ESCMID EUROPEAN SOCIETY OF CLINICAL MICROBIOLOGY AND INFECTIOUS DISEASES

ECCMID 2015
The Year in Clinical Microbiology
28 April 2015, 13.30 – 15.30, Hall A

References

1.1 Single-molecule long-read 16S sequencing to characterize the lung microbiome from mechanically ventilated patients with suspected pneumonia. J Clin Microbiol. 2014 Nov;52(11):3913-21. doi: 10.1128/JCM.01678-14. Epub 2014 Aug 20. Toma I, Siegel MO, Keiser J, Yakovleva A, Kim A, Davenport L, Devaney J, Hoffman EP, Alsubail R, Crandall KA, Castro-Nallar E, Pérez-Losada M, Hilton SK, Chawla LS, McCaffrey TA, Simon GL.

In critically ill patients, the development of pneumonia results in significant morbidity and mortality and additional health care costs. The accurate and rapid identification of the microbial pathogens in patients with pulmonary infections might lead to targeted antimicrobial therapy with potentially fewer adverse effects and lower costs. Major advances in next-generation sequencing (NGS) allow culture-independent identification of pathogens. The present study used NGS of essentially full-length PCR-amplified 16S ribosomal DNA from the bronchial aspirates of intubated patients with suspected pneumonia. The results from 61 patients demonstrated that sufficient DNA was obtained from 72% of samples, 44% of which (27 samples) yielded PCR amplimers suitable for NGS. Out of the 27 sequenced samples, only 20 had bacterial culture growth, while the microbiological and NGS identification of bacteria coincided in 17 (85%) of these samples. Despite the lack of bacterial growth in 7 samples that yielded amplimers and were sequenced, the NGS identified a number of bacterial species in these samples. Overall, a significant diversity of bacterial species was identified from the same genus as the predominant cultured pathogens. The numbers of NGS-identifiable bacterial genera were consistently higher than identified by standard microbiological methods. As technical advances reduce the processing and sequencing times, NGS-based methods will ultimately be able to provide clinicians with rapid, precise, culture-independent identification of bacterial, fungal, and viral pathogens and their antimicrobial sensitivity profiles.

1.2 Clinical detection and characterization of bacterial pathogens in the genomics era. Genome Med. 2014 Nov 29;6(11):114. doi: 10.1186/s13073-014-0114-2. eCollection 2014. Fournier PE, Dubourg G, Raoult D.

The availability of genome sequences obtained using next-generation sequencing (NGS) has revolutionized the field of infectious diseases. Indeed, more than 38,000 bacterial and 5,000 viral genomes have been sequenced to date, including representatives of all significant human pathogens. These tremendous amounts of data have not only enabled advances in fundamental biology, helping to understand the pathogenesis of microorganisms and their genomic evolution, but have also had implications for clinical microbiology. Here, we first review the current achievements of genomics in the development of improved diagnostic tools, including those that are now available in the clinic, such as the design of PCR assays for the detection of microbial pathogens, virulence factors or antibiotic-resistance determinants, or the design of optimized culture media for 'unculturable' pathogens. We then review the applications of genomics to the investigation of outbreaks, either through the design of genotyping assays or the direct sequencing of the causative strains. Finally, we discuss how genomics might change clinical microbiology in the future.

1.3 Microbial contamination in next generation sequencing: implications for sequence-based analysis of clinical samples. PLoS Pathog. 2014 Nov 20;10(11):e1004437. doi: 10.1371/journal.ppat.1004437. eCollection 2014. Strong MJ, Xu G, Morici L, Splinter Bon-Durant S, Baddoo M, Lin Z, Fewell C, Taylor CM, Flemington EK.

The high level of accuracy and sensitivity of next generation sequencing for quantifying genetic material across organismal boundaries gives it tremendous potential for pathogen discovery and diagnosis in human disease. Despite this promise, substantial bacterial contamination is routinely found in existing human-derived RNA-seq datasets that likely arises from environmental sources. This raises the need for stringent sequencing and analysis protocols for studies investigating sequence-based microbial signatures in clinical samples.

1.4 A cloud-compatible bioinformatics pipeline for ultrarapid pathogen identification from next-generation sequencing of clinical samples. Genome Res. 2014 Jul;24(7):1180-92. doi: 10.1101/gr.171934.113. Epub 2014 Jun 4. Naccache SN, Federman S, Veeraraghavan N, Zaharia M, Lee D, Samayoa E, Bouquet J, Greninger AL, Luk KC, Enge B, Wadford DA, Messenger SL, Genrich GL, Pellegrino K, Grard G, Leroy E, Schneider BS, Fair JN, Martínez MA, Isa P, Crump JA, DeRisi JL, Sittler T, Hackett J Jr, Miller S, Chiu CY.

Unbiased next-generation sequencing (NGS) approaches enable comprehensive pathogen detection in the clinical microbiology laboratory and have numerous applications for public health surveillance, outbreak investigation, and the diagnosis of infectious diseases. However, practical deployment of the technology is hindered by the bioinformatics challenge of analyzing results accurately and in a clinically relevant timeframe. Here we describe SURPI ("sequence-based ultrarapid pathogen identification"), a computational pipeline for pathogen identification from complex metagenomic NGS data generated from clinical samples, and demonstrate use of the pipeline in the analysis of 237 clinical samples comprising more than 1.1 billion sequences. Deployable on both cloud-based and standalone servers, SURPI leverages two state-of-the-art aligners for accelerated analyses, SNAP and RAPSearch, which are as accurate as existing bioinformatics tools but orders of magnitude faster in performance. In fast mode, SURPI detects viruses and bacteria by scanning data sets of 7-500 million reads in 11 min to 5 h, while in comprehensive mode, all known microorganisms are identified, followed by de novo assembly and protein homology searches for divergent viruses in 50 min to 16 h. SURPI has also directly contributed to real-time microbial diagnosis in acutely ill patients, underscoring its potential key role in the development of unbiased NGS-based clinical assays in infectious diseases that demand rapid turnaround times.

1.5 Emerging rapid resistance testing methods for clinical microbiology laboratories and their potential impact on patient management. Biomed Res Int. 2014;2014:375681. doi: 10.1155/2014/375681. Epub 2014 Sep 17. Frickmann H, Masanta WO, Zautner AE.

