microbiologist

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The magazine of the
Society for Applied Microbiology

INSIDE

Dental microbiology

Oral microbiology

Escape from the biofilm

Nanotechnology and the control of oral infections

Dental plaque

INTRODUCING

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Nancy Mendoza reviews the content of this issue

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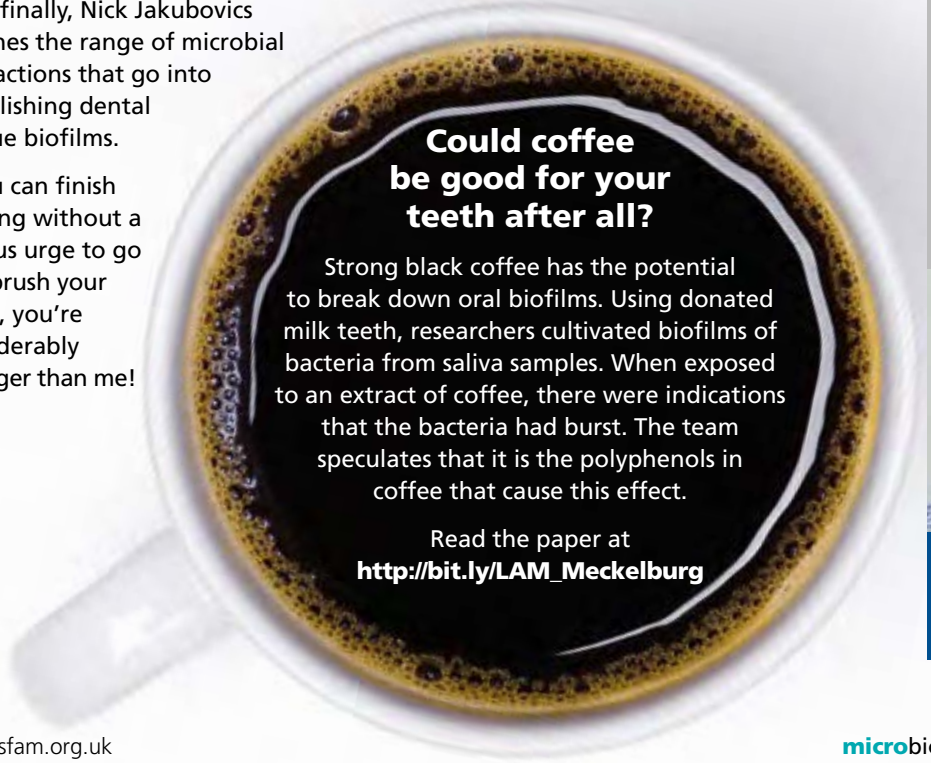
Help us to make sure *Microbiologist* delivers what you want

We very much hope that you enjoy this second edition of the new-look *Microbiologist*. Thanks to those of you who have been in touch with your comments already. We'd like to know more about what works well about the magazine and anything that doesn't work so well. Are there sections that you particularly look forward to? If we were to leave something out, what could you live without? Do you have any suggestions for new elements? Please take a few minutes to give us your feedback at **https://www.surveymonkey.com/s/SfAM_Microbiologist**

Dental microbiology

This edition focuses on dental microbiology which, when you consider that the human oral cavity is home to approximately 700 species of prokaryotes, is an unsurprisingly rich and varied area of study. In our first feature, Adam Roberts and Morgana Vianna of UCL's Eastman Dental Institute gives a fascinating overview of dental microbiology. Then, University of Manchester's Riina Rautemaa-Richardson describes some of the clinical problems that can occur when biofilms – a common factor of both dental caries and periodontal disease – are disrupted during dental procedures, resulting in the dissemination of infection. Robert Allaker and Kaveh Memarzadeh, who works on host-microbial interactions and antimicrobials at Queen Mary University of London, discusses the latest innovations in using nanotechnology approaches to disrupt oral biofilms. And, finally, Nick Jakubovics outlines the range of microbial interactions that go into establishing dental plaque biofilms.

If you can finish reading without a serious urge to go and brush your teeth, you're considerably stronger than me!



Could coffee be good for your teeth after all?

Strong black coffee has the potential to break down oral biofilms. Using donated milk teeth, researchers cultivated biofilms of bacteria from saliva samples. When exposed to an extract of coffee, there were indications that the bacteria had burst. The team speculates that it is the polyphenols in coffee that cause this effect.

Read the paper at **http://bit.ly/LAM_Meckelburg**

NEWS IN BRIEF

Antibiotic resistance

Only 6% of patients admit pushing GPs for antibiotics but they still over prescribe – Longitude Prize, **http://bit.ly/SFAM_PRESCRIBING**

Ebola vaccine by 2015

WHO says Ebola vaccine could be ready by the end of the year – too late? **http://bit.ly/SFAM_EBOLA**

Food poisoning research – 3Rs

Wax moth larvae have proven a successful model to quantify *Campylobacter* virulence. **http://bit.ly/SFAM_3RS**

Nancy Mendoza, *Editor*

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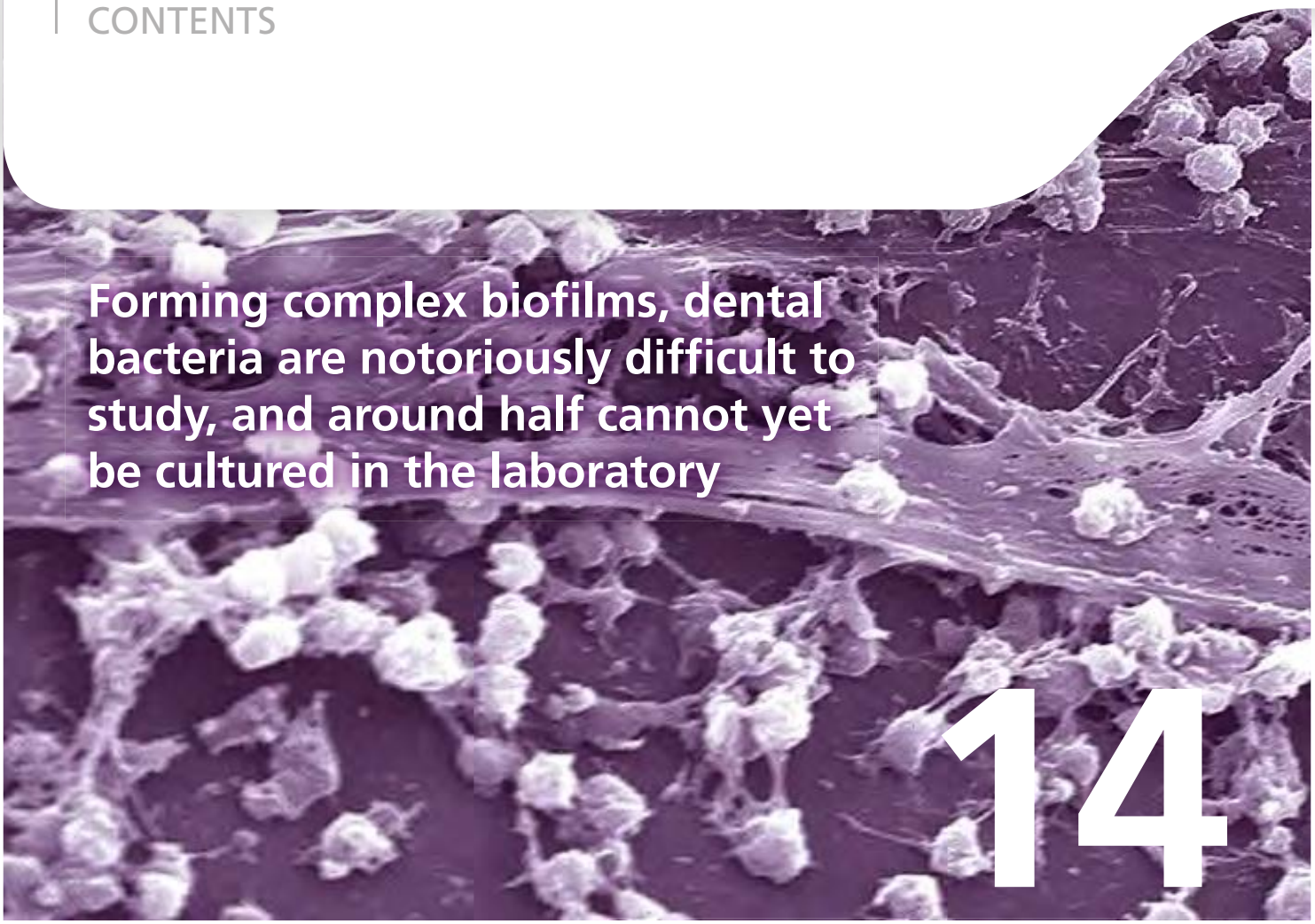

Forming complex biofilms, dental bacteria are notoriously difficult to study, and around half cannot yet be cultured in the laboratory

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An interview with *Sf*AM's new President

Nancy Mendoza interviews SfAM's incoming President ***Professor Christine Dodd*** *about her interest in microbiology and her plans for the Society during her presidency.*

Q. Did you always know you would pursue a career in microbiology? What first got you interested in the science of microorganisms?

A. I actually started off doing BSc Biological Sciences at Leicester University but changed to microbiology for my PhD when I went hard core and became a numerical taxonomist working on the Enterobacteriaceae and plasmids, and their effect on bacterial taxonomy. Microbial identification is still at the heart of a lot of what I do, both in research and teaching, and being given a sample to isolate and identify an organism from and ask *"What is it doing there?"* is still enormous fun – you are exploring the unknown!

Q. As President, you will be leading and representing the Society, but when, and how, did you first get involved with SfAM?

A. This is like asking a lady her age!! My first publication is from a Society meeting in 1978 when I presented a poster on ***A numerical taxonomic study of the genus Shigella*** (in those days all the abstracts were published), but it was when I moved to the University of Nottingham in 1985 and became a postdoc with Will Waites that I really got involved and regularly presented at meetings. I also helped with running the summer meeting at Nottingham in 1987 – this was when there were local organizers for meetings; I still know some Society Members from those days. I first became a Committee Member in 1990.

Q. Tell us a bit about your career to date – has being a Member of SfAM helped you, personally, in the development of your career?

A. I became a lecturer in Nottingham in 1989 and then it was just a typical university career with promotion to Senior Lecturer, Reader and then the Personal Chair in 2007. After that I have held management roles: Director of Learning and Teaching and most recently Head of Food Sciences. Of course all of that depends on building a research profile and reputation, and the Society conferences and its journals and other publications have been a significant contributor to that.

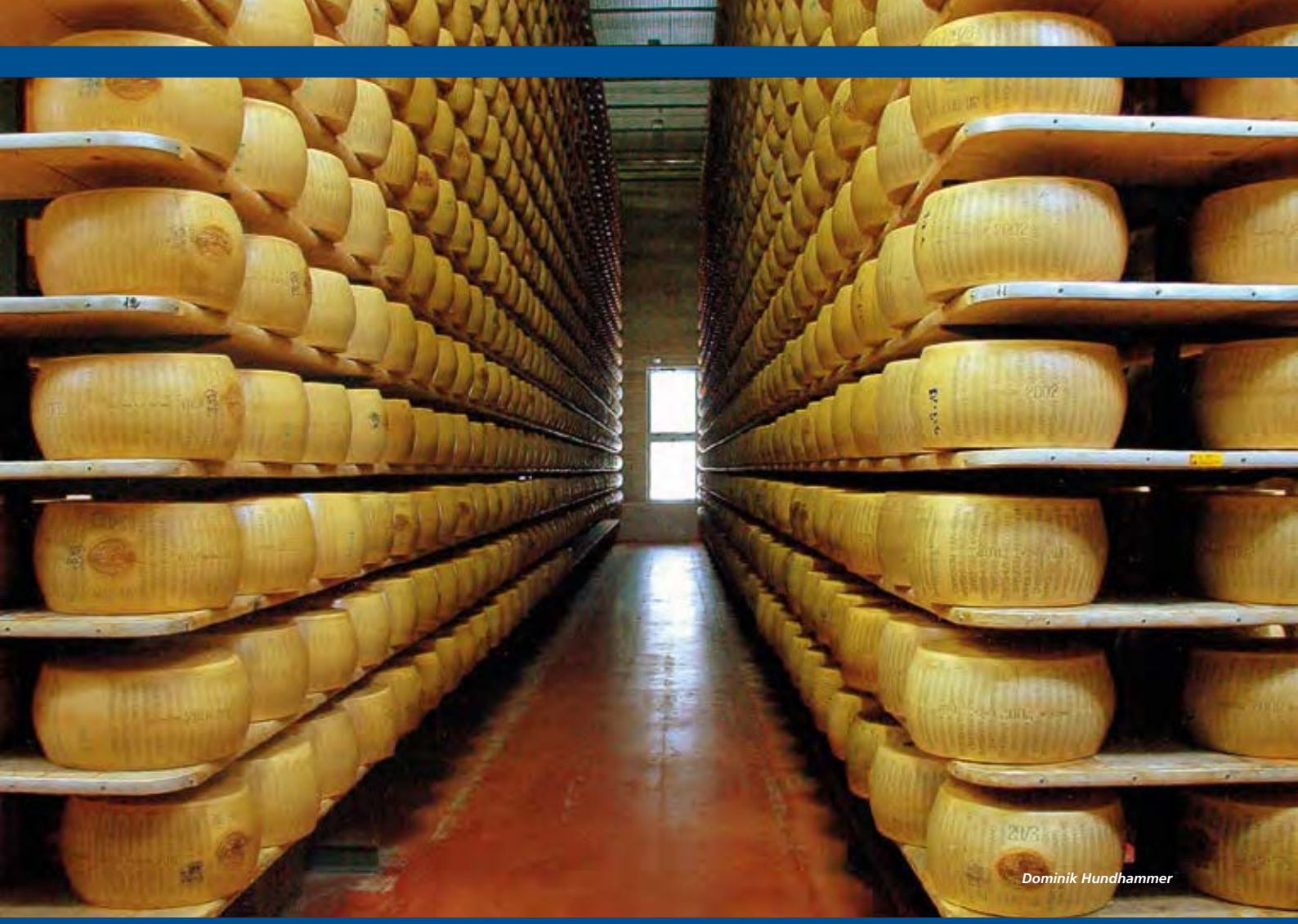
I know we hear a lot about the challenges faced by women in scientific careers, and I have had those moments when you suddenly realize you are the only woman in the room, but really I have experienced very few incidences which you might term discrimination. I think SfAM has always been good at involving and celebrating the achievement of women microbiologists, at all levels. In my early career, I was the first woman to win SfAM's W H Pierce Prize and there is a long history of women Presidents of the Society including my PhD supervisor, Dorothy Jones. Actually, looking back I have always worked somewhere with an SfAM President – Dorothy, Max Sussman and then Will Waites.

Q. What are you working on at the moment?

A. I always have a number of areas I'm involved in, but the really surprising thing was to find myself over the last few years back working on the Enterobacteriaceae and plasmids and the selective pressures influencing strain evolution. As I originally started out looking at R [antimicrobial resistance] plasmids in *Shigella* and their influence on its taxonomy I feel like I have come full circle. My other key area of research is microbial fermentation of foods, particularly cheese, so that goes back to the roots of SfAM (Ed: SfAM was started in 1931 by a group of dairy bacteriologists). This is an area which has shown great activity from the new analysis methods like metagenomics and metabolomics, something our Summer Conference will be covering next year.

Q. Is there a quote, saying, or piece of advice that you keep coming back to, professionally or personally? Where did you first find it?

A. I did come up with a number of these for my inaugural lecture to reflect my attitude to research but then people keep quoting them back at me so I have stopped using them! I guess what they all did reflect was having an open minded and positive attitude to doing new things. It can lead you to find yourself in a "*Why did I say I'd do this?*" situation but all these things build experience and you gain from it.



Dominik Hundhammer

Being given a sample to isolate and identify an organism from and ask *"What is it doing there?"* is still enormous fun – you are exploring the unknown!

Q. As incoming President, and a long-standing Member of the Society and its Committees, what would you say are SfAM's top three 'unique selling points'?

A. Everyone says it but it is still true – we are the friendly Society. That comes across as a very distinctive, lively and energetic atmosphere at our conferences and I think is the thing that makes people come back year after year. The second thing is our 'Applied' tag; the microbiology we cover has real relevance to everyday lives. Finally, I think we have always encouraged active engagement of our younger Members – and if you do that right they become long-term Members, like me.

Christine Dodd
President of the Society

Q. And finally, do you have any particular aims or goals for the Society during your presidency?

A. I'm not the sort of person who sets out to make a mark by imposing change for change's sake. The Society is in a very good position at the moment so I will be building on its current strengths. In terms of membership numbers, we have passed the 2,500 mark and have 3,000 in our sights. We'll be reviewing our recruitment and retention strategy to see if we could even exceed that number within the next few years, and we intend to include plans to develop a greater international membership, too. I'd also like to find more ways to involve our Members directly in our activities because that is the way we make the Society matter to them.

CEO's column

This is my first column since I attended the American Society for Microbiology meeting in Boston; with an S*f*AM team, I attended the trade exhibition. It was a great pleasure to meet so many existing Members who visited our stand and expressed their appreciation for the work undertaken by the Society. The meeting was highly successful and over 90 new Members registered. Thank you to all the new and existing Members who took time out to speak with us.

I am writing this a few days after the Society's Summer Conference in Brighton. The meeting proved to be very popular and the capacity for the conference rooms was reached several weeks before the meeting began. If you were one of the Members who tried to book late and could not, I do apologize. I suggest for next year's event (Dublin, June 29 – July 2 2015) delegates book as early as possible as once again I anticipate a very good attendance – we are returning to a Food Microbiology theme (Fermented Foods) as the sole topic for the meeting.

The 2014 Summer Conference proved very successful and it was a pleasure working with our joint organizers from the Med-Vet-Net Association.

A number of delegates received grants from the Society to attend the Summer Conference; if you would like to attend the meeting in 2015 why not consider applying for a grant to help with the cost? There are three relevant grants: Full Student Members can apply for a **Conference Studentship**; Full Ordinary Members with an accepted abstract can apply for a **President's Fund Grant**, which is up to £1200 (or local currency equivalent); or a Full Student or Ordinary Member can apply for a **Scientific Meeting Attendance Grant**. Full details of all grants can be found by visiting **www.sfam.org.uk/grants**.

The Trustee's Annual Report for year ending 31 December 2013 was approved at a meeting of the Trustees on the 30 June 2014 and accepted by the Members at the Society's Annual General Meeting on 02 July 2014 at The Grand Hotel, Brighton. Any Member can view the electronic version of the report by visiting the Members' area of the website (**www.sfam.org.uk/membersonly**). If an individual Member wishes to receive a hard copy please contact Julie Buchanan (**julieb@sfam.org.uk**) who will gladly send you one by post.

Some financial highlights of the Annual Report include the Society's overall assets exceeding £7 million for the first time. This compares with a value of just £6.2 million at the end of 2012. The increase is particularly impressive when you also see that we awarded a total of over £267,000 in grants to 169 individuals, compared with just over £197,000 and 153 respectively in 2012. Financial security ensures our ability to deliver the services you as Members expect. It also enables the Executive Committee to plan with confidence further development of new services in the future.

