ANTIMICROBIAL RESISTANCE CENTRE

Inspiring innovation in AMR research through interdisciplinary and international engagements

MICROBIOME GOES MAINSTREAM

IMPLICATIONS FOR HEALTH WORLDWIDE

A research symposium

17th February 2017, 09:00-17.30

John Snow Lecture Theatre

Followed by reception in the South Courtyard

London School of Hygiene & Tropical Medicine

Keppel Street, London WC1E 7HT

LONDON SCHOOL of HYGIENE &TROPICAL MEDICINE

Microbiome Goes Mainstream: implications for health research worldwide

Research into the microbiome has accelerated and has shifted from the margins of science to the mainstream. What are the implications for health research worldwide? The Antimicrobial Resistance Centre at LSHTM is hosting a one-day symposium on the microbiome. Bringing together researchers from across different areas and disciplines in health research, we will consider recent developments in our interests and understandings of the microbiome, learn about ongoing research at LSHTM on the microbiome, and scope out directions for further research and collaboration.

Objectives

1. Facilitate cross disciplinary dialogue
2. Enhance methodology and expertise
3. Create new research ideas and collaborations

Programme

9.00-9.30am: Registration, Manson foyer. Fill out and put up speed dating cards

9.30am – 11am: Session 1: Scope for microbiome research in health. Chair: Dr. Lisa Dawson

9.30 – 10.00: Keynote speaker 1: Hannah Landecker, UCLA Institute for Society and Genetics: *Science Inside Out: Piecing Together New Developments in Epigenetics and the Microbiome*

Speed talks (5min each): Illustrations of microbiome research at LSHTM

Talk 1: Eleanor Riley: "The Baby Biome Study"

Talk 2: Matthew Chico: The effect of sulphadoxine-pyrimethamine or azithromycin on the vaginal and Intestinal microbiome of pregnant women in Tanzania and Malawi

Talk 3: Helen Brotheron – via skype: The skin and intestinal microbiome of neonates and mothers and the effect of early continuous skin-to-skin contact

Talk 4: Lucy Pembrey/Neil Pearce: Understanding asthma phenotypes

Talk 5: Brian Greenwood: The microbiome and epidemic meningitis in Africa

Talk 6: Laith Yakob: Role of soil transmitted helminth parasites in disrupting the microbiota-gut-brain axis during childhood development

Talk 7: Mike Hudson: A 40 Year-Old Prospective Study of Large Bowel Cancer Revisited

Talk 8: Suzanna Francis: The effect of vaginal microbiota on the host immune response among women at high risk for HIV in Tanzania

Talk 9: Sarah-Jo Sinnott/Ketaki Bhate/Sinead Langan: Antibiotics for Acne – Anticipating Apocalypse

11.00 – 11.30: Morning coffee

11.30-13.20: Session 2: Developments in microbiome research. Chair: Prof. Eleanor Riley

11.30– 12.00: Keynote speaker 2 – Lindsay Hall, University of East Anglia: *The Early Life Gut Microbiota*

Research talks (10min each)

Talk 1: Martin Holland: Ocular surface microbiome and trachoma

Talk 2: Ozan Gundogdu: Assessment of the influence of intrinsic environmental and geographical factors on the bacterial ecology of pit latrines

Talk 3: Richard Stabler: Skin microbiome of an astronaut in relation to the gravity counter measure skinsuit

Talk 4: Christina Gill/Janneke van de Wijgert: The role of vaginal microbiome in HPV and cervical cancer among women with HIV in Africa

Talk 5: Ernest DiezBenavente: Microbiome bioinformatic pipelines: from raw data to useful insight

Talk 6: Michael Lewis: Experimental visceral leishmaniasis: Investigating roles for the gut and its microbiota in disease progression.

Panel questions and discussion

13.15- 14.15: Lunch (speed dating boards will be in the lunch area – refectory)

14.15-16.15: Session 3: Applications and implications of microbiome research. Chair: Prof. Brendan Wren

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14.20-14.50: <u>Keynote speaker 3 – Adam Roberts, Liverpool School of Tropical Medicine:</u> ***Factors influencing the human oral microbiome***

Research talks (10min each)

Talk 1: Lisa Dawson: The effects of *p-cresol* on the gut microbiome

Talk 2: Chrissy Roberts: Polymicrobial screening in the context of programmatic control of trachoma in the Western Pacific.

Talk 3: Hilary Browne: Utilisation of anaerobic culturing to investigate bacterial sporulation within the human intestinal microbiota

Talk 4: Matthew Rogers: Microbiome of sand flies in relation to their vectorial capacity for leishmaniasis

Talk 5: Tony Fletcher: Microbiome and environmental pollutants: perfluoroalkyl substances and the gut microbiome

Talk 6: Ron Behrens: Gut Bacteria changes in Travellers and the association with morbidity and anti-microbials. (Gutback)

Panel questions and discussion

16.30-17.00: Afternoon tea break with speed dating

17.00-18.00: Session 4: Collaborative research on the microbiome at LSHTM. Chair: Dr. Clare Chandler