Atypical and multidrug resistance, especially ESBL and carbapenemase expressing Enterobacteriaceae, is globally spreading. Therefore, it becomes increasingly difficult to achieve therapeutic success by calculated antibiotic therapy. Consequently, rapid antibiotic resistance testing is essential. Various molecular and mass spectrometry-based approaches have been introduced in diagnostic microbiology to speed up the providing of reliable resistance data. PCR- and sequencing-based approaches are the most expensive but the most frequently applied modes of testing, suitable for the detection of resistance genes even from primary material. Next generation sequencing, based either on assessment of allelic single nucleotide polymorphisms or on the detection of nonubiquitous resistance mechanisms might allow for sequence-based bacterial resistance testing comparable to viral resistance testing on the long term. Fluorescence in situ hybridization (FISH), based on specific binding of fluorescence-labeled oligonucleotide probes, provides a less expensive molecular bridging technique. It is particularly useful for detection of resistance mechanisms based on mutations in ribosomal RNA. Approaches based on MALDI-TOF-MS, alone or in combination with molecular techniques, like PCR/electrospray ionization MS or minisequencing provide the fastest resistance results from pure colonies or even primary samples with a growing number of protocols. This review details the various approaches of rapid resistance testing, their pros and cons, and their potential use for the diagnostic laboratory.

1.6 Proteome-based bacterial identification using matrix-assisted laser desorption ionization-time of flight mass spectrometry (MALDI-TOF MS): A revolutionary shift in clinical diagnostic microbiology. Biochim Biophys Acta. 2015 Jun;1854(6):528-537. doi: 10.1016/j.bbapap.2014.10.022. Epub 2014 Nov 1. Nomura F.

Rapid and accurate identification of microorganisms, a prerequisite for appropriate patient care and infection control, is a critical function of any clinical microbiology laboratory. Matrix-assisted laser desorption ionization-time of flight mass spectrometry (MALDI-TOF MS) is a quick and reliable method for identification of microorganisms, including bacteria, yeast, molds, and mycobacteria. Indeed, there has been a revolutionary shift in clinical diagnostic microbiology. In the present review, the state of the art and advantages of MALDI-TOF MS-based bacterial identification are described. The potential of this innovative technology for use in strain typing and detection of antibiotic resistance is also discussed. This article is part of a Special Issue entitled: Medical Proteomics.

1.7 Bacterial nanoscale cultures for phenotypic multiplexed antibiotic susceptibility testing. J Clin Microbiol. 2014 Sep;52(9):3310-7. doi: 10.1128/JCM.01161-14. Epub 2014 Jul 2. Weibull E, Antypas H, Kjäll P, Brauner A, Andersson-Svahn H, Richter-Dahlfors A.

An optimal antimicrobial drug regimen is the key to successful clinical outcomes of bacterial infections. To direct the choice of antibiotic, access to fast and precise antibiotic susceptibility profiling of the infecting bacteria is critical. We have developed a high-throughput nanowell antibiotic susceptibility testing (AST) device for direct, multiplexed analysis. By processing in real time the optical recordings of nanoscale cultures of reference and clinical uropathogenic Escherichia coli strains with a mathematical algorithm, the time point when growth shifts from lag phase to early logarithmic phase (Tlag) was identified for each of the several hundreds of cultures tested. Based on Tlag, the MIC could be defined within 4 h. Heatmap presentation of data from this high-throughput analysis allowed multiple resistance patterns to be differentiated at a glance. With a possibility to enhance multiplexing capacity, this device serves as a high-throughput diagnostic tool that rapidly aids clinicians in prescribing the optimal antibiotic therapy.

1.8 The identification of tuberculosis biomarkers in human urine samples. Eur Respir J. 2014 Jun;43(6):1719-29. doi: 10.1183/09031936.00175113. Epub 2014 Apr 17. Young BL, Mlamla Z, Gqamana PP, Smit S, Roberts T, Peter J, Theron G, Govender U, Dheda K, Blackburn J.

We aimed to determine whether shotgun proteomic approaches could be used to identify tuberculosis (TB)-specific biomarkers in the urine of well-characterised patients with active TB versus no TB. Patients with suspected TB (n=63) were classified as: definite TB (Mycobacterium tuberculosis positive culture, n=21); presumed latent-TB infection (LTBI) (M. tuberculosis negative culture, no radiological features of active TB, a positive QuantiFERON-TB Gold In-Tube (QFT-IT) test and a positive T-SPOT.TB test, n=24); and presumed non-TB/non-LTBI (M. tuberculosis negative culture, no radiological features of active TB, a negative QFT-IT test and a negative T-SPOT.TB test, n=18). Urine proteins, in the range of 3-50 kDa, were collected, separated by a one-dimensional SDS-PAGE gel and digested using trypsin, after which high-performance liquid chromatography-tandem mass spectrometry was used to identify the urinary proteome. 10 mycobacterial proteins were observed exclusively in the urine of definite TB patients, while six mycobacterial proteins were found exclusively in the urine of presumed LTBI patients. In addition, a gene ontology enrichment analysis identified a panel of 20 human proteins that were significant discriminators ($p<0.05$) for TB disease compared to no TB disease. Furthermore, seven common human proteins were differentially over- or under-expressed in the TB versus the non-TB group. These biomarkers hold promise for the development of new point-of-care diagnostics for TB.

1.9 The application of genomics to tracing bacterial pathogen transmission. Curr Opin Microbiol. 2015 Feb;23:62-7. doi: 10.1016/j.mib.2014.11.004. Epub 2014 Nov 22. Croucher NJ, Didelot X.

New sequencing technologies have made it possible to generate bacterial genomes at clinically relevant

timescales and price levels. The use of whole-genome sequencing (WGS) has proved useful for investigating transmission at different scales. WGS data are highly effective at determining whether individuals are part of the same transmission chain, making it possible to detect probable direct transmission events, delimit the extent of local nosocomial or community-based outbreaks, and identify worldwide patterns of spread and long-term dynamics of bacterial pathogens. Making the most of WGS data will probably always require associated detailed epidemiological data, but nevertheless it promises to become an increasingly valuable tool for infection control in the near future.

1.10 A high-resolution genomic analysis of multidrug-resistant hospital outbreaks of Klebsiella pneumoniae. EMBO Mol Med. 2015 Feb 20;7(3):227-39. doi: 10.15252/emmm.201404767. Chung The H, Karkey A, Pham Thanh D, Boinett CJ, Cain AK, Ellington M, Baker KS, Dongol S, Thompson C, Harris SR, Jombart T, Le Thi Phuong T, Tran Do Hoang N, Ha Thanh T, Shretha S, Joshi S, Basnyat B, Thwaites G, Thomson NR, Rabaa MA, Baker S.

Multidrug-resistant (MDR) Klebsiella pneumoniae has become a leading cause of nosocomial infections worldwide. Despite its prominence, little is known about the genetic diversity of K. pneumoniae in resource-poor hospital settings. Through whole-genome sequencing (WGS), we reconstructed an outbreak of MDR K. pneumoniae occurring on high-dependency wards in a hospital in Kathmandu during 2012 with a case-fatality rate of 75%. The WGS analysis permitted the identification of two MDR K. pneumoniae lineages causing distinct outbreaks within the complex endemic K. pneumoniae. Using phylogenetic reconstruction and lineage-specific PCR, our data predicted a scenario in which K. pneumoniae, circulating for 6 months before the outbreak, underwent a series of ward-specific clonal expansions after the acquisition of genes facilitating virulence and MDR. We suggest that the early detection of a specific NDM-1 containing lineage in 2011 would have alerted the high-dependency ward staff to intervene. We argue that some form of real-time genetic characterisation, alongside clade-specific PCR during an outbreak, should be factored into future healthcare infection control practices in both high- and low-income settings.

