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Speakers:
1 Luisa M. V. Peixe, Porto, Portugal
2 Herman Goossens, Edegem, Belgium
3 Gilbert Greub, Lausanne, Switzerland

References

1.1 Probable Person-to-Person Transmission of Legionnaires' Disease. Correia AM, Ferreira JS, Borges V, Nunes A, Gomes B, Capucho R, Gonçalves J, Antunes DM, Almeida S, Mendes A, Guerreiro M, Sampaio DA, Vieira L, Machado J, Simões MJ, Gonçalves P, Gomes JP. N Engl J Med. 2016 Feb 4;374(5):497-8. doi: 10.1056/NEJMc1505356.

1.2 Genomic analysis of diversity, population structure, virulence, and antimicrobial resistance in Klebsiella pneumoniae, an urgent threat to public health. Holt KE, Wertheim H, Zadoks RN, Baker S, Whitehouse CA, Dance D, Jenney A, Connor TR, Hsu LY, Severin J, Brisse S, Cao H, Wilksch J, Gorrie C, Schultz MB, Edwards DJ, Nguyen KV, Nguyen TV, Dao TT, Mensink M, Minh VL, Nhu NT, Schultsz C, Kuntaman K, Newton PN, Moore CE, Strugnell RA, Thomson NR. Proc Natl Acad Sci U S A. 2015 Jul 7;112(27):E3574-81. doi: 10.1073/pnas.1501049112. Epub 2015 Jun 22.

Klebsiella pneumoniae is now recognized as an urgent threat to human health because of the emergence of multidrug-resistant strains associated with hospital outbreaks and hypervirulent strains associated with severe community-acquired infections. K. pneumoniae is ubiquitous in the environment and can colonize and infect both plants and animals. However, little is known about the population structure of K. pneumoniae, so it is difficult to recognize or understand the emergence of clinically important clones within this highly genetically diverse species. Here we present a detailed genomic framework for K. pneumoniae based on whole-genome sequencing of more than 300 human and animal isolates spanning four continents. Our data provide genome-wide support for the splitting of K. pneumoniae into three distinct species, KpI (K. pneumoniae), KpII (K. quasipneumoniae), and KpIII (K. variicola). Further, for K. pneumoniae (KpI), the entity most frequently associated with human infection, we show the existence of >150 deeply branching lineages including numerous multidrug-resistant or hypervirulent clones. We show K. pneumoniae has a large accessory genome approaching 30,000 protein-coding genes, including a number of virulence functions that are significantly associated with invasive community-acquired disease in humans. In our dataset, antimicrobial resistance genes were common among human carriage isolates and hospital-acquired infections, which generally lacked the genes associated with invasive disease. The convergence of virulence and resistance genes potentially could lead to the emergence of untreatable invasive K. pneumoniae infections; our data provide the whole-genome framework against which to track the emergence of such threats.

1.3 The microbiome of the urinary tract--a role beyond infection. Whiteside SA, Razvi H, Dave S, Reid G, Burton JP. Nat Rev Urol. 2015 Feb;12(2):81-90. doi: 10.1038/nrurol.2014.361. Epub 2015 Jan 20.

Urologists rarely need to consider bacteria beyond their role in infectious disease. However, emerging evidence shows that the microorganisms inhabiting many sites of the body, including the urinary tract--which has long been assumed sterile in healthy individuals--might have a role in maintaining urinary health. Studies of the urinary microbiota have identified remarkable differences between healthy populations and those with urologic diseases. Microorganisms at sites distal to the kidney, bladder and urethra are likely to have a profound effect on urologic health, both positive and negative, owing to their metabolic output and other contributions. Connections between the gut microbiota and renal stone formation have already been discovered. In addition, bacteria are also used in the prevention of bladder cancer recurrence. In the future, urologists will need to consider possible influences of the microbiome in diagnosis and treatment of certain urological conditions. New insights might provide an opportunity to predict the risk of developing certain urological diseases and could enable the development of innovative therapeutic strategies.

Comment in Re: The Microbiome of the Urinary Tract--A Role Beyond Infection. [J Urol. 2015]

1.4 The new world of the urinary microbiota in women. Brubaker L, Wolfe AJ. Am J Obstet Gynecol. 2015 Nov;213(5):644-9. doi: 10.1016/j.ajog.2015.05.032. Epub 2015 May 21.

Emerging evidence challenges the long-held paradigm that the healthy bladder is sterile. These discoveries may provide new opportunities to address important women's health conditions, which include preterm labor and delivery, urinary tract infections, and common forms of urinary incontinence. Traditional tools for urinary bacterial assessment, which includes urinary dipsticks and standard urine cultures, have significant limitations that restrict the information that is available to clinicians. For example, the standard urine culture does not detect slow-growing bacteria that die in the presence of oxygen. Two new, complementary tools, however, can detect these and other organisms, which permits a more complete characterization of bacterial communities within the female bladder. Obstetrician-gynecologists should become familiar with these new approaches (expanded quantitative urine culture and 16S ribosomal RNA gene sequencing) that can detect previously unrecognized organisms. These advances are making it possible to answer previously intractable scientific and clinical questions. Traditional nomenclature used to describe the bacterial status in the bladder is quite dated and unsuited for the emerging information about the bacterial milieu of the female urinary tract. In the context of the sterile bladder paradigm, clinicians have learned about "uropathogens," "asymptomatic bacteriuria," and "urinary tract infection." Given that the lower urinary tract is not sterile, these terms should be reevaluated. Clinicians can already benefit from the emerging knowledge regarding urinary organisms that have previously gone undetected or unappreciated. For example, in some subpopulations of women with urinary symptoms, existing data suggest that the urinary bacterial community may be associated with women's health conditions of interest. This Clinical Opinion highlights the inadequacies of the current tools for urinary bacterial assessment, describes the new assessment tools, explains the current interpretation of the resulting data, and proposes potential clinical uses and relevance. A new world is opening to our view that will give us the opportunity to better understand urinary bacteria and the bladder in which they live. This new knowledge has significant potential to improve patient care in obstetrics and gynecology.

1.5 Fosfomycin for Treatment of Prostatitis: New Tricks for Old Dogs. Grayson ML, Macesic N, Trevillyan J, Ellis AG, Zeglinski PT, Hewitt NH, Gardiner BJ, Frauman AG. Clin Infect Dis. 2015 Oct 1;61(7):1141-3. doi: 10.1093/cid/civ436. Epub 2015 Jun 10.

Treatment options for prostatitis caused by multidrug-resistant gram-negative bacilli are limited. We report two cases cured with oral fosfomycin and provide a pharmacokinetic analysis of fosfomycin predose concentrations during treatment.

Comment in Editorial Commentary: Fosfomycin: The Current Status of the Drug. [Clin Infect Dis. 2015]

1.6 Oral Fosfomycin and Prostatitis. Davido B, Dinh A. Clin Infect Dis. 2016 Mar 1;62(5):671-2. doi: 10.1093/cid/civ966. Epub 2015 Dec 20.

1.7 Long-Term Fosfomycin-Tromethamine Oral Therapy for Difficult-To-Treat Chronic Bacterial Prostatitis. Los-Arcos I, Pigrau C, Rodríguez-Pardo D, Fernández-Hidalgo N, Andreu A, Larrosa N, Almirante B. Antimicrob Agents Chemother. 2015 Dec 14;60(3):1854-8. doi: 10.1128/AAC.02611-15.

This is a retrospective study of 15 difficult-to-treat (i.e., exhibiting previous failure, patient side effects, or resistance to ciprofloxacin and co-trimoxazole) chronic bacterial prostatitis infections (5 patients with multidrug-resistant Enterobacteriaceae [MDRE]) receiving fosfomycin-tromethamine at a dose of 3 g per 48 to 72 h for 6 weeks. After a median follow-up of 20 months, 7 patients (47%) had a clinical response, and 8 patients (53%) had persistent microbiological eradication; 4/5 patients with MDRE isolates achieved eradication. There were no side effects. Fosfomycin-tromethamine is a possible alternative therapy for chronic bacterial prostatitis.

1.8 Fosfomycin Resistance in Escherichia coli, Pennsylvania, USA. Alrowais H, McElheny CL, Spychala CN, Sastry S, Guo Q, Butt AA, Doi Y. Emerg Infect Dis. 2015 Nov;21(11):2045-7. doi: 10.3201/eid2111.150750.

Fosfomycin resistance in Escherichia coli is rare in the United States. An extended-spectrum β-lactamase-producing E. coli clinical strain identified in Pennsylvania, USA, showed high-level fosfomycin resistance caused by the fosA3 gene. The IncFII plasmid carrying this gene had a structure similar to those found in China, where fosfomycin resistance is commonly described.

1.9 Importation of Fosfomycin Resistance fosA3 Gene to Europe. Mendes AC, Rodrigues C, Pires J, Amorim J, Ramos MH, Novais Â, Peixe L. Emerg Infect Dis. 2016 Feb;22(2):346-8. doi: 10.3201/eid2202.151301.

1.10 Vaccination with Klebsiella pneumoniae-derived extracellular vesicles protects against bacteria-induced lethality via both humoral and cellular immunity. Lee WH, Choi HI, Hong SW, Kim KS, Gho YS, Jeon SG. Exp Mol Med. 2015 Sep 11;47:e183. doi: 10.1038/emm.2015.59.

The emergence of multidrug-resistant Klebsiella pneumoniae highlights the need to develop preventive measures to ameliorate Klebsiella infections. Bacteria-derived extracellular vesicles (EVs) are spherical nanometer-sized proteolipids enriched with outer membrane proteins. Gram-negative bacteria-derived EVs have gained interest for use as nonliving complex vaccines. In the present study, we evaluated whether K. pneumoniae-derived EVs confer protection against bacteria-induced lethality. K. pneumoniae-derived EVs isolated from in vitro bacterial culture supernatants induced innate immunity, including the upregulation of co-stimulatory molecule expression and proinflammatory mediator production. EV vaccination via the intraperitoneal route elicited EV-reactive antibodies and interferon-gamma-producing T-cell responses. Three vaccinations with the EVs prevented bacteria-induced lethality. As verified by sera and splenocytes adoptive transfer, the protective effect of EV vaccination was dependent on both humoral and cellular immunity. Taken together, these findings suggest that K. pneumoniae-derived EVs are a novel vaccine candidate against K. pneumoniae infections.

1.11 A novel mechanism for the biogenesis of outer membrane vesicles in Gram-negative bacteria. Roier S, Zingl FG, Cakar F, Durakovic S, Kohl P, Eichmann TO, Klug L, Gadermaier B, Weinzerl K, Prassl R, Lass A, Daum G, Reidl J, Feldman MF, Schild S. Nat Commun. 2016 Jan 25;7:10515. doi: 10.1038/ncomms10515.

Bacterial outer membrane vesicles (OMVs) have important biological roles in pathogenesis and intercellular interactions, but a general mechanism of OMV formation is lacking. Here we show that the VacJ/Yrb ABC (ATP-binding cassette) transport system, a proposed phospholipid transporter, is involved in OMV formation. Deletion or repression of VacJ/Yrb increases OMV production in two distantly related Gram-negative bacteria, Haemophilus influenzae and Vibrio cholerae. Lipidome analyses demonstrate that OMVs from VacJ/Yrb-defective mutants in H. influenzae are enriched in phospholipids and certain fatty acids. Furthermore, we demonstrate that OMV production and regulation of the VacJ/Yrb ABC transport system respond to iron starvation. Our results suggest a new general mechanism of OMV biogenesis based on phospholipid accumulation in the outer leaflet of the outer membrane. This mechanism is highly conserved among Gram-negative bacteria, provides a means for regulation, can account for OMV formation under all growth conditions, and might have important pathophysiological roles in vivo.