I attended the American Society for Microbiology meeting in Boston and over 90 new Members registered

Phil Wheat
S*f*AM *Chief Executive Officer*

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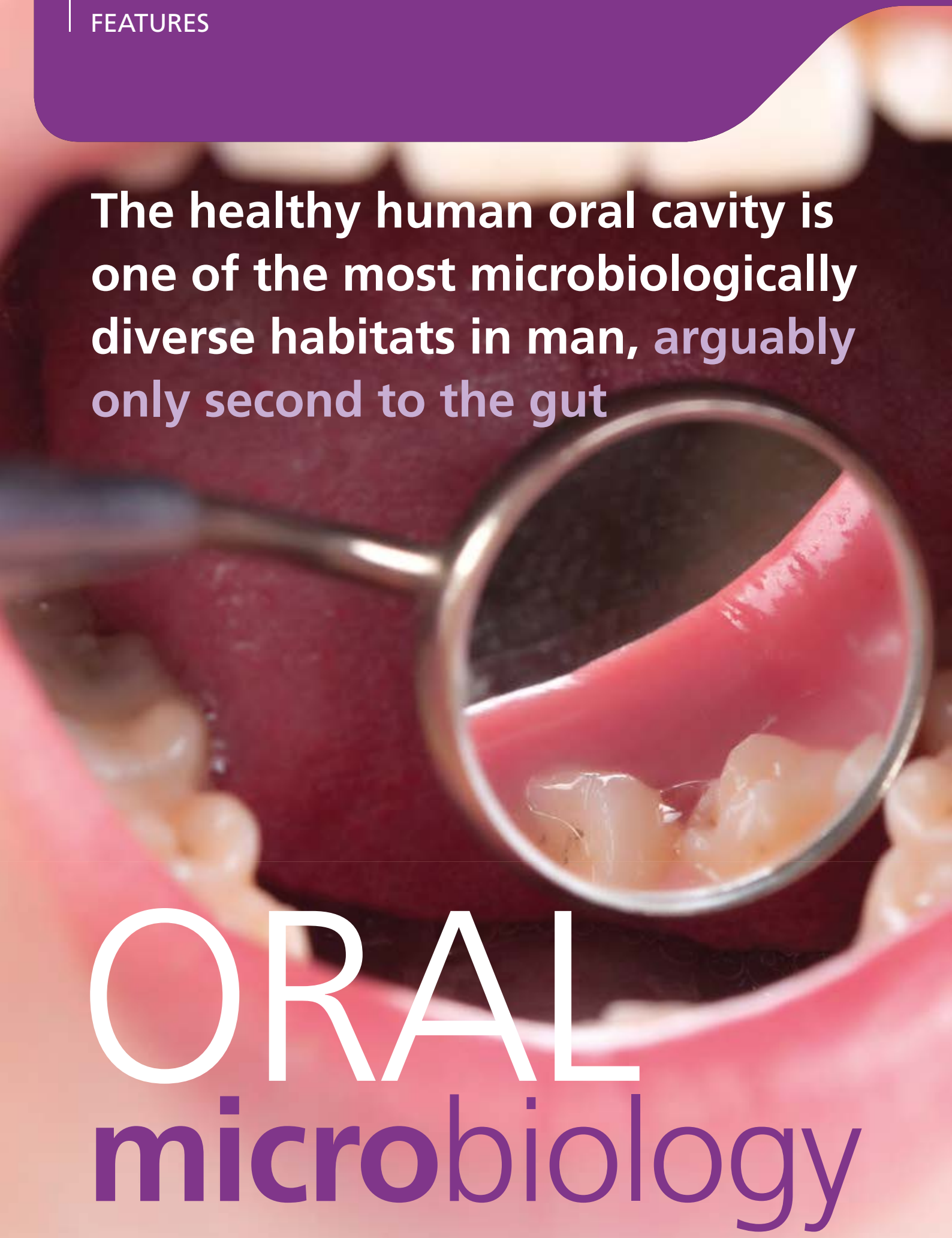

The healthy human oral cavity is one of the most microbiologically diverse habitats in man, arguably only second to the gut

ORAL microbiology

Introduction

The healthy human oral cavity is one of the most microbiologically diverse habitats in man, arguably only second to the gut in terms of microbial cell numbers and species diversity. It is recognized that the oral microbiota (oral microbiome) includes representatives from all three domains of life; bacteria, archaea and eukarya, however, *it is the* bacteria which are by far the most numerous colonizers, in both health and disease.

The oral cavity represents a unique, dynamic and challenging environment for microbial inhabitants, and contains multiple distinct ecological niches, ranging from the unique, non-shedding surfaces of the tooth to the various mucosal surfaces on the gums, tongue, cheek and palate. The oral environment is one of the most variable human sites readily colonized by microbes in terms of physicochemical characteristics such as nutrient availability, temperature changes, oxygen potential, shear forces, from the action of the tongue, chewing and eating, and the challenges and opportunities which arise with the constant influx of transient organisms on food.

Bacterial complexity

The numbers of bacterial species found in the human oral cavity is currently the subject of debate. With the advent of next generation sequencing and the ever-reducing costs of such experimentation, this figure is being constantly revised. Currently, approximately 700 bacterial species have been identified from the oral cavity and these have been curated in the Human Oral Microbiome Database (HOMD) available at: **www.homd.org**. Other studies have put this figure considerably higher, however, it must be noted that with next generation sequencing data there is a certain amount of variability in the interpretation of data depending on the various cut-off values used when deciphering the raw data. What is inescapable is that approximately only 50% of oral bacteria present can be currently cultivated in the laboratory.

Oral biofilms

Many, if not most, species of bacteria in this environment live as part of a multi-species biofilm, commonly known as dental plaque. Biofilm formation on the surface of the tooth begins as soon as the newly exposed surface is covered in the acquired pellicle. The pellicle contains many host proteins and is recognized by the "early colonizers" which bind to the pellicle-covered tooth surface. The early colonizers include, but are not limited to, *Streptococcus* spp., *Actinomyces* spp., *Capnocytophaga* spp., *Eikenella* spp., *Haemophilus* spp., *Prevotella* spp., *Propionibacterium* spp. and *Veillonella* spp. These pioneer microorganisms utilize oxygen and condition the environment, allowing colonization by other bacterial species. In subsequent phases, during maturation of the oral biofilm, it is often colonized by *Fusobacterium nucleatum* which is considered a "bridge" species between the early and late colonizers as it is able to aggregate to members of both groups as well as host molecules within the pellicle. The late colonizers include *Actinobacillus spp*., *Prevotella* spp., *Eubacterium* spp., *Porphorymonas* spp. and *Treponema* spp.

Biofilm resistance

Growth in a biofilm will lead to phenotypic changes in many of the inhabitants including the production of an extracellular polymeric substance (EPS) which is composed of carbohydrates, proteins and nucleic acids, and will often make up the majority of the biomass in a mature biofilm. The EPS affords the inhabitants protection against the external influences and stresses described above. Of clinical relevance is the intrinsic resistance to antimicrobial agents such as antibiotics exhibited by biofilm-growing cells compared to their planktonic, laboratory cultured, counterparts. MIC values have been recorded as being up to 1,000 times more for biofilm-growing cells. One explanation for this observation is the increased biomass of the EPS in mature biofilms which prevent antimicrobials, and other molecules, reaching cells deep within the biofilm. A similar effect may also be observed with bacterially derived excreted resistance molecules (such a ß-lactamases) which may form locally high concentrations due to impeded diffusion through the biofilm EPS. Biofilms exhibit gradients in nutrient and oxygen concentrations which will result in differing metabolic activities within cells occupying different regions within the biofilm.

Subsequently, this will lead to differing susceptibilities and transient resistances to agents which target metabolic processes such as protein synthesis. Finally, many species of bacteria exhibit differential gene expression when growing as part of a biofilm, which can lead to differences in, e.g., membrane transport systems resulting in a phenotypic decrease in antimicrobial sensitivity.

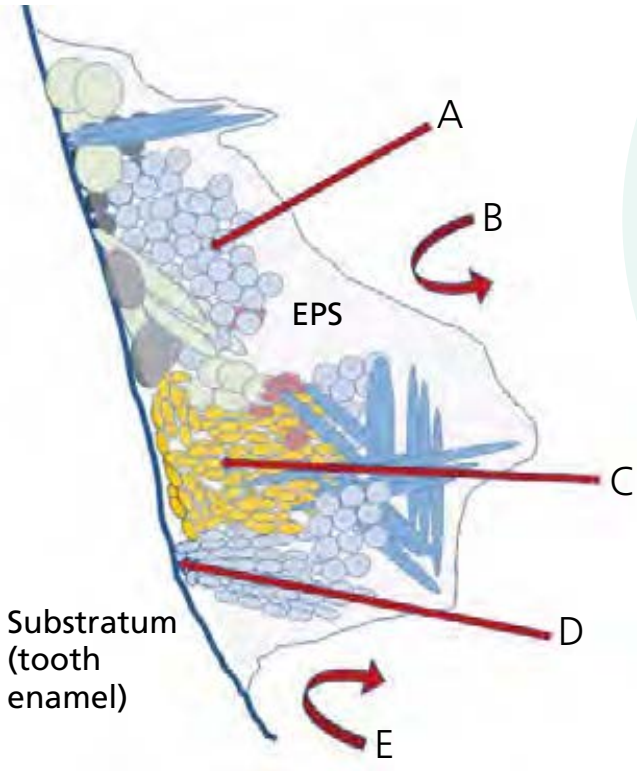

Figure 1. Dental plaque protective mechanisms. *Cartoon of a mixed species biofilm on the surface of a tooth. The coloured ovoids represent bacteria of different species. The shaded area around the bacterial cells represents the excreted EPS.* A: *cells within the biofilm may be in different metabolic states and therefore transient resistance may occur to antimicrobials which target metabolic processes such as protein synthesis.* B: *the EPS can exclude antibiotics leading to higher MICs.* C: *due to the EPS, bacterially derived molecules able to counteract antibiotics may form locally high concentrations within the biofilm.* D: *attachment may lead to transcriptional changes within the cell.* E: *the EPS will protect biofilm cells against physical forces such as the flow of saliva*

Biofilm interactions

Growth in a multi-species biofilm leads to some very specific beneficial interactions between some of the microbial inhabitants. Various syntrophic relationships have evolved, e.g., *Streptococcus oralis* and *Streptococcus sanguis* reach higher cell densities when grown together in a mucin-based chemostat compared with individual cell densities reached when growing as a single species, suggesting that there is complimentary metabolic activity allowing more efficient utilization of mucin. *Fuso. nucleatum* has been shown to aggregate with the anaerobic *Porphorymonas gingivalis* and *Prevotella nigrescens* which allow them to survive in oxygenated environments.

There are also more intricate relationships within oral biofilms. The biofilm environment is an environment which seems to be conducive to gene transfer leading to increased adaptability of oral bacteria. There are many experimental examples demonstrating horizontal gene transfer within model oral biofilms and circumstantial evidence from the genome sequence of oral bacteria indicates that this process is extremely pervasive in this environment. Indeed recent studies have confirmed that the normal oral flora is a reservoir for transferable antibiotic resistance and many other traits are also likely to be shared amongst the community.

Oral diseases

The bacteria within the oral cavity are responsible for a range of diseases including the most common diseases in the world, dental caries, apical periodontitis and periodontal disease, which are estimated to affect up to 90% of the global population. These dental diseases are interesting from a causative aspect as, unlike many other diseases which fit Koch's postulates, dental diseases are often polymicrobial in nature with no single causative species identified.

There are multiple theories regarding the aetiology of dental caries; all of which are concerned with monosaccharide and disaccharide metabolism. The most widely accepted is that a few acidogenic species, such as *Streptococcus mutans* and *Streptococcus sobrinus*, are actively involved in the disease. They lower the pH during carbohydrate metabolism leading to an

The bacteria within the oral cavity are responsible for a range of diseases including the most common diseases in the world, dental caries, apical periodontitis and periodontal disease, which are estimated to affect up to 90% of the global population

The **overall contribution that the oral microbiome makes to human health and disease is beginning to emerge**

imbalance of mineralization/de-mineralization in the enamel which results in lesions. This gives microbes and their metabolic by-products access to the underlying dentine. At this stage caries are irreversible.

If carious lesions are left untreated the microbes will invade the dental pulp causing necrosis and apical periodontitis will likely result. The bacteria isolated from these primary infections include *Actinomyces* spp., *Bifidobacterium* spp., *Eubacterium* spp., *Lactobacillus* spp., *Rothia* spp., *Streptococcus* spp. and *Prevotella* spp.

Periodontitis is a subsequent infection of the soft tissue anchoring the tooth. This is also a polymicrobial disease but the associated species are shifted towards anaerobic, protein-metabolizing bacteria such as *Tannerella forsythia*, *Porphyromonas gingivalis*, *Trepanema denticola* and *Aggregibacter actinomycetemcomitans* associated with the majority, but not all cases of the disease.

Concluding comments

The overall contribution that the oral microbiome makes to human health and disease is beginning to emerge. In addition to the ecological imbalances responsible for many oral diseases, e.g., caries and periodontitis described above, oral microbes have also been shown to be involved with disease at other body sites such as the heart, indicating that the oral cavity may act as a reservoir for bacteria with potential to metastasize to other regions of the body and cause disease. As a more in-depth understanding of the dynamic human oral microbiome emerges we will gain insight into its contribution to a range of diseases and will hopefully be in a position to ecologically manage it to a point which promotes health.

FURTHER READING

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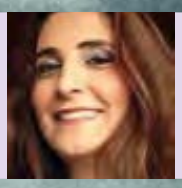

Adam P. Roberts left
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Morgana E. Vianna right
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DISSEMINATED ORAL INFECTIONS escape from the biofilm

One special feature sets the oral cavity apart from other body sites: the presence of natural non-shedding surfaces, i.e., tooth surfaces. These allow the formation of stable biofilms with substantial microbial diversity, dental plaque. In fact, a biofilm is the preferred lifestyle for most oral microbes. Therefore, a unique and diverse community of microbes resides in the oral cavity. Some 700 bacterial species have been isolated from the oral cavity by culture and over 19,000 species-level phylotypes by pyrosequencing. The majority of the sequences belong to seven phyla: Actinobacteria, Bacteroides, Firmicutes, Fusobacteria, Proteobacteria, Spirochetes and candidate division TM7. Of these, Actinobacteria, Fusobacteria and Spirochetes are dominant in dental plaque, while Bacteroides, Firmicutes and Proteobacteria are more abundant in saliva. The biofilm lifestyle makes the oral microbiota quite stable and it normally consists of microorganisms of low virulence. Hospitalized patients, however, are more frequently colonized with atypical pathogens, such as *Pseudomonas aeruginosa*. Thus, the oral cavity is a potential source of a wide range of microorganisms.

The prevalence of bacteraemia after dental procedures has ranged from 58% to 100%

Disseminated odontogenic infections

Teeth, and the periodontal structures supporting them, are the main source for spreading oral infections. Risk factors for disseminated odontogenic infections follow general risk factors for infection and include; various causes of immune suppression and deficiency, trauma and the presence of virulent strains. A range of oral microorganisms have been identified from blood cultures of septicaemic patients: *Streptococcus*, *Enterococcus* and *Staphylococcus* species, *Klebsiella pneumoniae*, and *Actinomyces*, *Bacteroides*, *Prevotella*, *Eubacterium* and *Clostridium* species. Odontogenic infections are often polymicrobial reflecting dispersal from multi-species biofilms.

Odontogenic sepsis can occur spontaneously or it can be a result of an invasive dental procedure. Practically all dental procedures can cause bacteraemia. The prevalence of bacteraemia after dental procedures has ranged from 58% to 100%. There is, however, variation in the incidence and intensity of bacteraemia depending on the invasiveness of the procedure. Thus, tooth extractions seem to result in the highest incidence of bacteraemia, followed by periodontal and orthodontic care. Lucas *et al*. showed that significant bacteraemia is detected after professional cleaning, but not after using a manual or electric toothbrush.

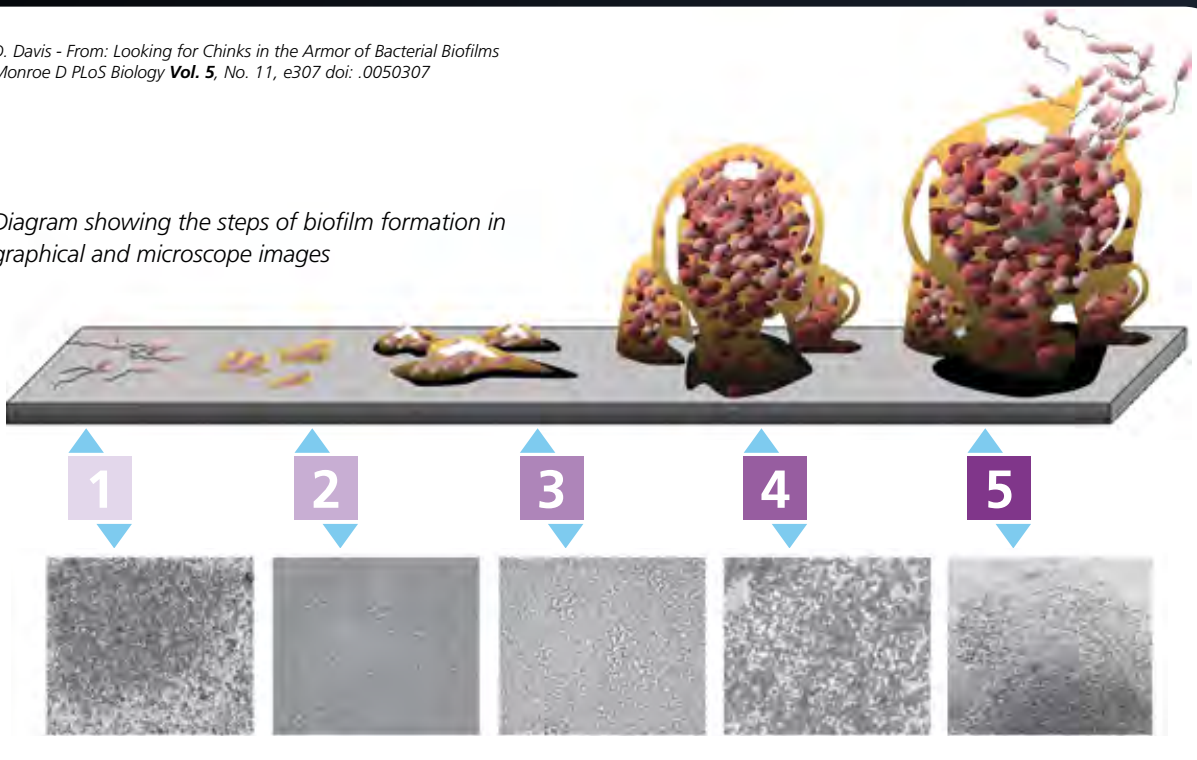
Chronic asymptomatic but potentially invasive oral infections are common both in patients who visit and do not visit the dentist regularly. The presence of such an infection, particularly gingivitis and periodontitis, is a risk factor for an unprovoked entry of oral microorganisms into the blood stream. In a recent study, DNA of bacteria typically causing root canal infections was detected in 78% of thrombus aspirates of non-selected patients with myocardial infarction.

Some 700 bacterial species have been isolated from the oral cavity

However, it appears that it is impossible to predict the intensity of bacteraemia following tooth extractions or periodontal procedures based on the severity of periodontal disease. Also, chewing and flossing have been shown not to provoke significant bacteraemia in immunocompetent patients with periodontal diseases. The immunological status of the patient appears to be more important than the infection status of the oral cavity or the insult to the tissues. As the immune system has an important role in abscess formation immunocompromised patients are more susceptible to systemic rather than local infection complications.

D. Davis - From: Looking for Chinks in the Armor of Bacterial Biofilms Monroe D PLoS Biology ***Vol. 5****, No. 11, e307 doi: .0050307*

Diagram showing the steps of biofilm formation in graphical and microscope images



Increase in severe odontogenic infections

An increase in the number of locally invasive odontogenic infections requiring hospital care as well as odontogenic sepsis has been recently observed in many countries. In the UK, the total number of admissions and bed days due to dental abscesses almost doubled between 1998–9 and 2005–6. On the other hand, Lee *et al*. reported an incidence of post-tooth extraction sepsis of 1.48 per 100,000 inhabitants and a sepsis mortality rate of 21% in Taiwan. We have reported four cases of vascular graft infections caused by oral microbes and one of these was lethal.

