University of Bristol, and glassblowers Kim George and Brian Jones from Wearside Glass studio in Sunderland. Together we created a number of alternative representations of viruses which are three-dimensional, transparent and created in glass. The first sculpture of the HIV virus was also made as an object to contemplate the global impact of the disease. However, the beauty of this alternative representation is at odds with the catastrophic human impact of the actual virus.

Over the past 12 years since this first *Glass Microbiology* artwork was conceived, I have developed over 20 sculptures in the series representing diseases and organisms such as malaria, smallpox, *E. coli* and Ebola. I have even created a fictional virus, '*Untitled Future Mutation*', a future virus that has yet to be born. Often these artworks have been made in response to times when a disease is topical. The 2009 flu pandemic was an outbreak of a new strain of H1N1 influenza virus. At the beginning of the outbreak I was diagnosed with swine flu. It was strange designing the sculpture with a fever whilst swallowing my Tamiflu tablets every few hours. I remember there was a lot of speculation in the media regarding whether or not the virus could wipe out a third of the global population. More recently, a zoo in Holland commissioned an Ebola artwork and this was finished just before the outbreak of the virus in Africa hit the media.

I am often approached by scientific research centres and education institutions who commission new virus artworks to celebrate and honour their research. They are also bought by private collectors and are acquired by public museums across the world. Works are included in the collections of The Metropolitan Museum, NYC; The Wellcome Collection, London and The Museum of Glass, Shanghai among others. In 2010, I received the 25th Rakow Award for the series from The Corning Museum of Glass, New York. The works are also regularly displayed in exhibitions around the globe, including a recent presentation in 2015 at ArtScience Museum, Singapore, alongside Leonardo Da Vinci's *Codex Atlanticus*. The *Glass Microbiology* artworks are exhibited in both art and science contexts and are considered a useful and inspiring tool for learning about microbiology as they reveal the properties of hidden structures of naturally occurring phenomena, making them tangible for a wider audience to contemplate and learn from.

Interestingly, photographs of my *Glass Microbiology* sculptures are now used as scientific illustrations for medical journals. In 2009, researchers from the US Institute of Medicine, who study potential clinical uses for the controversial smallpox (variola) virus, selected my photographs for the cover of their book, *Live Variola Virus: Considerations for Continuing Research*. The imagery has now become part of scientific visual language and the image has been used in American school textbooks. Photographs of the work have been used in the Lancet, the British Medical Journal and Nature Magazine, and even to illustrate microbiology reports and theses.



*HIV*

*T4 bacteriophage*

**Luke Jerram**
*Luke Jerram Ltd. www.lukejerram.com*

Our relationship with the microbial world has been one of constant misunderstanding. For much of human history, the cause of disease was attributed to an imbalance of humours, evil spirits and even 'bad air'. It was not until pioneering work by Pasteur and Koch that the cause for a number of our infectious ills was revealed: bacteria.

The fact that bacteria were identified as the cause of disease led to a century of germophobia. Early epidemiologists were concerned with eliminating disease, and bacteria were the cause for many of them. The obvious conclusion was to eliminate them from our lives as much as possible, and indeed, ensuring a clean environment has been shown to prevent the spread of more than 35 diseases. Thus, we had a revolution in hygiene, a revival of bathing (a habit which had gone out of fashion during the early 19th century), and the beginning of a fanatical devotion to washing our hands. This purge has actually been enormously successful; the mortality rate for the developed world is now a quarter of that during the Victorian era, and globally the average person now lives twice as long as they did in 1800.

However, it is gradually becoming apparent that hygiene has brought with it some unanticipated problems. Our bodies have lived with microbial exposure for hundreds of thousands of years, and this has led to a large number of co-dependencies, whereby the body's regulatory systems react inappropriately to the absence of certain bacterial strains. We see the same responses in other mammals (rats, mice, etc.), which suggests that some of the associations may be extremely ancient. Therefore, hygiene could be interrupting certain microbial exposures that could lead to a disruption in the immune, endocrine and even nervous systems, which may lead to allergies, hormonal imbalances, neurological developmental and behavioural changes.

The world we have built does not resemble the natural world; the natural world is saturated with bacteria. Microbes have been using the last 3–4 billion years evolving mechanisms to use a vast range of energy sources. This has enabled them to populate nearly every environment from the deep Earth to the atmosphere on our planet; we have even sent them to other planets, as they have hitchhiked on NASA spacecraft. The diversity of the Earth microbiome was recently predicted at approximately 1 trillion taxa. The number of bacteria that actively parasitize humans is a tiny fraction of this

**The fact that bacteria were identified as the cause of disease led to a century of germophobia**

diversity, and the number of known pathogenic bacteria also pales in comparison to the abundance of bacteria that form mutualistic or commensal relationships with us. However, it is important to understand that there are many unknown pathogens, and some commensal bacteria can become virulent in certain contexts.

Despite our best efforts, bacteria are a constant presence in and around us, as they have been for the entire history of multicellular life. Recent estimates suggest there are 1.3 bacterial cells for every human cell in our body, comprising between 0.5–1.2 kg of our body mass. They are basically a complex ecosystem inside and on the surface of the human body; including hundreds of species that can only live off other bacteria. While many of them are evolutionarily adapted to living inside us, many can survive without us – and there is compelling evidence to suggest that some will alter their metabolism to consume our tissues when we die. As with any ecosystem, the microbial community in the human intestine is quite stable. This stability is part of the reason the microbiome is seen as part of the host's defence against pathogenic disease. However, the bacteria in this ecosystem, as with any environment, fill many ecological roles, including competitive and cooperative. It is striking that when we look at the intestinal microbial community of Amerindians, who have supposedly never received a dose of antibiotics, their bacteria still maintain genes for antibiotic resistance. This is a perfect example of the arsenal of molecular weaponry used by the microbiome in this highly competitive environment.