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17.00-17.30: <u>Keynote speaker 4 - Jamie Lorimer, Oxford University, Oxford Interdisciplinary Microbiome Project</u> ***Making microbes public: participatory and interdisciplinary approaches to the microbiome***

Discussion, the way forward

17.45- 19.00: Speed dating reception

Participants encouraged to meet and exchange ideas via message boards in the café

TITLES AND ABSTRACTS

Keynote Speakers

Keynote speaker 1:

Hannah Landecker, Director of UCLA Institute for Society and Genetics

Hannah Landecker uses the tools of history and social science to study contemporary developments in the life sciences, and their historical taproots in the twentieth century. She has taught and researched in the fields of history of science, anthropology and sociology. At UCLA she is cross-appointed between the Institute for Society and Genetics, and the Sociology Department. She is currently working on a book called "American Metabolism," which looks at transformations to the metabolic sciences wrought by the rise of epigenetics, microbiomics, cell signaling and hormone biology. Landecker's work focuses on the social and historical study of biotechnology and life science, from 1900 to now. She is interested in the intersections of biology and technology, with a particular focus on cells, and the in vitro conditions of life in research settings.

Title: ***Science Inside Out: Piecing Together New Developments in Epigenetics and the Microbiome***

Abstract: Our rapidly expanding knowledge of the microbiome is full of unexpected findings and continuing unknowns. This is exciting for biomedical science and its publics, and is producing new narratives with new microbial protagonists and a changed sense of the interrelationships between humans and microbes. This talk gives an overview of recent work on the role of the microbiome in human physiology, in particular the fate of microbial metabolites in human cells and their interaction with cell signalling and chromatin regulation. These scientific developments are placed in historical context, allowing us to see how these developments on the one hand break with longstanding assumed distinctions between genetics and metabolism, yet on the other hand strengthen other dominant explanatory frameworks inherited from twentieth century life science, such as signal transduction. This map of continuities with and departures from the recent past of biomedical science should provide a useful anchor for assessing the unfolding frontier of knowledge about the microbiome.

Keynote speaker 2:

Lindsay Hall, Senior Lecturer, University of East Anglia

Lindsay Hall qualified with a BSc (Hons) in Microbiology from the University of Glasgow in 2003. She went on to study for a PhD in Microbiology and Immunology at the Wellcome Trust Sanger Institute under the supervision of Prof Gordon Dougan. Her thesis focused on designing and creating mucosal vaccines against Mycobacterium tuberculosis and characterising mucosal immune responses (specifically Natural Killer, [NK] cells) induced after oral and intranasal immunisations. Her PhD was part funded through the EU sixth framework programme entitled Mucosal Vaccines Against Poverty Related Diseases

(MUVAPRED). In 2007 Lindsay took up a postdoctoral position at the Alimentary Pharambiotic Centre, University College Cork in Ireland. During her time in Cork, Lindsay moved into a new mucosal immunology area; focused on intestinal inflammatory disorders. Her research utilised experimental Ulcerative colitis and enteric pathogen models to understand the protective role of early mucosal immune responses (including NK cells) during acute intestinal inflammation. In Cork, Lindsay also started to work on the bacterial communities that inhabit the gut, termed the microbiota. Specifically understanding the role of individual members (i.e. Bifidobacterium) and their products (exopolysaccharide capsules) in modulating the critical commensal-host interaction. She returned to the UK in October 2011 to take up a lecturing and Principle Investigator position within the Norwich Medical School, UEA. With her move to Norwich, Lindsay also took up a Research Leader position at the Institute of Food Research, working within the Gut Health and Food Safety programme. In 2013 Lindsay was awarded a 5 year Wellcome Trust New Investigator Award and is building her research team that studies the role of early life gut microbiota in resistance to enteric (gut) infections. Most recently (August 2014), Lindsay was promoted to senior lecturer in gastrointestinal sciences.

Title: ***The Early Life Gut Microbiota***

Abstract: The early life gut microbiota is essential for healthy development, including immune programming and infection resistance. However, this early colonisation process can be radically interrupted due to a variety of factors including mode of delivery and antibiotics, which is particularly pertinent within the context of preterm birth, which accounts for 1:10 live births globally and defined as < 37 weeks gestation. Within a disease context, this appears to be correlated to necrotising enterocolitis (NEC), a potentially fatal GI-associated condition. In this talk I will discuss some of our recent research which will explore: (i) how microbiota sample preparation and analysis methods significantly influences microbiota profiles obtained, (ii) how *probiotic* supplementation represents a powerful opportunity for strategically manipulating the wider early life microbiota (from birth up to 1 year) when bacterial assembly is disturbed within the context of preterm birth and (iii) how probiotic supplementation correlates to health (including immune, metabolite and disease) outcomes in preterm infants.