Antibiotic Resistance Funding

2.1 The Innovative Medicines Initiative's New Drugs for Bad Bugs programme: European public-private partnerships for the development of new strategies to tackle antibiotic resistance. Kostyanev T, Bonten MJ, O'Brien S, Steel H, Ross S, François B, Tacconelli E, Winterhalter M, Stavenger RA, Karlén A, Harbarth S, Hackett J, Jafri HS, Vuong C, MacGowan A, Witschi A, Angyalosi G, Elborn JS, deWinter R, Goossens H. J Antimicrob Chemother. 2016 Feb;71(2):290-5. doi: 10.1093/jac/dkv339. Epub 2015 Nov 15.

Antibiotic resistance (ABR) is a global public health threat. Despite the emergence of highly resistant organisms and the huge medical need for new drugs, the development of antibacterials has slowed to an unacceptable level worldwide. Numerous government and non-government agencies have called for public-private partnerships and innovative funding mechanisms to address this problem. To respond to this public health crisis, the Innovative Medicines Initiative Joint Undertaking programme has invested more than €660 million, with a goal of matched contributions from the European Commission and the European Federation of Pharmaceutical Industries and Associations, in the development of new antibacterial strategies. The New Drugs for Bad Bugs (ND4BB) programme, an Innovative Medicines Initiative, has the ultimate goal to boost the fight against ABR at every level from basic science and drug discovery, through clinical development to new business models and responsible use of antibiotics. Seven projects have been launched within the ND4BB programme to achieve this goal. Four of them will include clinical trials of new anti-infective compounds, as well as epidemiological studies on an unprecedented scale, which will increase our knowledge of ABR and specific pathogens, and improve the designs of the clinical trials with new investigational drugs. The need for rapid concerted action has driven the funding of seven topics, each of which should add significantly to progress in the fight against ABR. ND4BB unites expertise and provides a platform where the commitment and resources required by all parties are streamlined into a joint public-private partnership initiative of unprecedented scale.

2.2 Public funding for research on antibacterial resistance in the JPIAMR countries, the European Commission, and related European Union agencies: a systematic observational analysis. Kelly R, Zoubiane G, Walsh D, Ward R, Goossens H. Lancet Infect Dis. 2015 Dec 19. pii: S1473-3099(15)00350-3. doi: 10.1016/S1473-3099(15)00350-3. [Epub ahead of print]

BACKGROUND: Antibacterial resistant infections are rising continuously, resulting in increased morbidity and mortality worldwide. With no new antibiotic classes entering the market and the possibility of

returning to the pre-antibiotic era, the Joint Programming Initiative on Antimicrobial Resistance (JPIAMR) was established to address this problem. We aimed to quantify the scale and scope of publicly funded antibacterial resistance research across JPIAMR countries and at the European Union (EU) level to identify gaps and future opportunities.

METHODS: We did a systematic observational analysis examining antibacterial resistance research funding. Databases of funding organisations across 19 countries and at EU level were systematically searched for publicly funded antibacterial resistance research from Jan 1, 2007, to Dec 31, 2013. We categorised studies on the basis of the JPIAMR strategic research agenda's six priority topics (therapeutics, diagnostics, surveillance, transmission, environment, and interventions) and did an observational analysis. Only research funded by public funding bodies was collected and no private organisations were contacted for their investments. Projects in basic, applied, and clinical research, including epidemiological, public health, and veterinary research and trials were identified using keyword searches by organisations, and inclusion criteria were based on the JPIAMR strategic research agenda's six priority topics, using project titles and abstracts as filters.

FINDINGS: We identified 1243 antibacterial resistance research projects, with a total public investment of €1·3 billion across 19 countries and at EU level, including public investment in the Innovative Medicines Initiative. Of the total amount invested in antibacterial resistance research across the time period, €646·6 million (49·5%) was invested at the national level and €659·2 million (50·5%) at the EU level. When projects were classified under the six priority topics we found that 763 (63%) of 1208 projects funded at national level were within the area of therapeutics, versus 185 (15%) in transmission, 131 (11%) in diagnostics, 53 (4%) in interventions, and only 37 (3%) in environment and 39 (3%) in surveillance.

INTERPRETATION: This was the first systematic analysis of research funding of antibacterial resistance of this scale and scope, which relied on the availability and accuracy of data from organisations included. Large variation was seen between countries both in terms of number of projects and associated investment and across the six priority topics. To determine the future direction of JPIAMR countries a clear picture of the funding landscape across Europe and Canada is needed. Countries should work together to increase the effect of research funding by strengthening national and international coordination and collaborations, harmonising research activities, and collectively pooling resources to fund multidisciplinary projects. The JPIAMR have developed a publicly available database to document the antibacterial resistance research collected and can be used as a baseline to analyse funding from 2014 onwards.

FUNDING: JPIAMR and the European Commission.

New antibiotic class

2.3 A new antibiotic kills pathogens without detectable resistance. Ling LL, Schneider T, Peoples AJ, Spoering AL, Engels I, Conlon BP, Mueller A, Schäberle TF, Hughes DE, Epstein S, Jones M, Lazarides L, Steadman VA, Cohen DR, Felix CR, Fetterman KA, Millett WP, Nitti AG, Zullo AM, Chen C, Lewis K. Nature. 2015 Jan 22;517(7535):455-9. doi: 10.1038/nature14098. Epub 2015 Jan 7.

Erratum in: A new antibiotic kills pathogens without detectable resistance. [Nature. 2015]

Antibiotic resistance is spreading faster than the introduction of new compounds into clinical practice, causing a public health crisis. Most antibiotics were produced by screening soil microorganisms, but this limited resource of cultivable bacteria was overmined by the 1960s. Synthetic approaches to produce antibiotics have been unable to replace this platform. Uncultured bacteria make up approximately 99% of all species in external environments, and are an untapped source of new antibiotics. We developed several methods to grow uncultured organisms by cultivation in situ or by using specific growth factors. Here we report a new antibiotic that we term teixobactin, discovered in a screen of uncultured bacteria. Teixobactin inhibits cell wall synthesis by binding to a highly conserved motif of lipid II (precursor of peptidoglycan) and lipid III (precursor of cell wall teichoic acid). We did not obtain any mutants of Staphylococcus aureus or Mycobacterium tuberculosis resistant to teixobactin. The properties of this

compound suggest a path towards developing antibiotics that are likely to avoid development of resistance.

Comment in Antibacterial drugs: a new drug for resistant bugs. [Nat Rev Drug Discov. 2015]

Drug discovery: Early antibiotic from a cranberry bog. [Nature. 2015]

Antibiotics: An irresistible newcomer. [Nature. 2015]

Bacteria: Assessing resistance to new antibiotics. [Nature. 2015]

Novel antibacterial platform

2.4 Novel antibody-antibiotic conjugate eliminates intracellular S. aureus. Lehar SM, Pillow T, Xu M, Staben L, Kajihara KK, Vandlen R, DePalatis L, Raab H, Hazenbos WL, Morisaki JH, Kim J, Park S, Darwish M, Lee BC, Hernandez H, Loyet KM, Lupardus P, Fong R, Yan D, Chalouni C, Luis E, Khalfin Y, Plise E, Cheong J, Lyssikatos JP, Strandh M, Koefoed K, Andersen PS, Flygare JA, Wah Tan M, Brown EJ, Mariathasan S. Nature. 2015 Nov 19;527(7578):323-8. doi: 10.1038/nature16057. Epub 2015 Nov 4.

Staphylococcus aureus is considered to be an extracellular pathogen. However, survival of S. aureus within host cells may provide a reservoir relatively protected from antibiotics, thus enabling long-term colonization of the host and explaining clinical failures and relapses after antibiotic therapy. Here we confirm that intracellular reservoirs of S. aureus in mice comprise a virulent subset of bacteria that can establish infection even in the presence of vancomycin, and we introduce a novel therapeutic that effectively kills intracellular S. aureus. This antibody-antibiotic conjugate consists of an anti-S. aureus antibody conjugated to a highly efficacious antibiotic that is activated only after it is released in the proteolytic environment of the phagolysosome. The antibody-antibiotic conjugate is superior to vancomycin for treatment of bacteraemia and provides direct evidence that intracellular S. aureus represents an important component of invasive infections.

Comment in Antibiotics: Homed to the hideout. [Nature. 2015]

New antibiotic resistance mechanism

2.5 Emergence of plasmid-mediated colistin resistance mechanism MCR-1 in animals and human beings in China: a microbiological and molecular biological study. Liu YY, Wang Y, Walsh TR, Yi LX, Zhang R, Spencer J, Doi Y, Tian G, Dong B, Huang X, Yu LF, Gu D, Ren H, Chen X, Lv L, He D, Zhou H, Liang Z, Liu JH, Shen J. Lancet Infect Dis. 2016 Feb;16(2):161-8. doi: 10.1016/S1473-3099(15)00424-7. Epub 2015 Nov 19.

BACKGROUND: Until now, polymyxin resistance has involved chromosomal mutations but has never been reported via horizontal gene transfer. During a routine surveillance project on antimicrobial resistance in commensal Escherichia coli from food animals in China, a major increase of colistin resistance was observed. When an E coli strain, SHP45, possessing colistin resistance that could be transferred to another strain, was isolated from a pig, we conducted further analysis of possible plasmid-mediated polymyxin resistance. Herein, we report the emergence of the first plasmid-mediated polymyxin resistance mechanism, MCR-1, in Enterobacteriaceae.

METHODS: The mcr-1 gene in E coli strain SHP45 was identified by whole plasmid sequencing and subcloning. MCR-1 mechanistic studies were done with sequence comparisons, homology modelling, and electrospray ionisation mass spectrometry. The prevalence of mcr-1 was investigated in E coli and Klebsiella pneumoniae strains collected from five provinces between April, 2011, and November, 2014. The ability of MCR-1 to confer polymyxin resistance in vivo was examined in a murine thigh model.

FINDINGS: Polymyxin resistance was shown to be singularly due to the plasmid-mediated mcr-1 gene. The plasmid carrying mcr-1 was mobilised to an E coli recipient at a frequency of 10(-1) to 10(-3) cells per recipient cell by conjugation, and maintained in K pneumoniae and Pseudomonas aeruginosa. In an in-vivo model, production of MCR-1 negated the efficacy of colistin. MCR-1 is a member of the phosphoethanolamine transferase enzyme family, with expression in E coli resulting in the addition of phosphoethanolamine to lipid A. We observed mcr-1 carriage in E coli isolates collected from 78 (15%) of 523 samples of raw meat and 166 (21%) of 804 animals during 2011-14, and 16 (1%) of 1322 samples from inpatients with infection.

INTERPRETATION: The emergence of MCR-1 heralds the breach of the last group of antibiotics, polymyxins, by plasmid-mediated resistance. Although currently confined to China, MCR-1 is likely to emulate other global resistance mechanisms such as NDM-1. Our findings emphasise the urgent need for coordinated global action in the fight against pan-drug-resistant Gram-negative bacteria.

FUNDING: Ministry of Science and Technology of China, National Natural Science Foundation of China.

2.6 Colistin resistance gene mcr-1 harboured on a multidrug resistant plasmid. Malhotra-Kumar S, Xavier BB, Das AJ, Lammens C, Butaye P, Goossens H. Lancet Infect Dis. 2016 Mar;16(3):283-4. doi: 10.1016/S1473-3099(16)00012-8. Epub 2016 Jan 8.

2.7 Dissemination of the mcr-1 colistin resistance gene. Hu Y, Liu F, Lin IY, Gao GF, Zhu B. Lancet Infect Dis. 2016 Feb;16(2):146-7. doi: 10.1016/S1473-3099(15)00533-2. Epub 2015 Dec 18.

2.8 Early emergence of mcr-1 in Escherichia coli from food-producing animals. Shen Z, Wang Y, Shen Y, Shen J, Wu C. Lancet Infect Dis. 2016 Mar;16(3):293. doi: 10.1016/S1473-3099(16)00061-X.