# How clean is too clean?

More so than at any time in the history of our species we are spending most of our lives living in synthetic environments that we have created. We use the term 'built environment' to refer to the various buildings and infrastructure, such as houses, office buildings, transit systems, which we have built to make our lives more comfortable. However, for bacteria, this environment is a desert, as it is extremely dry and this lack of moisture appears to prevent bacterial accumulation. The microbiology of the built environment has been an active research topic for more than 150 years – with researchers primarily interested in understanding how to reduce the fungal and bacterial burden. This was logical because bacteria and fungi were known to cause disease and to affect the structural integrity of the building by rotting wood, concrete and even corroding metal. However, researchers and architects are starting to embrace a different perspective. They are actively trying to understand how to increase human exposure to the natural world, by weaving it into the structure of our buildings. What stimulated this paradigm shift?

In our modern world, we have removed most pathogens from our experience with improved hygiene and vaccines. Therefore, there is no longer a desperate need to sterilize the environments in which we live and work to reduce transmission of such diseases. The Hygiene Hypothesis explores the health benefits of our interaction with the biological world, most importantly the natural environmental bacteria that we have adapted to over our whole evolution, and which do not cause disease. Reducing our exposure to these beneficial organisms may be the underlying cause of many of the developed world's 'modern plagues', such as allergies, asthma and autism. The problem with the built environment is that although it is comfortable, it is also only really abundant in one subset of bacteria: our own.

We are constantly shedding bacteria whenever we touch, breathe, walk or even sit motionless, with approximately 38 million microbial cells being emitted into our immediate environment every hour. These microbial communities are predominantly from our skin; in fact, each person or family occupying a space emits their own unique signature, which could be used as trace evidence for forensic investigations. When moving home, the microbial population of a new property will quickly resemble that of the old house; the same as has been shown for hotel rooms, with no significant difference in microbial community composition seen between a hotel room and the occupant's other property within 24 hours.

This environment is very different from the one our species first evolved in, especially with regard to the microbial communities present there. While hygiene has ensured that our old enemies, cholera and typhoid have disappeared, there are microbes that have risen to prominence, with rare types of bacteria becoming predominant. These include major pathogens, such as *Staphylococcus aureus*, which is a leading cause of common skin infections, but has also picked up resistance to the antibiotic meticillin; whereupon it becomes known as MRSA. Certain viruses are also able to survive in the harsh expanse of the built environment, with flu remaining infectious for over a day on surfaces such as plastic or steel while only lasting around 15 minutes on hands.

People are still germophobic after more than 100 years of advertising telling us that dirt equals disease, and bacteria will kill you even if you cannot see them. So much so that our culture has become paranoid of the microbial world. The main concern most people have is that these environments can smell bad, and in their minds this equates to disease. Indeed, one of the best ways to get rid of bad smells is to eradicate the bacteria that are producing these odours. We do this to our bodies all the time, with antiperspirants that prevent us from sweating. These inhibit the microbial metabolism of sweat, preventing the production of volatile chemicals. However, if we take a close look at the environments which most people consider to be full of dangerous bacteria, it seems that the paranoia is likely unjustified.

For example, gyms are not a reservoir of MRSA or the cause of other *Staphylococcus* infections. Indeed, one study, while suggesting that rhinoviruses (flu) were present and resistant to cleaning, found no pathogenic bacteria at all. But if there are lots of humans in a gym, why are there not more human pathogens? The answer intuitively makes sense. Bacteria on gym surfaces are mostly from the people who use them, and most people at the gym are relatively healthy. The bacteria then have to survive in an environment with little food and water, and are constantly being replaced with bacteria from the next person to use the surface, giving them limited opportunities for reproduction and a slim chance of survival.

Kitchens are definitely a place where we should be careful, due to the preparation of raw food, which can carry virulent pathogens. Otherwise, there is generally no cause for concern. Kitchens are home to a diverse array of bacteria but less than 10% are even closely

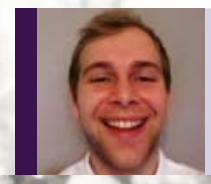
**Kitchens are definitely a place where we should be careful**

related to known pathogens; therefore, should you sterilize the surfaces in your kitchen? It is useful for ensuring that foodborne pathogens are controlled, and for reducing unwanted odours, but it is very unlikely to significantly reduce the spread of disease if you are careful with food.

The one place we should aim to be careful is in hospitals. Healthcare-associated infections and surgical-site infections are a major problem in our modern healthcare system. And yet, there is very limited evidence linking all infections to exposure to the hospital environment. Despite all existing protocols for cleaning and sterilization, people still develop infections in this environment.

One theory is that the majority of such infections occur due to organisms already present in the body of the patient, which become stressed by the procedures the patient is undergoing. This has been demonstrated using animal models, whereby organisms in the gut are induced to virulence by shifts in the gut environment that occur during and following a major surgical procedure. It is therefore possible that to effectively reduce the burden of healthcare-associated infections we will need to treat the microbiome along with the patient, reducing the stress that leads to virulence activation. However, another solution might just be to open the windows; this was used effectively by Florence Nightingale to impact infection rates on her wards, and has also been shown to affect the diversity of bacteria in the air of modern hospitals, which show a reduction in potential pathogens when a greater proportion of outdoor air is introduced into wards.

We are paranoid about diseases where few exist due to a centuries-long fight against infection. Our quest to eradicate disease from our lives may be inadvertently interrupting the exposure our bodies need (especially early in life) to develop healthy immune, endocrine and neurological systems. The environments we have built for ourselves may have contributed to this problem, surrounding us with organisms that are rare in the outside world. We urgently need more research to determine the most effective way to understand infection and the spread of disease in the built environment, and determine ways to reduce virulence activation in our own microbiome. But we also need to explore ways to embrace greater microbiological exposure in our lives, to stem the flow of pernicious germophobia and explore a safe way to re-engage with the natural world without opening ourselves back up to the chance of new infectious diseases.



**Miles Richardson** left **Jack A. Gilbert** right
*Department of Surgery*
*University of Chicago*


# London's MICROBIOTA

## *An occasional series on applied microbiology themes in the capital*

The merest glance at the audience of an S*f*AM meeting and the riot of style, colour and cutting-edge design on view will tell you that our Members take fashion very seriously. Few, therefore, will need any introduction to Soho's Carnaby Street, a magnet for the fashion-conscious since the 1960s. While there pursuing the latest sartorial trend, many will have noticed, at the junction of Carnaby Street and Broadwick Street, a large mural in the style of Diego Rivera. The mural commemorates Soho's huge cultural influence over the years with representations of many famous previous residents including Karl Marx, Dylan Thomas, Brendan Behan, Casanova, Mozart, Joshua Reynolds, Paul Verlaine and John Logie Baird. In the midst of these, it is possible to spot, looking rather modest and self-effacing, peeking out from behind a lady's turban, Dr John Snow.