1.11 Comparing whole-genome sequencing with Sanger sequencing for spa typing of methicillin-resistant Staphylococcus aureus. J Clin Microbiol. 2014 Dec;52(12):4305-8. doi: 10.1128/JCM.01979-14. Epub 2014 Oct 8. Bartels MD, Petersen A, Worning P, Nielsen JB, Larner-Svensson H, Johansen HK, Andersen LP, Jarløv JO, Boye K, Larsen AR, Westh H.

spa typing of methicillin-resistant Staphylococcus aureus (MRSA) has traditionally been done by PCR amplification and Sanger sequencing of the spa repeat region. At Hvidovre Hospital, Denmark, whole-genome sequencing (WGS) of all MRSA isolates has been performed routinely since January 2013, and an in-house analysis pipeline determines the spa types. Due to national surveillance, all MRSA isolates are sent to Statens Serum Institut, where the spa type is determined by PCR and Sanger sequencing. The purpose of this study was to evaluate the reliability of the spa types obtained by 150-bp paired-end Illumina WGS. MRSA isolates from new MRSA patients in 2013 (n = 699) in the capital region of Denmark were included. We found a 97% agreement between spa types obtained by the two methods. All isolates achieved a spa type by both methods. Nineteen isolates differed in spa types by the two methods, in most cases due to the lack of 24-bp repeats in the whole-genome-sequenced isolates. These related but incorrect spa types should have no consequence in outbreak investigations, since all epidemiologically linked isolates, regardless of spa type, will be included in the single nucleotide polymorphism (SNP) analysis. This will reveal the close relatedness of the spa types. In conclusion, our data show that WGS is a reliable method to determine the spa type of MRSA.

1.12 Genome sequencing defines phylogeny and spread of methicillin-resistant Staphylococcus aureus in a high transmission setting. Genome Res. 2015 Jan;25(1):111-8. doi: 10.1101/gr.174730.114. Epub 2014 Dec 9. Tong SY, Holden MT, Nickerson EK, Cooper BS, Köser CU, Cori A, Jombart T, Cauchemez S, Fraser C, Wuthiekanun V, Thaipadungpanit J,Hongsuwan M, Day NP, Limmathurotsakul D, Parkhill J, Peacock SJ.

Methicillin-resistant Staphylococcus aureus (MRSA) is a major cause of nosocomial infection. Whole-genome sequencing of MRSA has been used to define phylogeny and transmission in well-resourced healthcare settings, yet the greatest burden of nosocomial infection occurs in resource-restricted settings where barriers to transmission are lower. Here, we study the flux and genetic diversity of MRSA on ward and individual patient levels in a hospital where transmission was common. We repeatedly screened all patients on two intensive care units for MRSA carriage over a 3-mo period. All MRSA belonged to multilocus sequence type 239 (ST 239). We defined the population structure and charted the spread of MRSA by sequencing 79 isolates from 46 patients and five members of staff, including the first MRSA-positive screen isolates and up to two repeat isolates where available. Phylogenetic analysis identified a flux of distinct ST 239 clades over time in each intensive care unit. In total, five main clades were identified, which varied in the carriage of plasmids encoding antiseptic and antimicrobial resistance determinants. Sequence data confirmed intra- and interwards transmission events and identified individual patients who were colonized by more than one clade. One patient on each unit was the source of numerous transmission events, and deep sampling of one of these cases demonstrated colonization with a "cloud" of related MRSA variants. The application of whole-genome sequencing and analysis provides novel insights into the transmission of MRSA in under-resourced healthcare settings and has relevance to wider global health.

1.13 Investigation of a possible outbreak of carbapenem-resistant Acinetobacter baumannii in Odense, Denmark using PFGE, MLST and whole-genome-based SNPs. J Antimicrob Chemother. 2015 Mar 19. pii: dkv072. [Epub ahead of print] Hammerum AM, Hansen F, Skov MN, Stegger M, Andersen PS, Holm A, Jakobsen L, Justesen US.

OBJECTIVES: The objectives were to study a possible outbreak of carbapenem-resistant Acinetobacter baumannii by comparing three different typing methods (PFGE, MLST and whole-genome SNPs) and to compare the resistance gene profiles of the isolates.

METHODS: From December 2012 to October 2013, eight carbapenem-resistant A. baumannii were detected at Odense University Hospital, Odense, Denmark. These isolates were typed by PFGE, with ApaI and SmaI, respectively, and subjected to WGS. The WGS data were used for in silico extraction of MLST types using two different schemes, resistance genes and SNPs, to which 31 publicly available A. baumannii genomes were added.

RESULTS: Using ApaI, the eight isolates had four different PFGE profiles, which were further differentiated using SmaI, separating one of the profiles into two distinct PFGE types. Five ST2 (Pasteur MLST) OXA-23-producing isolates, two ST1 OXA-72-producing isolates and one ST158 OXA-23-producing isolate were detected. The five ST2 isolates were subdivided into ST195, ST208 and ST218 using the Oxford MLST scheme. The phylogenetic analysis based on the core genome showed that six of the eight Danish A. baumannii isolates were located in three distinct clusters. The two remaining isolates did not cluster with other Danish or international isolates included in the study. Isolates that clustered using PFGE, Oxford MLST and phylogenetic analysis also shared similar resistance gene profiles.

CONCLUSIONS: The SNP profile, Oxford MLST, PFGE and resistance gene profiles clearly indicated spread of three different A. baumannii strains.

1.14 Rapid High Resolution Genotyping of Francisella tularensis by Whole Genome Sequence Comparison of Annotated Genes ("MLST+"). PLoS One. 2015 Apr 9;10(4):e0123298. doi: 10.1371/journal.pone.0123298. eCollection 2015. Antwerpen MH, Prior K, Mellmann A, Höppner S, Splettstoesser WD, Harmsen D.

The zoonotic disease tularemia is caused by the bacterium Francisella tularensis. This pathogen is

considered as a category A select agent with potential to be misused in bioterrorism. Molecular typing based on DNA-sequence like canSNP-typing or MLVA has become the accepted standard for this organism. Due to the organism's highly clonal nature, the current typing methods have reached their limit of discrimination for classifying closely related subpopulations within the subspecies F. tularensis ssp. holarctica. We introduce a new gene-by-gene approach, MLST+, based on whole genome data of 15 sequenced F. tularensis ssp. holarctica strains and apply this approach to investigate an epidemic of lethal tularemia among non-human primates in two animal facilities in Germany. Due to the high resolution of MLST+ we are able to demonstrate that three independent clones of this highly infectious pathogen were responsible for these spatially and temporally restricted outbreaks.