Role of household members

2.9 Gut Colonization of Healthy Children and Their Mothers With Pathogenic Ciprofloxacin-Resistant Escherichia coli. Gurnee EA, Ndao IM, Johnson JR, Johnston BD, Gonzalez MD, Burnham CA, Hall-Moore CM, McGhee JE, Mellmann A, Warner BB, Tarr PI. J Infect Dis. 2015 Dec 15;212(12):1862-8. doi: 10.1093/infdis/jiv278. Epub 2015 May 12.

BACKGROUND: The reservoir of pathogenic ciprofloxacin-resistant Escherichia coli remains unknown.

METHODS: We conducted a prospective cohort study of 80 healthy twins and their mothers to determine the frequency of excretion of ciprofloxacin-resistant, potentially pathogenic E. coli. Stool specimens were cultured selectively for ciprofloxacin-resistant gram-negative bacteria. Isolates were categorized on the basis of additional resistance and virulence profiles. We also prospectively collected clinical metadata.

RESULTS: Fifteen children (19%) and 8 mothers (20%) excreted ciprofloxacin-resistant E. coli at least once. Overall, 33% of 40 families had at least 1 member whose stool specimen yielded ciprofloxacin-resistant E. coli on culture. Fifty-seven submitted stool specimens (2.8%) contained such organisms; clones ST131-H30 and ST405 accounted for 52 and 5 of the positive specimens, respectively. Length of hospital stay after birth (P = .002) and maternal colonization (P = .0001) were associated with subsequent childhood carriage of ciprofloxacin-resistant E. coli; antibiotic use, acid suppression, sex, mode of delivery,

and maternal perinatal antibiotic use were not. Ciprofloxacin-resistant E. coli were usually resistant to additional antibiotic classes, and all had virulence genotypes typical of extraintestinal pathogenic E. coli.

CONCLUSIONS: Healthy children and their mothers commonly harbor ciprofloxacin-resistant E. coli with pathogenic potential.

Comment in The Rise of Fluoroquinolone-Resistant Escherichia coli in the Community: Scarier Than We Thought. [J Infect Dis. 2015]

2.10 Transmission and microevolution of USA300 MRSA in U.S. households: evidence from whole-genome sequencing. Alam MT, Read TD, Petit RA 3rd, Boyle-Vavra S, Miller LG, Eells SJ, Daum RS, David MZ. MBio. 2015 Mar 10;6(2):e00054. doi: 10.1128/mBio.00054-15.

Methicillin-resistant Staphylococcus aureus (MRSA) USA300 is a successful S. aureus clone in the United States and a common cause of skin and soft tissue infections (SSTIs). We performed whole-genome sequencing (WGS) of 146 USA300 MRSA isolates from SSTIs and colonization cultures obtained from an investigation conducted from 2008 to 2010 in Chicago and Los Angeles households that included an index case with an S. aureus SSTI. Identifying unique single nucleotide polymorphisms (SNPs) and analyzing whole-genome phylogeny, we characterized isolates to understand transmission dynamics, genetic relatedness, and microevolution of USA300 MRSA within the households. We also compared the 146 USA300 MRSA isolates from our study with the previously published genome sequences of the USA300 MRSA isolates from San Diego (n = 35) and New York City (n = 277). We found little genetic variation within the USA300 MRSA household isolates from Los Angeles (mean number of SNPs ± standard deviation, 17.6 ± 35; π nucleotide diversity, 3.1 × 10(-5)) or from Chicago (mean number of SNPs ± standard deviation, 12 ± 19; π nucleotide diversity, 3.1 × 10(-5)). The isolates within a household clustered into closely related monophyletic groups, suggesting the introduction into and transmission within each household of a single common USA300 ancestral strain. From a Bayesian evolutionary reconstruction, we inferred that USA300 persisted within households for 2.33 to 8.35 years prior to sampling. We also noted that fluoroquinolone-resistant USA300 clones emerged around 1995 and were more widespread in Los Angeles and New York City than in Chicago. Our findings strongly suggest that unique USA300 MRSA isolates are transmitted within households that contain an individual with an SSTI. Decolonization of household members may be a critical component of prevention programs to control USA300 MRSA spread in the United States.

IMPORTANCE: USA300, a virulent and easily transmissible strain of methicillin-resistant Staphylococcus aureus (MRSA), is the predominant community-associated MRSA clone in the United States. It most commonly causes skin infections but also causes necrotizing pneumonia and endocarditis. Strategies to limit the spread of MRSA in the community can only be effective if we understand the most common sources of transmission and the microevolutionary processes that provide a fitness advantage to MRSA. We performed a whole-genome sequence comparison of 146 USA300 MRSA isolates from Chicago and Los Angeles. We show that households represent a frequent site of transmission and a long-term reservoir of USA300 strains; individuals within households transmit the same USA300 strain among themselves. Our study also reveals that a large proportion of the USA300 isolates sequenced are resistant to fluoroquinolone antibiotics. The significance of this study is that if households serve as long-term reservoirs of USA300, household MRSA eradication programs may result in a uniquely effective control method.

New determinant of antibiotic resistance

2.11 Antimicrobial resistance: the major contribution of poor governance and corruption to this growing problem. Collignon P, Athukorala PC, Senanayake S, Khan F. PLoS One. 2015 Mar 18;10(3):e0116746. doi: 10.1371/journal.pone.0116746. eCollection 2015.

OBJECTIVES: To determine how important governmental, social, and economic factors are in driving antibiotic resistance compared to the factors usually considered the main driving factors-antibiotic usage and levels of economic development.

DESIGN: A retrospective multivariate analysis of the variation of antibiotic resistance in Europe in terms of human antibiotic usage, private health care expenditure, tertiary education, the level of economic advancement (per capita GDP), and quality of governance (corruption). The model was estimated using a panel data set involving 7 common human bloodstream isolates and covering 28 European countries for the period 1998-2010.

RESULTS: Only 28% of the total variation in antibiotic resistance among countries is attributable to variation in antibiotic usage. If time effects are included the explanatory power increases to 33%. However when the control of corruption indicator is included as an additional variable, 63% of the total variation in antibiotic resistance is now explained by the regression. The complete multivariate regression only accomplishes an additional 7% in terms of goodness of fit, indicating that corruption is the main socioeconomic factor that explains antibiotic resistance. The income level of a country appeared to have no effect on resistance rates in the multivariate analysis. The estimated impact of corruption was statistically significant ($p< 0.01$). The coefficient indicates that an improvement of one unit in the corruption indicator is associated with a reduction in antibiotic resistance by approximately 0.7 units. The estimated coefficient of private health expenditure showed that one unit reduction is associated with a 0.2 unit decrease in antibiotic resistance.

CONCLUSIONS: These findings support the hypothesis that poor governance and corruption contributes to levels of antibiotic resistance and correlate better than antibiotic usage volumes with resistance rates. We conclude that addressing corruption and improving governance will lead to a reduction in antibiotic resistance.

Comment in Want a better way to control antibiotic resistance? Fight the corruption. [Pathog Glob Health. 2015]

Interventions to control AMR

2.12 Effects of national antibiotic stewardship and infection control strategies on hospital-associated and community-associated meticillin-resistant Staphylococcus aureus infections across a region of Scotland: a non-linear time-series study. Lawes T, Lopez-Lozano JM, Nebot CA, Macartney G, Subbarao-Sharma R, Dare CR, Wares KD, Gould IM. Lancet Infect Dis. 2015 Dec;15(12):1438-49. doi: 10.1016/S1473-3099(15)00315-1. Epub 2015 Sep 25.

BACKGROUND: Restriction of antibiotic consumption to below predefined total use thresholds might remove the selection pressure that maintains antimicrobial resistance within populations. We assessed the effect of national antibiotic stewardship and infection prevention and control programmes on prevalence density of meticillin-resistant Staphylococcus aureus (MRSA) infections across a region of Scotland.

METHODS: This non-linear time-series analysis and quasi-experimental study explored ecological determinants of MRSA epidemiology among 1,289,929 hospital admissions and 455,508 adults registered in primary care in northeast Scotland. Interventions included antibiotic stewardship to restrict use of so-called 4C (cephalosporins, co-amoxiclav, clindamycin, and fluoroquinolones) and macrolide antibiotics; a hand hygiene campaign; hospital environment inspections; and MRSA admission screening.

Total effects were defined as the difference between scenarios with intervention (observed) and without intervention (predicted from time-series models). The primary outcomes were prevalence density of MRSA infections per 1000 occupied bed days (OBDs) in hospitals or per 10,000 inhabitants per day (IDs) in the community.

FINDINGS: During antibiotic stewardship, use of 4C and macrolide antibiotics fell by 47% (mean decrease 224 defined daily doses [DDDs] per 1000 OBDs, 95% CI 154-305, p=0·008) in hospitals and 27% (mean decrease 2·52 DDDs per 1000 IDs, 0·65-4·55, p=0·031) in the community. Hospital prevalence densities of MRSA were inversely related to intensified infection prevention and control, but positively associated with MRSA rates in neighbouring hospitals, importation pressures, bed occupancy, and use of fluoroquinolones, co-amoxiclav, and third-generation cephalosporins, or macrolide antibiotics that exceeded hospital-specific thresholds. Community prevalence density was predicted by hospital MRSA rates and above-threshold use of macrolides, fluoroquinolones, and clindamycin. MRSA prevalence density decreased during antibiotic stewardship by 54% (mean reduction 0·60 per 1000 OBDs, 0·01-1·18, p=0·049) in hospital and 37% (mean reduction 0·017 per 10,000 IDs, 0·004-0·029, p=0·012) in the community. Combined with infection prevention and control measures, MRSA prevalence density was reduced by 50% (absolute difference 0·94 cases per 1000 OBDs, 0·27-1·62, p=0·006) in hospitals and 47% (absolute difference 0·033 cases per 10,000 IDs, 0·018-0·048, p<0·0001) in the community.

INTERPRETATION: Alongside infection control measures, removal of key antibiotic selection pressures during a national antibiotic stewardship intervention predicted large and sustained reductions in hospital-associated and community-associated MRSA.

FUNDING: NHS Grampian Research & Development Fund.

Comment in Putting your money where your mouth is: Scotland's attack on MRSA pays off. [Lancet Infect Dis. 2015]

Antibiotic susceptibility testing

2.13 Rapid electrochemical phenotypic profiling of antibiotic-resistant bacteria. Besant JD, Sargent EH, Kelley SO. Lab Chip. 2015 Jul 7;15(13):2799-807. doi: 10.1039/c5lc00375j. Epub 2015 May 26.

Rapid phenotyping of bacteria to identify drug-resistant strains is an important capability for the treatment and management of infectious disease. At present, the rapid determination of antibiotic susceptibility is hindered by the requirement that, in existing devices, bacteria must be pre-cultured for 2-3 days to reach detectable levels. Here we report a novel electrochemical approach that achieves rapid readout of the antibiotic susceptibility profile of a bacterial infection within one hour. The electrochemical reduction of a redox-active molecule is monitored that reports on levels of metabolically-active bacteria. Bacteria are captured in miniaturized wells, incubated with antimicrobials and monitored for resistance. This electrochemical phenotyping approach is effective with clinically-relevant levels of bacteria, and provides results comparable to culture-based analysis. Results, however, are delivered on a much faster timescale, with resistance profiles available after a one hour incubation period.

2.14 Antibiograms in five pipetting steps: precise dilution assays in sub-microliter volumes with a conventional pipette. Derzsi L, Kaminski TS, Garstecki P. Lab Chip. 2016 Feb 23;16(5):893-901. doi: 10.1039/c5lc01151e.