Originally from Yorkshire, Snow lived in Soho and its immediate environs from 1836 until his death in 1858. For much of that time he lived in nearby Frith Street, although it is with Broadwick Street that his name is particularly associated. Then called Broad Street, it has another claim to fame as the birthplace of the poet and artist William Blake, but for microbiologists everywhere it will always be linked with John Snow, the battle against cholera and the birth of epidemiology.

Snow's investigations into cholera were not his first encounter with applied microbiology. In 1836, while studying at the Hunterian School in Great Windmill

Street (also in Soho), he was encouraged by his chemistry lecturer to try a method for preserving bodies for dissection by injecting them with a solution of arsenic. In his own subsequent account in the Lancet in 1838 he records injecting the bodies with a solution of potassium arsenite and how effective it was. Regrettably though, he also had to note how one student dissecting a corpse preserved in this way fell ill with vomiting, stomach pains and diarrhoea – symptoms of arsenic poisoning – and that later, in the hot summer of 1837, similar symptoms were experienced by five of six students dissecting a similarly preserved body. Since he was one of the group of six students, he was prompted to investigate this further. His subsequent experiments established that decomposition in the bodies resulted in the conversion of the arsenic oxide to a volatile reduced form of arsenic that could be inhaled, though he was unaware that this was a microbiological process. As a result of his investigation, the use of arsenic for this purpose was discontinued.

The story of the 1854 Broad Street cholera outbreak has often been told. During an earlier outbreak in 1848 Snow had become convinced that cholera was spread by water but was unable to provide convincing evidence for this. During the Broad Street outbreak he carefully plotted cases and found that they centred on a water pump in Broad Street. Support came from the fact that workers in the Lion Brewery in Broad Street, where they drank beer, and residents of the nearby Poland Street Workhouse where they drank water from their own well, did not fall ill. Further evidence came from an apparently isolated case in distant Hampstead. Susannah Eley was a senior member of the Eley family which owned a factory in Broad Street making percussion caps for ammunition (the Eley name is still known in this context today). Having lived in Broad Street, she liked the water from the pump so much that she had some brought to her in Hampstead, with fatal results.


Armed with this accumulation of evidence, Snow and his allies decided to act. Though regarded as being a rather unconvincing public speaker, he managed to persuade the local Board of Guardians to remove the pump handle thereby isolating the primary source of the outbreak. It has been pointed out that by this time the outbreak had peaked and the number of cases was already declining rapidly but, as a dramatic gesture, it certainly adds to the story.

Snow identified cholera as being a contagious disease of the gut, spread by the faecal-oral route, primarily by water, but, as with his earlier arsenic investigation, he did not recognize microorganisms as the cause. An annular, fungus-like organism associated with cholera had been described in an earlier outbreak in Bristol but this had proved to be premature. In London, the microscopist Hassall had identified large numbers of what he termed vibriones in the rice-water stools from cholera victims but not in the water they consumed but thought these were a result rather than a cause of the disease. In fact, in the same year as the Broad Street outbreak, Filipo Pacini, an Italian anatomist working in Florence, published a description of the cholera bacillus and its association with disease, though this went largely unnoticed and it was not until Koch's work published in 1884 that the matter was finally settled.

The Broad Street pump stood just outside what is now the John Snow public house (a small irony in view of the fact that Snow was an ardent teetotaller). The original has long gone although a replica pump was installed nearby to commemorate the events of 1854. At the time of writing, this too has been removed due to construction work in Broadwick Street. I did hear that a previous replica has also disappeared inexplicably. Was this the result of an over enthusiastic re-enactment by epidemiologists not content with simply removing the handle? Did it coincide with the retirement party of S*f*AM's former Chief Executive in the John Snow pub? Or was it just scrap metal thieves? We'll probably never know.

**Martin Adams**
*SfAM President 2011–2014*

# The 85th Annual General Meeting of the Society for Applied Microbiology

was held on Wednesday 6 July 2016 at 17.15 in the Assembly Rooms, Edinburgh

**Present:**

39 Members attended the AGM. This included:

President, Christine Dodd (CD)
General Secretary, Clare Taylor (CT)
Treasurer, Steve Davies (SD)
Meetings Secretary, Andrew Sails (AS)

In attendance:

Lucy Harper (LH)
Paul Sainsbury (PS)
Clare Satchell (CS)

**1 Apologies for absence**

Carol Phillips

**2 84th Annual General Meeting**

The minutes of the 84th Annual General Meeting held in Dublin in 2015 were published in the September 2015 issue of *Microbiologist*. They were approved and accepted by those present.

*Proposed:* Mark Fielder
*Seconded:* John Rigarlsford

**3 Matters arising from the previous minutes**

None.

**4 Report of the Trustees of the Society 2015**

The President noted the success of the Society during the previous year, particularly with respect to the dedication shown by all the office staff. She announced the move of the Society Headquarters to Charles Darwin House in London and the departure of Julie Wright and Clare Satchell. Also noted was that Julie Buchanan will also leave at the end of 2016 but has kindly agreed to assist with this transition.

CD also bought to the attention of those present that Andrew Sails and Steve Davies would be stepping down as Meetings Secretary and Treasurer, respectively. CD thanked them both for the hard work and commitment they have both provided in fulfilling these roles for the last seven years.

The General Secretary gave a summary of the Society's communications and public engagement activities and highlighted the key successes of the social media channels and the Society's new blogging site, MicrobeBlog.

CT announced that the membership of the Society continued to increase during 2015 in line with the key areas of the organizational strategy.

CT then praised the continuing dedication shown by Members of the SfAM ECS Committee and explained that the increasing success of their activities is evident by attempts of others within the learned society sector to duplicate the format.