1.15 Recombination drives genome evolution in outbreak-related Legionella pneumophila isolates. Nat Genet. 2014 Nov;46(11):1205-11. doi: 10.1038/ng.3114. Epub 2014 Oct 5. Sánchez-Busó L, Comas I, Jorques G, González-Candelas F.

Legionella pneumophila is a strictly environmental pathogen and the etiological agent of legionellosis. It is known that non-vertical processes have a major role in the short-term evolution of pathogens, but little is known about the relevance of these and other processes in environmental bacteria. We report the whole-genome sequencing of 69 L. pneumophila strains linked to recurrent outbreaks in a single location (Alcoy, Spain) over 11 years. We found some examples where the genome sequences of isolates of the same sequence type and outbreak did not cluster together and were more closely related to sequences from different outbreaks. Our analyses identify 16 recombination events responsible for almost 98% of the SNPs detected in the core genome and an apparent acceleration in the evolutionary rate. These results have profound implications for the understanding of microbial populations and for public health interventions in Legionella outbreak investigations.

1.16 Rapid whole-genome sequencing for surveillance of Salmonella enterica serovar enteritidis. Emerg Infect Dis. 2014 Aug;20(8):1306-14. doi: 10.3201/eid2008.131399. den Bakker HC, Allard MW, Bopp D, Brown EW, Fontana J, Iqbal Z, Kinney A, Limberger R, Musser KA, Shudt M, Strain E, Wiedmann M, Wolfgang WJ.

For Salmonella enterica serovar Enteritidis, 85% of isolates can be classified into 5 pulsed-field gel electrophoresis (PFGE) types. However, PFGE has limited discriminatory power for outbreak detection. Although whole-genome sequencing has been found to improve discrimination of outbreak clusters, whether this procedure can be used in real-time in a public health laboratory is not known. Therefore, we conducted a retrospective and prospective analysis. The retrospective study investigated isolates from 1 confirmed outbreak. Additional cases could be attributed to the outbreak strain on the basis of whole-genome data. The prospective study included 58 isolates obtained in 2012, including isolates from 1 epidemiologically defined outbreak. Whole-genome sequencing identified additional isolates that could be attributed to the outbreak, but which differed from the outbreak-associated PFGE type. Additional putative outbreak clusters were detected in the retrospective and prospective analyses. This study demonstrates the practicality of implementing this approach for outbreak surveillance in a state public health laboratory.

1.17 Dynamics and associations of microbial community types across the human body. Ding T(1), Schloss PD(1). Nature. 2014 May 15;509(7500):357-60. doi: 10.1038/nature13178. Epub 2014 Apr 16.

A primary goal of the Human Microbiome Project (HMP) was to provide a reference collection of 16S ribosomal RNA gene sequences collected from sites across the human body that would allow microbiologists to better associate changes in the microbiome with changes in health. The HMP Consortium has reported the structure and function of the human microbiome in 300 healthy adults at 18 body sites from a single time point. Using additional data collected over the course of 12-18 months, we used Dirichlet multinomial mixture models to partition the data into community types for each body site and made three important observations. First, there were strong associations between whether individuals had been breastfed as an infant, their gender, and their level of education with their community types at several body sites. Second, although the specific taxonomic compositions of the oral and gut microbiomes were different, the community types observed at these sites were predictive of each

other. Finally, over the course of the sampling period, the community types from sites within the oral cavity were the least stable, whereas those in the vagina and gut were the most stable. Our results demonstrate that even with the considerable intra- and interpersonal variation in the human microbiome, this variation can be partitioned into community types that are predictive of each other and are probably the result of life-history characteristics. Understanding the diversity of community types and the mechanisms that result in an individual having a particular type or changing types, will allow us to use their community types to assess disease risk and to personalize therapies.

1.18 Transkingdom control of microbiota diurnal oscillations promotes metabolic homeostasis. Thaiss CA, Zeevi D, Levy M, Zilberman-Schapira G, Suez J, Tengeler AC, Abramson L, Katz MN, Korem T, Zmora N, Kuperman Y, Biton I, Gilad S, Harmelin A, Shapiro H, Halpern Z, Segal E, Elinav E. Cell. 2014 Oct 23;159(3):514-29. doi: 10.1016/j.cell.2014.09.048. Epub 2014 Oct 16.

All domains of life feature diverse molecular clock machineries that synchronize physiological processes to diurnal environmental fluctuations. However, no mechanisms are known to cross-regulate prokaryotic and eukaryotic circadian rhythms in multikingdom ecosystems. Here, we show that the intestinal microbiota, in both mice and humans, exhibits diurnal oscillations that are influenced by feeding rhythms, leading to time-specific compositional and functional profiles over the course of a day. Ablation of host molecular clock components or induction of jet lag leads to aberrant microbiota diurnal fluctuations and dysbiosis, driven by impaired feeding rhythmicity. Consequently, jet-lag-induced dysbiosis in both mice and humans promotes glucose intolerance and obesity that are transferrable to germ-free mice upon fecal transplantation. Together, these findings provide evidence of coordinated metaorganism diurnal rhythmicity and offer a microbiome-dependent mechanism for common metabolic disturbances in humans with aberrant circadian rhythms, such as those documented in shift workers and frequent flyers.

1.19 Detection of mecC-Methicillin-resistant Staphylococcus aureus isolates in river water: a potential role for water in the environmental dissemination. M. Concepción Porrero, Ewan M. Harrison, José Francisco Fernández-Garayzábal, Gavin K. Paterson, Alberto Díez-Guerrier, Mark A. Holmes and Lucas Domínguez. Environ Microbiol Rep. 2014 Dec;6(6):705-8.

Methicillin-resistant Staphylococcus aureus (MRSA) is a public health concern due to limited treatment options. The recent description of a mecA homologue, mecC in human and cattle, led to studies to detect this new variant in human and other animal species. Detection of mecC in wild boar and fallow deer in a Spanish game estate led us to further investigate the presence of mecC-MRSA at this location. Samples from cattle, wild animals, workers and river water were tested. A further three mecC-MRSA isolates were obtained from river water. Molecular characterization (multilocus sequence typing and spa typing) and antimicrobial susceptibility testing (broth microdilution) showed that isolates were similar to those detected in wild animals. Whole genome sequencing confirmed that the isolates from the river water and wild animals in the same geographic area were all closely related isolates of ST425 mecC-MRSA. The presence of mecC-MRSA in the river water highlights the potential role of water in the dissemination of mecC-MRSA.

1.20 An integrated catalog of reference genes in the human gut microbiome Li J, Jia H, Cai X, Zhong H, Feng Q, Sunagawa S, Arumugam M, Kultima JR, Prifti E, Nielsen T, Juncker AS, Manichanh C, Chen B, Zhang W, Levenez F, Wang J, Xu X, Xiao L, Liang S, Zhang D, Zhang Z, Chen W, Zhao H, Al-Aama JY, Edris S, Yang H, Wang J, Hansen T, Nielsen HB, Brunak S, Kristiansen K, Guarner F, Pedersen O, Doré J, Ehrlich SD, Bork P, Wang J; MetaHIT Consortium. Nat Biotechnol. 2014 Aug;32(8):834-41. doi: 10.1038/nbt.2942. Epub 2014 Jul 6.