We demonstrate a standalone microfluidic chip that allows us to carry out commonly executed antibiotic susceptibility assays in an array of nanoliter droplets. We eliminated the need for automation in performing an exemplary complicated liquid handling assay on a chip. Operations on droplets are hard-wired into the microfluidic chip. The liquid handling protocol can be executed with a simple and commonly available source of flow such as an automatic manual pipette. The system passively prepares a series of dilutions of a chemical compound and mixes them with portions of the sample. The precision of metering, merging, mixing, and splitting of discrete portions of liquid samples is rooted in the passive capillary

action in microfluidic traps and not in the precision of dosing with a pipette. We show an exemplary use of the device in the determination of the minimum inhibitory concentration (MIC) of ampicillin against E. coli ATCC 25922.

Diagnostics

2.15 A novel host-proteome signature for distinguishing between acute bacterial and viral infections. Oved K, Cohen A, Boico O, Navon R, Friedman T, Etshtein L, Kriger O, Bamberger E, Fonar Y, Yacobov R, Wolchinsky R, Denkberg G, Dotan Y, Hochberg A, Reiter Y, Grupper M, Srugo I, Feigin P, Gorfine M, Chistyakov I, Dagan R, Klein A, Potasman I, Eden E. PLoS One. 2015 Mar 18;10(3):e0120012. doi: 10.1371/journal.pone.0120012. eCollection 2015.

Bacterial and viral infections are often clinically indistinguishable, leading to inappropriate patient management and antibiotic misuse. Bacterial-induced host proteins such as procalcitonin, C-reactive protein (CRP), and Interleukin-6, are routinely used to support diagnosis of infection. However, their performance is negatively affected by inter-patient variability, including time from symptom onset, clinical syndrome, and pathogens. Our aim was to identify novel viral-induced host proteins that can complement bacterial-induced proteins to increase diagnostic accuracy. Initially, we conducted a bioinformatic screen to identify putative circulating host immune response proteins. The resulting 600 candidates were then quantitatively screened for diagnostic potential using blood samples from 1002 prospectively recruited patients with suspected acute infectious disease and controls with no apparent infection. For each patient, three independent physicians assigned a diagnosis based on comprehensive clinical and laboratory investigation including PCR for 21 pathogens yielding 319 bacterial, 334 viral, 112 control and 98 indeterminate diagnoses; 139 patients were excluded based on predetermined criteria. The best performing host-protein was TNF-related apoptosis-inducing ligand (TRAIL) (area under the curve [AUC] of 0.89; 95% confidence interval [CI], 0.86 to 0.91), which was consistently up-regulated in viral infected patients. We further developed a multi-protein signature using logistic-regression on half of the patients and validated it on the remaining half. The signature with the highest precision included both viral- and bacterial-induced proteins: TRAIL, Interferon gamma-induced protein-10, and CRP (AUC of 0.94; 95% CI, 0.92 to 0.96). The signature was superior to any of the individual proteins (P<0.001), as well as routinely used clinical parameters and their combinations (P<0.001). It remained robust across different physiological systems, times from symptom onset, and pathogens (AUCs 0.87-1.0). The accurate differential diagnosis provided by this novel combination of viral- and bacterial-induced proteins has the potential to improve management of patients with acute infections and reduce antibiotic misuse.

2.16 Serum Procalcitonin Measurement and Viral Testing to Guide Antibiotic Use for Respiratory Infections in Hospitalized Adults: A Randomized Controlled Trial. Branche AR, Walsh EE, Vargas R, Hulbert B, Formica MA, Baran A, Peterson DR, Falsey AR. J Infect Dis. 2015 Dec 1;212(11):1692-700. doi: 10.1093/infdis/jiv252. Epub 2015 Apr 24.

BACKGROUND: Viral lower respiratory tract illness (LRTI) frequently causes adult hospitalization and is linked to antibiotic overuse. European studies suggest that the serum procalcitonin (PCT) level may be used to guide antibiotic therapy. We conducted a trial assessing the feasibility of using PCT algorithms with viral testing to guide antibiotic use in a US hospital.

METHODS: Three hundred patients hospitalized with nonpneumonic LRTI during October 2013-April 2014 were randomly assigned at a ratio of 1:1 to receive standard care or PCT-guided care and viral PCR testing. The primary outcome was antibiotic exposure, and safety was assessed at 1 and 3 months.

RESULTS: Among the 151 patients in the intervention group, viruses were identified in 42% (63), and 83% (126) had PCT values of <0.25 μg/mL. There were no significant differences in antibiotic use or adverse events between intervention patients and those in the nonintervention group. Subgroup analyses revealed fewer subjects with positive results of viral testing and low PCT values who were discharged receiving antibiotics (20% vs 45%; P = .002) and shorter antibiotic durations among algorithm-adherent

intervention patients versus nonintervention patients (2.0 vs 4.0 days; P = .004). Compared with historical controls (from 2008-2011), antibiotic duration in nonintervention patients decreased by 2 days (6.0 vs 4.0 days; P < .001), suggesting a study effect.

CONCLUSIONS: Although antibiotic use was similar in the 2 arms, subgroup analyses of intervention patients suggest that physicians responded to viral and biomarker data. These data can inform the design of future US studies.

CLINICAL TRIALS REGISTRATION: NCT01907659.

Comment in Where Do We Go From Here? [J Infect Dis. 2015]

FROM DIAGNOSTIC MICROBIOLOGY TO EMERGING PATHOGENS (G. Greub): Automation

3.1.

Faron ML, Buchan BW, Vismara C, Lacchini C, Bielli A, Gesu G, Liebregts T, van Bree A, Jansz A, Soucy G, Korver J, Ledeboer NA. Automated Scoring of Chromogenic Media for Detection of Methicillin-Resistant Staphylococcus aureus by Use of WASPLab Image Analysis Software. J Clin Microbiol. 2016 Mar;54(3):620-4.

Recently, systems have been developed to create total laboratory automation for clinical microbiology. These systems allow for the automation of specimen processing, specimen incubation, and imaging of bacterial growth. In this study, we used the WASPLab to validate software that discriminates and segregates positive and negative chromogenic methicillin-resistant Staphylococcus aureus (MRSA) plates by recognition of pigmented colonies. A total of 57,690 swabs submitted for MRSA screening were enrolled in the study. Four sites enrolled specimens following their standard of care. Chromogenic agar used at these sites included MRSASelect (Bio-Rad Laboratories, Redmond, WA), chromID MRSA (bioMérieux, Marcy l'Etoile, France), and CHROMagar MRSA (BD Diagnostics, Sparks, MD). Specimens were plated and incubated using the WASPLab. The digital camera took images at 0 and 16 to 24 h and the WASPLab software determined the presence of positive colonies based on a hue, saturation, and value (HSV) score. If the HSV score fell within a defined threshold, the plate was called positive. The performance of the digital analysis was compared to manual reading. Overall, the digital software had a sensitivity of 100% and a specificity of 90.7% with the specificity ranging between 90.0 and 96.0 across all sites. The results were similar using the three different agars with a sensitivity of 100% and specificity ranging between 90.7 and 92.4%. These data demonstrate that automated digital analysis can be used to accurately sort positive from negative chromogenic agar cultures regardless of the pigmentation produced.

Comment: First article reporting the use of a smart imaging system allowing automated detection of MRSA growth on chromogenic agar

3.2.

Quiblier C, Jetter M, Rominski M, Mouttet F, BÃ¶ttger EC, Keller PM, Hombach M. Performance of Copan WASP for Routine Urine Microbiology. J Clin Microbiol. 2016 Mar;54(3):585-92.

This study compared a manual workup of urine clinical samples with fully automated WASPLab

processing. As a first step, two different inocula (1 and 10 µl) and different streaking patterns were compared using WASP and InoqulA BT instrumentation. Significantly more single colonies were produced with the10-µl inoculum than with the 1-µl inoculum, and automated streaking yielded significantly more single colonies than manual streaking on whole plates ($P < 0.001$). In a second step, 379 clinical urine samples were evaluated using WASP and the manual workup. Average numbers of detected morphologies, recovered species, and CFUs per milliliter of all 379 urine samples showed excellent agreement between WASPLab and the manual workup. The percentage of urine samples clinically categorized as positive or negative did not differ between the automated and manual workflow, but within the positive samples, automated processing by WASPLab resulted in the detection of more potential pathogens. In summary, the present study demonstrates that (i) the streaking pattern, i.e., primarily the number of zigzags/length of streaking lines, is critical for optimizing the number of single colonies yielded from primary cultures of urine samples; (ii) automated streaking by the WASP instrument is superior to manual streaking regarding the number of single colonies yielded (for 32.2% of the samples); and (iii) automated streaking leads to higher numbers of detected morphologies (for 47.5% of the samples), species (for 17.4% of the samples), and pathogens (for 3.4% of the samples). The results of this study point to an improved quality of microbiological analyses and laboratory reports when using automated sample processing by WASP and WASPLab.

Comment: Improved quality thank to automation

3.3.

Croxatto A, Dijkstra K, Prod'hom G, Greub G. Comparison of Inoculation with the InoqulA and WASP Automated Systems with Manual Inoculation. J Clin Microbiol. 2015 Jul;53(7):2298-307.

The quality of sample inoculation is critical for achieving an optimal yield of discrete colonies in both monomicrobial and polymicrobial samples to perform identification and antibiotic susceptibility testing. Consequently, we compared the performance between the InoqulA (BD Kiestra), the WASP (Copan), and manual inoculation methods. Defined mono- and polymicrobial samples of 4 bacterial species and cloudy urine specimens were inoculated on chromogenic agar by the InoqulA, the WASP, and manual methods. Images taken with ImagA (BD Kiestra) were analyzed with the VisionLab version 3.43 image analysis software to assess the quality of growth and to prevent subjective interpretation of the data. A 3- to 10-fold higher yield of discrete colonies was observed following automated inoculation with both the InoqulA and WASP systems than that with manual inoculation. The difference in performance between automated and manual inoculation was mainly observed at concentrations of >10(6) bacteria/ml. Inoculation with the InoqulA system allowed us to obtain significantly more discrete colonies than the WASP system at concentrations of >10(7) bacteria/ml. However, the level of difference observed was bacterial species dependent. Discrete colonies of bacteria present in 100- to 1,000-fold lower concentrations than the most concentrated populations in defined polymicrobial samples were not reproducibly recovered, even with the automated systems. The analysis of cloudy urine specimens showed that InoqulA inoculation provided a statistically significantly higher number of discrete colonies than that with WASP and manual inoculation. Consequently, the automated InoqulA inoculation greatly decreased the requirement for bacterial subculture and thus resulted in a significant reduction in the time to results, laboratory workload, and laboratory costs.

Comment: Improved quality, reduced time to results and reduced costs thank to automation

3.4.

Wang H, Fan YY, Kudinha T, Xu ZP, Xiao M, Zhang L, Fan X, Kong F, Xu YC. A Comprehensive Evaluation of Bruker Biotyper MS and Vitek MS Matrix-Assisted Laser Desorption/Ionization Time-of-Flight Mass Spectrometry Systems for the Identification of Yeasts - Part of the National China Hospital Invasive Fungal Surveillance Net (CHIF-NET) Study, 2012-2013. J Clin Microbiol. 2016 Feb 24.

Among the 2,683 yeast isolates representing 41 different species (25 Candida and Candida-related species and 16 non-Candida yeast species) collected in the National China Hospital Invasive Fungal Surveillance Net program (CHIF-NET) (2012-2013), Bruker Biotyper MALDI-TOF MS system exhibited significantly higher accuracy rates than the Vitek MS system for identification of all yeast isolates (98.8% vs. 95.4%, p <0.001 by Pearson's chi-square test) and for all Candida and Candida-related species isolates (99.4% vs. 95.5%, p <0.001).