# 2016 *Sf*AM **AGM**

The Meetings Secretary gave a summary of activities and gave special thanks to the Events Manager, Sally Hawkes. AS also thanked Ken Nealson for giving the *Environmental Microbiology* Lecture. AS thanked the Society for the last seven years in his role as Meetings Secretary and wished his successor the best of luck.

The Treasurer reported that the Society's value has continued to rise and that net assets now stand at £7650711. SD was proud to report that £220K worth of individual grants had been awarded to Members and that Members had also benefited from a large subsidy of £120K provided for the Society's meetings. SD thanked the Society for the last seven years in his role as Treasurer and wished his successor the best of luck.

**5 Adoption of the Annual Report 2015**

Copies of the Annual Report of the Society for 2015 had been distributed previously.

*Proposed:* Basil Jarvis
*Seconded:* Mark Fielder

**6 Election of new Members (including honorary Members), deaths and resignations**

A list of the names of applicants for membership and a list of deaths has appeared in the *Microbiologist* throughout the previous year. The Society also holds a summary list of new Members and resignations throughout the previous year.

**7 Nomination and election of the Vice President, Mark Fielder**

CD announced Professor Mark Fielder was nominated as Vice President and the Members agreed unanimously with this decision.

*Proposed:* Philip Wheat
*Seconded:* Valerie Edwards-Jones
Mark Fielder was duly elected.

**8 Nomination and election of the Meetings Secretary, Ian Feavers**

CD announced Professor Ian Feavers was nominated as Meetings Secretary and the Members agreed unanimously with this decision.

*Proposed:* Andrew Sails
*Seconded:* Brendan Gilmore
Ian Feavers was duly elected.

**9 Nomination and election of the Treasurer, Philip Wheat**

CD announced Mr Philip Wheat was nominated as Treasurer and the Members agreed unanimously with this decision.

*Proposed:* Steve Davies
*Seconded:* Geoff Hanlon
Philip Wheat was duly elected.

**10 Nomination and election of new Ordinary Committee Members**

Brendan Gilmore and John Threlfall had come to the end of their terms and this created two vacancies for new Ordinary Committee Members. CD thanked them for their contributions to the continued success of the Society.

There had been two nominations for the two vacancies:

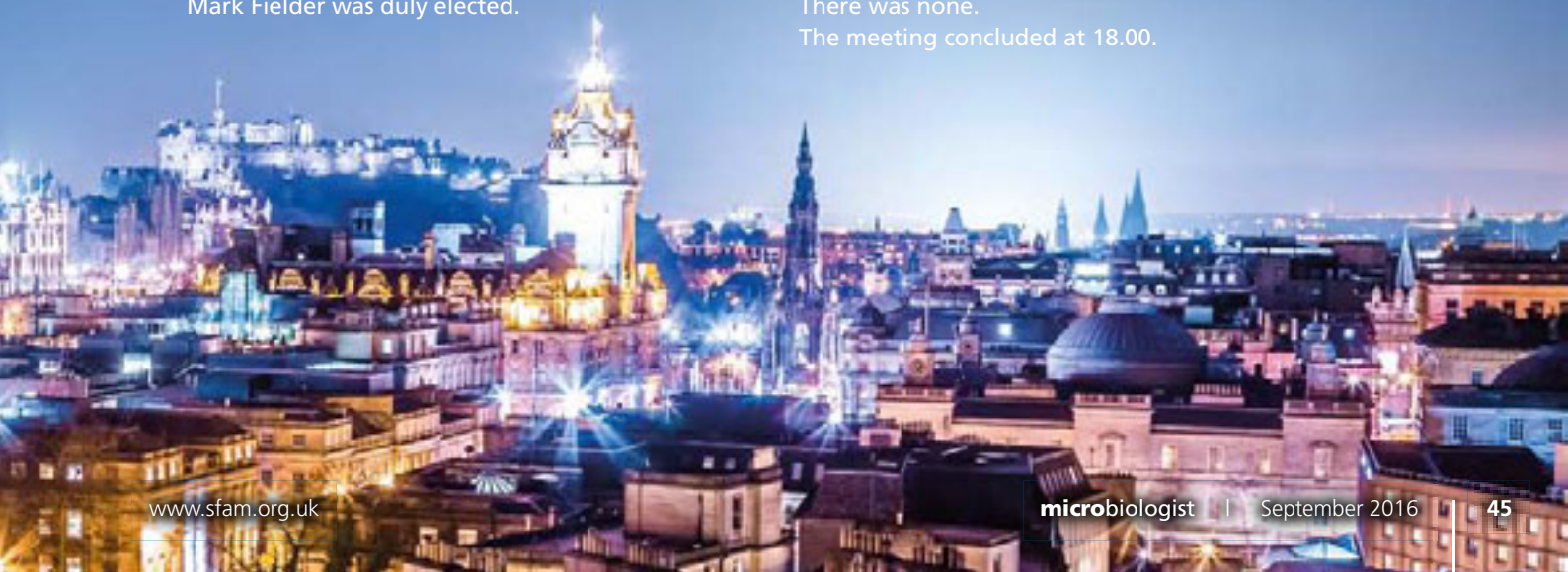
Simon Gould
*Proposed:* Mark Fielder
*Seconded:* Ali Ryan

Stephen Forsythe
*Proposed:* Christine Dodd
*Seconded:* Tim Aldsworth

Both were unanimously elected.

**11 Any other business**

There was none.
The meeting concluded at 18.00.