Many analyses of the human gut microbiome depend on a catalog of reference genes. Existing catalogs for the human gut microbiome are based on samples from single cohorts or on reference genomes or protein sequences, which limits coverage of global microbiome diversity. Here we combined 249 newly

sequenced samples of the Metagenomics of the Human Intestinal Tract (MetaHit) project with 1,018 previously sequenced samples to create a cohort from three continents that is at least threefold larger than cohorts used for previous gene catalogs. From this we established the integrated gene catalog (IGC) comprising 9,879,896 genes. The catalog includes close-to-complete sets of genes for most gut microbes, which are also of considerably higher quality than in previous catalogs. Analyses of a group of samples from Chinese and Danish individuals using the catalog revealed country-specific gut microbial signatures. This expanded catalog should facilitate quantitative characterization of metagenomic, metatranscriptomic and metaproteomic data from the gut microbiome to understand its variation across populations in human health and disease.

1.21 A new antibiotic kills pathogens without detectable resistance. Losee L. Ling, Tanja Schneider, Aaron J. Peoples, Amy L. Spoering, Ina Engels, Brian P. Conlon, Anna Mueller, Till F.Schaberle, DallasE.Hughes, SlavaEpstein, MichaelJones, LinosLazarides, Victoria A.Steadman, Douglas R.Cohen, Cintia R. Felix, K. Ashley Fetterman, William P. Millett, Anthony G. Nitti, Ashley M. Zullo, Chao Chen & Kim Lewis. Nature. 2015 Jan 22;517(7535):455-9. doi: 10.1038/nature14098. Epub 2015 Jan 7.

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.

2.1 Safety and clinical outcomes of carbapenem de-escalation as part of an antimicrobial stewardship program in an ESBL-endemic setting. Kaung Yuan Lew et al. J Antimicrob Chemother 2015; 70: 1219–1225

Objectives: To evaluate the safety and clinical outcomes of patients who received carbapenem de-escalation as guided by an antimicrobial stewardship programme (ASP) in a setting where ESBL-producing Enterobacteriaceae are endemic.

Methods: Patients receiving meropenem or imipenem underwent a prospective ASP review for eligibility for de-escalation according to defined institutional guidelines. Patients in whom carbapenem was de-escalated or not de-escalated, representing the acceptance and rejection of the ASP recommendation, respectively, were compared. The primary outcome was the clinical success rate; secondary outcomes included the 30 day readmission and mortality rates, the duration of carbapenem therapy, the incidence of adverse drug reactions due to antimicrobials, the acquisition of carbapenem-resistant Gram-negative bacteria and the occurrence of Clostridium difficile-associated diarrhoea (CDAD).

Results: The de-escalation recommendations for 300 patients were evaluated; 204 (68.0%) were accepted. The patient demographics and disease severity were similar. The clinical success rates were similar [de-escalated versus not de-escalated, 183/204 (89.7%) versus 85/96 (88.5%), P=0.84], as was the survival at hospital discharge [173/204 (84.8%) versus 79/96 (82.3%), P=0.58]. In the de-escalated group, the duration of carbapenem therapy was shorter (6 versus 8 days, P<0.001), the rate of adverse drug reactions was lower [11/204 (5.4%) versus 12/96 (12.5%), P=0.037], there was less diarrhoea [9/204 (4.4%) versus 12/96 (12.5%), P=0.015], there was a lower incidence of carbapenem-resistant Acinetobacter baumannii acquisition [4/204 (2.0%) versus 7/96 (7.3%), P=0.042] and there was a lower incidence of CDAD [2/204 (1.0%) versus 4/96 (4.2%), P=0.081].

Conclusions: This study suggests that the ASP-guided de-escalation of carbapenems led to comparable clinical success, fewer adverse effects and a lower incidence of the development of resistance. This approach is safe and practicable, and should be a key component of an ASP.

2.2 Global antibiotic consumption 2000 to 2010: an analysis of national pharmaceutical sales data. Thomas P Van Boeckel et al. Lancet Infect. Dis. 2014; 14: 742–50

Background Antibiotic drug consumption is a major driver of antibiotic resistance. Variations in antibiotic resistance across countries are attributable, in part, to diff erent volumes and patterns for antibiotic consumption. We aimed to assess variations in consumption to assist monitoring of the rise of resistance and development of rational-use policies and to provide a baseline for future assessment.

Methods With use of sales data for retail and hospital pharmacies from the IMS Health MIDAS database, we reviewed trends for consumption of standard units of antibiotics between 2000 and 2010 for 71 countries. We used compound annual growth rates to assess temporal differences in consumption for each country and Fourier series and regression methods to assess seasonal differences in consumption in 63 of the countries.

Findings Between 2000 and 2010, consumption of antibiotic drugs increased by 36% (from 54 083 964 813 standard units to 73 620 748 816 standard units). Brazil, Russia, India, China, and South Africa accounted for 76% of this increase. In most countries, antibiotic consumption varied significantly with season. There was increased consumption of carbapenems (45%) and polymyxins (13%), two last-resort classes of antibiotic drugs.

Interpretation The rise of antibiotic consumption and the increase in use of last-resort antibiotic drugs raises serious concerns for public health. Appropriate use of antibiotics in developing countries should be encouraged. However, to prevent a striking rise in resistance in low-income and middle-income countries with large populations and to preserve antibiotic efficacy worldwide, programs that promote rational use through coordinated efforts by the international community should be a priority.

2.3 Determinants of antibiotic dispensing without a medical prescription: a cross-sectional study in the north of Spain. Maruxa Zapata-Cachafeiro et al. J. Antimicrob. Chemother. 2014; 69: 3156–3160

Objectives. Antibiotic resistance is a major public health concern and is greatly exacerbated by inappropriate antibiotic use at a community level. The aim of this study was to ascertain which attitudes of community pharmacists were related to inappropriate antibiotic dispensing.

Methods. We conducted a cross-sectional study of community pharmacists in a region situated in northern Spain (n=393). Personal interviews were conducted using a self-administered questionnaire. The degree of agreement with each item of knowledge and attitude was measured using an unnumbered, horizontal visual analogue scale, with replies being scored from 0 (total disagreement) to 10 (total agreement). The data were analyzed using logistic regression.

Results. Of the total of 286 pharmacists (72.8%) who completed the questionnaire, 185 (64.7%) acknowledged having undertaken dispensing of antibiotics without a medical prescription (DAwMP). Attitudes such as patient complacency, external responsibility, indifference and insufficient knowledge were shown to be related to DAwMP. In contrast, no association was found with any of the pharmacists' personal or professional traits.