Confirm that Bruker MALDI-TOF MS exhibit a very high quality of identification with > 99% reliable identification of Candida species and 98.8% overall for fungi

3.5.

Veenemans J, Welker M, van Belkum A, Saccomani MC, Girard V, Pettersson A, Verhulst C, Kluytmans-Vandenbergh M, Kluytmans J. Comparison of MALDI-TOF MS and AFLP for strain typing of ESBL-producing Escherichia coli. Eur J Clin Microbiol Infect Dis. 2016 Feb 27.

Typing of bacterial isolates using matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) potentially provides an efficient on-site method to monitor the spread of antibiotic-resistant bacteria and rapidly detect outbreaks. We compared MALDI-MS typing results to those of amplified fragment length polymorphism (AFLP) in a collection of 52 ESBL-producing Escherichia coli, isolated in a Dutch nursing home with an on-going outbreak of ST131 E. coli. Specific MALDI types were defined based on spectral data from four replicate colony samples of isolates grown on Columbia agar using multivariate statistical procedures. Type-specific superspectra were computed for four E .coli MALDI-types and tested for the potential of rapid and automated typing. The effect of different incubation conditions on typing performance was tested by analysing five isolates incubated for 24 h and 48 h on five different media. Types defined based on MALDI spectra were largely in agreement with the AFLP results, although some MALDI types comprised of more than one AFLP type. In particular, isolates belonging to ST131 showed distinct mass patterns. The proportion of isolates correctly assigned was substantially lower for isolates incubated on Sabouraud-dextrose and Drigalski agars for 24 h, and for those incubated for 48 h (all media). Our results show that the identification of type-specific peaks potentially allows direct typing of isolates belonging to specific clonal lineages. Both incubation time and media affected type assignment, suggesting that there is a need for a careful standardization of incubation time and culturing conditions when developing MALDI-typing schemes for E. coli.

Comment: MALDI-TOF based typing may be accurate, but only if performed in standardized conditions and using specific database & analytical bioinformatic algorithms.

3.6.

Kehrmann J, Wessel S, Murali R, Hampel A, Bange FC, Buer J, Mosel F. Principal component analysis of MALDI TOF MS mass spectra separates M. abscessus (sensu stricto) from M. massiliense isolates. BMC Microbiol. 2016 Mar 1;16(1):24.

BACKGROUND: The discrimination of the members of the Mycobacterium abscessus complex is of clinical interest because one of the subspecies, M. massiliense, exhibits higher rates of response to antibiotic treatment for lung infection than do the other members of that complex. M. abscessus complex contains three subspecies that are laborious to identify; therefore, a routine diagnostic tool would be worthwhile.

RESULTS: We used principal component analysis, hierarchical cluster analysis, and single-peak analysis to examine peak lists derived from matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI TOF MS) mass spectra of 50 clinical M. abscessus complex isolates, including 28 M. abscessus (sensu stricto), 19 M. massiliense, and 3 M. bolletii isolates grown in mycobacterium growth indicator tube liquid medium and prepared with a bead-based protocol. Principal component analysis but not hierarchical cluster analysis separated M. abscessus (sensu stricto) isolates and M. massiliense isolates into two clusters. Furthermore, single-peak analysis displayed 4 discriminating peaks that separated M. abscessus (sensu stricto) from M. massiliense isolates. M. bolletii isolates did not exhibit specific peaks but resembled the M. abscessus (sensu stricto) peak profile and also grouped within this principal component analysis cluster. Principal component analysis of all peak lists with the exclusion of the four discriminating peaks again separated M. abscessus (sensu stricto) from M. massiliense isolates, thus relativizing the importance of these peaks for subspecies identification.

CONCLUSIONS: Principal component analysis of peak lists derived from MALDI TOF mass spectra is a robust and convenient method of discriminating M. massiliense isolates from the other members of the M. abscessus complex.

Comment: New bioinformatic analytical pipelines such as PCA might help identifying using MALDI-TOF closely related species

3.7.

Yssouf A, Almeras L, Berenger JM, Laroche M, Raoult D, Parola P.

Identification of tick species and disseminate pathogen using hemolymph by MALDI-TOF MS. Ticks Tick Borne Dis. 2015 Jul;6(5):579-86.

BACKGROUND: Matrix Assisted Laser Desorption/Ionization Time-of-Flight Mass Spectrometry (MALDI-TOF MS) is increasingly emerging tool for identification of arthropods including tick vectors using whole or body part of specimens. The challenges of the present study were to assess MALDI-TOF MS profiling for the both identification of tick species and Rickettsia spp. in infected ticks using hemolymph as protein mixture.

METHODS: Firstly, hemolymph protein mixture from legs of 5 tick species, Rhipicephalus sanguineus, Rhipicephalus bursa, Dermacentor marginatus, Hyalomma marginatum rufipes and Amblyomma variegatum infected by Rickettsia africae were submitted to MALDI-TOF MS to assess tick species identification ability. Secondly, hemolymph MS spectra from Rh. sanguineus infected or not by Rickettsia c. conorii were compared to detect protein profiles changes. Finally, leg hemolymph MS spectra from new specimens of the 5 tick species were tested blindly including ticks infected by R. c. conorii. Discriminating mass peaks distinguishing the R. c. conorii infected and non-infected Rh sanguineus were determined.

RESULTS: Consistent and reproducible MS profiles were obtained into each tick species. Comparison of MS spectra revealed distinct hemolymph protein profiles according to tick species. MS spectra changes were observed between hemolymphs from R. c. conorii-infected and non-infected Rh. sanguineus specimens, revealing 17 discriminating mass peaks. Clustering analysis based on MS protein profiles highlighted that hemolymph samples were grouped according to tick species. All tick hemolymph samples

blindly tested against our home-made arthropod MS reference database were correctly identified at the species distinguishing also R. c. conorii-infected from Rickettsia-free Rh. sanguineus specimens.

CONCLUSION: The present study demonstrated the use of hemolymph MS profiles for dual identification of tick species and associated pathogens. This concomitant identification could be helpful for tick entomological diagnosis, notably for specimens removed directly on patients..

Comment: MALDI-TOF may also be used to successfully identify the tick species & the bacterial pathogens present in the tick hemolymphs, by simply starting from the tick hemolymph

3.8.

Buckwalter SP, Olson SL, Connelly BJ, Lucas BC, Rodning AA, Walchak RC, Deml SM, Wohlfiel SL, Wengenack NL. Evaluation of Matrix-Assisted Laser Desorption Ionization-Time of Flight Mass Spectrometry for Identification of Mycobacterium species, Nocardia species, and Other Aerobic Actinomycetes. J Clin Microbiol. 2016 Feb;54(2):376-84.

The value of matrix-assisted laser desorption ionization-time of flight mass spectrometry (MALDI-TOF MS) for the identification of bacteria and yeasts is well documented in the literature. Its utility for the identification of mycobacteria and Nocardia spp. has also been reported in a limited scope. In this work, we report the specificity of MALDI-TOF MS for the identification of 162 Mycobacterium species and subspecies, 53 Nocardia species, and 13 genera (totaling 43 species) of other aerobic actinomycetes using both the MALDI-TOF MS manufacturer's supplied database(s) and a custom database generated in our laboratory. The performance of a simplified processing and extraction procedure was also evaluated, and, similar to the results in an earlier literature report, our viability studies confirmed the ability of this process to inactivate Mycobacterium tuberculosis prior to analysis. Following library construction and the specificity study, the performance of MALDI-TOF MS was directly compared with that of 16S rRNA gene sequencing for the evaluation of 297 mycobacteria isolates, 148 Nocardia species isolates, and 61 other aerobic actinomycetes isolates under routine clinical laboratory working conditions over a 6-month period. MALDI-TOF MS is a valuable tool for the identification of these groups of organisms. Limitations in the databases and in the ability of MALDI-TOF MS to rapidly identify slowly growing mycobacteria are discussed.

Comment: MALDI-TOF is an accurate tool to identify mycobacteria and Nocardia; incomplete databases remains one of the major limitation of the MALDI-TOF.

3.9.

Charretier Y, Dauwalder O, Franceschi C, Degout-Charmette E, Zambardi G, Cecchini T, Bardet C, Lacoux X, Dufour P, Veron L, Rostaing H, Lanet V, Fortin T, Beaulieu C, Perrot N, Dechaume D, Pons S, Girard V, Salvador A, Durand G, Mallard F, Theretz A, Broyer P, Chatellier S, Gervasi G, Van Nuenen M, Roitsch CA, Van Belkum A, Lemoine J, Vandenesch F, Charrier JP. Rapid Bacterial Identification, Resistance, Virulence and Type Profiling using Selected Reaction Monitoring Mass Spectrometry.

Sci Rep. 2015 Sep 9;5:13944

Mass spectrometry (MS) in Selected Reaction Monitoring (SRM) mode is proposed for in-depth characterisation of microorganisms in a multiplexed analysis. Within 60-80 minutes, the SRM method performs microbial identification (I), antibiotic-resistance detection (R), virulence assessment (V) and it provides epidemiological typing information (T). This SRM application is illustrated by the analysis of the human pathogen Staphylococcus aureus, demonstrating its promise for rapid characterisation of bacteria from positive blood cultures of sepsis patients.

Comment: Mass spectrometry may be in the future a powerful tool allowing short time to results for additional applications such as virulence factors, antibiotic susceptibility and typing when applied on positive blood cultures

Blood cultures

3.10.

Jo SJ, Park KG, Han K, Park DJ, Park YJ. Direct Identification and Antimicrobial Susceptibility Testing of Bacteria From Positive Blood Culture Bottles by Matrix-Assisted Laser Desorption/Ionization Time-of-Flight Mass Spectrometry and the Vitek 2 System. Ann Lab Med. 2016 Mar;36(2):117-23.

BACKGROUND: We evaluated the reliability and accuracy of the combined use of matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) bacterial identification and Vitek 2 antimicrobial susceptibility testing (AST) for bacteria from positive blood culture bottles.

METHODS: Direct identification and AST were performed in parallel to the standard methods in monomicrobial positive blood culture bottles. In total, 254 isolates grown on aerobic and/or anaerobic bottles were identified with MALDI-TOF Vitek MS (bioMérieux, France), and 1,978 microorganism/antimicrobial agent combinations were assessed. For isolates from anaerobic bottles, an aliquot of the culture broth was centrifuged, washed, and filtered through a nylon mesh. For isolates from aerobic/pediatric bottles, a lysis step using 9.26% ammonium chloride solution and 2% saponin solution was included.

RESULTS: The overall correct identification rate was 81.8% (208/254) and that for gram-positive/gram-negative isolates was 73.9%/92.6%, respectively, and it was 81.8%, 87.6%, and 57.9% for isolates from aerobic, anaerobic, and pediatric bottles, respectively. Identification was not possible in 45 cases, and most of these isolates were streptococci (N=14) and coagulase-negative staphylococci (N=11). Misidentification occurred only in one case. Compared with standard methods, direct AST showed 97.9% (1,936/1,978) agreement with very major error of 0.25%, major error of 0.05%, and minor error of 1.8%.

CONCLUSIONS: This simple and cost-effective sample preparation method gives reliable results for the direct identification and AST of bacteria. For the identification of streptococci and coagulase-negative staphylococci, the method should be further improved.

Comment: MALDI-TOF and AB Susceptibility testing directly on positive blood culture pellet after lysis with saponin and ammonium chloride provides reliable results in >99% of available identification and for 98.7% of AST results

3.11.

Wattal C, Oberoi JK.Microbial identification and automated antibiotic susceptibility testing directly from positive blood cultures using MALDI-TOF MS and VITEK 2. Eur J Clin Microbiol Infect Dis. 2016 Jan;35(1):75-82.