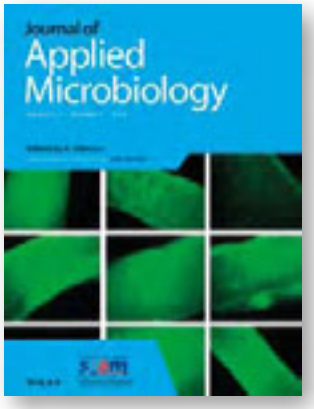
# Journal**WATCH**

## Highlights and featured articles from the S*f*AM journals

### Journal of Applied Microbiology

**www.journalappliedmicro.com**

#### A review of melanized (black) fungal contamination in pharmaceutical products—incidence, drug recall and control measures




R. Vijayakumar, M. Saleh Al-Aboody and T. Sandle

The aim of this study was to describe the incidence of contamination of pharmaceutical products by melanized fungi and to consider control measures in relation to bioburden and cleanrooms. This study reviews and analyses pharmaceutical product recalls and offers incidence rates of fungal detection from typical cleanrooms. The recalls include some serious cases which resulted in the loss of life. Of the different types of fungal contamination incidences, some of the most damaging have been due to melanized fungi ('black mould'), such as *Exserohilum rostratum*. The focus of the article is on melanized fungi. The study concludes that, from the review of recent pharmaceutical product recalls, fungal contamination is either increasingly common within cleanroom environments or the accuracy of sampling and the level of reporting has risen. The prevalence of melanized fungi in pharmaceutical facilities rests on specific virulence factors particular to these types of fungi, which are outlined. The article identifies a gap in the way that such fungi are screened for using available cultural methods. The article provides some control strategies, including assessing the suitability of disinfectants and biocides for reducing the risk of melanized fungal incidences within the pharmaceutical facility. Understanding the fungal risk to pharmaceutical products remains a poorly understood and often overlooked aspect of pharmaceutical microbiology. This article helps to identify this risk and offer some guidance to those involved with pharmaceutical products manufacture in relation to bio-contamination control strategies.

http://onlinelibrary.wiley.com/doi/10.1111/jam.12888/full

#### Assessing the activity of microbicides against bacterial spores: knowledge and pitfalls

M. J. Leggett, P. Setlow, S. A. Sattar and J.-Y. Maillard

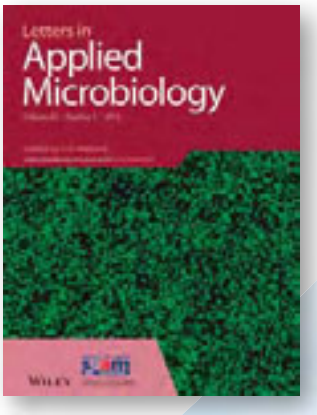
Bacterial endospores (spores) have a higher intrinsic resistance to microbicides compared with other microbial forms, most likely due to their impermeable outer layers and low water content. Though structural differences between the spores of various bacterial species may account for observed variations in their resistance to microbicides, flaws in methods for testing the sporicidal activity of microbicides often exaggerate the differences. This has major implications when considering the selection of one or more surrogates to assess microbicides against clinically relevant spore-formers such as *Clostridium difficile*. The mounting significance of *Cl. difficile* as a pathogen is leading to a corresponding increase in the number of commercially available microbicidal formulations claiming activity against its spores without proper differentiation between the product's sporistatic and sporicidal actions. In this review, we critically assess the situation and the implications of product claims on the field use of microbicidal products.

http://onlinelibrary.wiley.com/doi/10.1111/jam.13061/full

### Letters in Applied Microbiology

**www.lettersappliedmicro.com**

#### Transfer of antibiotic resistance from *Enterococcus faecium* of fermented meat origin to *Listeria monocytogenes* and *Listeria innocua*




M. Jahan and R. A. Holley

*Listeria monocytogenes* is an important foodborne pathogen that can cause infection in children, pregnant women, the immunocompromised and the elderly. Antibiotic resistance in this species would represent a significant public health problem since the organism has a high fatality/case ratio and resistance may contribute to failure of therapeutic treatment. This study was designed to explore whether the *in vitro* transferability of antibiotic resistance from enterococci to *Listeria* spp. could occur. It was found that 2/8 *Listeria* strains were able to acquire tetracycline resistance from *Enterococcus faecium*. *Listeria monocytogenes* GLM-2 acquired the resistance determinant *tet*(M) and additional streptomycin resistance through *in vitro* mating with *Ent. faecium* S27 isolated from commercial fermented dry sausage. Similarly, *Listeria innocua* became more resistant to tetracycline, but the genetic basis for this change was not confirmed. It has been suggested that enterococci may transfer antibiotic resistance genes via transposons to *Listeria* spp., and this may explain, in part, the origin

of their antibiotic resistance. Thus, the presence of enterococci in food should not be ignored since they may actively contribute to enhanced antibiotic resistance of *L. monocytogenes* and other pathogens.

http://onlinelibrary.wiley.com/doi/10.1111/lam.12553/abstract

### Detection of pathogenic *E. coli* and microbiological quality of chilled shrimp sold in street markets

L. J. Barbosa, L. F. Ribeiro, L. F. Lavezzo, M. M. C. Barbosa, G. A. M. Rossi and L. A. do Amaral

Foodborne illnesses caused by *E. coli* are one of the most important gastrointestinal diseases and therefore represent a public health risk. The presence of *E. coli* in water or in products such as shrimp indicates faecal contamination. However, indicator microorganisms can be used to evaluate the microbiological quality of food sold in markets. This study focused on detecting isolates of *E. coli* containing the genes *stx1A*, *stx2A*, *eae*, *LTI*, *STa*, *STb*, *aggR* and *pCVD432* in chilled shrimp sold in street markets in the municipality of São Paulo, Brazil, and to assess the microbiological quality of this product. Enteropathogenic and enterotoxigenic *E. coli* pathotypes were detected on the surface of two chilled shrimp samples. *Salmonella* spp. were not isolated. In addition, contamination of surface and muscle of the shrimp samples was found to be correlated. The detection of EPEC and ETEC pathotypes in chilled shrimp sold in street markets in Brazil provides useful epidemiological information for public health authorities to improve food safety and public health.