Keynote speaker 3:

Adam Roberts, Senior Lecturer, Liverpool School of Tropical Medicine

Adam's research is concentrated on investigating the genetic basis for transferable antimicrobial resistance and virulence, predominantly in the Firmicutes. In addition he has a long standing interest in mobile genetic elements (MGEs) found throughout all domains of life. Adam is currently involved in projects investigating the contribution of mobile genetic elements to, and the molecular basis of virulence of Clostridium difficile. Additionally he has projects investigating the emergence and persistence of antibiotic-resistant bacteria in humans, the molecular evolution of mobile genetic elements and their biological cost to the host cell, mobile genetic elements and resistance genes in microbial metagenomes from human, animal and environmental samples and horizontal gene transfer in clinically important biofilms. His group is currently funded by the BBSRC, the Schlumberger Foundation flagship program; Faculty for the Future, DEFRA, Commonwealth Scholarships, The EU, The Saudi Arabian, Malaysian and Thai Governments, The Eastman Foundation for Oral Research and Training (EFFORT) and UCL.

Title: ***Factors influencing the human oral microbiome***

Abstract: The human oral microbiome is affected by multiple factors, including the environment and host genetics. In this study, we analysed the oral microbiota of an extended family of Ashkenazi individuals living across several cities and investigated associations with both shared household and host genetic similarities. We found that environmental effects dominated over genetic ones. We found only weak associations between host genetic relatedness and microbiome dissimilarity, indicating that environment rather than host genetics is the dominant factor affecting the composition of the oral microbiome in closely-related individuals. Our results support the concept that there is a consistent, global core microbiome, but that small-scale effects due to shared living environment significantly affect community composition.

Keynote speaker 4:

Jamie Lorimer, Oxford University, Oxford Interdisciplinary Microbiome Project

Jamie's current research focuses on the microbiome - the invisible life in, on and around us. In an ESRC-funded Transformative Research Project, entitled Good germs, Bad germs, Jamie is collaborating with colleagues in the School to develop a participatory model for mapping the domestic microbiome. This project aims to take the science of metagenomics out of the lab to allow members of the public to visualize and experiment upon the life in their kitchens. Working with the Food Standards Agency, the project explores how hygiene practices are shaped by an awareness of the inevitability of microbial life in domestic spaces. In other work on the microbiome, Jamie is exploring the emergence of pro-biotic approaches to managing human and environmental health - focusing in particular on the rise of helminthic and other forms of biotherapy for tackling autoimmune and allergic disease. These research interests emerge from over a decade of work exploring the geographies of Nature. This research focuses on the implications of the Anthropocene for contemporary environmental thought and practice. The public recognition of humans as a planet changing force challenges forms of science and policy premised on the separation of Nature from Society. It also poses questions to the category Human as the sole locus of agency and value. Jamie's work explores 'more-than-human' and 'multinatural' alternatives. Jamie has recently drawn this work together in a research monograph.

Title: ***Making microbes public: participatory and interdisciplinary approaches to the microbiome***

Abstract: The rise of the microbiome as a field of science and translation raises important questions about the place of microbiology in society. This paper reports from an ongoing, interdisciplinary research project that examines the social implications of the microbiome, and develops participatory approaches to microbiology. The project is entitled Good Germs, Bad Germs and involves a collaboration between social scientists, biologists and a group of households in Oxford. We use next generation sequencing to map the microbiomes of domestic kitchens, and allow our participants to design their own experiments in domestic hygiene. The project tracks the social and biological effects of these experiments over the course of year.

This talk starts with a critical review of recent efforts to take the science of metagenomics out of the lab and to engage publics with the microbiome. It then outlines the methodology for the Good Germs study. It presents some of early findings of the project, reflecting on the potential of 'thinking with microbes' for scientists, citizens and policymakers concerned with questions of health and hygiene.

Session 1: Illustrations of microbiome research at LSHTM

Eleanor Riley, Immunology and Infection Department, Eleanor.Riley@lshtm.ac.uk

The Baby Biome Study

Faecal microbiome of neonates and infants in relation to immune development and risk of allergy and autoimmunity in UK.

Mathew Chico, Department of Disease Control**,** Mathew.Chico@lshtm.ac.uk

The effect of sulphadoxine-pyrimethamine or azithromycin on the vaginal and Intestinal microbiome of pregnant women in Tanzania and Malawi

We will investigate the intestinal and vaginal microbiome among pregnant women as part of an individually-randomized, three-arm, superiority trial (N = 4,680) in Kenya, Malawi, and Tanzania comparing: (1) intermittent preventive treatment of malaria in pregnancy (IPTp) using sulphadoxine-pyrimethamine (SP) versus, (2) IPTp using dihydroartemisinin-piperaquine (DP) versus, (3) IPTp-DP plus azithromycin. Stool and vaginal swab samples will be collected from a random selection of participants at sites in Malawi and Tanzania at enrolment and, again, at 32-35 gestational weeks. Stool samples from the newborns of these same women will be collected at 28-day post-partum.

Degrees of similarity between pairs of maternal-infant faecal microbiota samples will be computed using the phylogeny-based UniFrac metric across treatment groups. In addition to analysis of whole community similarity between mother and infant, computational methods will be applied to identify bacterial species shared between mothers and infants, and the degree to which this varies by treatment group. The effect of SP or azithromycin on the vaginal microbiome will be assessed using MiSeq sequencing analysis. The study will determine whether exposure to study drug during pregnancy alters the composition of the microbiota or the relationship between maternal and infant microbiota structure.