The study addresses the utility of Matrix Assisted Laser Desorption/Ionisation Time-Of-Flight mass spectrometry (MALDI-TOF MS) using VITEK MS and the VITEK 2 antimicrobial susceptibility testing (AST) system for direct identification (ID) and timely AST from positive blood culture bottles using a lysis-filtration method (LFM). Between July and December 2014, a total of 140 non-duplicate mono-microbial blood cultures were processed. An aliquot of positive blood culture broth was incubated with lysis buffer before the bacteria were filtered and washed. Micro-organisms recovered from the filter were first identified using VITEK MS and its suspension was used for direct AST by VITEK 2 once the ID was known. Direct ID and AST results were compared with classical methods using solid growth. Out of the 140 bottles tested, VITEK MS resulted in 70.7 % correct identification to the genus and/ or species level. For the 103 bottles where identification was possible, there was agreement in 97 samples (94.17 %) with

classical culture. Compared to the routine method, the direct AST resulted in category agreement in 860 (96.5 %) of 891 bacteria-antimicrobial agent combinations tested. The results of direct ID and AST were available 16.1 hours before those of the standard approach on average. The combined use of VITEK MS and VITEK 2 directly on samples from positive blood culture bottles using a LFM technique can result in rapid and reliable ID and AST results in blood stream infections to result in early institution of targeted treatment. The combination of LFM and AST using VITEK 2 was found to expedite AST more reliably.

Comment: MALDI-TOF and AB Susceptibility testing directly on positive blood culture reduce significantly the time to results

3.12.

Dolch ME, Janitza S, Boulesteix AL, Graßmann-Lichtenauer C, Praun S, Denzer W, Schelling G, Schubert S. Gram-negative and -positive bacteria differentiation in blood culture samples by headspace volatile compound analysis. J Biol Res (Thessalon). 2016 Mar 12;23:3.

BACKGROUND: Identification of microorganisms in positive blood cultures still relies on standard techniques such as Gram staining followed by culturing with definite microorganism identification. Alternatively, matrix-assisted laser desorption/ionization time-of-flight mass spectrometry or the analysis of headspace volatile compound (VC) composition produced by cultures can help to differentiate between microorganisms under experimental conditions. This study assessed the efficacy of volatile compound based microorganism differentiation into Gram-negatives and -positives in unselected positive blood culture samples from patients.

METHODS: Headspace gas samples of positive blood culture samples were transferred to sterilized, sealed, and evacuated 20 ml glass vials and stored at -30 °C until batch analysis. Headspace gas VC content analysis was carried out via an auto sampler connected to an ion-molecule reaction mass spectrometer (IMR-MS). Measurements covered a mass range from 16 to 135 u including CO2, H2, N2, and O2. Prediction rules for microorganism identification based on VC composition were derived using a training data set and evaluated using a validation data set within a random split validation procedure.

RESULTS: One-hundred-fifty-two aerobic samples growing 27 Gram-negatives, 106 Gram-positives, and 19 fungi and 130 anaerobic samples growing 37 Gram-negatives, 91 Gram-positives, and two fungi were analysed. In anaerobic samples, ten discriminators were identified by the random forest method allowing for bacteria differentiation into Gram-negative and -positive (error rate: 16.7 % in validation data set). For aerobic samples the error rate was not better than random.

CONCLUSIONS: In anaerobic blood culture samples of patients IMR-MS based headspace VC composition analysis facilitates bacteria differentiation into Gram-negative and -positiveTyping of schemes for E. coli.

Comment: Innovative approach potentially enabling in the future to automatically differentiate Gram positive from Gram negative bacteria growing in a blood culture system

Bacterial culture

3.13.

Peel TN, Dylla BL, Hughes JG, Lynch DT, Greenwood-Quaintance KE, Cheng AC, Mandrekar JN, Patel R. Improved Diagnosis of Prosthetic Joint Infection by Culturing Periprosthetic Tissue Specimens in Blood Culture Bottles. MBio. 2016 Jan 5;7(1).

Despite known low sensitivity, culture of periprosthetic tissue specimens on agars and in broths is routine. Culture of periprosthetic tissue samples in blood culture bottles (BCBs) is potentially more convenient, but it has been evaluated in a limited way and has not been widely adopted. The aim of this study was to compare the sensitivity and specificity of inoculation of periprosthetic tissue specimens into blood culture bottles with standard agar and thioglycolate broth culture, applying Bayesian latent class modeling (LCM) in addition to applying the Infectious Diseases Society of America (IDSA) criteria for prosthetic joint infection. This prospective cohort study was conducted over a 9-month period (August 2013 to April 2014) at the Mayo Clinic, Rochester, MN, and included all consecutive patients undergoing revision arthroplasty. Overall, 369 subjects were studied; 117 (32%) met IDSA criteria for prosthetic joint infection, and 82% had late chronic infection. Applying LCM, inoculation of tissues into BCBs was associated with a 47% improvement in sensitivity compared to the sensitivity of conventional agar and broth cultures (92.1 versus 62.6%, respectively); this magnitude of change was similar when IDSA criteria were applied (60.7 versus 44.4%, respectively; P = 0.003). The time to microorganism detection was shorter with BCBs than with standard media (P < 0.0001), with aerobic and anaerobic BCBs yielding positive results within a median of 21 and 23 h, respectively. Results of our study demonstrate that the semiautomated method of periprosthetic tissue culture in blood culture bottles is more sensitive than and as specific as agar and thioglycolate broth cultures and yields results faster.

IMPORTANCE: Prosthetic joint infections are a devastating complication of arthroplasty surgery. Despite this, current microbiological techniques to detect and diagnose infections are imperfect. This study examined a new approach to diagnosing infections, through the inoculation of tissue samples from around the prosthetic joint into blood culture bottles. This study demonstrated that, compared to current laboratory practices, this new technique increased the detection of infection. These findings are important for patient care to allow timely and accurate diagnosis of infection.

Comment: Improved sensitivity by inoculating tissue samples from around the prosthetic joint into blood culture bottles, as compared to current agar-bsed & thioglycolate broth-based cultures.

3.14.

Calderaro A, Martinelli M, Montecchini S, Motta F, Covan S, Larini S, Medici MC, Arcangeletti MC, Chezzi C, De Conto F. Higher recovery rate of microorganisms from cerebrospinal fluid samples by the BACTEC culture system in comparison with agar culture. Diagn Microbiol Infect Dis. 2016 Apr;84(4):281-6.

The aim of this study was to assess the diagnostic value of the BACTEC FX blood culture (BC) system as compared to the agar culture (AC) of cerebrospinal fluid samples (CSF), evaluating the recovery rate and the time to detection of microorganisms in a 3.5-year period. From December 2011 to May 2015, 1326 CSF samples (694 patients) were submitted to both AC and BC. Among the 150 positive samples (96 patients), 165 microorganisms were detected: 81 by both the protocols, 77 by BC alone, and 7 by AC alone, demonstrating a higher detection rate of BC (95.8%) than AC (53.3%). Although BC presents some disadvantages, it is able to improve the yield of clinically significant microorganisms, and it could potentially reduce the reporting time as compared to AC. The results obtained highlighted the necessity of a combined approach for the successful detection of central nervous system microbial infections.

Comment: Improved sensitivity by inoculating CSF into blood culture bottles

3.15.

Dione N, Khelaifia S, La Scola B, Lagier JC, Raoult D. A quasi-universal medium to break the aerobic/anaerobic bacterial culture dichotomy in clinical microbiology. Clin

Microbiol Infect. 2016 Jan;22(1):53-8.

In the mid-19th century, the dichotomy between aerobic and anaerobic bacteria was introduced. Nevertheless, the aerobic growth of strictly anaerobic bacterial species such as Ruminococcus gnavus and Fusobacterium necrophorum, in a culture medium containing antioxidants, was recently demonstrated. We tested aerobically the culture of 623 bacterial strains from 276 bacterial species including 82 strictly anaerobic, 154 facultative anaerobic, 31 aerobic and nine microaerophilic bacterial species as well as ten fungi. The basic culture medium was based on Schaedler agar supplemented with 1 g/L ascorbic acid and 0.1 g/L glutathione (R-medium). We successively optimized this media, adding 0.4 g/L uric acid, using separate autoclaving of the component, or adding haemin 0.1 g/L or α-ketoglutarate 2 g/L. In the basic medium, 237 bacterial species and ten fungal species grew but with no growth of 36 bacterial species, including 22 strict anaerobes. Adding uric acid allowed the growth of 14 further species including eight strict anaerobes, while separate autoclaving allowed the growth of all tested bacterial strains. To extend its potential use for fastidious bacteria, we added haemin for Haemophilus influenzae, Haemophilus parainfluenzae and Eikenella corrodens and α-ketoglutarate for Legionella pneumophila. This medium allowed the growth of all tested strains with the exception of Mycobacterium tuberculosis and Mycobacterium bovis. Testing primoculture and more fastidious species will constitute the main work to be done, but R-medium coupled with a rapid identification method (matrix-assisted laser desorption/ionization time-of-flight mass spectrometry) will facilitate the anaerobic culture in clinical microbiology laboratories.

Comment: A new growth medium that challenges the definition of anaerobes

Metagenomics & microbiome

3.16.

Decuypere S, Meehan CJ, Van Puyvelde S, De Block T, Maltha J, Palpouguini L, Tahita M, Tinto H, Jacobs J, Deborggraeve S. Diagnosis of Bacterial Bloodstream Infections: A 16S Metagenomics Approach. PLoS Negl Trop Dis. 2016 Feb 29;10(2):e0004470.

BACKGROUND: Bacterial bloodstream infection (bBSI) is one of the leading causes of death in critically ill patients and accurate diagnosis is therefore crucial. We here report a 16S metagenomics approach for diagnosing and understanding bBSI.

METHODOLOGY/PRINCIPAL FINDINGS: The proof-of-concept was delivered in 75 children (median age 15 months) with severe febrile illness in Burkina Faso. Standard blood culture and malaria testing were conducted at the time of hospital admission. 16S metagenomics testing was done retrospectively and in duplicate on the blood of all patients. Total DNA was extracted from the blood and the V3-V4 regions of the bacterial 16S rRNA genes were amplified by PCR and deep sequenced on an Illumina MiSeq sequencer. Paired reads were curated, taxonomically labeled, and filtered. Blood culture diagnosed bBSI in 12 patients, but this number increased to 22 patients when combining blood culture and 16S metagenomics results. In addition to superior sensitivity compared to standard blood culture, 16S metagenomics revealed important novel insights into the nature of bBSI. Patients with acute malaria or recovering from malaria had a 7-fold higher risk of presenting polymicrobial bloodstream infections compared to patients with no recent malaria diagnosis (p-value = 0.046). Malaria is known to affect epithelial gut function and may thus facilitate bacterial translocation from the intestinal lumen to the blood. Importantly, patients with such polymicrobial blood infections showed a 9-fold higher risk factor for not surviving their febrile illness (p-value = 0.030).

CONCLUSIONS/SIGNIFICANCE: Our data demonstrate that 16S metagenomics is a powerful approach for the diagnosis and understanding of bBSI. This proof-of-concept study also showed that appropriate control samples are crucial to detect background signals due to environmental contamination.

Comment: PCR-based metagenomics might possibly in the future be a useful approach to

improve the diagnosis of bacteremia, providing that accurate negative controls and interpretation of the results are done

3.17. Korem T, Zeevi D, Suez J, Weinberger A, Avnit-Sagi T, Pompan-Lotan M, Matot E, Jona G, Harmelin A, Cohen N, Sirota-Madi A, Thaiss CA, Pevsner-Fischer M, Sorek R, Xavier RJ, Elinav E, Segal E. Growth dynamics of gut microbiota in health and disease inferred from single metagenomic samples. Science. 2015 Sep 4;349(6252):1101-6

Metagenomic sequencing increased our understanding of the role of the microbiome in health and disease, yet it only provides a snapshot of a highly dynamic ecosystem. Here, we show that the pattern of metagenomic sequencing read coverage for different microbial genomes contains a single trough and a single peak, the latter coinciding with the bacterial origin of replication. Furthermore, the ratio of sequencing coverage between the peak and trough provides a quantitative measure of a species' growth rate. We demonstrate this in vitro and in vivo, under different growth conditions, and in complex bacterial communities. For several bacterial species, peak-to-trough coverage ratios, but not relative abundances, correlated with the manifestation of inflammatory bowel disease and type II diabetes.