http://onlinelibrary.wiley.com/doi/10.1111/lam.12562/abstract

## Microbial Biotechnology

**www.microbialbiotech.com**

### Enzymatic hydrolysis of biomass from wood


C. Álvarez, F. M. Reyes-Sosa and B. Díez

Current research and development in cellulosic ethanol production has been mainly focused on agricultural residues and dedicated energy crops such as corn stover and switchgrass; however, woody biomass remains a very important feedstock for ethanol production. The precise composition of hemicellulose in the wood is strongly dependent on the plant species, therefore different types of enzymes are needed based on hemicellulose complexity and type of pretreatment. In general, hardwood species have much lower recalcitrance to enzymes than softwood. For hardwood, xylanases, beta-xylosidases and xyloglucanases are the main hemicellulases involved in degradation of the hemicellulose backbone, while for softwood the effect of mannanases and beta-mannosidases is more relevant. Furthermore, there are different key accessory enzymes involved in removing the hemicellulosic fraction and increasing accessibility of cellulases to the cellulose fibres improving the hydrolysis process. A diversity of enzymatic cocktails has been tested using from low to high densities of biomass (2–20% total solids) and a broad range of results has been obtained. The performance of recently developed commercial cocktails on hardwoods and softwoods will enable a further step for the commercialization of fuel ethanol from wood.

http://onlinelibrary.wiley.com/doi/10.1111/1751-7915.12346/full

### Biocontrol agents promote growth of potato pathogens, depending on environmental conditions

J. A. Cray, M. C. Connor, A. Stevenson, J. D. R. Houghton, D. E. N. Rangel, L. R. Cooke and J. E. Hallsworth

There is a pressing need to understand and optimize biological control so as to avoid over-reliance on the synthetic chemical pesticides that can damage environmental and human health. This study focused on interactions between a novel biocontrol strain, *Bacillus* sp. JC12GB43, and potato-pathogenic *Phytophthora* and *Fusarium* species. In assays carried out *in vitro* and on the potato tuber, the bacterium was capable of near-complete inhibition of pathogens. This *Bacillus* was sufficiently xerotolerant (water activity limit for growth = 0.928) to out-perform *Phytophthora infestans* (~0.960) and challenge *Fusarium coeruleum* (~0.847) and *Fusarium sambucinum* (~0.860) towards the lower limits of their growth windows. Under some conditions, however, strain JC12GB43 stimulated proliferation of the pathogens: for instance, *Fusarium coeruleum* growth rate was increased under chaotropic conditions *in vitro* (132 mM urea) by > 100% and on tubers (2 M glycerol) by up to 570%. Culture-based assays involving macromolecule-stabilizing (kosmotropic) compatible solutes provided proof-of-principle that the *Bacillus* may provide kosmotropic metabolites to the plant pathogen under conditions that destabilize macromolecular systems of the fungal cell. Whilst unprecedented, this finding is consistent with earlier reports that fungi can utilize metabolites derived from bacterial cells. Unless the antimicrobial activities of candidate biocontrol strains are assayed over a full range of field-relevant parameters, biocontrol agents may promote plant pathogen infections and thereby reduce crop yields. These findings indicate that biocontrol activity, therefore, ought to be regarded as a mode-of-behaviour (dependent on prevailing conditions) rather than an inherent property of a bacterial strain.

http://onlinelibrary.wiley.com/doi/10.1111/1751-7915.12349/full

## Environmental Microbiology

**www.env-micro.com**

### Analysis of dsDNA and RNA viromes in methanogenic digesters reveals novel viral genetic diversity

M. Calusinska, M. Marynowska, X. Goux, E. Lentzen and P. Delfosse

Although viruses are not the key players of the anaerobic digestion process, they may affect the dynamics of bacterial and archaeal populations involved in biogas production. Until now, viruses have received very little attention in this specific habitat; therefore, as a first step towards their characterization, we optimized a virus filtration protocol from anaerobic sludge. Afterwards, to assess dsDNA and RNA viral diversity in sludge samples from nine different reactors fed either with waste water, agricultural residues or solid municipal waste plus agro-food residues, we


performed metagenomic analyses. As a result we showed that, while the dsDNA viromes (21 assigned families in total) were dominated by dsDNA phages of the order *Caudovirales*, RNA viruses (14 assigned families in total) were less diverse and were, for the main part, plant-infecting viruses. Interestingly, less than 2% of annotated contigs were assigned as putative human and animal pathogens. Our study greatly extends the existing view of viral genetic diversity in methanogenic reactors and shows that these viral assemblages are distinct not only among the reactor types but also from nearly 30 other environments already studied, including the human gut, fermented food, deep sea sediments and other aquatic habitats.

http://onlinelibrary.wiley.com/doi/10.1111/1462-2920.13127/full

### Functional metagenomic selection of ribulose 1,5-bisphosphate carboxylase/oxygenase from uncultivated bacteria

V. A. Varaljay, S. Satagopan, J. A. North, B. Witte, M. N. Dourado, K. Anantharaman, M. A. Arbing, S. H. McCann, R. S. Oremland, J. F. Banfield, K. C. Wrighton and F. R. Tabita

Ribulose 1,5-bisphosphate carboxylase/oxygenase (RubisCO) is a critical yet severely inefficient enzyme that catalyses the fixation of virtually all of the carbon found on Earth. Here, we report a functional metagenomic selection that recovers physiologically active RubisCO molecules directly from uncultivated and largely unknown members of natural microbial communities. Selection is based on $CO_2$-dependent growth in a host strain capable of expressing environmental DNA, precluding the need for pure cultures or screening of recombinant clones for enzymatic activity. Seventeen functional RubisCO-encoded sequences were selected using DNA extracted from soil and river autotrophic enrichments, a photosynthetic biofilm and a subsurface groundwater aquifer. Notably, three related form II RubisCOs were recovered which share high sequence similarity with metagenomic scaffolds from uncultivated members of the *Gallionellaceae* family. One of the *Gallionellaceae* RubisCOs was purified and shown to possess $CO_2/O_2$ specificity typical of form II enzymes. X-ray crystallography determined that this enzyme is a hexamer, only the second form II multimer ever solved and the first RubisCO structure obtained from an uncultivated bacterium. Functional metagenomic selection leverages natural biological diversity and billions of years of evolution inherent in environmental communities, providing a new window into the discovery of $CO_2$-fixing enzymes not previously characterized.