Conclusions. This study confirms that, albeit unlawful, DAwMP is a common practice in Spanish pharmacies. DAwMP was seen to be usually associated with some of the attitudes evaluated.

2.4 Outpatient treatment for upper respiratory tract infections: an evaluation of factors associated with antibiotic misuse. Schtoeck JL et al. Antimicrob. Agents Chemother. Accepted Manuscript Posted Online 13 April 2015

Background: The Centers for Disease Control and Prevention has promoted the appropriate use of antibiotics since 1995 when it initiated the National Campaign for Appropriate Antibiotic Use in the Community. This study examines upper respiratory tract infections included in the campaign to determine

the degree to which antibiotics were appropriately prescribed and subsequent admission rates in a veteran population.

Methods: This study is a retrospective chart review conducted among outpatients with a diagnosis of a respiratory tract infection between January 2009 and December 2011 for bronchitis, pharyngitis, sinusitis, and non-specific upper respiratory infections.

Results: The study found 595 (35.8%) patients were treated appropriately and 1067 (64.3%) patients received therapy considered inappropriate based on the Get Smart Campaign criteria. Overall subsequent readmission rate were 1.5%. The majority (77.5%) of patients were prescribed an antibiotic. The most common antibiotics prescribed were azithromycin (39.0%), amoxicillin/clavulanate (13.2%) and moxifloxacin (7.5%). A multivariate regression analysis demonstrated significant predictors of appropriate treatment included presence of tonsillar exudates (OR 0.6 CI 0.3-0.9), fever (OR 0.6 CI 0.4-0.9), and lymphadenopathy (OR 0.4 CI 0.3-0.6), while penicillin allergy (OR 2.9 CI 1.7-4.7) and cough (OR 1.6 CI 1.1-2.2) were significant predictors for inappropriate treatment.

Discussion: Poor compliance with the Get Smart Campaign was found in outpatients for respiratory infections. Results from this study demonstrate the overprescribing of antibiotics, while providing a focused view of improper prescribing. This article provides evidence the current efforts are insufficient to curtail inappropriate antibiotic use.

2.5 Bacteriostatic versus bactericidal antibiotics for patients with serious bacterial infections: systematic review and meta-analysis. Johannes Nemeth et al. J. Antimicrob. Chemother. 2015; 70: 382–395

Objectives. Antibiotics are commonly classified into bactericidal and bacteriostatic agents based on their antimicrobial action. We aimed to assess whether this distinction is clinically relevant.

Methods. OVID MEDLINE, EMBASE, The Cochrane Central Register of Controlled Trials (CENTRAL) and relevant references and conference proceedings using the Web of Science and Scopus databases were searched for randomized controlled trials comparing bactericidal with bacteriostatic antibiotics for treatment of severe infections. Main outcome measures were clinical cure rates and overall mortality. Abstracts of studies selected in the database search were screened by one reviewer; full-text screening and data extraction were performed by three independent reviewers.

Results. Thirty-three studies were included. Approximately half of patients were treated with bacteriostatic monotherapy. Infections covered were pneumonia (n=13), skin and soft tissue infections (n=8), intraabdominal infections (n=4) and others (n=8). Neither clinical cure rates [risk ratio (RR), 0.99; 95% CI, 0.97–1.01; P=0.11] nor mortality rates (RR, 0.91; 95% CI, 0.76–1.08; P=0.28) were different between patients treated with bactericidal drugs and those treated with bacteriostatic drugs. Subgroup analyses showed a benefit for clinical cure rates associated with linezolid and increased mortality associated with tigecycline. In meta-regression, clinical cure rates remained higher in patients treated with linezolid (P=0.01); tigecycline displayed a close to significant association with increased mortality (P=0.05) if compared with other bacteriostatic agents.

Conclusions. The categorization of antibiotics into bacteriostatic and bactericidal is unlikely to be relevant in clinical practice if used for abdominal infections, skin and soft tissue infections and pneumonia. Because we were not able to include studies on meningitis, endocarditis or neutropenia, no conclusion regarding these diseases can be drawn.

2.6 Antibiotic resistance patterns of more than 120 000 clinical Escherichia coli isolates in Southeast Austria, 1998–2013. A. Badura et al. Clin Microbiol Infect. 2015 Feb 20. pii: S1198-743X(15)00300-6. doi: 10.1016/j.cmi.2015.02.012. [Epub ahead of print]

Antibiotic resistance patterns of more than 120 000 clinical Escherichia coli isolates were retrospectively analyzed. Isolates originated from both hospitalized patients and outpatients from the region of southeast Austria from 1998 to 2013. Except for amoxicillin/clavulanic acid, nitrofurantoin and piperacillin/tazobactam, all of the antibiotics analyzed showed increasing proportions of resistant isolates over time, which were most prominent for ampicillin (from 25.4% in 1998 to 40% in 2013), cefotaxime (0.1% to 6.7%), ceftazidime (0.3% to 14.2%), ciprofloxacin (4.3% to 16.7%) and trimethoprim/sulfamethoxazole (14.6% to 24.8%). There was a marked increase in extended-spectrum β-

lactamase–positive isolates (0.1% to 6.3%) starting in 2005, with male patients and hospital-related patients showing a higher increase than female patients and outpatients. Proportions of resistant isolates for most antibiotics were generally higher for male patients and hospital-related patients. Amikacin, nitrofurantoin and trimethoprim/sulfamethoxazole showed a marked increase in resistance proportions among male subjects aged 10 to 19 years which were absent for female subjects, indicating a strong modulation potential of host characteristics.

2.7 Nitrofurantoin retains antimicrobial activity against multidrug-resistant urinary Escherichia coli from US outpatients. G. V. Sanchez et al. J. Antimicrob. Chemother. 2014; 69: 3259-3262

Objectives. To examine the prevalence of multidrug-resistant (MDR) urinary Escherichia coli among US outpatients and to assess the antimicrobial activity of oral antibiotics commonly used to treat urinary tract infections (UTIs) against MDR isolates.

Methods. Antimicrobial susceptibility testing data from outpatient urine cultures in The Surveillance Network (TSN) Database USA were analyzed. Six antimicrobial agents from six separate drug classes were included: ampicillin, cefalotin, ciprofloxacin, nitrofurantoin, trimethoprim/sulfamethoxazole and amoxicillin/clavulanate. Isolates were categorized as resistant to one, two, three, four, five and six agents and compared for the years 2001 and 2010. Phenotypes of MDR isolates were assessed to determine antimicrobial activity of recommended therapy for UTIs.

Results. Prevalence of MDR E. coli increased from 9.1% in 2001 (n=29198) to 17.0% in 2010 (n=32742) (P <0.0001). In isolates that demonstrated resistance to three, four or five antimicrobial agents in 2010, resistance to nitrofurantoin was observed in only 2.1%, 7.5% and 24.1% of isolates, respectively. Conversely, widespread resistance was observed for trimethoprim/sulfamethoxazole (62.6%, 88.6% and 97.9% for isolates resistant to three, four and five agents, respectively) and ciprofloxacin (48.9%, 84.3% and 98.2% for isolates resistant to three, four and five agents, respectively).