Comment: Metagenomics may provide an estimate of the growth rate of the bacteria by comparing peak-to-through ratio of the coverage; this opens important future study that will help understanding the growth dynamics of human microbiota.

3.18.

Franzosa EA, Huang K, Meadow JF, Gevers D, Lemon KP, Bohannan BJ, Huttenhower C. Identifying personal microbiomes using metagenomic codes. Proc Natl Acad Sci U S A. 2015 Jun 2;112(22):E2930-8.

Community composition within the human microbiome varies across individuals, but it remains unknown if this variation is sufficient to uniquely identify individuals within large populations or stable enough to identify them over time. We investigated this by developing a hitting set-based coding algorithm and applying it to the Human Microbiome Project population. Our approach defined body site-specific metagenomic codes: sets of microbial taxa or genes prioritized to uniquely and stably identify individuals. Codes capturing strain variation in clade-specific marker genes were able to distinguish among 100s of individuals at an initial sampling time point. In comparisons with follow-up samples collected 30–300 d later, ∼30% of individuals could still be uniquely pinpointed using metagenomic codes from a typical body site; coincidental (false positive) matches were rare. Codes based on the gut microbiome were exceptionally stable and pinpointed >80% of individuals. The failure of a code to match its owner at a later time point was largely explained by the loss of specific microbial strains (at current limits of detection) and was only weakly associated with the length of the sampling interval. In addition to highlighting patterns of temporal variation in the ecology of the human microbiome, this work demonstrates the feasibility of microbiome-based identifiability—a result with important ethical implications for microbiome study design.

Comment: The microbiota is highly stable over time and somehow represents a fingerprint of a given individual

3.19.

Lim ES, Zhou Y, Zhao G, Bauer IK, Droit L, Ndao IM, Warner BB, Tarr PI, Wang D, Holtz LR. Early life dynamics of the human gut virome and bacterial microbiome in infants. Nat Med. 2015 Oct;21(10):1228-34.

The early years of life are important for immune development and influence health in adulthood. Although it has been established that the gut bacterial microbiome is rapidly acquired after birth, less is known about the viral microbiome (or 'virome'), consisting of bacteriophages and eukaryotic RNA and DNA viruses, during the first years of life. Here, we characterized the gut virome and bacterial microbiome in a longitudinal cohort of healthy infant twins. The virome and bacterial microbiome were more similar between co-twins than between unrelated infants. From birth to 2 years of age, the eukaryotic virome and the bacterial microbiome expanded, but this was accompanied by a contraction of and shift in the bacteriophage virome composition. The bacteriophage-bacteria relationship begins from birth with a high predator-low prey dynamic, consistent with the Lotka-Volterra prey model. Thus, in contrast to the stable microbiome observed in adults, the infant microbiome is highly dynamic and associated with early life changes in the composition of bacteria, viruses and bacteriophages with age.

Comment: The gut microbiota of very young infants is highly dynamic, contrasting with the high stability of the microbiota later in life

3.20.

Bäckhed F, Roswall J, Peng Y, Feng Q, Jia H, Kovatcheva-Datchary P, Li Y, Xia Y, Xie H, Zhong H, Khan MT, Zhang J, Li J, Xiao L, Al-Aama J, Zhang D, Lee YS, Kotowska D, Colding C, Tremaroli V, Yin Y, Bergman S, Xu X, Madsen L, Kristiansen K, Dahlgren J, Wang J. Dynamics and Stabilization of the Human Gut Microbiome during the First Year of Life. Cell Host Microbe. 2015 Jun 10;17(6):852.

The gut microbiota is central to human health, but its establishment in early life has not been quantitatively and functionally examined. Applying metagenomics analysis on fecal samples from a large cohort of Swedish infants and their mothers, we characterized the gut microbiome during the first year of life and assessed the impact of mode of delivery and feeding on its establishment. In contrast to vaginally delivered infants, the gut microbiota of infants delivered by C-section showed significantly less resemblance to their mothers. Nutrition had a major impact on early microbiota composition and function, with cessation of breast-feeding, rather than introduction of solid food, being required for maturation into an adult-like microbiota. Microbiota composition and ecological network had distinctive features at each sampled stage, in accordance with functional maturation of the microbiome. Our findings establish a framework for understanding the interplay between the gut microbiome and the human body in early life.

Comment: Impact of the mode of delivery on the microbiota composition of newborns

3.21.

Loman NJ, Quick J, Simpson JT. A complete bacterial genome assembled de novo using only nanopore sequencing data. Nat Methods. 2015 Aug;12(8):733-5.

We have assembled de novo the Escherichia coli K-12 MG1655 chromosome in a single 4.6-Mb contig using only nanopore data. Our method has three stages: (i) overlaps are detected between reads and then corrected by a multiple-alignment process; (ii) corrected reads are assembled using the Celera Assembler; and (iii) the assembly is polished using a probabilistic model of the signal-level data. The assembly reconstructs gene order and has 99.5% nucleotide identity.

Comment: Nanopore technology: a promising sequencing approach, even for de novo sequencing ?!

3.22.

Quick J, Ashton P, Calus S, Chatt C, Gossain S, Hawker J, Nair S, Neal K, Nye K, Peters T, De Pinna E, Robinson E, Struthers K, Webber M, Catto A, Dallman TJ, Hawkey P, Loman NJ. Rapid draft sequencing and real-time nanopore sequencing in a hospital outbreak of Salmonella. Genome Biol. 2015 May 30;16:114.

BACKGROUND: Foodborne outbreaks of Salmonella remain a pressing public health concern. We recently detected a large outbreak of Salmonella enterica serovar Enteritidis phage type 14b affecting more than 30 patients in our hospital. This outbreak was linked to community, national and European-wide cases. Hospital patients with Salmonella are at high risk, and require a rapid response. We initially investigated this outbreak by whole-genome sequencing using a novel rapid protocol on the Illumina MiSeq; we then integrated these data with whole-genome data from surveillance sequencing, thereby placing the outbreak in a national context. Additionally, we investigated the potential of a newly released sequencing technology, the MinION from Oxford Nanopore Technologies, in the management of a hospital outbreak of Salmonella.

RESULTS: We demonstrate that rapid MiSeq sequencing can reduce the time to answer compared to the standard sequencing protocol with no impact on the results. We show, for the first time, that the MinION can acquire clinically relevant information in real time and within minutes of a DNA library being loaded. MinION sequencing permits confident assignment to species level within 20 min. Using a novel streaming phylogenetic placement method samples can be assigned to a serotype in 40 min and determined to be part of the outbreak in less than 2 h.

CONCLUSIONS: Both approaches yielded reliable and actionable clinical information on the Salmonella outbreak in less than half a day. The rapid availability of such information may facilitate more informed epidemiological investigations and influence infection control practices.

Comment: Nanopore technology may be used for outbreaks investigation, one of the main current application of bacterial genomics

3.23.

Bradley P, Gordon NC, Walker TM, Dunn L, Heys S, Huang B, Earle S, Pankhurst LJ, Anson L, de Cesare M, Piazza P, Votintseva AA, Golubchik T, Wilson DJ, Wyllie DH, Diel R, Niemann S, Feuerriegel S, Kohl TA, Ismail N, Omar SV, Smith EG, Buck D, McVean G, Walker AS, Peto TE, Crook DW, Iqbal Z. Rapid antibiotic-resistance predictions from genome sequence data for Staphylococcus aureus and Mycobacterium tuberculosis. Nat Commun. 2015 Dec 21;6:10063.

The rise of antibiotic-resistant bacteria has led to an urgent need for rapid detection of drug resistance in clinical samples, and improvements in global surveillance. Here we show how de Bruijn graph representation of bacterial diversity can be used to identify species and resistance profiles of clinical isolates. We implement this method for Staphylococcus aureus and Mycobacterium tuberculosis in a software package ('Mykrobe predictor') that takes raw sequence data as input, and generates a clinician-friendly report within 3 minutes on a laptop. For S. aureus, the error rates of our method are comparable to gold-standard phenotypic methods, with sensitivity/specificity of 99.1%/99.6% across 12 antibiotics (using an independent validation set, n=470). For M. tuberculosis, our method predicts resistance with sensitivity/specificity of 82.6%/98.5% (independent validation set, n=1,609); sensitivity is lower here, probably because of limited understanding of the underlying genetic mechanisms. We give evidence that minor alleles improve detection of extremely drug-resistant strains, and demonstrate feasibility of the use of emerging single-molecule nanopore sequencing techniques for these purposes.

Comment: Predicting antibiotic resitance based on genomic data: a challenge

3.24

Hutchison CA 3rd, Chuang RY, Noskov VN, Assad-Garcia N, Deerinck TJ, Ellisman MH, Gill J, Kannan K, Karas BJ, Ma L, Pelletier JF, Qi ZQ, Richter RA, Strychalski EA, Sun L, Suzuki Y, Tsvetanova B, Wise KS, Smith HO, Glass JI, Merryman C, Gibson DG, Venter JC. Design and synthesis of a minimal bacterial genome. Science. 2016 Mar 25;351(6280):aad6253.

We used whole-genome design and complete chemical synthesis to minimize the 1079-kilobase pair synthetic genome of Mycoplasma mycoides JCVI-syn1.0. An initial design, based on collective knowledge of molecular biology combined with limited transposon mutagenesis data, failed to produce a viable cell. Improved transposon mutagenesis methods revealed a class of quasi-essential genes that are needed for robust growth, explaining the failure of our initial design. Three cycles of design, synthesis, and testing, with retention of quasi-essential genes, produced JCVI-syn3.0 (531 kilobase pairs, 473 genes), which has a genome smaller than that of any autonomously replicating cell found in nature. JCVI-syn3.0 retains almost all genes involved in the synthesis and processing of macromolecules. Unexpectedly, it also contains 149 genes with unknown biological functions. JCVI-syn3.0 is a versatile platform for investigating the core functions of life and for exploring whole-genome design.

Comment: Microbiology is entering the synthetic biology era

New species, emerging pathogens & outbreaks

3.25. Zangenah S, Abbasi N, Andersson AF, Bergman P. Whole genome sequencing identifies a novel species of the genus Capnocytophaga isolated from dog and cat bite wounds in humans. Sci Rep. 2016 Mar 7;6:22919.

C. canimorsus and C. cynodegmi are dog and cat commensals which can be transmitted to humans via bites or scratches and can cause sepsis, meningitis, endocarditis, and eye- or wound infections. Recently an additional Capnocytophaga species was identified as part of the oral flora of healthy dogs and was given the name "C. canis". We previously identified a Capnocytophaga isolate that could not be typed with available diagnostic tests including MALDI-TOF, 16S rRNA sequencing or species-specific PCR. This strain and 21 other Capnocytophaga spp isolated in Sweden from clinical blood- or wound-cultures were subjected to whole genome sequencing using the Illumina platform. Phylogenetic analysis revealed that the previously non-typable isolate belongs to the putative new species "C. canis". Since this strain was isolated from a wound it also shows that members of "C. canis" have the potential to be pathogenic. In addition, our phylogenetic analysis uncovered an additional species of Capnocytophaga, which can be transmitted from dogs and cats to humans, suggesting a speciation within the Capnocytophaga family that has not been observed before. We propose the name of "C. stomatis" for this putative novel species.