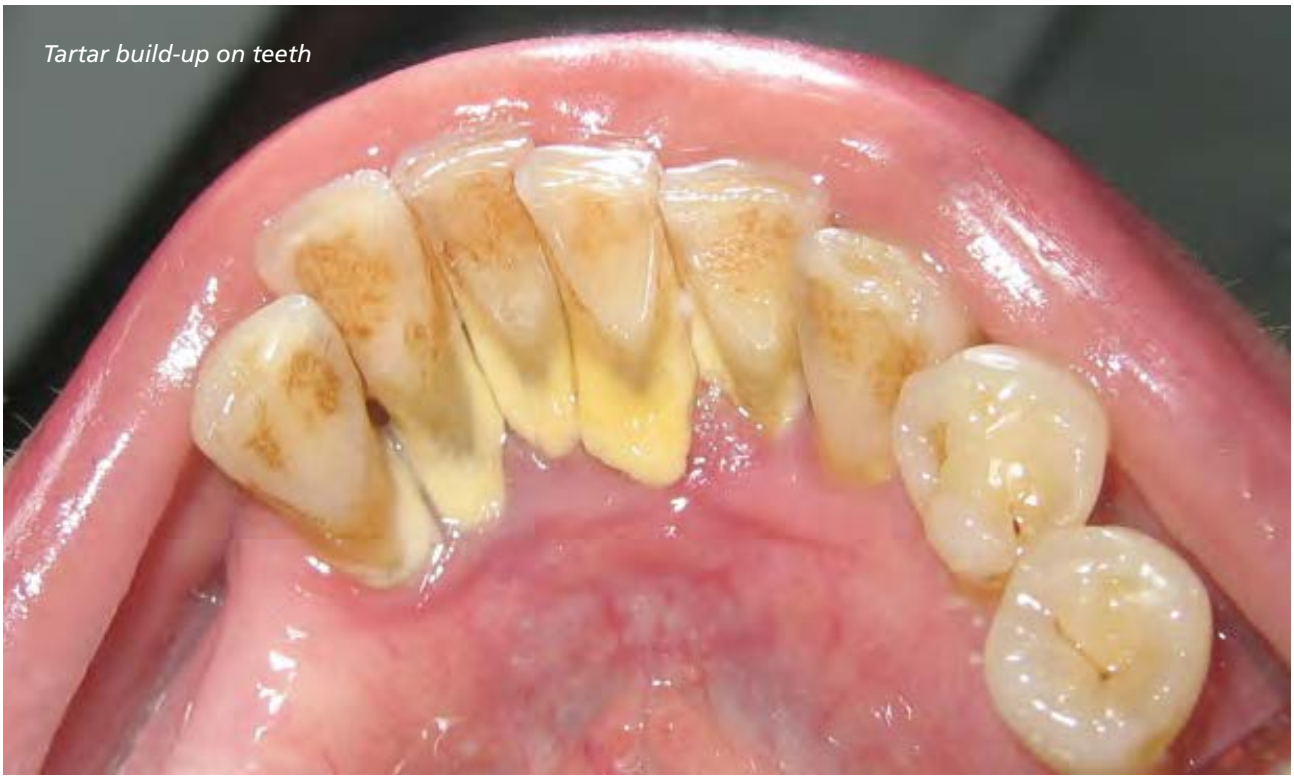
These severe outcomes have been associated with an inadequate surgical intervention and abscess drainage, inadequate disruption of biofilms, the presence of microbial strains and species resistant to standard antimicrobial therapy, as well as to the inability of dental practitioners to assess the immunological status of the patient. Many of these issues are for the dental practitioners to act upon. However, it is the responsibility of microbiology laboratories to follow the trends in oral infections.

The management of dental and odontogenic infections should always target the biofilm

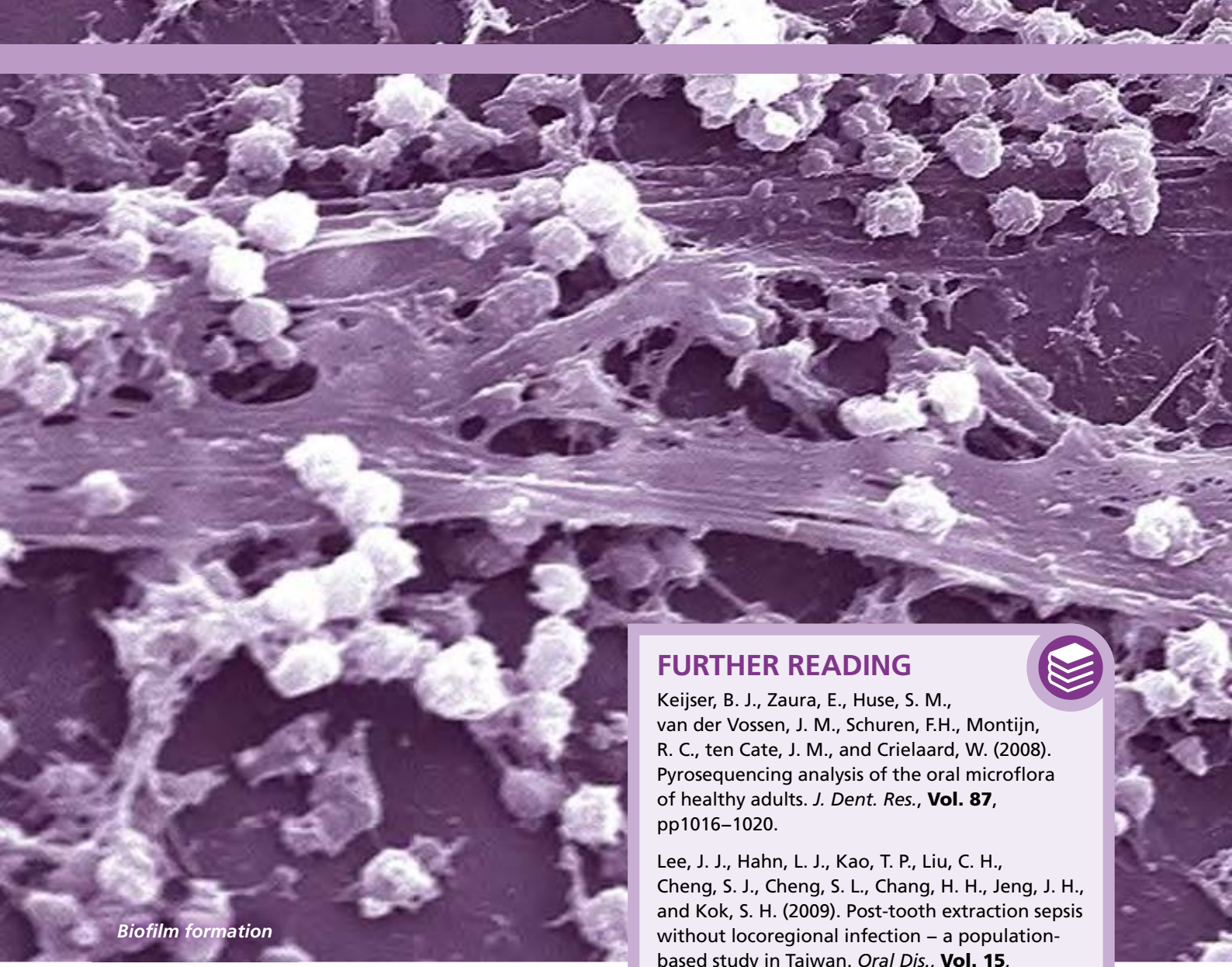
Diagnostic challenges

It is not common practice for dentists to take samples for microbiological investigations. Therefore, there is a clear lack of data regarding the incidence of strains and species resistant to standard antimicrobial therapy. There are a number of reasons why dentists do not take samples, but one major reason is that many laboratories are not familiar with processing oral samples and the reports they provide are, therefore, often not that useful for dentists.

On the rare occasion of receiving pus from the oral cavity it would be very important for the laboratory to try to analyse it as well as possible in order to gather local epidemiological data. This includes identification of dominant species (typically streptococci and anaerobes) and performing susceptibility testing on the isolates. It would also be important to report any atypical growth such as staphylococci or Gram-negative aerobes as these are not covered by the standard empiric therapy. There is some evidence that resistance of oral microbes to penicillin and macrolides is increasing, and that staphylococci may have a greater role in severe odontogenic infections than generally appreciated. However, more evidence is required prior to updating treatment guidelines whereby dental practitioners need to be encouraged to take samples for diagnostic purposes. There are a few oral microbiologists in the country whose expertise is available for all colleagues including microbiology laboratory staff. Their contact details are available on the General Dental Council's Specialist list.



Tartar build-up on teeth



Biofilm formation

Management

Most dental abscesses can be managed by general dental practitioners and with surgical procedures only. In the case of significant swelling and signs of systemic inflammation, antibiotic therapy targeted at oral streptococci and anaerobes should be considered in addition to the surgical procedure. However, if the course of infection is aggressive, the infection poses a threat to the airways or there is a risk of systemic or disseminated spread of infection, the patient should be evaluated and treated at a hospital. Of the patients admitted to the hospital, up to 30% require intensive care.

The most important feature of oral infections to remember is that they are polymicrobial biofilm infections. It is well known that biofilm infections do not respond well to antimicrobial therapy and there is plenty of evidence of poor outcomes of managing dental infections with antimicrobials alone. Therefore, it is important for all healthcare professionals to be aware of the patient's urgent need for the attention of a dentist or maxillo-facial surgeon. The management of dental and odontogenic infections should always target the biofilm. The systemic response to the infection is more severe in the absence of dental treatment.

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NANOTECHNOLOGY and the control of oral infections

Nanotechnology represents the ability to image, manipulate and model functionalities on the nanometre scale. This discipline includes the use of nanoparticles which can be classified as particles of less than 100 nm. Those particles with an antimicrobial function have received considerable attention within a range of diverse fields, including medicine and dentistry. Properties of nanoparticles, for example their active surface area, chemical reactivity and biological activity, are often far removed from those of a greater size. These characteristics should allow them to closely interact with microbial surfaces, and thus elicit an antimicrobial effect that is not solely due to released components. Metallic and other nanoparticles are now being combined with polymers and other base materials, and coated onto surfaces to ultimately provide a variety of potential applications within the oral cavity.

The prevention of dental caries and periodontal diseases is traditionally targeted at the mechanical or non-specific control of dental plaque as this is the precipitating factor. Antimicrobial approaches, including the use of antimicrobial agents, represent a valuable addition to mechanical plaque control. Such strategies should ideally prevent plaque biofilm

The use of nanotechnology offers the possibility to control the formation of oral biofilms

formation without affecting the biological equilibrium within the oral cavity, which is inhabited by more than 700 different species of bacteria at 10^8–10^9 bacteria per ml saliva or mg dental plaque. The use of nanotechnology offers the possibility to control the formation of oral biofilms through the application of nanoparticles with biocidal, anti-adhesive and delivery capabilities.

Nanoparticle-based implant coatings should also offer both osteoconductive and antimicrobial functionalities to prevent dental implant failure. Such implant systems are increasingly being used to replace missing teeth and most integrate with bone without complications. However, peri-implantitis is a major cause of dental implant failure whereby the induced inflammatory changes in the soft tissues surrounding the implant lead to a progressive destruction of the supporting bone. Current forms of treatment are often inadequate, with chronic infection often requiring implant removal and expensive resective and regenerative procedures in an attempt to restore and reshape the supporting tissue.

Antimicrobial nanoparticles and control of oral biofilms

Metals have been used for centuries as antimicrobial agents. Silver, copper, gold, titanium and zinc have attracted particular attention, each having different properties and spectra of activity. Indeed, many oral products, including toothpastes, now incorporate powdered (micron-sized) zinc citrate or acetate to control the formation of dental plaque. With respect to nanoparticulate metals, the antimicrobial properties of silver and copper have received the most attention. Both of these have been coated onto or incorporated into various test materials, including the denture material Poly (methyl methacrylate)(PMMA).

Nanoparticulate metal oxides (Figure 1) have been of particular interest as antimicrobial agents as they can be prepared with extremely high surface areas and unusual crystal morphologies that have a high number of edges, corners and other potentially reactive sites. On the other hand, certain

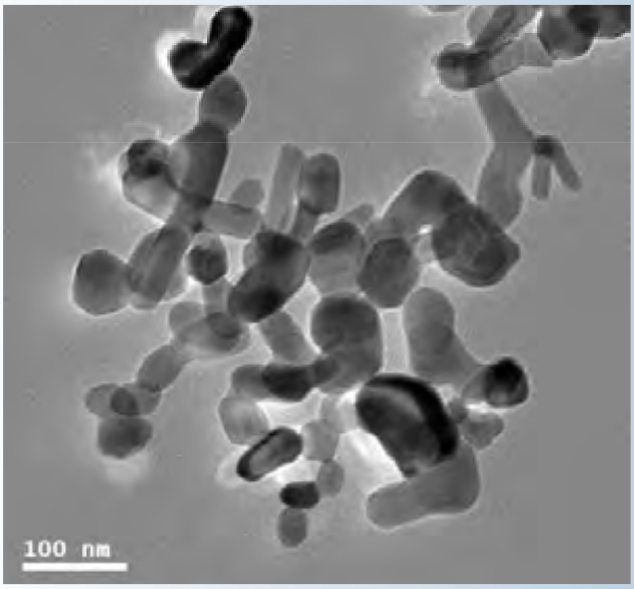

Figure 1. *Transmission electron microscopy image of ZnO nanoparticles*

metal oxides are now coming under close scrutiny because of their potential toxic effects to eukaryotic cells. Oxides under consideration as antimicrobial agents include those of copper, zinc, titanium and tungsten. Studies have shown that some nanoparticulate metal oxides, such as ZnO, have a degree of selective toxicity to bacteria (Figure 2) with a minimal effect on human cells at the concentrations employed. Bacteria are far less likely to acquire resistance to metal nanoparticles than they are to other conventional and narrow-spectrum antibiotics. This is thought to occur because

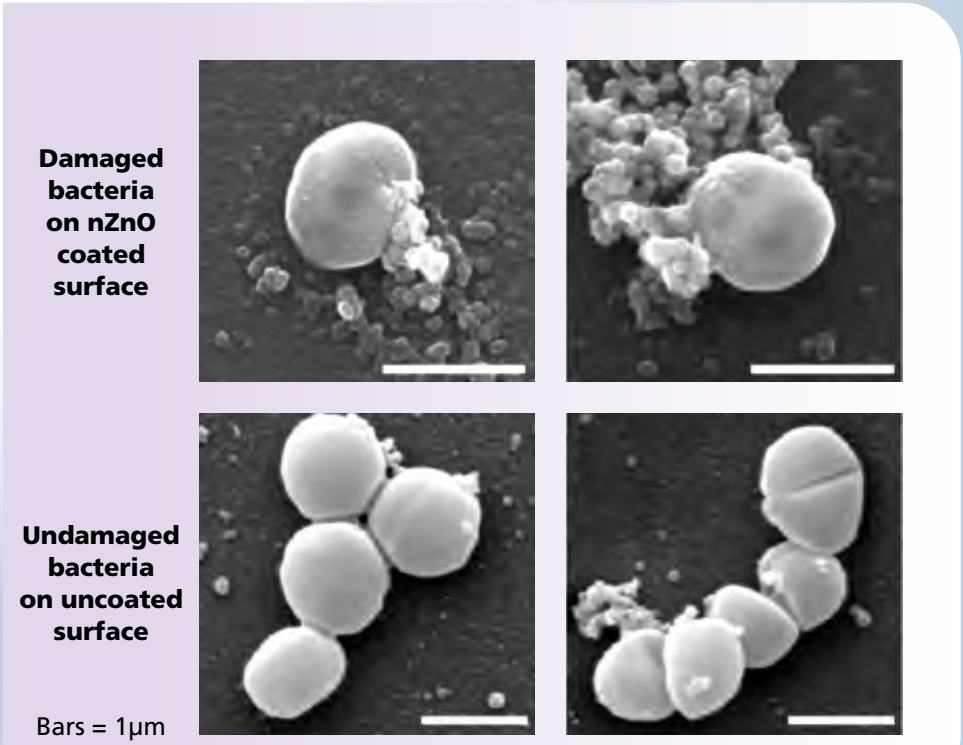

Figure 2. *Effects of nano zinc oxide (nZnO) on Staphylococcus aureus*

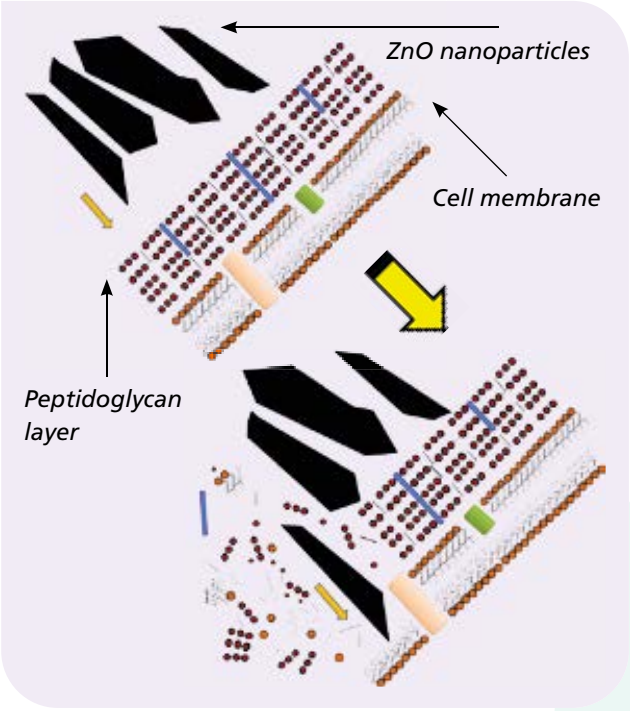

Figure 3. *Hypothetical contact-based activity of ZnO nanoparticles against the cell wall of Gram-positive species*

metals may act on a broad range of microbial targets (Figure 3), and many mutations would have to occur in order for the microorganisms to resist their antimicrobial activity.

Quaternary ammonium poly (ethylene imine) (QA-PEI) antimicrobial nanoparticles have also been developed and incorporated into dental composite resins. This approach may be particularly beneficial when compared with the currently used composite resins for hard tissue restoration, which are known to possess several disadvantages including the development of biofilms on both teeth and restorative material.

Anti-adhesive nanoparticles and oral biofilm control

Particles of a nano and micro size, based upon the element silicon, have been designed to rapidly deliver antimicrobial and anti-adhesive capabilities to the desired site within the oral cavity. Companies use silica (often classed as 'micro fine', but with a particle size within the definition of nanoparticles) in toothpastes, and some have actively sought new directions in this area through the use of porous silicon and nanocrystalline silicon technology to carry and deliver antimicrobials, for example, triclosan.

Other novel systems based upon silica have been investigated with respect to the control of oral biofilms. The use of nitric oxide-releasing silica nanoparticles to eradicate biofilm growth has been described. Bioactive glasses of the SiO_2-Na_2O-CaO-P_2O_5 system have been shown to possess antimicrobial activity through the release of ionic alkaline species over time. Those in the form of amorphous nanoparticles, with a size range of 20 to 60 nm, may show an advantage over micron-sized material as the decrease in glass particle size should increase the active exchange surface of glass and surrounding liquid. This should then substantially increase ionic release into suspension and enhance antimicrobial efficacy. Chitosan, a biopolymer derived by the deacetylation of chitin occurring in the exoskeleton of crustaceans is positively charged and soluble in acidic to neutral solution, enabling it to bind to mucosal surfaces. Both chitosan nanoparticles and microparticles have been investigated as a potential platform for the local delivery of drugs within the oral cavity.