Co-investigators and institutions: Daniel Chandramohan, LSHTM, Nigel Klein, University College London, Feiko ter Kuile, Liverpool School of Tropical Medicine, Per Ashorn, University of Tampere (Finland), Ken Maleta, College of Medicine, University of Malawi (Malawi), John Lusinga, National Institute of Medical Research (Tanzania)

Funder: EDCTP

Helen Brotherton (via Skype) Department of Infectious Disease Epidemiology, Helen.Brotherton@lshtm.ac.uk

The skin and intestinal microbiome of neonates and mothers and the effect of early continuous skin-to-skin contact

PhD student with Professor Lawn in IDE looking at early kangaroo mother care for newborns <2000g and part of my project plan is to look at the skin and intestinal microbiome of neonates and mothers and the effect of early continuous skin-to-skin contact.

Lucy Pembrey/Neil Pearce, Department of Infectious Disease Epidemiology
Lucy.Pembrey@lshtm.ac.uk

Understanding asthma phenotypes

Fifteen years ago, it was widely believed that asthma was an allergic/atopic disease caused by allergen exposure in infancy; this produced atopic sensitization and continued exposure resulted in eosinophilic airways inflammation, bronchial hyper-responsiveness and reversible airflow obstruction. It is now clear that this model is at best incomplete. Less than one-half of asthma cases involve allergic (atopic) mechanisms, and most asthma in low-and-middle income countries is non-atopic. Westernization may be contributing to the global increases in asthma prevalence, but this process appears to involve changes in asthma susceptibility rather than increased exposure to "established" asthma risk factors. Understanding why these changes are occurring is essential in order to halt the growing global asthma epidemic. This will require a combination of epidemiological, clinical and basic science studies in a variety of environments.

A key task is to reclassify asthma phenotypes. These are important to: (i) better understand the aetiological mechanisms of asthma; (ii) identify new causes; and (iii) identify new therapeutic measures. There are major opportunities to address these issues using new techniques for sample collection from the airways (sputum induction, nasal lavage), new methods of analysis (microbiome, epigenetics), and new bioinformatics methods for integrating data from multiple sources and levels. There is an unprecedented potential to go beyond the old atopic/non-atopic categorization of phenotypes.

We will therefore conduct analyses to re-examine and reclassify asthma phenotypes. The key features are the inclusion of: (i) both high and low prevalence centres from both high income countries and low-and-middle income countries; (ii) much more detailed biomarker information than has been used for previous studies of asthma phenotypes, including microbiome data; and (iii) new bioinformatics methods for integrating data from multiple sources and levels.

Co-investigators and institutions: MSD, LSHTM
Funder: ERCAdvanced Grant

Brian Greenwood, Department of Disease Control, Brian.Greenwood@lshtm.ac.uk

The microbiome and epidemic meningitis in Africa

Major epidemics of meningococcal meningitis have occurred at irregular intervals in countries of the African Sahel and sub-Sahel (the African meningitis belt) for over 100 years. A characteristic feature of these epidemics is their seasonality; epidemics peak at the height of the dry season and stop spontaneously with the coming of the rains. We have shown recently that the incidence of infection, as measured by the acquisition of asymptomatic nasopharyngeal carriage, is only increased modestly during the dry season. Consequently, epidemics must be associated with a change in the ratio of cases of meningitis to that of asymptomatic infections. Why this is the case is unknown. One factor could be a change in the efficacy of the mucosal immune response during the dry season but, alternatively, the overall nasopharyngeal microbiome might change with season with the microbiome present during the dry season favouring invasion of the nasopharyngeal mucosa by Neisseria meningitides thus accounting for the occurrence of epidemics of meningitis just during the dry season. This needs to be investigated.

Laith Yakob, Department of Disease Control, Laith.Yakob@lshtm.ac.uk

The role of gastrointestinal parasites in disrupting the microbiota-gut-brain axis during childhood development

Signalling between the brain and the gastrointestinal tract is regulated at neural, hormonal, and immunological levels. The gut microbiota is instrumental in the normal development, maturation and function of the brain. Recent studies have shown that parasitic worm infections can alter the normal gut microbiota. We synthesise the current evidence linking helminth infections with altered brain development using a loop analytic framework.