Comment: New technologies (including NGS) enables the discovery of new species, including pathogenic ones

3.26.

Cassir N, Benamar S, Khalil JB, Croce O, Saint-Faust M, Jacquot A, Million M, Azza S, Armstrong N, Henry M, Jardot P, Robert C, Gire C, Lagier JC, Chabrière E, Ghigo E, Marchandin H, Sartor C, Boutte P, Cambonie G, Simeoni U, Raoult D, La Scola B. Clostridium butyricum Strains and Dysbiosis Linked to Necrotizing Enterocolitis in Preterm Neonates. Clin Infect Dis. 2015 Oct 1;61(7):1107-15.

BACKGROUND: Necrotizing enterocolitis (NEC) is the most common and serious gastrointestinal disorder among preterm neonates. We aimed to assess a specific gut microbiota profile associated with NEC.
METHODS: Stool samples and clinical data were collected from 4 geographically independent neonatal intensive care units, over a 48-month period. Thirty stool samples from preterm neonates with NEC (n = 15) and controls (n = 15) were analyzed by 16S ribosomal RNA pyrosequencing and culture-based methods. The results led us to develop a specific quantitative polymerase chain reaction (qPCR) assay for Clostridium butyricum, and we tested stool samples from preterm neonates with NEC (n = 93) and controls (n = 270). We sequenced the whole genome of 16 C. butyricum strains, analyzed their phylogenetic relatedness, tested their culture supernatants for cytotoxic activity, and searched for secreted toxins.
RESULTS: Clostridium butyricum was specifically associated with NEC using molecular and culture-based methods (15/15 vs 2/15; P < .0001) or qPCR (odds ratio, 45.4 [95% confidence interval, 26.2-78.6]; P < .0001). Culture supernatants of C. butyricum strains from preterm neonates with NEC (n = 14) exhibited significant cytotoxic activity (P = .008), and we identified in all a homologue of the β-hemolysin toxin gene shared by Brachyspira hyodysenteriae, the etiologic agent of swine dysentery. The corresponding protein was secreted by a NEC-associated C. butyricum strain.
CONCLUSIONS: NEC was associated with C. butyricum strains and dysbiosis with an oxidized, acid, and poorly diversified gut microbiota. Our findings highlight the plausible toxigenic mechanism involved in the pathogenesis of NEC.

Comment: A possible role of Clostridium butyricum in necrotizing enterocolitis : when a so-called beneficial bacterial species is unmasked as a novel pathogen

3.27.

Senneby E, Göransson L, Weiber S, Rasmussen M. A population-based study of aerococcal bacteraemia in the MALDI-TOF MS-era. Eur J Clin Microbiol Infect Dis. 2016

The purpose of this study was to determine the incidence of aerococcal bacteraemia in the MALDI-TOF MS-era, to describe the clinical presentation and to determine the MIC values of aerococci for ten antibiotics. Aerococci in blood cultures were identified through searches in the laboratory database for the years 2012-2014. MALDI-TOF MS, sequencing of the 16S rRNA gene and a PYR test were used for species identification. Patients' medical charts were systematically reviewed. Etests were used to determine MIC values. Seventy-seven patients were identified (Aerococcus urinae n = 49, Aerococcus viridans n = 14, Aerococcus sanguinicola n = 13 and Aerococcus christensenii n = 1) corresponding to incidences of 14 cases per 1,000,000 inhabitants per year (A. urinae) and 3.5 cases per 1,000,000 inhabitants per year (A. sanguinicola and A.viridans). A. urinae was in pure culture in 61 %, A. sanguinicola in 46 % and A. viridans in 36 % of the cases. The A. urinae and A. sanguinicola patients were old and many had urinary tract disorders, and a majority had a suspected urinary tract focus of the bacteraemia. Eighty percent of the A. urinae patients were men. Five A. urinae patients were diagnosed with infective endocarditis. Six patients died within 30 days. Most isolates had low MICs to penicillins and carbapenems. MALDI-TOF MS has led to an increased identification of aerococcal bacteremia. A. urinae remains the most common Aerococcus in blood cultures and in aerococcal IE.

Comment: MALDI-TOF has dramatically increased our awareness of the importance of Aerococcus as a urinary tract pathogen and Aerococcus is a good example of a novel pathogenic agent emerging due to the implementation of a new diagnostic technology.

3.28.

Faria NR, Azevedo RD, Kraemer MU, Souza R, Cunha MS, Hill SC, Thézé J, Bonsall MB, Bowden TA, Rissanen I, Rocco IM, Nogueira JS, Maeda AY, Vasami FG, Macedo FL, Suzuki A, Rodrigues SG, Cruz AC, Nunes BT, Medeiros DB, Rodrigues DS, Nunes Queiroz AL, Silva EV, Henriques DF, Travassos da Rosa ES, de Oliveira CS, Martins LC, Vasconcelos HB, Casseb LM, Simith DB, Messina JP, Abade L, Lourenço J, Alcantara LC, Lima MM, Giovanetti M, Hay SI, de Oliveira RS, Lemos PD, Oliveira LF, de Lima CP, da Silva SP, Vasconcelos JM, Franco L, Cardoso JF, Vianez-Júnior JL, Mir D, Bello G, Delatorre E, Khan K, Creatore M, Coelho GE, de Oliveira WK, Tesh R, Pybus OG, Nunes MR, Vasconcelos PF. Zika virus in the Americas: Early epidemiological and genetic findings. Science. 2016 Mar 24.

Brazil has experienced an unprecedented epidemic of Zika virus (ZIKV), with ~30,000 cases reported to date. ZIKV was first detected in Brazil in May 2015 and cases of microcephaly potentially associated with ZIKV infection were identified in November 2015. Using next generation sequencing we generated seven Brazilian ZIKV genomes, sampled from four self-limited cases, one blood donor, one fatal adult case, and one newborn with microcephaly and congenital malformations. Phylogenetic and molecular clock analyses show a single introduction of ZIKV into the Americas, estimated to have occurred between May-Dec 2013, more than 12 months prior to the detection of ZIKV in Brazil. The estimated date of origin coincides with an increase in air passengers to Brazil from ZIKV endemic areas, and with reported outbreaks in Pacific Islands. ZIKV genomes from Brazil are phylogenetically interspersed with those from other South American and Caribbean countries. Mapping mutations onto existing structural models revealed the context of viral amino acid changes present in the outbreak lineage; however no shared amino acid changes were found among the three currently available virus genomes from microcephaly cases. Municipality-level incidence data indicate that reports of suspected microcephaly in Brazil best correlate with ZIKV incidence around week 17 of pregnancy, although this does not demonstrate causation. Our genetic description and analysis of ZIKV isolates in Brazil provide a baseline for future studies of the evolution and molecular epidemiology in the Americas of this emerging virus.T

Comment: Another major outbreak that underlines the importance of having trained microbiologists and a reactive collaborative network to implement the appropriate measures, including development of accurate diagnostic tools

3.29.

Cauchemez S, Besnard M, Bompard P, Dub T, Guillemette-Artur P, Eyrolle-Guignot D, Salje H, Van Kerkhove MD, Abadie V, Garel C, Fontanet A, Mallet HP. Association between Zika virus and microcephaly in French Polynesia, 2013-15: a retrospective study. Lancet. 2016 Mar 15.

BACKGROUND: The emergence of Zika virus in the Americas has coincided with increased reports of babies born with microcephaly. On Feb 1, 2016, WHO declared the suspected link between Zika virus and microcephaly to be a Public Health Emergency of International Concern. This association, however, has not been precisely quantified.

METHODS: We retrospectively analysed data from a Zika virus outbreak in French Polynesia, which was the largest documented outbreak before that in the Americas. We used serological and surveillance data to estimate the probability of infection with Zika virus for each week of the epidemic and searched medical records to identify all cases of microcephaly from September, 2013, to July, 2015. Simple models were used to assess periods of risk in pregnancy when Zika virus might increase the risk of microcephaly and estimate the associated risk.

FINDINGS: The Zika virus outbreak began in October, 2013, and ended in April, 2014, and 66% (95% CI

62-70) of the general population were infected. Of the eight microcephaly cases identified during the 23-month study period, seven (88%) occurred in the 4-month period March 1 to July 10, 2014. The timing of these cases was best explained by a period of risk in the first trimester of pregnancy. In this model, the baseline prevalence of microcephaly was two cases (95% CI 0-8) per 10 000 neonates, and the risk of microcephaly associated with Zika virus infection was 95 cases (34-191) per 10 000 women infected in the first trimester. We could not rule out an increased risk of microcephaly from infection in other trimesters, but models that excluded the first trimester were not supported by the data

Comment: A work supporting a clear relationship between zikavirus and microcephaly when infection occurred during first trimester

3.30.

Cao-Lormeau VM, Blake A, Mons S, Lastère S, Roche C, Vanhomwegen J, Dub T, Baudouin L, Teissier A, Larre P, Vial AL, Decam C, Choumet V, Halstead SK, Willison HJ, Musset L, Manuguerra JC, Despres P, Fournier E, Mallet HP, Musso D, Fontanet A, Neil J, Ghawché F. Guillain-Barré Syndrome outbreak associated with Zika virus infection in French Polynesia: a case-control study. Lancet. 2016 Feb 29.

BACKGROUND: Between October, 2013, and April, 2014, French Polynesia experienced the largest Zika virus outbreak ever described at that time. During the same period, an increase in Guillain-Barré syndrome was reported, suggesting a possible association between Zika virus and Guillain-Barré syndrome. We aimed to assess the role of Zika virus and dengue virus infection in developing Guillain-Barré syndrome.

METHODS: In this case-control study, cases were patients with Guillain-Barré syndrome diagnosed at the Centre Hospitalier de Polynésie Française (Papeete, Tahiti, French Polynesia) during the outbreak period. Controls were age-matched, sex-matched, and residence-matched patients who presented at the hospital with a non-febrile illness (control group 1; n=98) and age-matched patients with acute Zika virus disease and no neurological symptoms (control group 2; n=70). Virological investigations included RT-PCR for Zika virus, and both microsphere immunofluorescent and seroneutralisation assays for Zika virus and dengue virus. Anti-glycolipid reactivity was studied in patients with Guillain-Barré syndrome using both ELISA and combinatorial microarrays.

FINDINGS: 42 patients were diagnosed with Guillain-Barré syndrome during the study period. 41 (98%) patients with Guillain-Barré syndrome had anti-Zika virus IgM or IgG, and all (100%) had neutralising antibodies against Zika virus compared with 54 (56%) of 98 in control group 1 ($p<0{\cdot}0001$). 39 (93%) patients with Guillain-Barré syndrome had Zika virus IgM and 37 (88%) had experienced a transient illness in a median of 6 days (IQR 4-10) before the onset of neurological symptoms, suggesting recent Zika virus infection. Patients with Guillain-Barré syndrome had electrophysiological findings compatible with acute motor axonal neuropathy (AMAN) type, and had rapid evolution of disease (median duration of the installation and plateau phases was 6 [IQR 4-9] and 4 days [3-10], respectively). 12 (29%) patients required respiratory assistance. No patients died. Anti-glycolipid antibody activity was found in 13 (31%) patients, and notably against GA1 in eight (19%) patients, by ELISA and 19 (46%) of 41 by glycoarray at admission. The typical AMAN-associated anti-ganglioside antibodies were rarely present. Past dengue virus history did not differ significantly between patients with Guillain-Barré syndrome and those in the two control groups (95%, 89%, and 83%, respectively).

INTERPRETATION: This is the first study providing evidence for Zika virus infection causing Guillain-Barré syndrome. Because Zika virus is spreading rapidly across the Americas, at risk countries need to prepare for adequate intensive care beds capacity to manage patients with Guillain-Barré syndrome.

Comment: A major work providing evidences for a causative role of the Zika virus in Guillain-Barré syndrome