The application of nano-scaled hydroxyapatite (nHA) particles has been shown to impact on oral biofilm formation and can also provide a re-mineralization capability. Biomimetic approaches based upon HA nanocrystals which resemble the structure at the nano scale of abraded dental enamel crystallites, in theory should allow adsorbed particles to interact with bacterial adhesins, reduce bacterial adherence and hence impact on biofilm formation. A number of oral healthcare products, including toothpastes and mouth rinses, have been developed containing nano-sized apatite particles with and without protein-based additives. It is suggested that the efficacy of these compounds can be attributed to the size-specific effects of the apatite nanoparticulates. Casein phosphopeptide (CPP)-amorphous calcium phosphate (ACP) nanocomplex (Recaldent™/ MI Paste™) is a particular technology based upon ACP and stabilized by casein phosphopeptide (CPP). Use of this technology has demonstrated anticariogenic activity under both *in vitro* and *in vivo* test conditions.

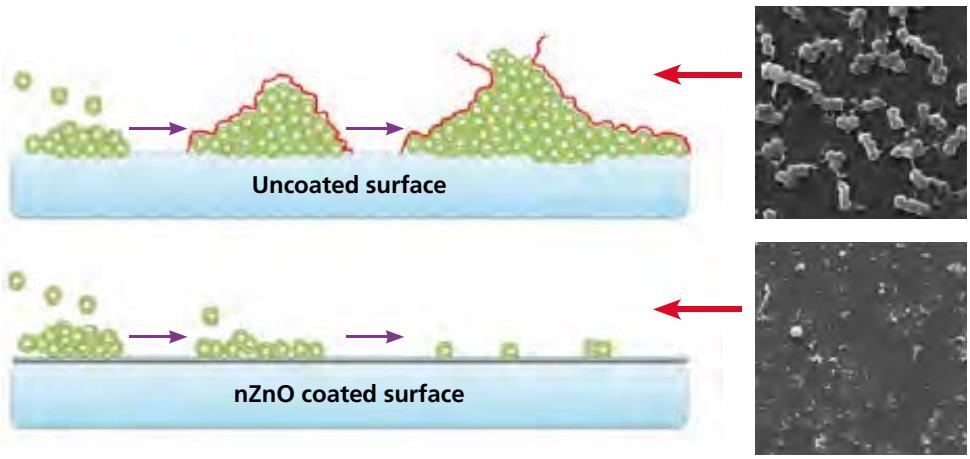

Figure 4. *Prevention of biofilm formation on nZnO coated surface*

With reference to dental implants, numerous companies market novel synthetic HA materials as the 'optimal' available osteoconductive implant coating and some companies have developed nano scaled varieties. Furthermore, combined nHA and nano zinc oxide (nZnO) coatings have shown much potential as regards antimicrobial activity and biocompatibility (Figures 4 and 5).

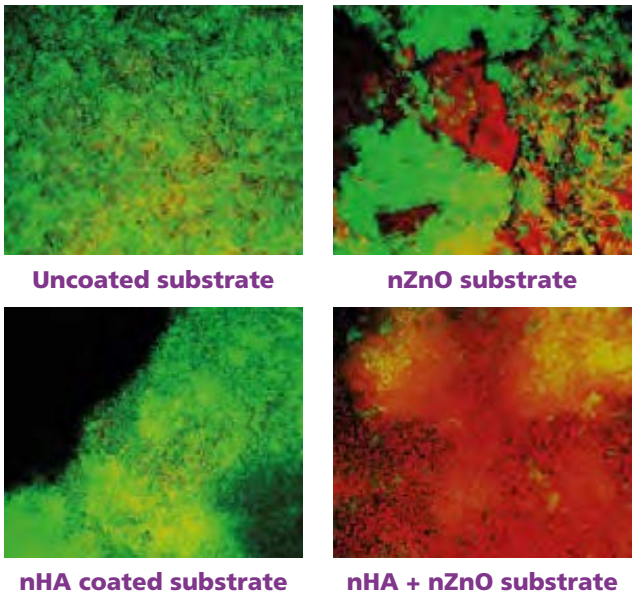

Figure 5. *Confocal images demonstrating anti-oral biofilm activity of nano zinc oxide alone and in synergy with nano hydroxyapatite (nHA). Green indicates viable bacteria and red indicates non-viable bacteria. (Supplied by E. Abdulkareem, Eastman Dental Institute)*

Biocompatibility of nanoparticles within the oral cavity

Although the development and application of nanotechnology is of considerable interest, knowledge regarding the possible toxicity of nanotechnology products to humans is limited. In order to fully understand the mechanism of toxicity, a thorough knowledge of the toxico-kinetic properties of nanoparticles is required. Toxicology and biodynamic studies suggest that silica, silicon and chitosan nanoparticles are relatively safe if introduced via the oral route.

The safe use of nanotechnology and the design of nanomaterials for biological applications involve a thorough understanding of the interface between these materials and biological systems. The interface comprises three interacting components: (i) the surface of the nanoparticle, (ii) the solid-liquid interface and the effects of the surrounding medium and (iii) the contact zone with biological substrates. The nanoparticle characteristics of most importance as regards interaction with biological systems, whether mammalian or microbial, are chemical composition, surface function, shape and number of sides, porosity and surface crystallinity, size heterogeneity, roughness, and hydrophobicity or hydrophilicity. In order to help prevent aggregation of nanoparticles, stabilizing (capping) agents that bind to the entire nanoparticle surface can be used; these include water-soluble polymers, oligosaccharides and polysaccharides, sodium dodecyl sulfate, polyethylene glycol and glycolipids.

An understanding of the interface between biological systems and nanomaterials should enable design features to be used to control the exposure, bioavailability and bio catalytic activities. A number of possible approaches are starting to be identified including the changing ability to aggregate, application of surface coatings, and altering charge density and oxidative state. However, this may well compromise the intended selective toxicity of antimicrobial nanoparticles. It remains to be determined how potential mammalian toxicity issues will fully impact on the use of nanotechnology in the control of oral infections.

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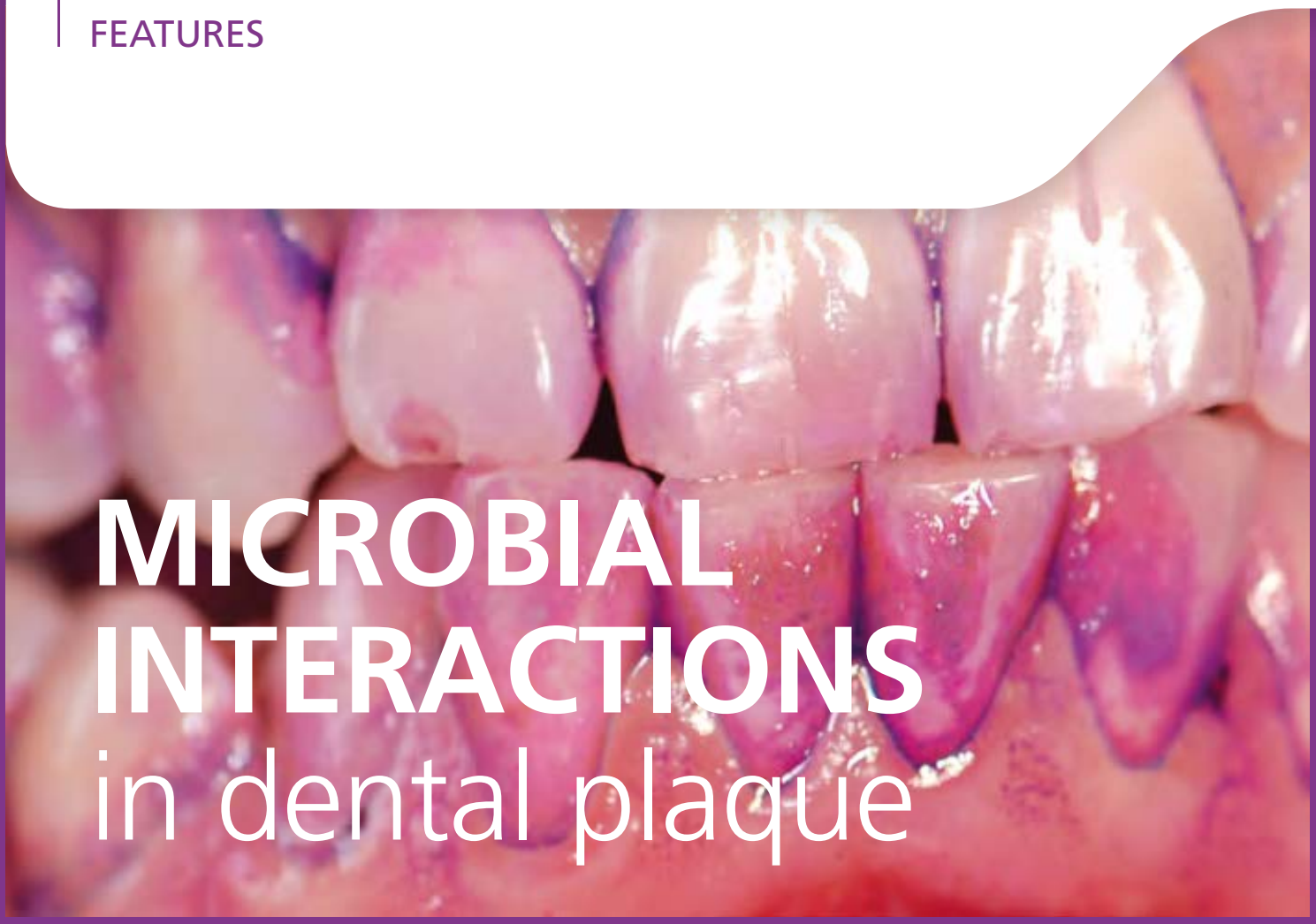

MICROBIAL INTERACTIONS in dental plaque

Figure 1. *Dental plaque after careful cultivation for 2 days, stained with a disclosing agent*

Despite decades of research effort, it is estimated that we can still only culture around 50% of the 200 or so dental plaque microorganism species in the laboratory. Yet, with very little effort we all culture the entire microbial consortium in our mouths every day! The key to this is the dependence that microorganisms have for one another to survive, and a better understanding of microbial interactions within dental plaque may potentially lead to new targets for disease control.

Throughout the last century a great deal of work was targeted towards finding a single pathogen associated with dental caries, but both dental caries and

Despite decades of research effort, it is estimated that we can still only culture around 50% of the 200 or so dental plaque microorganism species in the laboratory

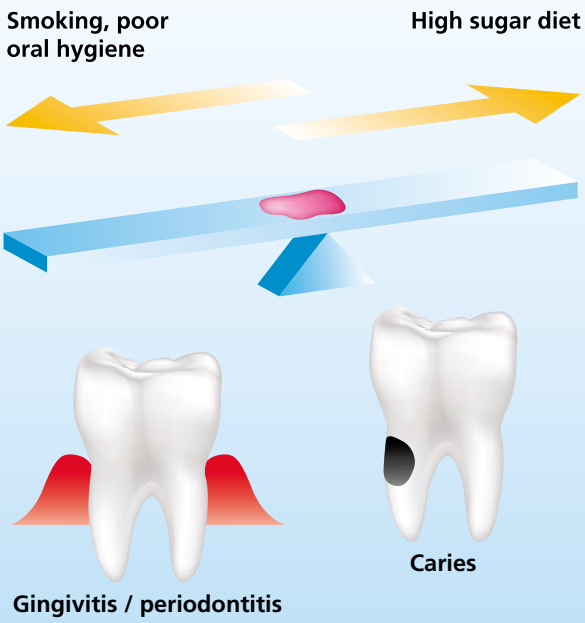

Figure 2. *A simplified version of the ecological plaque hypothesis. Health-associated dental plaque is a microbial homeostasis that may shift to a disease-associated community in response to changes in the host or environment*

periodontitis are almost certainly better explained by an 'ecological plaque hypothesis'. This says that disease is attributed to shifts in the microbial population and the overgrowth of selected species (but not just a single organism). A simplified version of the hypothesis is presented in Figure 2.

According to this theory, dental plaque reaches a 'microbial homeostasis' after around 24 h if left undisturbed. From this point, the microbial population in dental plaque is stable until it is perturbed by changes in host, bacterial or environmental factors such as eating sugary snacks or smoking.

The formation of dental plaque on a clean tooth surface begins with the attachment of primary colonizing bacteria, such as *Streptococcus*, *Neisseria*, *Haemophilus*, *Actinomyces* or *Veillonella* species, to the salivary pellicle that coats tooth surfaces. These species promote the colonization of other organisms through cell-cell adhesion, cell-cell signalling or by modifying the local environment, for example, by reducing the oxygen tension.

The adhesion of microorganisms to pre-bound species is known as 'coadhesion', whereas microbial cell-cell binding in the fluid phase is termed 'coaggregation'. Coadhesion and coaggregation interactions only occur between compatible partners, and involve the specific recognition of protein or carbohydrate receptors on one organism by protein adhesins on the other.

As dental plaque accumulates, an extracellular macromolecular matrix is produced that enhances the adhesion of microbial cells to one another and to the tooth. Even when physically dislodged by scraping with a toothpick, dental plaque microorganisms remain bound to one another and can be visualized as large aggregates under a microscope (Figure 3).

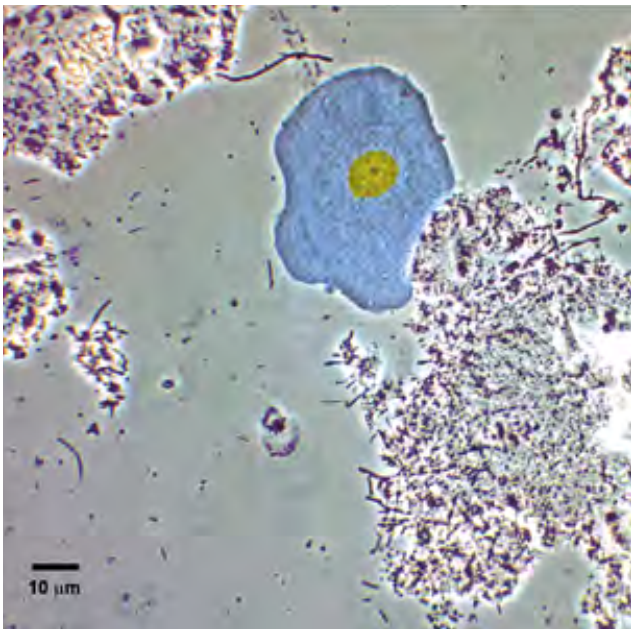

Figure 3. *Dental plaque removed from the mouth of a volunteer using a toothpick. Individual microbial cells are small compared with the epithelial cell (coloured blue with yellow nucleus), but together the microbial aggregate is large*

Microbial cell-cell binding helps to keep cells in close proximity to one another and consequently may promote a wide range of distance-critical interactions including the exchange of metabolites, signals or genetic information (Figure 4).

For example, *Veillonella* spp. require lactate for growth, and this nutrient is readily available from *Streptococcus* spp. which secrete lactate as a waste product from carbohydrate metabolism. Studies have shown that coaggregating strains of *Strep. mutans* and *Veillonella alcalescens* colonize the teeth of a rat more effectively than non-coaggregating strains.

The major cell-cell signals in dental plaque are thought to be autoinducer-2, a product of the *luxS* gene that is present in many oral bacteria, and peptides that are produced by Gram-positive species and are often linked to the development of a genetically competent state. The physical proximity of different species to one another is likely to enhance the exchange of signals, and will increase the efficiency of DNA transfer.

The exchange of transposon constructs has been shown to occur in model oral biofilms. There is also extensive

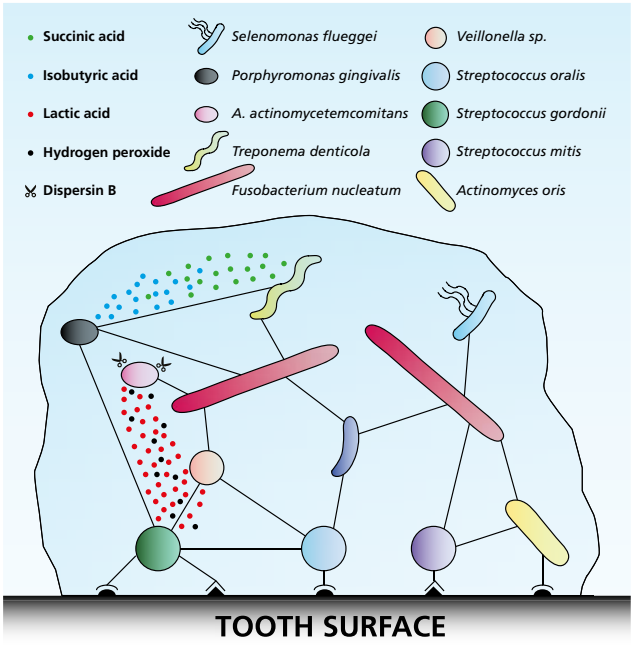

Figure 4. *An illustration of the varied interactions in dental plaque. The primary colonizing bacteria adhere to salivary receptors and recruit later colonizers through specific interbacterial interactions. Metabolic exchange occurs in two directions between Porphyromonas gingivalis and Treponema denticola. Lactic acid produced by streptococci such as Strep. gordonii promotes the growth of Aggregatibacter actinomycetemcomitans and Veillonella sp. At the same time, streptococcal H_2O_2 triggers the production of the matrix-degrading enzyme dispersin B*

evidence in the genome sequences of oral bacteria that indicate genetic exchange has taken place. Somewhat worryingly, the development of penicillin resistance in *Strep. pneumoniae* has been attributed to the transfer of a penicillin-binding protein gene (*pbp2x*) from oral streptococci.

Not all interactions between microorganisms are beneficial to the species involved, and cell-cell binding may also promote competition. Many oral bacteria produce small peptide bacteriocins that kill selected competitors, and under laboratory conditions, oral streptococci produce hydrogen peroxide (H_2O_2) at concentrations that inhibit the growth of different species. It appears that this inhibition is likely to be part of a network of controls that dictate the spatial organization of organisms in the biofilm and maintain a fine balance of competition.

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It will be a long time before all the different intermicrobial interactions in dental plaque are resolved, but even without a complete knowledge of these processes it may be possible to develop strategies that support good oral health. For example, pre-biotic approaches that enhance the production of H_2O_2 by oral streptococci may help to control the growth of other, less desirable, microorganisms.

There is also evidence that our ability to culture microorganisms can be improved by taking intermicrobial interactions into account. For example, the isolation of *Fretibacterium fastidiosum*, a previously uncultured member of the phylum Synergistetes, from dental plaque was reported recently using an enrichment strategy that selectively depleted different members of the community. In the end, *Fretibacterium fastidiosum* was shown to grow in the laboratory when cross-streaked with other bacteria or when incubated with bacterial extracts.

Despite the apparent paradox, the isolation of new strains is critical in order to understand why these strains are so difficult to isolate in the first place. Studies using mixed species of bacteria were once shunned in the laboratory as being too complex to interpret. With the development of massively parallel technologies and sophisticated bioinformatics, it is now realistic to start deciphering these interactions even in complex communities such as dental plaque.