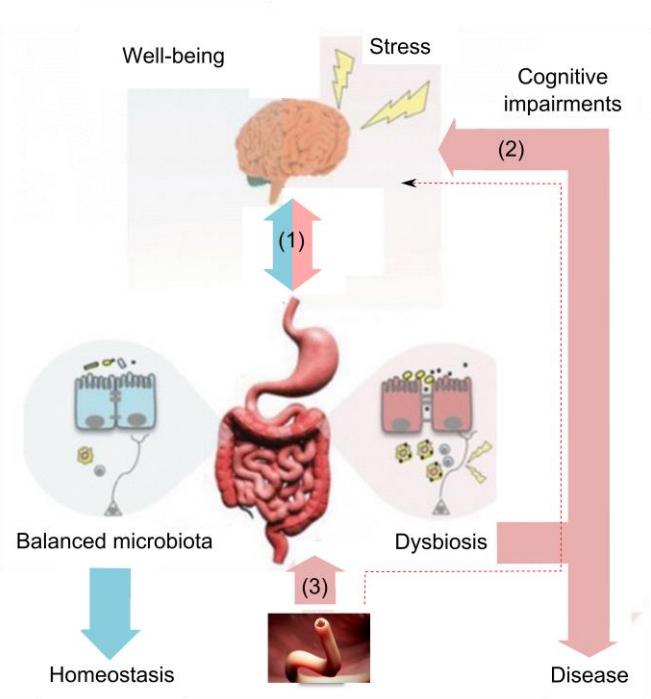


Figure legend: The microbiota-gut-brain axis and its interactions with soil-transmitted helminths (STH): (1) shows the bidirectional communication between the gut and the brain, which occurs through multiple pathways that include hormonal, neural and immune mediators; (2) shows the impact of gut microbiota dysbiosis on cognition; (3) shows the impact of helminth infection on the gut microbiota. The dotted arrow shows the hypothesized pathway leading from STH infection to cognitive impairments.

Co-investigators and institutions: Ricardo Soares-Magalhaes, University of Queensland

Mike Hudson hudsonmj@gmail.com

A 40 Year-Old Prospective Study of Large Bowel Cancer Revisited

Some 40 years ago, a collection of lyophilised stool samples was archived for subsequent bacteriological and chemical analysis to test prospectively a hypothesis linking diet and bacterial metabolism to the causation of colorectal cancer – a hypothesis supported by case-control studies. The stools were collected from some 7,200 healthy adult volunteers in three UK populations in a study supported by the UK Cancer Research Campaign. However, an interim analysis of bile acid and cholesterol metabolites in cases and control samples after 15 years failed to substantiate the hypothesis. We intend to evaluate the utility of new approaches for the analysis of faecal bacterial populations and for chemical analysis to this unique stool archive to further test the hypothesis linking dietary fat, bile acids and bacterial metabolism to risk of large bowel cancer and to extend this to other epidemiologically-related cancers and to coronary heart disease. Indeed, a pilot study has shown that freeze-dried stool stored at room temperature for over 35 years yields microflora DNA that can be sequenced for microbiome studies. *Co-investigators and institutions:* Saheer Gharbia (PHE), Elaine Holmes (Imperial College) and Andy Haines (LSHTM)

Suzanna Francis, Department of Infectious Disease Epidemiology**,** Suzanna.Francis@lshtm.ac.uk

The effect of vaginal microbiota on the host immune response among women at high risk for HIV in Tanzania

Vaginal dysbiosis is characterised by high diversity communities and has been associated with HIV-1 infection. One mechanism for this association is the inflammatory immune response induced by vaginal dysbiosis. Using previously collected cervicovaginal lavage cell pellets from 215 women at high risk for HIV infection in Tanzania, we sequenced the V1-V2 16S rRNA gene to assess bacterial community composition and structure. We used matching supernatants to determine concentration of 33 soluble immune and antimicrobial proteins and peptides by Luminex. Five microbial clusters were identified: one cluster each dominated by *Lactobacillus crispatus* (C1) and *L. iners* (C2); one dominated by *Gardnerella vaginalis* (C4); and two clusters with mixed facultative anerobes, one with with *L. iners* (C3) and one without *L. iners* (C5). These clusters are similar to clusters reported from other studies among women at high risk in East Africa. Strong differences between clusters were found for IL-1α, IL-1β and IP-10 with increasing concentrations of IL-1α and IL-1β with increasing diversity (C1→C5), and decreasing concentrations of IP-10 with increasing diversity. Vaginal microbiota are modulators of the host inflammatory responses and may increase susceptibility to HIV.

Co-investigators and institutions: Ernest Diez-Benavente[2], Josef Wagner[2], Julia Makinde[3], Nuno Sepulveda[2], Robert Butcher[2], Tania Crucitti[5], Janneke van de Wijgert[6], Martin Holland[2], Taane Clark[1,2], Robin Shattock[4], Richard Hayes[1], David Mabey[2]

[1]Faculty of Epidemiology and Population Health, [2]Faculty of Infectious and Tropical Diseases, [3]Wellcome Trust Sanger Institute, Cambridge UK, [4]Imperial College London, Department of Medicine, Section of Virology, Group of Mucosal Infection and Immunity, London, [5]Institute of Tropical Medicine, Antwerp, Belgium; [6]Institute of Infection and Global Health, University of Liverpool, Liverpool.

Funder: The collection of cervical vaginal lavage samples was funded by the European and Developing Countries Clinical Trials Partnership (project code: CT_ct_05_32070_002). Luminex and salary support for analysis was funded by the Medical Research Council (MRC) and Department for International Development (DFID) (G1002369). The sequencing was funded by the Wellcome Trust.

Sarah-Jo Sinnott, Sarah-Jo.Sinnott@lshtm.ac.uk , **Sinead Langan**, Sinead.Langan@LSHTM.ac.uk, **Ketaki Bhate**, Ketaki.Bhate@nottingham.ac.uk , Department of Non-Communicable Disease Epidemiology

Antibiotics for Acne – Anticipating Apocalypse

Acne vulgaris is one of the most common skin conditions, with prevalence reaching almost 100% among adolescents. Antibiotics, both oral and topical, are a mainstay in the treatment of acne. Little is known about its impact on the development of global antimicrobial resistance.