http://onlinelibrary.wiley.com/doi/10.1111/1462-2920.13138/full

**Melissa McCulloch**
*Wiley-Blackwell*

## Environmental Microbiology Reports

**www.env-micro-reports.com**

### Virus and calcium: an unexpected tandem to optimize insecticide efficacy


V. Apaire-Marchais, M. Ogliastro, F. Chandre, C. Pennetier, V. Raymond and B. Lapied

The effective control of insect pests is based on the rational use of the most efficient and safe insecticide treatments. To increase the effects of classical insecticides and to avoid the ability of certain pest insects to develop resistance, it is essential to propose novel strategies. Previous studies have shown that calcium-dependent phosphorylation/dephosphorylation is now considered as a new cellular mechanism for increasing the target sensitivity to insecticides. Because it is known that virus entry is correlated with intracellular calcium concentration rise, this report attempts to present the most important data relevant to the feasibility of combining an insect virus such as baculovirus or densovirus with an insecticide. In this case, the insect virus is not used as a bioinsecticide but acts as a synergistic agent able to trigger calcium rise and to activate calcium-dependent intracellular signalling pathways involved in the increase of the membrane receptors' and/or ion channels' sensitivity to insecticides. This virus–insecticide mixture represents a promising alternative to optimize the efficacy of insecticides against insect pests while reducing the doses.

http://onlinelibrary.wiley.com/doi/10.1111/1758-2229.12377/full

### Partitioning of fungal assemblages across different marine habitats

T. C. Jeffries, N. J. Curlevski, M. V. Brown, D. P. Harrison, M. A. Doblin, K. Petrou, P. J. Ralph and J. R. Seymour

Fungi are a highly diverse group of microbes that fundamentally influence the biogeochemistry of the biosphere, but we currently know little about the diversity and distribution of fungi in aquatic habitats. Here we describe shifts in marine fungal community composition across different marine habitats, using targeted pyrosequencing of the fungal Internal Transcribed Spacer (ITS) region. Our results demonstrate strong partitioning of fungal community composition between estuarine, coastal and oceanic samples, with each habitat hosting discrete communities that are controlled by patterns in salinity, temperature, oxygen and nutrients. Whereas estuarine habitats comprised a significant proportion of fungal groups often found in terrestrial habitats, the open ocean sites were dominated by previously unidentified groups. The patterns observed here indicate that fungi are potentially a significant, although largely overlooked, feature of the ocean's microbiota, but greater efforts to characterize marine species are required before the full ecological and biogeochemical importance of marine fungi can be ascertained.

http://onlinelibrary.wiley.com/doi/10.1111/1758-2229.12373/full

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**Full Ordinary** gives access to our many grants and awards, online access to the *Journal of Applied Microbiology*, *Letters in Applied Microbiology*, *Environmental Microbiology*, *Environmental Microbiology Reports* and *Microbial Biotechnology*, copies of *Microbiologist*, preferential registration rates at Society meetings, and access to the Members-only area of the website.

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You can apply for membership online (**www.sfam.org.uk/join**) or offline. To apply offline, please contact the Membership Officer, Julie Buchanan on **+44 (0)207 685 2596**, or email **julieb@sfam.org.uk**.

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We would like to warmly **welcome** the following new Members to the Society.

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# Corporate NEWS

The latest news, views and microbiological developments from our Corporate Members

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We also offer a wide range of animal disease challenge models for testing the efficacy of experimental vaccines to support market authorisation. They include bacterial, viral and parasitological diseases of many farm animal and poultry species. Our scientists have extensive experience of working on Eimeria species and, using our dedicated poultry accommodation, we offer coccidiosis testing, anticoccidial sensitivity testing, safety, efficacy and reversion to virulence trials and Eimeria oocysts suspensions.

APHA Scientific can also provide Bacterial Identification and Characterisation, Virus Discovery, Pathology and Bio-Imaging ideal for host-pathogen interactions and other additional services including DNA sequencing.

### Further Information

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## Cherwell Laboratories – Microbiological media, Cleanroom monitoring and bio-decontamination expertise

Cherwell Laboratories manufactures microbiological growth media at its ISO 9001:2008 registered site in Bicester, UK. They supply customers from the pharmaceutical, biotechnology, medical device, cosmetic, R&D and food and beverage industries with a range of products which include:

**Redipor® Prepared media** – A comprehensive range of prepared media including Petri dishes, contact plates, gamma irradiated media, injection vials, broth in bags and ampoules: all available in a variety of packaging options and with flexible order quantities. Cherwell also specialise in offering bespoke prepared media solutions to meet customer specific requirements.

**SAS Air Samplers** – A selection of robust, reliable air samplers designed for specific environmental monitoring purposes, including portable hand held units, a compressed air sampling device and an isolator specific unit. Our calibration and repair service is also available, both at the Cherwell factory and as an onsite visit from one of our engineers.

**Cleanroom Bio-decontamination** – Suitable for use in pharmaceutical cleanrooms and other critical areas, the combination of dry fog technology and cold sterilants ensures effective and efficient bio-decontamination for surfaces, confined spaces and cleanroom suites. The range incorporates the highly effective Minncare® Dry Fog 2 and Mini Dry Fog systems plus Minncare and Actril Cold Sterilants.

### Further Information

**Visit: www.cherwell-labs.co.uk**
Tel: +44(0)1869 355500
Email: sales@cherwell-labs.co.uk

## Say Goodbye to Manual Plating with WASP Touch

Customer response to WASP Touch, the new spiral plater from Don Whitley Scientific, has been very positive and the device is selling well. Even though spiral plating was invented over 40 years ago, there are some microbiologists who have yet to experience the advantages of spiral plating over manual methods.

Manual plating requires the repetitive creation of a series of dilutions and plates to obtain one good plate for subsequent reading. All those plates (laboriously produced) then have to be incubated (taking up incubator space). One plate is selected for counting and the rest are discarded.

WASP Touch greatly reduces the need for serial dilutions as one plate replaces three conventional dilutions, saves time, effort and consumables – and speeds up the whole process. WASP Touch incorporates a colour touchscreen, sanitizing station (patent pending) and Automated Intelligent Monitoring Software, making it extremely simple to use.

Reading a spiral plate is very easy as you don't have to count the entire plate. Whitley Spiral Counting Tables and grid will help you to manually count plates quickly and efficiently. However, spiral plates are ideally suited for use with automated colony counters, such as the aCOLyte or ProtoCOL, also available from Don Whitley Scientific.