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HISTORICAL PERSPECTIVES

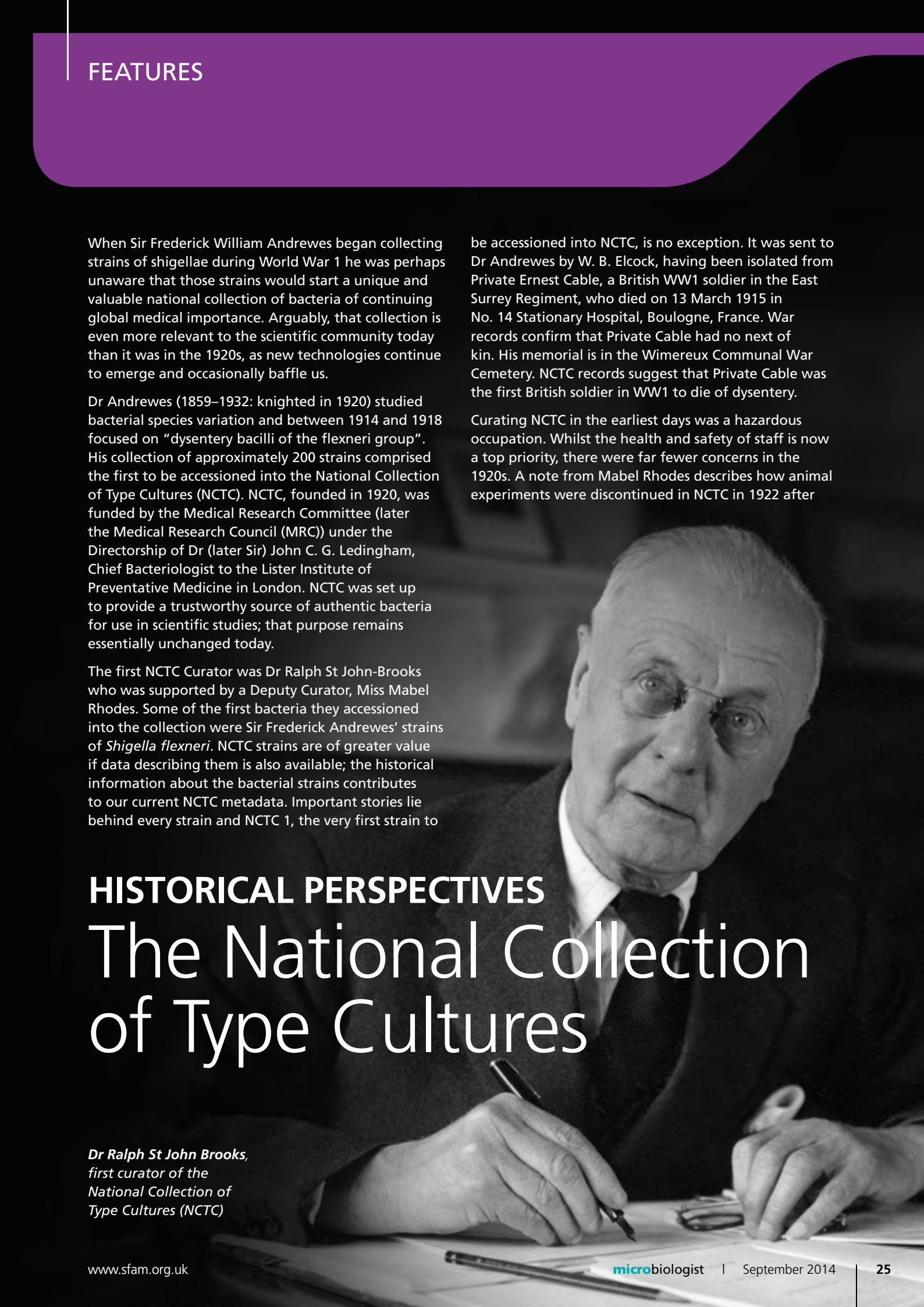
The National Collection of Type Cultures

When Sir Frederick William Andrewes began collecting strains of shigellae during World War 1 he was perhaps unaware that those strains would start a unique and valuable national collection of bacteria of continuing global medical importance. Arguably, that collection is even more relevant to the scientific community today than it was in the 1920s, as new technologies continue to emerge and occasionally baffle us.

Dr Andrewes (1859–1932: knighted in 1920) studied bacterial species variation and between 1914 and 1918 focused on "dysentery bacilli of the flexneri group". His collection of approximately 200 strains comprised the first to be accessioned into the National Collection of Type Cultures (NCTC). NCTC, founded in 1920, was funded by the Medical Research Committee (later the Medical Research Council (MRC)) under the Directorship of Dr (later Sir) John C. G. Ledingham, Chief Bacteriologist to the Lister Institute of Preventative Medicine in London. NCTC was set up to provide a trustworthy source of authentic bacteria for use in scientific studies; that purpose remains essentially unchanged today.

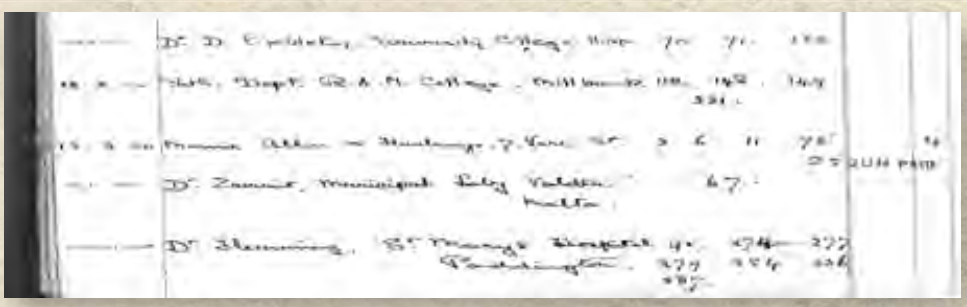
The first NCTC Curator was Dr Ralph St John-Brooks who was supported by a Deputy Curator, Miss Mabel Rhodes. Some of the first bacteria they accessioned into the collection were Sir Frederick Andrewes' strains of *Shigella flexneri*. NCTC strains are of greater value if data describing them is also available; the historical information about the bacterial strains contributes to our current NCTC metadata. Important stories lie behind every strain and NCTC 1, the very first strain to be accessioned into NCTC, is no exception. It was sent to Dr Andrewes by W. B. Elcock, having been isolated from Private Ernest Cable, a British WW1 soldier in the East Surrey Regiment, who died on 13 March 1915 in No. 14 Stationary Hospital, Boulogne, France. War records confirm that Private Cable had no next of kin. His memorial is in the Wimereux Communal War Cemetery. NCTC records suggest that Private Cable was the first British soldier in WW1 to die of dysentery.

Curating NCTC in the earliest days was a hazardous occupation. Whilst the health and safety of staff is now a top priority, there were far fewer concerns in the 1920s. A note from Mabel Rhodes describes how animal experiments were discontinued in NCTC in 1922 after



***Dr Ralph St John Brooks**, first curator of the National Collection of Type Cultures (NCTC)*

a strain of *Brucella* (now *Francisella*) *tularensis* was passaged through guinea pigs by rubbing a piece of infected guinea pig spleen onto the scarified skin of a live healthy guinea pig. This resulted in the Curator, Assistant Curator and another microbiologist, Dr H. Schulze, becoming seriously ill with tularaemia. Mabel Rhodes was on sick leave for more than a year and records indicate that none of the three ever fully recovered. Nevertheless, Ralph St John-Brooks was appointed as Director of NCTC when John Ledingham was promoted to Director of the Lister Institute in 1930.



An excerpt from the first NCTC order book showing Alexander Fleming was a customer in 1920 (bottom line).

Nearly 2000 cultures were distributed by NCTC in 1920–1921, free-of-charge, and mostly to scientists working in public institutions. NCTC sent growing bacteria sub-cultured from the stock cultures onto Dorset egg agar sealed with paraffin wax. Today, most NCTC cultures are distributed as viable freeze-dried (lyophilized) cultures in evacuated glass ampoules, a result of the freeze-drying experiments that began in 1933 to preserve the collection. By 1934 every strain in the collection had been freeze-dried in duplicate. The ampoules, together with the rest of the culture collection and records, were transferred from London to the Lister Farm Laboratories in Elstree, Hertfordshire in 1939, a fortuitous move since the Chelsea site was bombed in WW2. Freeze-dried cultures were relatively easy to handle and post. Restrictions on hazardous bacteria in the postal system were not introduced until the Vienna Convention (1964). Records show that 6397 cultures were distributed in 1938, of which 45% were posted to researchers, 20% to colleges and 35% to "technology workers". Of those cultures, 46 were posted outside the UK (Europe 23; Asia 11; Africa 6; North America 2; South America 2; Australasia 2). This period (1920s and 1930s) coincides with the emergence of private pharmaceutical and medical technology companies. Nominal charges (one shilling per culture) were introduced in 1947 although reduced rates were offered for "hospitals, scientific institutes and genuine research workers" and charges were often waived. NCTC moved from Elstree to the Central Public Health Laboratory (CPHL) of the Public Health Laboratory Service (PHLS) in Colindale, London, in July 1949. The administration and finance of NCTC passed from the MRC to the PHLS Board in 1960. However, nominal fees for cultures remained in place until 1970 when a more robust pricing structure was introduced to cover at least some of NCTC's operational costs.

Biosecurity is of paramount importance to NCTC today, but also caused problems in the early days. Mabel Rhodes sent an NCTC culture to a colleague in "the Argentine" in the 1930s who, unbeknown to her, was viewed by the UK Government as a Nazi sympathizer. She was accused subsequently of "trading with the enemy". Miss Rhodes was not required to consider the Specified Animal Pathogens Order 1998 (SAPO), the Anti-terrorism, Crime and Security Act (2001) or Biological Agents and Genetically Modified Organisms (Contained Use) 2010, just a few examples of the legislation that affects our daily work today.

Variety of culture containers

NCTC expanded rapidly, despite the early setbacks of mass staff illness and two World Wars. An initial centralized policy had resulted in the collection consisting of a wide range of microorganisms (bacteria, yeasts and fungi) of medical, veterinary, agricultural and economic importance, a practice continued today by more generalized collections. However, a larger, broader-focused collection needed wide-ranging expertise if it were to remain scientifically valid, so in 1947 Dr Samuel T. Cowan, who succeeded Dr St John-Brookes as Curator, decided NCTC should focus specifically on bacteria of medical and veterinary interest. He was concerned that collections preserved by non-specialists might result in stock cultures being replaced by contaminants. Non-medical cultures were transferred to other institutes with different expertise, leaving approximately 3,000 cultures remaining in

the NCTC. Dr Cowan then requested a review of the collection's documentation and data, and improvements to the characterization processes for the cultures. Our ongoing strategy for the NCTC to this day includes continuous improvement of strain data and the characterization and authentication procedures.

A large-scale freeze-drying and characterization project for every organism in the collection started in 1949 and took 10 years to complete. This project included the introduction of a manual data recording system, using individual cards for every strain to record colony morphology, biochemical test results and freeze-drying records. This initiative resulted in NCTC being recognized as the foremost collection in the world with a vision to "encourage uniformity in the scientific work of microbiologists of all nations". Contributions by the Curator to the First International Code for Nomenclature (1947) eventually led to the publication of Cowan and Steel's '*Manual for Identification of Medical Bacteria*' (1965). NCTC also offered a bacterial identification testing service and was awarded a research grant by the Department of Health and Social Security in 1965 to develop a computer-based identification system, the first such grant to be awarded to the PHLS. By contrast, NCTC's most recently awarded grant came from the Wellcome Trust in 2013, enabling us to progress a five-year project that includes whole genome sequencing of the entire NCTC collection in collaboration with the Wellcome Trust Sanger Institute. The 'NCTC 3000 Project' team will also develop a web-based portal, combining the taxonomic backbone for species identification with easy access to other relevant electronic resources such as NCTC strain metadata, external sequence databases and publication websites.

Over time, microbial collections arose in other countries and in 1947 the Permanent Committee of the British Commonwealth Collections of Microorganisms was set up to coordinate the work of all the culture collections in the British Commonwealth. The World Federation of Culture Collections (WFCC) was established with UNESCO support in the 1970s and pioneered the development of an international database on culture resources worldwide, the World Data Centre for Microorganisms (WDCM) which also publishes the WDCM Reference Strain catalogue. However, publishing culture collection data is not a novel concept. The first NCTC catalogue was published in 1922 by H. M. Stationery Office, with subsequent editions available until the 1990s. The current catalogue is electronic only and accessible via the website: **www.phe-culturecollections.org.uk**.

Julie E. Russell
Head of Culture Collections
Public Health England

The Queen visiting NCTC in 1985, a rack of freeze-dried cultures in the foreground

PHLS was subsumed by the Health Protection Agency (HPA) in 2003, and HPA became part of Public Health England in 2013. By 2005, in addition to operating NCTC, HPA was also responsible for three other collections (pathogenic viruses, fungi and cell cultures) so the four collections were brought together under the leadership of a single Bioresource Centre Director who has a remit to ensure that each collection retains the expertise necessary for its maintenance and scientific development, whilst consolidating operations across the collections wherever practical.

NCTC reflects the history of clinical bacterial infections with a wealth of strains that, like NCTC 1, caused infections before the widespread use of antibiotics. The genomic data for those historical organisms is of particular interest to scientists studying microbial evolution. The entire genome of NCTC 1 was recently sequenced by the Wellcome Trust Sanger Institute and will be published later this year.

Acknowledgements

The author acknowledges all the people who have developed and maintained NCTC over the past 94 years, especially those who kept the meticulous records that made this article possible. Particular thanks also to the current NCTC Scientific and Operational Team headed by Ana Deheer-Graham, the NCTC Taxonomist, Professor Haroun Shah, and the PHE Culture Collections staff who support NCTC.

BIOFocus

A strong **united voice** to influence political decisions

Some argue that charities have no role to play in the political arena and that science and politics do not mix; at the Society of Biology, however, we believe the opposite is true – we have an essential role to play in national politics. Of course, as a charity we cannot be partisan in our opinions. We must set out the relevant facts and what we believe to be the implications of varying policy agendas, engaging with all parties wherever possible. But, we can also be proactive in describing what we believe will be good for biology and science more widely, encouraging all the parties to adopt our proposals.

This autumn is a critical time for UK politics. On 18 September there is the vote on the potential independence for Scotland, and it is the time of the last party conferences before the UK national elections on 7 May 2015. It is critically important that the value of science in terms of its contribution to social infrastructure, employment, wealth creation and innovation is fully reflected by all party manifestoes and in the decision over Scottish independence.

To ensure that the Society is best placed to present the views of the sector and engage with the political classes we have long been developing our links with both Westminster and the devolved administrations. Our Director of Parliamentary Affairs, Stephen Benn (email: stephenbenn@societyofbiology.org), who works not only on behalf of the Society of Biology but

also the whole scientific community, partly funded by SfAM, spends most of his day engaging with politicians directly, walking the corridors of power, helping to ensure the scientific community is listened to. Part of this work is designed to raise the profile of science, technology and engineering, and specifically biology, wherever possible, through innovative formats in the Palace of Westminster. But, of course, we also continue to develop collaborative policy responses which are hugely valued by both officials and parliamentarians. They are valued because of their independence, non-partisan and collaborative nature representing a broad spectrum of views in, hopefully, a balanced and evidence-based way. Part of the value of the Society of Biology is being able to bring together views from all quarters to present clear messages. SfAM continues to be a great supporter of our policy and public engagement work, and is always one of the first professional bodies to engage with new initiatives.

This year's largest scientific event in Westminster – LINKS Day – provided another opportunity to link members of parliament with the scientific community through the theme of "Science and Public Trust". It builds on two other major Society of Biology led events "Voice of the Future" and "SET for Britain", and complements our support for Holyrood, Stormont and Senedd events organized through the Royal Society of Chemistry. These events provide an ideal opportunity to engage with politicians and policymakers, and to explain how we can support their work.

A lot of policy time over recent months has been dominated by the reform of A-levels and the worrying lack of new anti-infective treatments as antibiotic resistance continues to grow. These are very specific, and important, issues. We rely on the expertise of our membership to help make sure we get the evidence and arguments right, and we are always keen to hear if SfAM, or any of our other member organizations, have key concerns you would like us to help with.

However, for the debate around independence in Scotland and the general election next year, we also need to ensure we argue strongly for biology, and science more widely, in terms of funding, the right education and training framework, building our research base and an appropriate policy framework to support science-based industries whilst increasing public engagement with our subjects.

With that in mind, we are developing our own mini-manifesto and will try to ensure all existing and prospective parliamentary candidates are aware of our arguments, backed by evidence wherever possible. The Society would love to hear the views of SfAM Members and we will actively be seeking opportunities for you to contribute. To help make the case for the economic value of science we have supported a piece of collaborative research through CaSE (Campaign for Science and Engineering) with the striking conclusion that every pound spent on research generates 20 pence return year-on-year in perpetuity and more when additional private investment is attracted.

To add further weight to our credibility as a biology advocate, we all need to grow our memberships as much as possible and demonstrate to biologists, of all flavours, that a strong united voice really can influence political decisions.

Every pound spent on research generates 20 pence return year-on-year in perpetuity

Mark Downs FSB
Chief Executive, Society of Biology

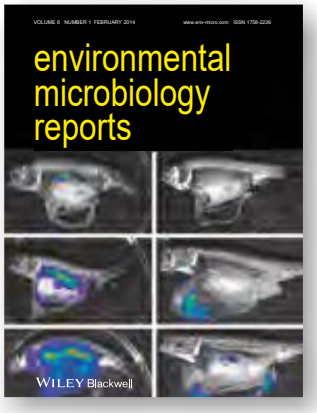
Journal**WATCH**

Highlighted Articles from the *Sf*AM journals

Environmental Microbiology Reports

Metagenomic analysis on seasonal microbial variations of activated sludge from a full-scale wastewater treatment plant over four years

Ju *et al*.



A metagenomic technique was employed to characterize the seasonal dynamics of activated sludge (AS) communities in a municipal wastewater treatment plant (WWTP) over four years. The results indicated that contrary to *Eukaryota* (mainly *Rotifera* and *Nematoda*), the abundance of bacteria and archaea (mainly *Euryarchaeota*) were significantly higher in winter than summer. Two-way analysis of variance and canonical correspondence analysis revealed that many functionally important genera followed strong seasonal variation patterns driven by temperature and salinity gradients. However, SEED subsystems annotation showed that functional categories in AS exhibited no significant difference between summer and winter, indicating that compared with its microbial components, the functional profiles of AS were much more stable. Taken together, our study provides novel insight into the microbial community variations in AS and discloses their correlations with influential factors in WWTPs.
http://bit.ly/EMR_Ju

sigmaS, a major player in the response to environmental stresses in *E. coli*: role, regulation and mechanisms of promoter recognition

Landini *et al*.

Bacterial cells often face hostile environmental conditions, to which they adapt by activation of stress responses. In *E. coli*, environmental stresses resulting in significant reduction in growth rate stimulate the expression of the *rpoS* gene, encoding the alternative σ factor σ^S. The σ^S protein associates with RNA polymerase, and through the transcription of genes belonging to the *rpoS* regulon allows the activation of a 'general stress response', which protects the bacterial cell from harmful environmental conditions. Each step of this process is finely tuned in order to cater to the needs of the bacterial cell. This report discusses the function of the *rpoS* gene in the general stress response, and reviews the current knowledge on regulation of *rpoS* expression and on promoter recognition by σ^S.
http://bit.ly/EMR_Landini

Environmental Microbiology

Synthetic microbial ecosystems: an exciting tool to understand and apply microbial communities

De Roy *et al*.