Funding:
SL: Wellcome Trust Senior Research Fellowship in Clinical Science Award (starting March 2017)
SJS: Sir Henry Wellcome Post-Doctoral Fellowship Award
KB: Using this project to apply for clinical doctoral funding

Session 2: Developments in microbiome research

Martin Holland, Department of Clinical Research, Martin.Holland@lshtm.ac.uk

Ocular surface microbiome and trachoma

Trachoma is a blinding disease caused by infection with Chlamydia trachomatis (Ct). Individuals in endemic communities are repeatedly infected with Ct throughout childhood causing inflammation that may progress to conjunctival scarring and blindness in adults. In trachoma endemic communities the clinical signs of disease can also be associated with non-chlamydial bacteria such as Streptococcus pneumoniae or Haemophilus influenza. In adults with scarring, Ct is rarely identified but infection with other bacterial species such as S. pneumoniae, H. influenza, coagulase-negative Staphylococcus and Corynebacterium species is more frequent in scarred eyes than normal eyes. The isolation of bacteria such as S. pneumoniae from trachomatous eyes suggests colonisation exacerbates clinical signs or that those with conjunctival inflammation are more susceptible to colonization and scarring. However, increased numbers of bacteria regarded as commensal, such as Corynebacterium species, in trachomatous eyes are of interest as dysbiosis of the microbiota could contribute to disease. We used deep sequencing of 16S-PCR amplicons from trachomatous and healthy eyes to investigate the composition of ocular bacterial communities. Since community composition is thought to be important in shaping host immune and inflammatory responses we also measured the host conjunctival response. We discuss how the bacterial ecology of the conjunctiva and the host response contribute to the development of ocular pathology.

Ozan Gundogdu, Department of Pathogen Molecular Biology, Ozan.Gundogdu@lshtm.ac.uk

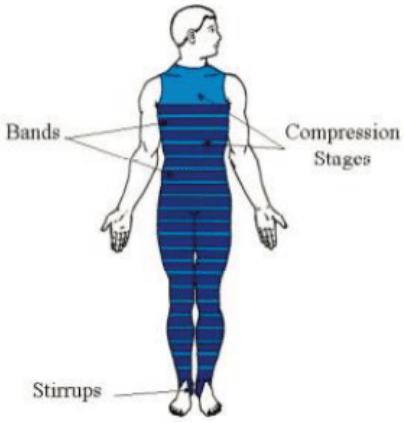
Assessment of the influence of intrinsic environmental and geographical factors on the bacterial ecology of pit latrines

Improving the rate and extent of faecal decomposition in basic forms of sanitation such as pit latrines would benefit around 1.7 billion users worldwide, but to do so requires a major advance in our understanding of the biology of these systems. As a critical first step, bacterial diversity and composition was studied in 30 latrines in Tanzania and Vietnam using pyrosequencing of 16S rRNA genes, and correlated with a number of intrinsic environmental factors such as pH, temperature, organic matter content/composition and geographical factors. Clear differences were observed at the operational taxonomic unit, family and phylum level in terms of richness and community composition between latrines in Tanzania and Vietnam. The results also clearly show that environmental variables, particularly substrate type and availability, can exert a strong structuring influence on bacterial communities in latrines from both countries. This work describes the bacterial ecology of pit latrines in combination with inherent latrine characteristics at an unprecedented level of detail. As such, it provides useful baseline information for future studies that aim to understand the factors that affect decomposition rates in pit latrines.

Richard Stabler, Department of Pathogen Molecular Biology, Richard.Stabler@lshtm.ac.uk

Skin microbiome of an astronaut in relation to the gravity counter measure SkinSuit

The microgravity environment aboard the International Space Station (ISS) results in bone mineral density loss and atrophy of muscles. In consequence, astronauts experience a lengthening of the spine, back pain and some evidence of intervertebral disc damage. The MIT designed SkinSuit is a lightweight compression suit which replicates gravity loading equivalent to that on Earth and has greater comfort. Studies on the SkinSuit material indicate these may be prone to sweat-induced problems of odour generation, discolouration and loss of textile performance. Space farers on extended missions and individuals who have remained non-ambulatory for a significant amount of time suffer changes to their natural bacterial skin flora, with increases in potentially harmful bacteria and decreases in protective commensals. We evaluated fluctuations in the bacterial flora of Earth bound volunteers and an ESA astronaut during a ten-month pre-flight training period, an eight-day ISS mission and one-month recovery period. Alterations in the skin microbiota, without loss of the complexity and diversity, were associated with changes in astronaut location during pre-flight training. The terrestrial and flown studies indicated that short term SkinSuit wear is unlikely to deleteriously impact on the bacterial population of the skin.



Co-investigators and institutions: Helena Rosado, Ronan Doyle, David Negus, David A. Green, Rafael Franco-Cendejas, Cadi Davies, Andreas Mogensen, Jonathan Scott and Peter W. Taylor
Funding: European Space Agency

Christina Gill/Janneke van de Wijgert, cgill@liv.ac.uk

The role of vaginal microbiome in HPV and cervical cancer among women with HIV in Africa

There is a strong association between persistent infection with human papilloma virus (HPV) and cervical cancer, with virtually all cervical cancers being positive for high-risk types of the virus. The progressive immunosuppression associated with HIV increases both the risk of acquisition and persistence of HPV infection. Furthermore, HIV infection has been associated with an increased incidence of pre-cancerous cervical lesions.