Conclusions Because of its consistent antimicrobial activity against MDR E. coli, nitrofurantoin remains a reliable first-line agent for the empirical treatment of acute uncomplicated cystitis.

2.8 Detection of carbapenemase activity directly from blood culture vials using MALDI-TOF MS: a quick answer for the right decision. Cecilia G. Carvalhaes. J. Antimicrob. Chemother. 2014; 69: 2132–2136

Objectives. Recently, matrix-assisted laser desorption ionization–time-of-flight mass spectrometry (MALDI-TOF MS) was successfully applied for the detection of carbapenemase activity directly from Gram-negative colonies. Based on this principle, we evaluated the performance of MALDI-TOF MS for rapid detection of carbapenemase activity directly from positive blood culture vials.

Methods. A total of 100 blood culture vials were randomly selected. MALDI-TOF MS carbapenemase assay results were confirmed by the detection of carbapenemase-encoding genes.

Results. A total of 110 bacterial isolates were recovered. The MALDI-TOF MS carbapenemase assay identified 21 of 29 (72.4%) of the carbapenemase-producing isolates directly from the blood culture vials, especially those encoding KPC-2 (100%) and SPM-1 (100%), after a 4 h incubation period. Although the majority of OXA-23-producing Acinetobacter baumannii isolates were not identified on day 1, all isolates were identified as carbapenemase producers directly from the colony on the next day.

Conclusions. The MALDI-TOF MS carbapenemase assay is a feasible and rapid test to identify carbapenemase activity directly from blood culture vials. It may contribute to faster readjustment of empirical antimicrobial therapy and implementation of infection control measures.

2.9 Rapid Detection of Extended-Spectrum-β-Lactamase-Producing Enterobacteriaceae from Urine Samples by Use of the ESBL NDP Test. Dortet L et al. J. Clin. Microbiol. 2014: 52: 3701-3706

From June to September 2012, 500 urine samples were recovered from patients with urinary tract infections (UTI) due to Gram-negative bacilli (≥104 leukocytes/ml and ≥105 Gram-negative isolates/ml)

who visited the University hospital Bicêtre (France). They were challenged with extended-spectrum-β-lactamase (ESBL)-producing Enterobacteriaceae (ESBL-E) using the rapid diagnostic ESBL NDP test. Results of the ESBL NDP test were compared to the results of the double-disc susceptibility test (DDST) performed on solid-agar plates and molecular identification of the β-lactamase genes. Among the 450 nonduplicate urine samples, 11.3% were positive for ESBL-E by using the DDST, the ESBL determinants being mostly of the CTX-M type (CTX-M-15) according to molecular testing. Results of the ESBL NDP test were obtained within 15 min. The sensitivity and specificity of the ESBL NDP test were 98% and 99.8%, respectively, whereas the positive and negative predictive values of this test were 98% and 99.8%, respectively. A perfect correlation between cefotaxime resistance and positivity of the ESBL NDP test was observed. Therefore, the ESBL NDP test offers a powerful tool for a rapid identification of ESBL-E and associated resistance to expanded-spectrum cephalosporins. It may be useful in particular for guiding first-line antibiotic therapy.

2.10 The β-Lacta Test for Direct Detection of Extended-Spectrum-β-Lactamase-Producing Enterobacteriaceae in Urine. Salah Gallach et al. J. Clin. Microbiol. 2014 52: 3792-3794

With the β-Lacta test, production of extended-spectrum β-lactamases (ESBLs) was assayed in 200 urine samples showing Gram-negative bacilli during direct microscopic examination. While 168 samples tested negative, all samples yielding ESBL-producing Enterobacteriaceae after culture gave positive (n = 30) or uninterpretable (n = 2) results. The sensitivity and specificity of ESBL detection were 94% and 100%, respectively.

2.11 CarbAcineto NP Test for Rapid Detection of Carbapenemase-Producing Acinetobacter spp. Laurent Dortet et al. J. Clin. Microbiol. 2014; 52: 2359-2364

Multidrug-resistant Acinetobacter baumannii isolates, particularly those that produce carbapenemases, are increasingly reported worldwide. The biochemically based Carba NP test, extensively validated for the detection of carbapenemase producers among Enterobacteriaceae and Pseudomonas spp., has been modified to detect carbapenemase production in Acinetobacter spp. A collection of 151 carbapenemase-producing and 69 non-carbapenemase-producing Acinetobacter spp. were tested using the Carba NP test and a modified Carba NP protocol (the CarbAcineto NP test) in this study. The CarbAcineto NP test requires modified lysis conditions and an increased bacterial inoculum compared to those of the original Carba NP test. The Carba NP test detects metallo-β-lactamase producers but failed to detect the production of other carbapenemase types among Acinetobacter spp. In contrast, the newly designed CarbAcineto NP test, which is rapid and reproducible, detects all types of carbapenemases with a sensitivity of 94.7% and a specificity of 100%. This cost-effective technique offers a reliable and affordable technique for identifying carbapenemase production in Acinetobacter spp., which is a marker of multidrug resistance in those species. Its use will facilitate the recognition of these carbapenemases and prevent their spread.

2.12 Rapid detection of Enterobacteriaceae producing extended-spectrum-beta-lactamases directly from positive blood cultures by matrix-assisted laser desorption ionization-time of flight mass spectrometry. M. Oviano et al. Clin. Microbiol. Infect. 2014; 20: 1146–1157

Bacteria that produce extended-spectrum b-lactamases (ESBLs) are an increasing healthcare problem and their rapid detection is a challenge that must be overcome in order to optimize antimicrobial treatment and patient care. Matrix-assisted laser desorption ionization-time of flight (MALDI-TOF) mass spectrometry (MS) has been used to determine resistance to b-lactams, including carbapenems in Enterobacteriaceae, but the methodology has not been fully validated as it remains time-consuming. We aimed to assess whether MALDI-TOF can be used to detect ESBL-producing Enterobacteriaceae from positive blood culture bottles in clinical practice. In the assay, 141 blood cultures were tested, 13 of them were real bacteraemias and 128 corresponded to blood culture bottles seeded with bacterial clinical isolates. Bacteraemias were analysed by MALDI-TOF after a positive growth result and the 128 remaining blood cultures 24 h after the bacterial seeding. b-lactamase activity was determined through the profile of peaks associated with the antibiotics cefotaxime and ceftazidime and its hydrolyzed forms.

Clavulanic acid was added to rule out the presence of non-ESBL mechanisms. Overall data show a 99% (103 out of 104) sensitivity in detecting ESBL in positive blood cultures. Data were obtained in 90 min (maximum 150 min). The proposed methodology has a great impact on the early detection of ESBL-producing Enterobacteriaceae from positive blood cultures, being a rapid and efficient method and allowing early administration of an appropriate antibiotic therapy.