Many microbial ecologists have described the composition of microbial communities in a plenitude of environments, which has improved our understanding of microorganisms and ecosystems. However, the factors and processes that influence the behaviour and functionality of an ecosystem largely remain black boxes when using

conventional approaches. Therefore, synthetic microbial ecology has gained a lot of interest in the last few years. Because of their reduced complexity and increased controllability, synthetic communities are often preferred to examine ecological theories. However, besides their use for basic research, synthetic ecosystems also found their way towards different applications, such as industrial fermentation and bioremediation. Here, we review why and how synthetic microbial communities are applied for research purposes and for which applications they have been and could be successfully used.
http://bit.ly/EMI_DeRoy

Stress responses go three-dimensional – the spatial order of physiological differentiation in bacterial macrocolony biofilms

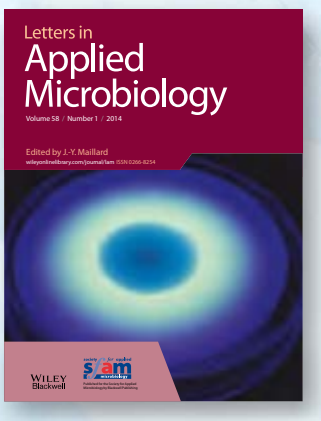
Serra and Hengge

In natural habitats, bacteria often occur in multicellular communities characterized by a robust extracellular matrix of proteins, amyloid fibres, exopolysaccharides and extracellular DNA. These biofilms show pronounced stress resistance including a resilience against antibiotics that causes serious medical and technical problems. This review summarizes recent studies that have revealed clear spatial physiological differentiation, complex supracellular architecture and striking morphology in macrocolony biofilms. Using *E. coli* as a model system, this review also describes our detailed current knowledge about the underlying molecular control networks – prominently featuring sigma factors, transcriptional cascades and second messengers – that drive this spatial differentiation and points out directions for future research.
http://bit.ly/EMI_Serra-Hengge

Letters in Applied Microbiology

Carriage of meticillin-resistant staphylococci by healthy companion animals in the US

Davis *et al*.



In this study, antimicrobial-resistant coagulase-negative and coagulase-positive staphylococci were isolated from various body sites on healthy dogs and cats. Resistance to 14 antimicrobials was observed including resistance to oxacillin; the majority of staphylococci were also multidrug resistant. Results from this study suggest that healthy dogs and cats may act as reservoirs of antimicrobial-resistant bacteria that may be transferred to people by simple interaction with the animals. Such carriage poses an underlying risk of infection, which should be considered during handling of healthy dogs and cats by pet owners and veterinary personnel.
http://bit.ly/LAM_Davis

Antimicrobial activity of fluorescent Ag nanoparticles

Bera *et al*.

This study aims to demonstrate the size- and shape-dependent antimicrobial activity of Ag nanoparticles. It is shown for the first time that fluorescent Ag nanoparticles of 1.5 nm have superior antimicrobial activity with respect to the larger particles. The shape and size of the particles actually control their activity. The smaller particles can easily penetrate the cell wall and have pronounced activity. These findings may be useful in the development of potential antimicrobial agents.
http://bit.ly/LAM_Bera

Journal of Applied Microbiology

Bacterial community dynamics during the early stages of biofilm formation in a chlorinated experimental drinking water distribution system: implications for drinking water discolouration

Douterelo *et al*.

This study aimed to characterize bacterial communities during the early stages of biofilm formation and their role in water discolouration in a fully representative, chlorinated, experimental drinking water distribution system (DWDS). Shifts in the bacterial community structure were observed along with an increase in cell coverage, bacterial richness and diversity. Species related to *Pseudomonas* spp. and *Janthinobacterium* spp. dominated the process of initial attachment. Based on fingerprinting results, the hydraulic regimes did not affect the bacteriological composition of biofilms, but they did influence their mechanical stability. This study gives a better insight into the early stages of biofilm formation in DWDS and will contribute to the improvement of management strategies to control the formation of biofilms and the risk of discolouration.
http://bit.ly/JAM_Douterelo

Characterization of the gut microbiota of three commercially valuable warm water fish species

Larsen *et al*.

Due to the strong influence of the gut microbiota on fish health, dominant bacterial species in the gut are strong candidates for probiotics. This study aimed to characterize the gut microbiota of channel catfish *Ictalurus punctatus*, largemouth bass *Micropterus salmoides* and bluegill *Lepomis macrochirus* to provide a baseline for future probiotic studies. The overwhelming dominance of the genus *Cetobacterium* in all species warrants further investigation into its role in the fish gut microbiota. This study provides the first characterization of the gut microbiota of three economically significant fish and establishes a baseline for future probiotic trials.
http://bit.ly/JAM_Larsen

Microbial Biotechnology

Construction of leaky strains and extracellular production of exogenous proteins in recombinant *E. coli*

Chen *et al*.

In this study, a strategy of the construction of leaky strains for the extracellular production of target proteins was exploited, in which the genes *mrcA*, *mrc*B, *pal* and *lpp* (as a control) from *E. coli* were knocked out using single- and/or double-gene deletion methods. The results demonstrated that the resultant leaky strains were genetically stable and had a similar growth profile in the complex media as compared with the original strain, and the secretory levels of target proteins into Modified R media from the strains with a double-gene deletion (up to 88.9%/mrcA lpp-pth) are higher than the excretory levels from the strains with a single-gene deletion (up to 71.1%/lpp-pth) and the host *E. coli* JM109 (DE3) (near zero). The highest level of extracellular production of Trx-hPTH in fermenters is up to 680 mg/l.
http://bit.ly/MBT_Chen

Discovering new bioactive molecules from microbial sources

Monciardini *et al*.

There is an increased need for new drug leads to treat diseases in humans, animals and plants. A dramatic example is represented by the need for novel and more effective antibiotics to combat multidrug-resistant microbial pathogens. Natural products represent a major source of approved drugs and still play an important role in supplying chemical diversity, despite a decreased interest by large pharmaceutical companies. Novel approaches must be implemented to decrease the chances of rediscovering the tens of thousands of known natural products. This review presents an overview of natural product screening, focusing particularly on microbial products.
http://bit.ly/MBT_Monciardini

Melissa McCulloch
Wiley-Blackwell

The sign test

The sign test is a simple non-parametric test which can be used on paired data, i.e., two related samples, matched samples or repeated measurements on the same sample. It was developed by Wilcoxon before the more powerful and familiar 'Wilcoxon signed-rank test' described in a previous StatNote (StatNote 4, Hilton & Armstrong, 2006).

StatNote 38

Introduction

The sign test is useful in three circumstances. First, where there is no scale available for measuring a variable but it is necessary to distinguish grades of merit. For example, a sample of observers compared two hospital wards for cleanliness and therefore, possible microbial contamination, by assigning one as cleaner than the other. If such rankings are made by a random sample of observers, inferences can be made about the ranking in the population from which the sample was drawn, even though no parameters describing the distribution of the data can be established or even assumed. Hence, if there was no effective difference between the two wards, it would be expected that half the observers on average would rank each ward as 'better' than the other. Second, the sign test can be used as a simple non-parametric test to compare two paired samples using any continuous data when it is necessary to avoid the assumption of normality. Hence, previous StatNotes (StatNotes 35–36, Hilton & Armstrong, 2013, 2014) have described scenarios in which microbiological data are unlikely to be normally distributed and in which the sign test may be appropriate. Third, the sign test can be used as a quick substitute for either the paired 't' test (StatNote 3, Hilton & Armstrong, 2005) or Wilcoxon signed-rank test (StatNote 4, Hilton & Armstrong, 2006).

This StatNote describes the use of the sign test with reference to two scenarios: (1) to compare the cleanliness of two hospital wards as assessed by a sample of observers and (2) to compare bacterial contamination on cloths and sponges from a domestic kitchen.

Scenarios

Cleanliness of a hospital ward

Twelve observers, selected at random, assessed the degree of cleanliness of two hospital wards (A and B) to identify the ward they considered to be cleaner than the other. The data are paired because each observer scored each pair of wards independently of the others. Each observer ranked the ward they considered cleaner as a '2' and the remaining ward as a '1', and the data are illustrated in Table 1. If there was no effective difference in the rankings between the two wards, then it would be expected that half the observers, on average, would rank each ward as cleaner than the other.

Table 1. *Assessment of the degree of cleanliness of two hospital wards by 12 observers. Is there a difference in the cleanliness of the two wards? (If a ward was considered cleaner than the other it was given a score of 2, the remaining ward a score of 1.)*

Observer	Ward A	Ward B	Sign of (B –A)
1	1	2	+
2	1	2	+
3	2	1	-
4	1	2	+
5	1	2	+
6	1	2	+
7	1	2	+
8	1	2	+
9	2	1	-
10	1	2	+
11	1	2	+
12	1	2	+

1 *Subtract each pair of scores, i.e., B - A*
2 *Ignore the magnitude of the difference, retaining only their signs*
3 *Add the number of '+' and '-' differences*
4 *For n < 20, take the smaller number of like signs (N) to Snedecor and Cochran (1980) Table A8 to obtain a P value*
5 *Hence, N = 2 and P < 0.05*
6 *If 'n' is larger than 20, calculate: $Z_c = 2.02$, P < 0.05*

Microbial contamination on cloths and sponges

To illustrate a second application of the sign test, we return to the scenario described in StatNote 4 (Hilton & Armstrong, 2006). Given the intrinsic structural and compositional differences between cloths and sponges, a study investigated if one material provided a more favourable environment for bacterial survival than the other. Data were obtained on the number of bacteria on a single pair of cloths and sponges sampled on 10 separate occasions and are summarized in Table 2. In this scenario, the data are paired because a single pair of cloths and sponges was sampled and compared on each occasion. Previously, these data were analysed using the Wilcoxon signed-rank test.

How is the test carried out?

To carry out the sign test, either the number of observers ranking each of the two wards as better is counted (Scenario 1), or with quantitative or data ranked on an ordinal scale (Scenario 2), differences between the members of a pair of values are replaced by '+' or '-', the size of the difference being ignored. If the sample size is small (n < 20), then the smaller number of like signs (N) is used as the test statistic. If two numbers of a pair are equal and their difference zero, they can be included as 0.5 in 'N'. Snedecor and Cochran (1980) give a table (Table A8) of the smaller number of like signs that is required for statistical significance using the sign test or the test can be made via most statistical software. If the sample size is larger than 20, then N is determined as for small sample sizes, and then either Z_c or chi-square (χ^2) is calculated as follows:

$Z_c = (|2N - n| - 1)/\sqrt{n}$ ..... (1)

Or alternatively

$\chi^2 = Z2_c = (|2N - n| - 1)^2/\sqrt{n}$ ..... (2)

Statistical significance is judged using either the 'Z' or 'χ^2' distribution respectively.

Interpretation

In the first scenario, 10/12 observers rated the cleanliness of the second ward as better than the first, and only 2/12 observers *vice versa*. Hence, the smaller number of like signs is 2 and this value is taken to Table A8 (Snedecor & Cochran, 1980). Hence, for n = 12 at the 5% level of probability (P), N should be 2 or less for statistical significance which indicates a significant difference between the wards, i.e., ward B was assessed as cleaner than ward A. To confirm the result and illustrate the use of the 'Z' distribution, using equation (1), $Z_c = 2.02$, which is greater than 1.96, the value of 'Z' which has to be exceeded at P = 0.05 for statistical significance.

In the second scenario, the smaller number of like signs is 1 and this value is taken to Table A8 (Snedecor & Cochran, 1980). For n = 8, this is sufficient for significance at the 5% level of probability. Hence, the sign test confirms the result of the Wilcoxon signed-rank test applied previously to these data (StatNote 4, Hilton & Armstrong, 2006) that sponges harbour more bacteria than cloths.

Table 2. *Comparison of bacteria on pairs of cloths and sponges sampled on 10 occasions (two dependent groups). Is bacterial contamination similar on cloths and sponges?*

Occasion	Cloth (A)	Sponge (B)	A – B	+/-
1	1×10^4	4.6×10^6	-4.5×10^6	-
2	3.3×10^7	9.8×10^7	-6.5×10^7	-
3	5.7×10^7	1.3×10^8	-7.3×10^7	-
4	1.9×10^7	1.3×10^8	1.11×10^8	-
5	1.2×10^4	6.0×10^2	$+1.1 \times 10^4$	+
6	8.8×10^2	4.7×10^7	-4.7×10^7	-
7	2.6×10^6	1.4×10^8	-1.14×10^7	-
8	3.3×10^7	1.2×10^8	-8.7×10^7	-
9	8.7×10^6	2.1×10^8	-2.0×10^8	-
10	7.6×10^7	1.1×10^8	-3.4×10^7	-

7 *Subtract each pair of counts A - B*
8 *Ignore the magnitude of the difference, retaining only their signs*
9 *Add the number of '+ 'and '-' differences*
10 *For n < 20, take the smaller number of like signs to Snedecor and Cochran (1980) Table A8 to obtain a P value*
11 *Hence, n = 8, N = 1 and P < 0.05*

Assumptions of the sign test

The sign test is popular with investigators because it makes very few assumptions regarding the data, *viz.* only that the differences between pairs are independent, come from the same type of population and that it is possible to rank the values in order. When using a 't' test, however, it is necessary to assume normality and a specific hypothesis, i.e., that the sample means are equivalent for both treatments. The Wilcoxon signed-rank test, in addition to the assumptions of the sign test, also assumes that although distributions do not have to be normal, they do need to be relatively symmetrical.

In sampling from a normal distribution, the efficiency of the sign test is approximately 65% of that of the paired 't' test. In addition, if the null hypothesis is false, and the means differ by an amount 'd', the sign test would require n = 18 pairs to achieve the same statistical 'power' as a 't' test carried out on a sample of n = 12, i.e., the sign test saves time, but at the expense of loss of sensitivity. Hence, the sign test makes fewer assumptions regarding the data, but if the data do fit the assumptions for either a Wilcoxon signed-rank test or paired sample 't' test, then these tests would be more powerful.

Conclusions

The sign test is an alternative non-parametric test to the Wilcoxon signed-rank test when testing the difference between two groups of paired data. It can be used on both discrete and continuous data, and is a rapid and easy method of assessing the difference between two groups. Nevertheless, it is less sensitive and has less power than the parametric 't' test or Wilcoxon signed-rank test but makes fewer assumptions regarding the data.

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A. C. Hilton *Pharmaceutical Sciences*
R. A. Armstrong *Vision Sciences*
Aston University, Birmingham, B4 7ET, UK.

*Sf*AM Spring Meeting 2014

MORNING Session

The theme for the Spring Meeting was **Control of infection: current status and future prospects**. It was held at the Sheffield Hilton Hotel on 30 April. This popular annual event attracted delegates from a variety of healthcare settings including NHS hospitals and the private healthcare sector. It was also relevant to biomedical science courses so there were many academic delegates too.

During the registration period there was an opportunity to visit the trade stands of the five exhibitors.

The morning session, which was chaired by S*f*AM's Treasurer, Steve Davies, consisted of four presentations representing different aspects of Infection Control.

In her talk **Antibiotic resistance: implications for infection control**, Helena Parsons, Sheffield Teaching Hospitals, addressed the challenges faced by medical microbiologists as they work alongside clinical colleagues. The most alarming current situation is the emergence of carbapenemase-producing Gram-negative bacilli.

The limited treatment options for these organisms demand stringent infection control measures, but these must be balanced with the effective delivery of activities such as rehabilitation. Cases from a spinal injuries unit were described to exemplify some of the issues. Cleaning of equipment such as cough assist machines and wheelchairs presented challenges and compromises were necessary.

Viruses and their infection control implications were addressed by Mike Ankcorn, Sheffield Teaching Hospitals. The scene was set by a 1948 video highlighting the risks of sneezing. Infection control is dependent on our understanding of transmission which in turn guides our use of interventions. Mathematical models of public health measures in US pandemics have shown that early interventions have an impact on mortality figures, but their success leads to them being removed and a second wave of higher mortality follows.

One of the conclusions of the 2011 Cochrane update was that face masks were the best physical intervention. In the UK, however, masks are not as acceptable as in the Far East and the Department of Health only recommends their use as part of a bundle.

Emma Bell, in her talk **Challenges for the antimicrobial application of bacteriophage technologies**, described the products of a spin-out company from the University of Strathclyde. They use corona discharge technology to irreversibly immobilize bacteriophages onto the surface of various materials. This overcomes some of the limitations of free bacteriophages, namely instability and lack of patent protection.

Two applications were described. Firstly, bacteriophage-coated sutures were compared with normal sutures for MRSA-infected wounds in rats. Healing was observed with the coated sutures but not the normal sutures. Secondly, bacteriophage-coated dressings were found to be effective for pig skin burns. In order to target the most common wound pathogens a cocktail of bacteriophages was used.

Surprisingly, 9 of the 17 respondents had been unaware that they'd had an infection

The most alarming current situation is the emergence of carbapenemase-producing Gram-negative bacilli

Although promising, there are regulatory challenges for the application of bacteriophage therapy and avoidance of the term 'bacterial viruses' may be necessary to facilitate their acceptance.

Patient experience of surgical infection was the final presentation of the morning session. Judith Tanner, De Montfort University, conducted a study involving interviews with patients from three hospitals who had surgical site infections (SSIs). Surprisingly, 9 of the 17 respondents had been unaware that they'd had an infection. A recurring theme was the misunderstanding that the symptoms were thought to be part of the 'normal' post-operative process. Healthcare professionals often downplayed their significance with such assurances as, "*Don't worry, it's not MRSA!*"

Despite the SSIs being associated with considerable psychological and financial costs, there was evidence of a high level of goodwill among patients, many of whom believed that they were to blame.

Louise Hill-King
Frimley Park Hospital NHS Foundation Trust

AFTERNOON Session

The afternoon session opened with a talk by **Stephanie Dancer**, entitled **How clean is my hospital?** With nosocomial infections an ever-increasing concern in the UK and antimicrobial resistance on the rise, hospital cleanliness is becoming an increasing focus in the fight against infectious diseases. Unfortunately, hospital cleaning has historically lacked a scientific approach and there are currently no objective standards for cleaning. This is in contrast to the food industry, where environmental monitoring of cleanliness is routinely performed by visual perception, ATP measurement or microbiological analysis. Studies have begun to show correlations between levels of cleanliness and infection rates in hospitals. However, the development of appropriate standards for hospital cleanliness is likely to require a major multi-centre study powered to correlate the application of standard procedures to reductions in infection rates, and currently this is some way off.

Marcel Jaspars then gave an overview of some large-scale approaches to identify novel natural products for microbial control and other applications. Natural products have unrivalled chemical diversity as well as a proven track record of success, particularly in the case of anti-infectives. To search for novel chemical structures, microorganisms have been recovered from extreme environments such as Northern Norway, the ocean depths or the hyper-arid Atacama Desert in South America. Already, a variety of novel chemical structures have been identified. Even though many of these only have relatively weak activity in biological assays, they may act as a starting point for further modifications leading to more effective drugs that are distinct from current classes of molecules.

New approaches to antimicrobial therapy are not restricted to small molecules and **Val Edwards-Jones** presented some of the promising alternatives. These range from bacterial interference using prebiotics or probiotics, to photodynamic therapy, essential oils and even the long-established approach of using maggots

AFTERNOON Session

Infection control is dependent on our understanding of transmission which in turn guides our use of interventions

for debriding wounds. There is increasing evidence that different antimicrobials can work synergistically to control microorganisms. For example, silver appears to potentiate the activity of many different antimicrobials. There is increasing awareness amongst politicians and the general public of the threat of antimicrobial resistance, and extra funding in this area will hopefully accelerate the development of improved strategies for the control of infections.

Martin Kiernan wrapped up the afternoon with some fascinating insights into the daunting task of preventing infections in hospitals. Infectious disease targets in hospitals have been a contentious issue, particularly since they are reinforced by heavy fines when they are not met. However, the recent evidence shows that these targets are having a huge impact. For example, the numbers of *Clostridium difficile* infections in many Hospital Trusts have reduced 10-fold since targets were introduced. Unfortunately, there are still problems with reporting and it is often difficult to trace the root cause of a hospital-acquired infection. The patterns of infection are continually changing and constant vigilance is needed, as well as improved methods for pinpointing the routes of transmission of infectious agents in hospitals. Overall, the afternoon session provided an optimistic message that improvements in hospital prevention measures and the development of new antimicrobial approaches should help to keep us armed in the war against infectious disease for some time yet.