Several studies have found a positive association between bacterial vaginosis (a condition typified by a vaginal microbiota made up of a diverse array of anaerobic bacteria with low numbers of lactobacilli) and both HPV infection and cervical cancer. However, with the help of molecular techniques we are only now beginning to explore the bacterial microbiome in HPV and cervical cancer in any detail.

The VMB-HARP is a sub-study of the HARP (HPV in Africa Research Partnership) study, which is coordinated by Professor Philippe Mayaud at LSHTM. The VMB-HARP study has two main aims. First, to determine the association between the type of vaginal microbiome (determined by 16s rRNA amplicon sequencing on the Illumina MiSeq platform) and incidence, persistence and clearance of high-risk HPV in HIV-infected African women. Second, to determine the association between the type of vaginal microbiome and the presence of CIN2+ lesions (prevalent or incident) among HIV-infected African women. A better understanding of how the VMB impacts on the natural history of HPV infection and cervical cancer in women living with HIV could ultimately lead to improved management and treatment of these conditions in this high-risk group. Preliminary data from the VMB-HARP study will be presented.
Co-investigators and institutions: Professor Janneke van de Wijgert and Dr Alistair Darby (University of Liverpool) and the HPV in Africa Research Partnership (HARP) coordinated by Professor Philippe Mayaud (LSHTM)
Funder: Institute of Infection and Global Health, University of Liverpool

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Ernest Diez Benavente, Department of Pathogen Molecular Biology**,**
Ernest.DiezBenavente@lshtm.ac.uk

Microbiome bioinformatic pipelines: from raw data to useful insight

In most cases when a new method to generate biological data is successfully developed a wave of data analysis methodologies and protocols follows closely flooding the gap between data and useful information with tones of options that make the process of choosing the best approach difficult and tedious. Here we present some examples from a hands-on experience on the process of installing the bioinformatic pipelines needed for the analysis of this type of data and the conclusions we extracted from it.

Michael Lewis, Department of Pathogen Molecular Biology, Michael.Lewis@lshtm.ac.uk

Experimental visceral leishmaniasis: Investigating roles for the gut and its microbiota in disease progression.

Leishmania donovani causes visceral leishmaniasis (VL), which is typically fatal without treatment. The course of disease varies substantially between individuals and over 90% of infected people are persistently asymptomatic. The reasons for this heterogeneity have proved difficult to define. We hypothesized that host microbiota is a factor affecting the outcome of L. donovani infection. Using mice and hamsters as models of asymptomatic and fatal VL respectively, we assessed the impact of antibiotic-induced dysbiosis on chronic pathology and immune responses in a range of target tissues. Dysbiosis was associated with prolonged survival in hamsters but did not influence the infection in mice. Antibiotic-treated hamsters also had significantly reduced hepatomegaly and splenomegaly compared with untreated controls. However, parasite loads in the liver and spleen were not

significantly different, nor were they correlated overall with disease progression. Direct parasitism of the GI tract was also a feature of VL in hamsters but not mice. Together these data show that absence of the normal gut microbiota leads to improved outcomes for hamsters infected with L. donovani. Possible explanations for this phenomenon include altered nutritional status, immune responses, haematopathology or risk of secondary bacterial infections.

Session 3: Applications and implications of microbiome research

Lisa Dawson, Department of Pathogen Molecular Biology, Lisa.Dawson@lshtm.ac.uk

The effects of p-cresol on the gut microbiome

Clostridium difficile is a Gram-positive spore-forming anaerobe and a major cause of antibiotic-associated diarrhoea. Disruption of the commensal microbiota, such as through treatment with broad-spectrum antibiotics, is a critical precursor for colonisation by *C. difficile* and subsequent disease. Furthermore, failure of the gut flora to recover colonisation resistance can result in recurrence of infection. An unusual characteristic of *C. difficile* among gut bacteria is its ability to produce the bacteriostatic compound *para*-cresol through fermentation of tyrosine. Here, we demonstrate that a mutant deficient in *p*-cresol production displays a fitness defect in a mouse relapse model of *C. difficile* infection. We demonstrate by 16S rRNA sequencing that colonisation by the *p*-cresol mutant results in a distinctly altered composition of the mouse gut microbiota, with a greater representation of Gammaproteobacteria.

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Polymicrobial screening in the context of programmatic control of trachoma in the Western Pacific.

Clinical signs of trachoma are highly prevalent in the Solomon Islands and a national programme of mass drug administration (MDA) began there in 2014. We will show how we used poly-microbial screening in combination with targeted PCR diagnostics, serological tests and survey for clinical signs, to demonstrate that ocular Chlamydia trachomatis (Ct) infection was rare and that trachoma was not a public health problem in the Solomon Islands in 2013. The poly-microbial screen provided a simple route to evidence against the involvement of other bacterial infections or dysbiosis in driving the trachoma like symptoms on these islands. In response to this data, the Solomon Islands national MDA programme has been halted. We recommend that global trachoma control programmes should confirm ocular Ct endemicity before commencing with MDA.