### Further Information

**Visit: www.dwscientific.co.uk**
Tel: +44(0)1274 595728
Email: sales@dwscientific.co.uk

## Lab M Launches MMGA Pre-Poured Media Plate

The new plate is formulation compliant to ISO 16649-1 and performance compliant to ISO 11133 for the recovery of stressed and injured *E. coli* in food, water and feed samples.

MMGA, Mineral Modified Glutamate Agar, is the resuscitation medium specified by ISO 16649-1 and is used as an additional step prior to enumeration. Processing and environmental factors such as heating, freezing and dehydrating can cause organisms to become stressed and difficult to detect. By using MMGA you can enhance the recovery of stressed or injured organisms.

"*Our MMGA plate has been developed in response to changes in industry preferred testing methods,*" said Melanie Patterson, Lab M's Market Development Manager. "*It is supplied ready-to-use so there is no need to weigh, autoclave or pour any media. Simply place a filter membrane onto the plate, add your sample and incubate for 4 hours, then transfer the membrane straight to our TBGA (TBX) plate for incubation and enumeration. The quality of our products is extremely important to us and by complying with ISO 11133 it also means that there is minimal internal QC required by the customer.*"

Lab M also offers both its MMGA and TBGA (TBX) media in a DCM format. For more information on Lab M's range of media, visit www.labm.com.

### Further Information

**Visit: www.labm.com**
Tel: +44(0)161 820 3833
Email: info@labm.com

## Effective Surveillance in the Food and Pharmaceutical Industries

MWE has launched a new brochure detailing its ranges of leading products for microbiological sampling of surfaces in clean and sterile areas in the food and pharmaceutical industries.

NRS II Transwabs® are pre-wetted swabs with neutralising media including NRS and Letheen Broth. Polywipes™ are blue sponge swabs pre-wetted with a similar range of media, and suitable for larger surfaces such as benches and conveyor belts. Both NRS II Transwabs[1] and Polywipes can

be used in ISO 18583 based programmes. SteriKit™ and Steriswab™ are also premoistened swab systems designed for sampling sterile areas in pharmaceutical manufacture.

Isolation Transwabs® provide a self-contained "warning bell" method for the early detection of particular pathogens such as *Salmonella* and *Listeria*.

### Further Information

**Visit: www.mwe.co.uk**
Tel: +44(0)1225 810361
Email: sales@mwe.co.uk

## NCIMB project studies application of bacteriophages to oilfield biofilm control

NCIMB is undertaking a research project to evaluate the potential of bacteriophages as a means of controlling biofilms in oilfield systems. Microbial biofilms can cause significant economic and environmental issues in oilfield systems, including souring of oil and contribution to corrosion of concrete and metal surfaces. While chemical biocides can be effective, poor diffusion of chemical into the biofilm can limit success in preventing biofilm regrowth.

NCIMB's identification services manager Vikki Mitchell, explains more about what is involved in the work, which has been funded by Innovate UK: "*The project aims to isolate and identify bacteriophages that are selective to biofilm-causing organisms in North Sea reservoirs. High throughput sequencing is being used to study microbial populations in reservoir samples and provide information on the relative abundance of genera of interest.*

"*The efficacy of isolated bacteriophages will then be tested against pure and mixed-culture microbial enrichments from the same samples before testing of the bacteriophage against static biofilms*".

Potential benefits of the project could include more effective biofilm control and reduced environmental impact through a reduction in the use of chemical biocides.

NCIMB curates the UK's National Collection of Industrial Food and Marine Bacteria and provides ID services to a diverse range of industry sectors.

### Further Information

**Visit: www.ncimb.com**
Tel: +44(0)1224 711100
Email: enquiries@ncimb.com

## Protos 3 Colony Counter and Rapid ID System from Synbiosis

## Saves Microbiologists Time and Provides Accurate, Traceable Results

Synbiosis has introduced a vibrant new automated colony counting and chromogenic identification system, Protos 3. This system allows walk-away colony counts, as well as accurate identification of colonies cultured on chromogenic plates.

What makes the new Protos 3 outperform other commercial colony counters is the system's ability to count colonies in seconds and identify microbial species by their colour on chromogenic plates. This is a great time saver, providing accurate, objective and fully traceable GLP compliant results.

The stylish, yet practical Protos 3, which comes in bright red, attaches easily to a computer and requires minimal training to set up. Users simply input their plate identification and click. The Protos 3, featuring a highly sensitive CCD camera combined with unique three colour LED lighting, rapidly images an infinite number of colony colours on one plate and detects colonies as small as 0.043mm.

The Protos 3's powerful software then generates true to life counts and plate images, which can be transferred and stored in Excel. This GLP compliant process, with its full audit trail eliminates keying and image transfer errors providing accurate, objective data, which can be reviewed anywhere and anytime.

### Further Information

**Visit: www.synbiosis.com/protos-3/**
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## New Pre-Weigh Dehydrated Culture Media Format Enables More Streamlined Workflow for Microbiology Laboratories

To help microbiology laboratories that are under growing pressure from higher test volumes and demands for faster time to results, a new dehydrated culture media format provides a convenient solution to media preparation.

Thermo Scientific™ Pre-Weigh Dehydrated Culture Media are supplied in 'rip and tip' pouches, available in both 1L and 8L sizes to fit microbiology laboratories' culture media batch sizes. A choice of media in the new format offers reduced hands-on time and increased accuracy to help maximize laboratory productivity. The convenient pouches contain the same high quality media as standard Thermo Scientific Dehydrated Culture Media packs. In-house trials have demonstrated that using the new pre-weigh format can deliver a saving of over two hours every week when preparing 45L of media each day.

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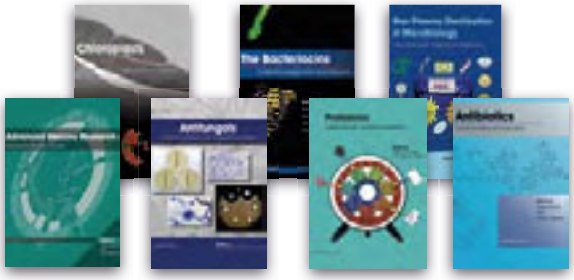

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