Micropod

Nancy Mendoza grabs a few minutes with two of the speakers at the S*f*AM Spring Meeting in Sheffield for the micropod podcast. Judith Tanner tells us why bed jackets, wooly socks and clean showers are important elements of the fight against surgical site infections, and Marcel Jaspars has been looking for new compounds, including antibiotics, in some very unusual places. You can download the podcast here: **http://bit.ly/1qgrVH6**

Nick Jakubovics
Newcastle University

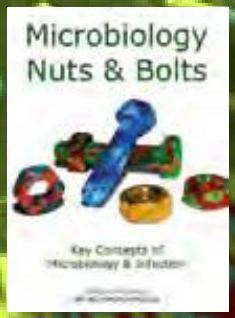
Microbiology Nuts and Bolts

David Garner
CreateSpace Independent Publishing Platform
(13 May 2013)

ISBN-13: 978-1484123911

Paperback £27.99 (RRP)

288 pages
Reviewed by Louise Hill-King



Microbiology Nuts and Bolts is a pocket-sized paperback book. The target readership encompasses doctors on the front line of medical microbiology and those studying for, or working, in allied professions such as nursing, pharmacy and biomedical sciences. The contents of the book will equip doctors to effectively manage their patients as it addresses the scenarios they face day-to-day on the wards, in clinics and in GP surgeries. Likewise, it will help those in allied professions understand their aspect of the subject within the wider context of medical microbiology as a whole.

The accessible style of writing and use of tables and bullet points presents the information clearly in a digestible form. Many of the tables can be used as standalone quick-reference tools. Indeed, the two figures containing the most critical information are reproduced at the very end of the book so they can be accessed even more quickly: a "Golden Hour" management flowchart for adult sepsis and a table summarizing guidelines for commonly used first-line antibiotics for a range of infections.

The main body of the book is divided into six sections: ***Basic Concepts***, ***Microbiology***, ***Infection Control***, ***Clinical Scenarios***, ***Antibiotics*** and ***Emergencies***.

The *Basic Concepts* chapter covers issues such as colonization, normal flora and an introduction to diagnosing infections.

The *Microbiology* chapter addresses the importance of completing laboratory request forms and provides information regarding appropriate samples and sample containers. A particularly useful section of this chapter gives guidance regarding interpretation of laboratory results.

The *Infection Control* chapter outlines the use of personal protective equipment and best practice for when and how to use side rooms. It also describes control measures for specific problems such as TB, *Clostridium difficile*-associated disease (CDAD), MRSA and viral haemorrhagic fever (VHF).

The *Clinical Scenarios* chapter is subdivided according to the anatomical sites which are affected: *Respiratory Infections; Head and Neck Infections; Urogenital Infections; Skin, Soft Tissue, Bone and Joint Infections; Gastrointestinal Infections* and *Other Infections*. Each subsection covers clinical features, causes, appropriate investigations, treatment and prognosis.

The *Antibiotics* chapter provides lots of detailed information on individual agents as well as guidelines for empirical therapy. It also explains therapeutic drug monitoring and the detection of antibiotic resistance.

The final chapter on *Emergencies* summarizes the application of Early Warning Scores for categorizing patients according to the severity of various parameters. Specific emergencies are then described in turn, including sepsis, meningitis, spinal epidural abscesses and toxic shock syndrome (TSS).

Throughout the book, common mistakes and myths are highlighted in coloured textboxes to help readers recognize and avoid pitfalls in everyday practice.

I recommend this book as an invaluable resource for junior doctors seeking to build on their medical training by furthering their knowledge of medical microbiology. Similarly, it would be of great benefit to BSc and MSc students of microbiology and other related disciplines who wish to broaden their understanding of infection.

14 January 2015, The Royal Society, London

*Sf*AM Winter Meeting

14 January 2015

10:00 – 10:30	**Tea, coffee and registration**
Chair:	Christine Dodd, *Sf*AM *President*
10:30 – 11:15	**The Denver Russell Memorial Lecture:** ***Vibrio vulnificus*: a killer lurking on our beaches?** James Oliver, *University of North Carolina, USA*
11:15 – 11:50	**Urban UK flood water: microbiology and much, much more** Lorna Fewtrell, *Aberystwyth University, UK*
11:50 – 12:25	**Potential microbiological risks in recreational water activities** Frances Lucy, *Institute of Technology, Sligo, Ireland*
12:25 – 13:30	**Lunch**
Chair:	*TBC*
13:30 – 14:05	**Contamination of bivalve shellfish with enteric viruses** David Lees, *Centre for Environment, Fisheries and Aquaculture Science, Weymouth, Dorset, UK*
14:05 – 14:40	**Microbial risks associated with spring waters and private supplies** Paul Hunter, *University of East Anglia, UK*
14:40 – 15:00	**Tea and coffee**
15:00 – 15:35	**Biofilm problems in dental unit water systems and its practical control** David Coleman, *University of Dublin, Ireland*
15:35 – 16:10	**Pseudomonas in hospital waters** Jimmy Walker, *Public Health England, UK*

Water, water everywhere
but is it safe?

MEMBERSHIP Benefits & Options

Benefits

The Society for Applied Microbiology is the voice of applied microbiology within the UK and was founded in 1931. Society Members play a leading role in shaping the future of applied microbiology, and enjoy many benefits, including:

- The opportunity to apply for one of our many grants or funds.
- Eligibility to win any of our awards or nominate a candidate for the S*f*AM Communications Award.
- Access to our five peer-reviewed journals: *Journal of Applied Microbiology* (JAM), *Letters in Applied Microbiology* (LAM), *Environmental Microbiology*, *Environmental Microbiology Reports* and *Microbial Biotechnology*.
- Free access to the entire collection of digitized back files for JAM and LAM dating back to 1938.
- A topical quarterly magazine, *Microbiologist*.
- Substantially reduced rates for attendance at S*f*AM meetings and conferences.
- Networking with worldwide professionals in over 80 countries
- Access to private Members' area of the S*f*AM website.
- Monthly email bulletins with the latest news from S*f*AM.
- Invitation to the annual *Environmental Microbiology* and *Journal of Applied Microbiology* lectures.
- Fostering cross disciplinary research.
- A 35% discount on the extensive Wiley-Blackwell collection of titles.

Detailed information about all these benefits and more can be found on the Society website at: **www.sfam.org.uk/membership**.

GRANTS & AWARDS

Many grants, awards and prizes are available to Members including the W H Pierce Memorial Prize and prizes for student oral presentations and posters at the Summer Conference. In addition to these substantial awards, the Society has funds to assist Members in their careers as microbiologists. These include the President's Fund, Conference Studentships, Sponsored Lecture Grants and the popular Students into Work Scheme.

Full details of all the Society's grants and awards, together with application forms, can be found on the website at **www.sfam.org.uk/grants**.

JOURNALS

The Society publishes two monthly journals: *Journal of Applied Microbiology* and *Letters in Applied Microbiology*. We also produce this quarterly colour magazine, *Microbiologist*, which contains features, topical news stories and full details of our meetings. The Society is also a partner with Wiley-Blackwell in the monthly journals: *Environmental Microbiology*, *Environmental Microbiology Reports* and *Microbial Biotechnology*. See more at **www.sfam.org.uk/journals**.

All Full and Student Members receive free access to the online versions of the Society's journals, and can also submit papers to our journals via an online submission service.

MEETINGS

We hold three annual meetings: the Winter Meeting is a one-day meeting with parallel sessions on topical subjects; the Spring Meeting is a one-day meeting tailored for personnel in clinical microbiology; and the Summer Conference is held every June/July and comprises a main symposium, a poster session, the AGM and a lively social programme. All Members are invited to our prestigious annual lectures held to commemorate the success of two of our journals: *Environmental Microbiology* and the *Journal of Applied Microbiology*. We also hold *ad hoc* meetings on topical subjects and enter into joint ventures with other organizations on topics of mutual interest.

WEBSITE

www.sfam.org.uk is the best source of detailed information on the Society and its many activities. It has a fully interactive Members-only area (**www.sfam.org.uk/membersonly**) where you can find archive issues of *Microbiologist*, exclusive S*f*AM documentation and much more.

Membership OPTIONS

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.

Full Student confers the same benefits as Full Membership at a specially reduced rate for full-time students not in receipt of a taxable salary.

Associate is only open to those with an interest in applied microbiology without it being a prime aspect of their job. For example, school teachers and those taking a career break, on maternity leave, or working temporarily in other areas. It does not provide access to any journals or Society grants and awards.

Honorary membership of the Society is by election only and this honour is conferred on persons of distinction in the field of applied microbiology. Honorary Members have access to our online journals.

Retired is available to Full Members once they have retired from their employment. Retired Members are entitled to all the benefits of Full Membership except grants and access to the Society's journals.

eAffiliate: this category of membership is open to microbiologists residing in Band I developing countries and is free of charge. It is an online only membership and provides access to the eAffiliate bursary only.

eStudent: this category of membership is open to undergraduate students only. It is an online only membership and is free of charge. This category of membership does not provide access to the Society's grants or journals.

Corporate is open to all companies with an interest in microbiology. Corporate Members benefits include:

- Quarter page advertisement in each issue of *Microbiologist* (which can be upgraded to a larger size at discounted rates).
- The opportunity to publish press releases, company news, etc., in each issue of *Microbiologist*.
- FREE banner advert on the Society website with a direct link to your company site.
- Up to three Members of company staff attending Society meetings at Members' rate (this means a 50% discount on non-Member registration rate).

Join us!

You can apply for membership online (**www.sfam.org.uk/join**) or offline. To apply offline, please contact the Membership & Finance Co-ordinator, Julie Wright on **+44 (0)1234 326846**, or email **julie@sfam.org.uk**.

Membership CHANGES

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

AUSTRIA
S. Hedge

BELGIUM
D. Debecker
S. Lebeer
A. Strens

BRAZIL
M. Ishii

BURKINA FASO
Y. Maiga

CANADA
S. Memon

DENMARK
J. Kjeldgaard
K. Krogfelt
A. Moodley

EGYPT
S. A. Selim

FRANCE
C. Henri
A. Jestin
S. Lozach

GERMANY
O. Sammra
D. Tischler

GHANA
F. K. Bright

GREECE
D. Komodromos

HUNGARY
T. Magyar

INDIA
S. Pattnaik
P. Raghunathan
N. Rokana

IRELAND
A. K. Allen
M. McElligot
C. O'Donovan
K. Ward

MALAYSIA
D. Benacer

MALTA
K. Montebello

MEXICO
A. Garcia-Heredia
P. I. Martinez
F. G. Moreno
J. Perez Garza
S. Simental Magana
J. C. Suarez

NIGERIA
L. Abia-Bassey
O. O. Adeogun
A. E. Adeyemo
O. Agwu
S. Akagha
S. B. Akinde
A. Asuquo
F. C. Chieze
S. K. Dike
S. Dimo
E. E. Duru
J. Ekpete
F. O. Ekundayo
C. I. Eleazar
U. Eze
H. Ezeudo
S. C. Eziuzor
F. O. Faro
O. Fawole
T. Femi-ola
I.-A. Ibangha
A. Iluyomade
Y. O. Isa
T. Lawal
N. I. Lerum
P. E. Martins
I. A. Mbachu
M. C. Mbagwu
I. A. Ndubuisi
V. Njoku
O. P. Nkpecha
I. Nsa
J. Nweze
J. A. Nweze
A. Obayiuwana
H. O. Ocean
A. T. Odeyemi
C. K. Odoh
C. Ojobor
O. Okafor
T. R. Omodara
M. O. Omoigberale
P. Omojasola
J. Onoja
R. Owuna
I. P. Udoh

NORWAY
J. Jarp

PAKISTAN
N. Haider
A. I. Khan

POLAND
E. Bilska-Zajac
H. Korpysa-Dzirba
I. Kozyra

PUERTO RICO
M. Andino Norat
E. Colon
T. Goire
R. Irizarry-Caro
J. Irizarry-Caro
J. Melendez
D. Rodriguez
G. Sanz
L. Trajillo

SOUTH AFRICA
A. Badmos
N. F. Tanih

SPAIN
M. C. Porrero
M. Ugarte-Ruiz

SRI LANKA
V. Liyanapathirana

SWEDEN
S. Borjesson
A. Lindberg

THAILAND
J. Salje

THE NETHERLANDS
A. Brahma
M. Heijne
S. Jahfari
A. Van de Giessen

UK
H. Abdule
S. Ahmed
M. Alanazi
R. Al-Neama
L. Ball
J. Bereza
S. Brindley
J. Brookes
K. Brown
L. Calvert
F. Cawkell
D. Cook
A. Correia
L. Cullen
A. Dapchi
S. Davis
D. Davis
N. Dawit
S. A. Diaz
M. Dudek
J. C. Ekediegwu
G. Eltringham
L. Etunyi
L. Fahmy
H. Fallatah
S. H. Farnham
P. B. Flynn
C. Frapwell
E. Fraser
R. C. Furniss
A. Gates
M. Giles
S. Gilmore
L. C. Gordon
C. Heller
L. Hink
A. Holland
G. P. Hunt
D. Ibrahim
R. Ifeanyi-Dibie
T. Iqbal
M. D. Johnston
P. Joyce
B. Kelan
J. Kosciuch
D. Kumar
D. Leybourne
J. Mattock
L. McKechnie
A. McMahon
J. Megaw
A. Menendez Rivero
A. J. Montgomery
E. J. Morgan
D. Morrison
J. Mundy
C. Murrell
D. Nichols
C. A. Okoye
A. Omotola
O. Onamade
C. O'Reilly
F. H. Osman
L. Owen
M. Parr
A. Pearson
R. K. Pendaer
L. Perrin
V. Pooja
R. Pothi
J. Powles
M. N. Radzuan
J. Roe
A. Saleh
A. Sawko
A. D. Scimone
R. Seipke
R. Shah
P. Shibu
S. Sinebe
J. Spencer
N. R. Stanley-Wall
G. Stewart
A. Talbot
N. Temperton
L. Vieira Goncalves
A. Ward
C. Weerasinghe
Y. Zhu

USA
E. Bryant
L. Christopher
J. Cisneros
M. DeGear
L. Ellis
G. Flock
M. Folmsbee
S. Garrett
E. Goluch
T. Hassan
D. Kamath
P. Kochan
R. Kumar
B. Lambert
N. Laskar
S. Markland
S. McBride
J. Millar
S. Mooyottu
S. Morrison
S. Patel
S. Post
L. Powell
B. Quinones
D. Ramjee
R. L. Rodriguez
K. Ross
J. Skoglund
J. Stamos
V. Thulsiraj
A. Zimmer-Faust

CORPORATE
Bruker UK Ltd

2014 *Sf*AM AGM

The 83rd Annual General Meeting of the Society for Applied Microbiology was held on **Wednesday 2 July 2014 at 17:50 at The Grand Hotel, Brighton**

Present:

34 members attended the AGM. This included:

President, Martin Adams (MA)
General Secretary, Mark Fielder (MF)
Treasurer, Steve Davies (SD)
Meetings Secretary, Andy Sails (AS)

In attendance:

Philip Wheat (PW)
Lucy Harper (LH)
Nancy Mendoza (NM)

1. Apologies for absence

Susan Passmore
Basil Jarvis

2. 82nd Annual General Meeting

The minutes of the 82nd Annual General Meeting held in Cardiff in 2013 were published in the September 2013 issue of *Microbiologist*. They were approved and accepted by those present.

Proposed: Sally Cutler
Seconded: Christine Dodd

3. Matters arising from the previous minutes

None

4. Report of the Trustees of the Society 2013

The President noted the success of the Society during the previous year, particularly with respect to membership.

The General Secretary reported an increase in membership of 4% and a trend for innovation in the Society.

MF also noted that there has been good uptake of grants and this is the end of the first year of the first PhD Studentship. He announced that S*f*AM will soon begin a new round of applications for the PhD studentship.

MF gave a summary of public engagement activities.

MF acknowledged the death of Louise Fielding – a much valued member of the Society.

The Meetings Secretary gave a summary of activities and thanked the Events Organizer, Sally Hawkes. AS requested ideas for future meetings.

The Treasurer reported that the Society's value has continued to rise to over £7M by 31 December 2013 – this is thanks to the hard work of Editors and the stewardship of the Society's portfolio. At the same time, the Society has continued to subsidize meetings and award increasing numbers of grants.

SD acknowledged the impact of a raised profile of the Society, thanks to Communications activities.

Bernard Dixon (BD) asked a question of the Trustees: has S*f*AM considered involvement with the Edinburgh Science Festival? BD suggested is a good opportunity for S*f*AM's public engagement activities. All agreed to consider this.

5. Adoption of the Annual Report 2013

Copies of the Annual Report of the Society for 2013 had been distributed previously.

Proposed: John Rigarlsford
Seconded: Sam Law

The Annual Report 2013 was duly adopted.

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 President, Professor Christine Dodd

MA highlighted the end of his term and thanked the Society staff and officers for an enjoyable term.

After serving a year as Vice President, Professor Christine Dodd was nominated as the next President. The meeting agreed unanimously with this decision.

Proposed: Arthur Gilmore
Seconded: Tim Aldsworth

Professor Christine Dodd was duly elected.

CD highlighted the good position the Society is in financially, and in terms of membership, and new activities, following MA's term of office.

8. Nomination and election of the General Secretary, Mark Fielder

MF had come to the end of his term and was eligible to stand for a further year.

Proposed: Andy Sails
Seconded: Sally Cutler

Mark Fielder was duly elected.

9. Nomination and election of the Meetings Secretary, Andy Sails

AS had come to the end of his term and was eligible to stand for a further year.

Proposed: Steve Davies
Seconded: Mark Fielder

Andy Sails was duly elected.

10. Nomination and election of the Treasurer, Steve Davies

SD had come to the end of his term and was eligible to stand for a further year.

Proposed: Christine Dodd
Seconded: Andy Sails

Steve Davies was duly elected.

11. Nomination and election of new Ordinary Committee Members

There had been two nominations for two vacancies:

Tim Aldsworth

Proposed: Christine Dodd
Seconded: Cath Rees

Linda Thomas

Proposed: John Threlfall
Seconded: Mark Fielder

Both were unanimously elected.

10. Any other business

None

The meeting concluded at 18:10

ROLLING with the changes

When I graduated from high school and went to University I had no desire to study biology; in fact, I disliked it very much. This may have been because at my high school the football coaches (American football that is) taught biology and didn't do a very good job of it. When I entered the University of Louisiana, I was a chemistry major. Like all science majors I was required to take introductory biology classes, the first two of which were taught in a large stadium-styled lecture hall with about 350 other students and my dislike for biology was confirmed. In my second year I was required to take an elective biology class and I chose microbiology, which seemed to be the least boring of the bunch. When I enrolled in the class I had no idea that my life would be forever changed. I was fascinated by microbiology especially microbial physiology, genetics and pathogenesis. After taking this class I was hooked and switched my major from chemistry to microbiology. However, I did go on to receive a minor in chemistry.

After graduating with a B.S., I found a job working at the U. S. Food and Drug Administration (FDA) in Atlanta, Georgia where I tested food, drugs and cosmetics for microbial contamination. The FDA had just hired about 10 new microbiologists, all of whom were fresh out of university, so it made for a collegiate place to work. I learned a lot about regulatory work, especially about the mountain of paperwork that went along with it. Most of my time was spent on the food end of the lab, examining various types of fruits, nuts, spices and seafood for pathogens such as *Salmonella*, *Listeria* and *Campylobacter*. It was a really good job, and it paid very well for a new graduate, but after a few years I wanted to do something more than the repetitive "grind and find", in particular I wanted to do

The only thing I know for certain in life is that it is going to change; you can either roll with it or it will roll over you

US Department of Agriculture (USDA), Agricultural Research Service

research. I spoke with our scientific advisor, who came from the University of Georgia once a month to give us lectures, about leaving my job and going to graduate school. With his help I applied to the University of Georgia and was accepted into the Masters programme. I was a little nervous about leaving my good-paying Government job and testing my luck at graduate school, but my supervisor told me that I could take a two-year leave of absence and if graduate school didn't work out I could come back to work: how could I lose?