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Utilisation of anaerobic culturing to investigate bacterial sporulation within the human intestinal microbiota

Our knowledge of the human intestinal microbiota, its composition and the role it plays in human health has expanded greatly in recent years. Sequence-based, culture-independent approaches have driven this expansion in knowledge, however, to progress to phenotypic analysis, culturing and isolation of the relevant microbial species is required. While generally considered recalcitrant to culture, we have developed a process to isolate and archive a substantial proportion of this microbial community, including many novel taxa. Using our targeted phenotypic culturing approach, we have demonstrated that spore-forming bacteria are phylogenetically diverse and abundant within the human gut. Resilient, aero-tolerant spores may therefore provide a previously unappreciated means for host-to-host transmission of a large proportion of the intestinal microbiota. Whole genome sequencing of the isolated and archived bacterial species coupled with in vitro analysis now permits investigation of sporulation dynamics within the human gut. Our targeted culturing approach can be utilised to investigate other important phenotypes within the microbiota and ultimately paves the way to develop bacterial based therapies that can resolve intestinal disorders.

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Microbiome of sand flies in relation to their vectorial capacity for leishmaniasis

To date little is known of the interaction between communities of gut bacteria in sand fly vectors and *Leishmania* infection. Our aim was to analyse the sand fly gut microbiota of newly emerged *Lutzomyia longipalpis* adult flies by 454 pyrosequencing and real-time quantitative PCR and to identify the ability of these microorganisms to influence *Leishmania mexicana* colonisation and transmission from vectors. We found that sand flies emerge as adults with one of two distinct, sex- and diet-independent clusters of bacteria which show a predominance or absence of *Ochrobactrum* in Cluster 1 and Cluster 2, respectively, in roughly equal proportions. To analyse transmission from these flies in closer detail we determined the dose size and composition from single *L. mexicana*-infected *Lu. longipalpis* bites using our new metacyclic-specific RT-qPCR. This revealed that C2 flies deposited more parasites per bite that were enriched for metacyclic promastigotes. Co-infection of C1 or C2 bacteria with a low dose of *L. mexicana* metacyclics demonstrated that C2 bacteria significantly enhanced cutaneous infection in BALB/c mice. Collectively, we show that the emergent assembly of midgut bacteria has a profound influence on *Leishmania* metacyclogenesis, transmission and infection.

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Microbiome and environmental pollutants: perfluoroalkyl substances and the gut microbiome

Perfluoroalkyl substances (PFAS eg PFOA and PFOS), have been associated with endocrine and other effects. They have a long half-life in humans 3-5 years, serum PFAS is a stable biomarker and a preferred exposure measure of choice. The long half-life of PFAS reflects relatively low net excretion and this in turn is due to passage into, then active reabsorption, via kidneys and gut. If factors determining these excretion pathways were also associated with disease or relevant clinical markers of interest, then this could lead to confounding between measured serum and these outcomes. An impaired gut microbiome has been implicated in the etiology of obesity and evidence of an association between serum PFOS and obesity could be affected by microbiome status in two ways: if PFOS exposure affected gut flora, microbiome status could be on the causal pathway; if the gut microbiome affected both PFOS excretion and obesity it could be acting as a confounder. Preliminary data point to the latter. In a study of 67000 individuals with serum PFAS measurements, serum PFOS was lower for those taking antibiotics which could be explained by the effect on the gut microbiome. Whether the microbiome is a mechanism or confounder, requires fecal and serum samples and a population with PFOS contrasts. A possible design for this in a population with some high PFOS exposure, will be outlined.

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Gut Bacteria changes in Travellers and the association with morbidity and anti-microbials. (Gutback)

Studies on the gut resistome, including using targeted faecal PCR approaches, have demonstrated high rates of acquisition of resistant organisms associated with travel. An estimated 125 Million visits to these regions with high faecal microbiome prevalence of multi resistant enterobacteriaceae (MRE) in and approximately one quarter of these travellers (or more) encounter an episode of traveller's diarrhoea. Many gram negative resistance elements are imported from these high prevalence countries an and a consistent risk factor for carriage of MRE, is an episode of diarrhoea, and/or the use of antibiotics. Longitudinal analyses of resistance mutation acquisition, prevalence and persistence would provide useful data to quantify the risk of resistance attributable to host microbiota, sociodemographic factors and use of presumptive antimicrobials. The Gutback study aims to explore the presence of antimicrobial resistance in faecal microbiota before and after travel to a high-risk country using novel whole genome sequencing and to identify microbiome changes during travel, effects of antimicrobial treatments and acquired illness on the microbiome and the persistence of resistance elements.

Seventy travellers have been recruited before travel, then followed 2 weeks after return and 6 months later providing a faecal sample at each visit. Detailed information was collected on travel, morbidity and use of medication. The serial samples have DNA extracted and analysed using a 16S rRNA gene and MiSeq shotgun sequencing to identify organisms and resistance markers present in the faecal material. This is to be analysed and the microbiome changes over time will be calculated and factors associated with change and persistence estimated.

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