At the University of Georgia I enrolled in the Masters programme and began to study the oxidation of mercury metal vapour by peroxidases produced by *E. coli*. After two years of graduate school I realized that I was not going to go back to the FDA and enrolled in the PhD program. For my PhD I studied the plant pathogenic bacterium *Ralstonia solanacearum*, the aetiological agent of a wilt disease in over 100 species of plants including the economically important crops tomato, potato and chillies. I was specifically interested in virulence factor production *in planta*, especially an extracellular polysaccharide (EPS1) that is produced in the plant's xylem and induces wilting. I developed an enzyme linked immunosorbent assay (ELISA) against EPS1 and quantified the levels of it produced in resistant and susceptible tomato cultivars. I liked being a graduate student, but one day my advisor informed me that I was graduating so I began to look for a Postdoctoral fellowship. I found one at California Pacific Medical Center in San Francisco, California studying the pathogenesis of the mycobacteria, specifically *M. avium* and *M. tuberculosis.* I had to learn quite a bit about the human host, which is obviously very different from tomato plants. However, the basis of pathogenesis was essentially the same; the bacterium had to attach, invade, evade the host defences, produce virulence factors, etc.

After about three years as a postdoc I wanted to find a "real job" and happened to find one about 15 miles away at the US Department of Agriculture (USDA), Agricultural Research Service. They were interested in *M. avium* subsp. *paratuberculosis* (MAP), the aetiological agent of bovine paratuberculosis, a gastrointestinal disease which causes wasting and eventually death in infected cattle. The fact that MAP was shown to be secreted in milk, resistant to pasteurization and was linked to Crohn's Disease in humans was of great concern. However, after about three years the USDA was no longer interested in MAP. The new concern was the transfer of pathogens from animal waste to crop plants, either by use as a fertilizer or from storm runoff from confined animal feeding operations (CAFOs) to crop fields. So my focus changed once again and I began to study different low-cost methods to treat animal wastes to eliminate pathogens. A side project from these studies was the use of animal wastes to produce bio-fuels and to reduce the levels of volatile organic compounds that were emitted from them. These projects lasted for about six years until the USDA was no longer interested in them and wanted to shift my lab's efforts to studying the contamination of crop plants, such as almonds and cantaloupes by *Salmonella*, which I am studying now. My time at the University of Georgia studying wilt disease of tomato has served me well with this new assignment. Even though *Salmonella* does not cause a disease in the plants, it does form intimate associations with plants that are only now beginning to be elucidated.

While I am sure that in another four or five years there will be a new topic that will be of interest to the USDA and they will once again change my research focus, I look forward to it. Every time I have changed my research programme I have learned more and more fascinating aspects of microbiology. The only thing I know for certain in life is that it is going to change; you can either roll with it or it will roll over you.

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Jeff McGarvey

The PECS Committee

SUMMER Conference summary

This year at the Summer Conference in Brighton, there was plenty to be involved in. On the first night of the conference, we held the ice-breaker session entitled 'Roll with it', where attendees were asked to pull off several squares of toilet roll – the more pieces, the more the person had to say about themselves. It was a great way to start the conference, to make new friends, and find out about each other. After the ice-breaker there was the Quiz Night, which is always hilarious but extremely competitive. This year, finally, one of the PECS teams managed to win a place, so we already cannot wait for next year!

On the second day of the conference we always have a session dedicated to the student and early career scientist delegates. We have listened to your previous feedback, and this year's session was designed to enhance your CV skills. The 'CV clinic' was a vibrant, energetic and interactive session where delegates had an opportunity to present their CV to experts from academia, industry, science communication and career specialists. The session received excellent feedback from the attendees. We would like to take this opportunity to say a big thank you to all the experts for their time and dedication during the session.

As always, we encourage all students and early career scientists to relay any ideas that they may have for future events to our Committee; the best way to achieve this is by joining our steering group, which you can do by emailing **pecs@sfam.org.uk.**

After two very intense days of great science, everyone was invited to come along to watch a World Cup football game on Brighton beach. It was a time for much needed relaxation and social interaction. On Wednesday afternoon student delegates presented their work; as usual, the standard of talks was extremely high and a little birdie told us that judges struggled to decide who was the best, so a very well done to all who presented their work at the conference! We hope that you had a great time at this conference, and will join us at our next one in October.

UPCOMING EVENT **PECS RESEARCH CONFERENCE**

Thursday 13 October 2014 at the Royal Society of Medicine, London

Following the success of our two previous autumn meetings, the Postgraduate and Early Career Scientist Committee are proud to announce the third annual Research Conference. This year we are focusing on "Current and Advanced Methods in Microbiology". As always we will have a selection of mini lectures from renowned scientists in the field, but also give the floor to upcoming scientists to disseminate their research. This is a perfect opportunity to share common ideas and explore new ones. There will also be a trade show – a chance to explore new technology and meet with different laboratory suppliers.

Of course you can attend this conference just to observe, but if you are keen to share your current research, the deadline for abstract submission is 12 September. As previously, your area of interest does not have to be related to the topic of the conference. Be sure to state whether you would like to participate in an oral or poster presentation format; all details can be found on the S*f*AM website under the PECS tab. There will be prizes for the best oral and poster presentations so be sure to bring your A-game!

We really look forward to seeing you then, and hope that you find the day as enjoyable as it is educational and informative.

2014 Public Engagement Grant article

The University of Manchester hosts the Science Spectacular every year, as part of National Science and Engineering Week. This year, I decided that microbiology deserved to feature in the line-up of stands and activities for the first time and applied for an *Sf*AM Public Engagement Grant. We lured in the crowds with the promise of seeing the bacteria on their teeth, cuddly microbes and nitrile gloves – surprisingly effective!

We offered three different activities, all designed to explore the impact of microbes on our lives. The first activity was 'Pin the Microbes on the Body'. We used fluffy toys of four human commensals, a poster with different body sites labelled and flashcards with information about the habitats and food sources these microbes need to flourish. The aim for the students was to work out where on the body each microbe commonly lives, introducing the students to the idea that the human body is a collection of habitats for microbes.

The second activity was to blow up a balloon using yeast. This introduced the students to the concept that microbes can be incredibly useful to us. It was also a way of getting students to understand that microbes are cells, they need food and oxygen, respire and produce waste gases. The experiment got students thinking about hypotheses and collecting data. They drew two marks on their balloons, and then measured the distance between them over time. We were amazed at how many students came back to check up on the progress of their balloons and record the expansion. It wasn't long before we had balloons all over the table, slowly filling up and growing. There was, of course, the odd bubbly, yeasty accident, but most of the balloons and bottles behaved themselves.

The most involved of the experiments we ran was to stain bacteria from the teeth and look at them under the microscope. Once the students' clothes and hands were protected, they collected a sample of microbes from their teeth using sterile swabs and fixed them onto a slide. We did a series of stains and washes, and then each student had the opportunity to see the bacteria from their teeth. Every single student claimed to have brushed their teeth that morning, so it was great to have the chance to chat about how quickly some bacteria can grow, and how important dental hygiene is. The wonder and amazement on the students' faces when they saw their bacteria under the microscope was really exciting to see.

Although the fair was exhausting, it was definitely worth the effort. We had some great feedback from attendees. I'd like to thank Danielle Weaver, Emily White, Adrian Jervis, Nader Masri and Edward Horton, and the team who run the science fair every year in particular Emma Lewis for her support.

Helen Frost
Manchester University

STUDENTS into WORK Grant report

Am I eligible – can I apply?

Yes, if you are a Full Member who can offer an undergraduate microbiology student the chance to obtain work experience. If you would like to read about the experience of a student who has benefited from this grant, you can do so, below.

For further information see **www.sfam.org.uk/siw**

Investigating the effect of cold recovery on the isolation of *Listeria*

As a second-year BSc Food Microbiology student at The University of Nottingham, I was awarded an SfAM Students into Work Grant. This funded an eight-week project in an Advisory Committee on Dangerous Pathogens (ACDP) Containment Level 2 laboratory within the microbiology and good safety group. My project was to follow-up some results gained by a previous MRes student, to see if chilling foods after a heat treatment would allow sub-lethally damaged *Listeria monocytogenes* cells to recover from the effects of the heat shock. This would be particularly important in the food industry as many pasteurized foods are refrigerated after heat treatment for transportation and storage. If bacterial levels are tested directly after pasteurization, and the heat-injured cells have not yet recovered, then the measured levels of bacteria may be deemed acceptable. However, if there is subsequent bacterial recovery and growth during refrigeration then the product may actually contain unacceptable levels at point of purchase. For *L. monocytogenes*, its presence in ready-to-eat foods, even in very low numbers is not acceptable, because of the organism's ability to grow under refrigeration conditions. *L. monocytogenes* causes foodborne illness, typically experienced by healthy adults as self-limiting gastroenteritis with vomiting, abdominal pains and diarrhoea. However, in the immunocompromised, it results in the highest mortality rate of all the common, foodborne, bacterial pathogens.

Natalie working in the Microbiology and Food Safety Lab at Nottingham

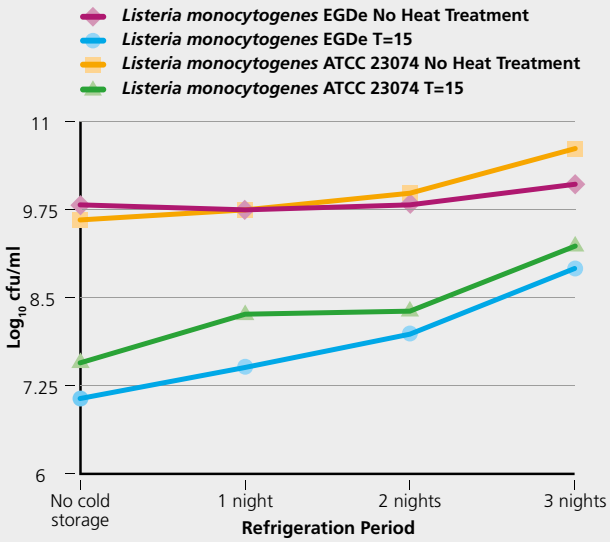
To begin the project, the *Listeria* strains were recovered from frozen culture and characterized to confirm that the strains were the ones required. As a new researcher this gave me a chance to learn how to culture the bacteria and how to perform molecular identification using a multiplex speciation PCR test. Two of the strains that were chosen were *L. monocytogenes* ATCC 23074 and *L. monocytogenes* EGDe, which are well-studied lab strains that belong to the main two serotypes that are known to cause most outbreaks of listeriosis. In addition, a food factory isolate of *L. monocytogenes* was chosen to represent a strain that had recently been recovered from the environment rather than being a lab-adapted strain.

After this, experiments were designed to investigate the effect of chilling on the recovery of *Listeria*. First, the conditions to simulate pasteurization were established. Since milk is a commonly pasteurized product where

Listeria is found, and it is also a product that is chilled after pasteurization, this was chosen as the test food substance. UHT milk was used since this could be spiked with *Listeria* without a high background of other bacteria interfering with the results. To heat treat the bacteria, 1ml of semi-skimmed UHT milk inoculated to an approximate level of 108 cfu/ml was transferred into a glass vial and then the samples were heated in a water bath to represent high-temperature short-time pasteurization (72°C for a maximum of 30 sec). When the temperature inside the vials reached 72°C, samples were taken out at intervals and the number of bacteria surviving immediately after the treatment were determined using viable counts, on two different types of non-selective agar: brain heart infusion (BHI) agar and tryptone soya yeast extract (TSYE) agar.

To determine whether chilling the samples would allow the *Listeria* to recover and the number of cells detected to increase, the milk samples were refrigerated and then the viable counts repeated after either a short incubation in the refrigerator (90–270 min) or after either 24 hours or 48 hours incubation at low temperature. In addition, samples were plated on Oxford agar which would be expected to have a detrimental effect on the recovery of heat-injured cells due to the selective agents in this type of agar.

On average, the heat treatment produced a 2.3 $\log_{10}$ cfu/ml reduction in *Listeria* counts on TSYE agar plates. Interestingly, on samples tested with no refrigeration, lower counts were seen on BHI agar compared with TSYE agar whereas, after 24 hours or more of refrigeration, there was no significant difference in counts on these two agars. This result suggests that the more nutrient rich agar (BHI) puts some stress on heat-injured cells, and that TSYE agar aids the recovery of damaged cells. The change in counts recorded on the two agars after refrigeration suggests that the cells were damaged. However, after refrigeration the cells recovered and therefore were able to grow equally as well on both agars. There was shown to be no significant recovery of cells when samples were refrigerated for 270 minutes or less ($p = > 0.05$) for all three strains tested. However, as seen in Figure 1,



Viable counts of *L. monocytogenes* EGDe and *L. monocytogenes* ATCC 23074 on TSYE agar, after either no heat treatment (◆, ■) or 15s at 72°C (◆, ■) with varying lengths of refrigeration. Viable counts calculated using the Miles-Misra method.

Figure 1. *Viable counts of Listeria monocytogenes EGDe and Listeria monocytogenes ATCC 23074 on TSYE*

when refrigerated for 24 hours, there were significant increases in the numbers of *L. monocytogenes* ATCC 23074 and *L. monocytogenes* EGDe, and potentially the increase in numbers could be accounted for by growth since *L. monocytogenes* is able to grow at refrigeration temperature. However, the increase in numbers was far greater than that of the non-heat treated controls, suggesting that this is recovery of injured cells rather than cell growth. Since most pasteurized foods are refrigerated for more than 24 hours, this study indicates the potential for injured bacteria (which are not detected by viable count), to recover during the chill storage and result in a risk to health.

Undertaking this research helped me to turn the theoretical knowledge I gained in my degree into practical experience, and expand skills both within and relating to my discipline. My thanks go to Benjamin Swift for his invaluable help in both scientific practices and familiarizing me with laboratory logistics. I would also like to thank Cath Rees and Christine Dodd for giving me the chance to take part in this project, their extensive knowledge of this area provided me with essential guidance in undertaking my research. Finally, thanks to SfAM for funding the project.

Natalie Barratt

FURTHER READING

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Corporate NEWS

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

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WASPs are being used by customers to test for safe levels of bacteria in a variety of food stuffs such as milk, dairy, fish, poultry and frozen food. It is also used in pharmaceuticals, cosmetics, the water industry and for mouth and skin bacteria.

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Lab M has launched µPrep™ Half Fraser Broth ISO (+FAC), the latest addition to the company's µPrep range of convenient, ready-to-reconstitute microbiological culture media. Designed for use in the isolation of Listeria spp. from foodstuffs, this complete primary enrichment broth is supplied sterile in a highly robust bag, which simply requires the addition of water. There is no mess and no autoclaving is required, ensuring the speedy preparation of 20 litres of complete Half Fraser Broth ISO (+FAC). The medium is based on the formulation of ISO 11290 and meets the performance requirements of ISO/TS 11133.

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To find out how µPrep™ Half Fraser Broth ISO (+FAC) can help streamline your laboratory's workflow visit http://bit.ly/LabMHFB.

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Leatherhead Food Research: Food Safety & Product Integrity

Leatherhead's food safety portfolio provides a comprehensive range of products and services to help food and drink companies maintain the highest possible standards of safety and stability in their products. We provide a broad array of microbiological food testing, analysis and consultation covering microbiological food safety, training and advice, and bespoke testing, if required.

Our main focus areas include:

- Shelf life and challenge testing (including *Cl. botulinum*)
- Microbial inactivation kinetics (to aid food processing)
- Molecular diagnostics including speciation of bacteria, yeasts and moulds, and horsemeat
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- Antimicrobial screening and alternative natural preservation technologies
- HACCP training and studies including troubleshooting and audits
- Biofilm study and control

- Method development and validation of rapid detection methods
- Validating and advice on cleaning and disinfection procedures

Additionally, our Food Safety & Product Integrity Department is supported by expertise from across Leatherhead. For example, we can check the safety/shelf life of a reformulation, whilst ensuring that it meets the required sensory and nutritional properties. We can also provide regulatory advice so that the product meets the required legislation for the countries in which is it sold.

Further Information

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New Accessions at NCIMB

Interesting new accessions to the National Collection of Industrial, Food and Marine Bacteria include bacteria discovered on a medieval wall painting in Austria and an extremely radiation resistant isolate from a Finnish paper mill.

Georgina muralis which has been added to the collection was discovered on murals dating as far back as the twelfth century in the Church of St Georgen, Styria, Austria. Prolonged dampness and salts have resulted in biofilms developing on several of the medieval church's paintings containing bacteria including Georgina muralis. Unfortunately over hundreds of years this has resulted in damage to the wall paintings, some of which depict St George famously battling dragons.

Deinobacterium chartae was isolated from a biofilm growing in the wire section of a Finnish paper mill producing folding boxboard. Bacteria growing in paper machines can result in numerous problems especially if the biofilms detach from the machines leading to holes and spots in the produced paper. Deinobacterium chartae could be commercially significant due to its extremely radiation resistant nature and may have applications to similar bacteria in the cleaning up of toxic waste and testing hypotheses about life in extreme environments, among other things.

For further information on depositing a strain with NCIMB, or to obtain these new strains contact Dr Samantha Law. s.law@ncimb.com

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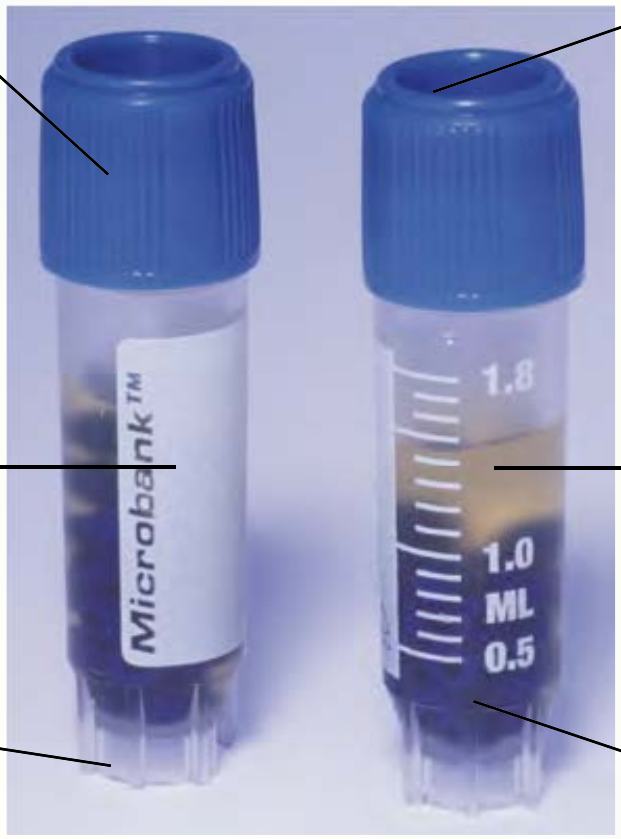
Large 2 ml vials with triple depth external threaded cap which reduces the possibility of contamination. Wider tube diameter provides more room for mixing to ensure beads are properly coated.

Available in five colours to provide laboratories with a system to colour-code different bacterial species.



Larger writing area allows for complete coding and reference data.

Specially formulated preservative ensures longer survival of fastidious bacteria and higher quantitative recoveries.

Industry-standard robust cryovial can withstand snap freezing with liquid nitrogen.

Chemically treated beads improve bacterial adhesion.

Microbank™ World Wide Performance Portfolio

Microbank™ has enjoyed many years of success as the method of choice for storage and retrieval of bacterial and fungal cultures. Extensive reference data are available from customers, centres of excellence, and reference collection sites around the world detailing up to 21 year's successful storage of an extensive range of cultures. Full details can be obtained in the Microbank™ World Wide Performance Portfolio available on the Pro-Lab website.

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