2.13 Skin and soft tissue infections in intercontinental travelers and the import of multi-resistant Staphylococcus aureus to Europe. D. Nurjadi et al. Clin. Microbiol. Infect. 2015; Jan 28 pii: S1198-743

Staphylococcus aureus is emerging globally. Treatment of infections is complicated by increasing antibiotic resistance. We collected clinical data and swabs of returnees with skin and soft tissue infections (SSTI) at 13 travel-clinics in Europe (www.staphtrav.eu). Sixty-two percent (196/318) SSTI patients had S. aureus-positive lesions, of which almost two-thirds (122/196) were Panton–Valentine leukocidin (PVL) positive. PVL was associated with disease severity, including hospitalization for SSTI (OR 5.2, 95% CI 1.5–18.2). In returnees with SSTI, longer travel and more intense population contact were risk factors for nasal colonization with PVL-positive S. aureus. Imported S. aureus frequently proved resistant to trimethoprim-sulfamethoxazole (21%), erythromycin (21%), tetracycline (20%), ciprofloxacin (13%), methicillin (12%) and clindamycin (8%). Place of exposure was significantly ($p < 0.05$) associated with predominant resistance phenotypes and spa genotypes: Latin America (methicillin; t008/CC24/304), Africa (tetracycline, trimethoprim-sulfamethoxazole; t084/CC84, t314/singleton, t355/CC355), South Asia (trimethoprim-sulfamethoxazole, ciprofloxacin; t021/CC21/318), South-East Asia (clindamycin; t159/CC272). USA300-like isolates accounted for 30% of all methicillin-resistant S. aureus imported to Europe and were predominantly (71%) acquired in Latin America. Multi-resistance to non-β-lactams were present in 24% of imports and associated with travel to South Asia (OR crude 5.3, 95% CI 2.4–11.8), even after adjusting for confounding by genotype (ORadjusted 3.8, 95% 1.5–9.5). Choosing randomly from compounds recommended for the empiric treatment of severe S. aureus SSTI, 15% of cases would have received ineffective antimicrobial therapy. These findings call for the development of regionally stratified guidance on the antibiotic management of severe imported S. aureus disease and put the infected and colonized traveller at the centre of interventions against the global spread of multi-resistant S. aureus.

2.14 Antimicrobials Increase Travelers' Risk of Colonization by Extended-Spectrum Beta lactamase-Producing Enterobacteriaceae. Anu Kantele et al. Clin. Infect. Dis. 2015; 60: 837-46

Background. More than 300 million travelers visit regions with poor hygiene annually. A significant percentage of them become colonized by resistant intestinal bacteria such as extended-spectrum beta-lactamase–producing Enterobacteriaceae (ESBL-PE) and may transmit the strains to others and to medical care settings when they return home. Despite the threats to global healthcare caused by an upsurge in antimicrobial resistance, no effort has been centered on prevention of colonization while traveling.

Methods. Stool samples were collected from 430 Finns before and after traveling outside Scandinavia. All specimens were analyzed for ESBL- and carbapenemase-producing Enterobacteriaceae (CPE). Questionnaires were used to survey volunteers about use of antimicrobials as well as other potential risk factors. The results were subjected to multivariable analysis.

Results. Twenty-one percent (90/430) of the travelers became colonized by ESBL-PE and none by CPE. Geographic region, occurrence of travelers' diarrhea (TD), age, and use of antimicrobial (AB) for TD were identified as independent risk factors predisposing to contracting ESBL-PE. Eleven percent of those in subgroup TD−AB−, 21% in TD+AB−, and 37% in TD+AB+ acquired ESBL-PE. The risk proved to be highest in South Asia (46%); 23% became colonized in subgroup TD−AB−, 47% in TD+AB−, and 80% in TD+AB+. In Southeast Asia, the rates were 14%, 37%, and 69%, respectively.

Conclusions. TD and antimicrobials for TD proved to be independent risk factors, with up to 80% of TD+AB+travelers contracting ESBL-PE. In modern pre-travel counseling for those visiting high-risk regions, travelers should be advised against taking antibiotics for mild or moderate TD.

2.15 High Rates of Antimicrobial Drug Resistance Gene Acquisition after International Travel, the Netherlands. Christian J.H. von Wintersdorff et al. Emerg Infect Dis. 2014; 20: 649-57

We investigated the effect of international travel on the gut resistome of 122 healthy travelers from the Netherlands by using a targeted metagenomic approach. Our results confirm high acquisition rates of the ESBL encoding gene blaCTX-M, documenting a rise in prevalence from 9.0% before travel to 33.6% after travel ($p<0.001$). The prevalence of quinolone resistance encoding genes qnrB and qnrS increased from 6.6% and 8.2% before travel to 36.9% and 55.7% after travel, respectively (both $p<0.001$). Travel to Southeast Asia and the Indian subcontinent was associated with the highest acquisition rates of qnrS and both blaCTX-M and qnrS, respectively. Investigation of the associations between the acquisitions of the blaCTX-M and qnr genes showed that acquisition of a blaCTX-M gene was not associated with that of a qnrB ($p = 0.305$) or qnrS ($p = 0.080$) gene. These findings support the increasing evidence that travelers contribute to the spread of antimicrobial drug resistance.

2.16 Zero tolerance for healthcare-associated MRSA bacteraemia: is it realistic? M. Estée Török et al. J. Antimicrob. Chemother. 2014; 69: 2238–2245

Background. The term ‘zero tolerance’ has recently been applied to healthcare-associated infections, implying that such events are always preventable. This may not be the case for healthcare-associated infections such as methicillin-resistant Staphylococcus aureus (MRSA) bacteraemia.

Methods. We combined information from an epidemiological investigation and bacterial whole-genome sequencing to evaluate a cluster of five MRSA bacteraemia episodes in four patients in a specialist hepatology unit.

Results. The five MRSA bacteraemia isolates were highly related by multilocus sequence type (ST) (four isolates were ST22 and one isolate was a single-locus variant, ST2046). Whole-genome sequencing demonstrated unequivocally that the bacteraemia cases were unrelated. Placing the MRSA bacteraemia isolates within a local and global phylogenetic tree of MRSA ST22 genomes demonstrated that the five bacteraemia isolates were highly diverse. This was consistent with the acquisition and importation of MRSA from the wider referral network. Analysis of MRSA carriage and disease in patients within the hepatology service demonstrated a higher risk of both initial MRSA acquisition compared with the nephrology service and a higher risk of progression from MRSA carriage to bacteraemia, compared with patients in nephrology or geriatric services. A root cause analysis failed to reveal any mechanism by which three of five MRSA bacteraemia episodes could have been prevented.

Conclusions This study illustrates the complex nature of MRSA carriage and bacteraemia in patients in a specialized hepatology unit. Despite numerous ongoing interventions to prevent MRSA bacteraemia in healthcare settings, these are unlikely to result in a zero incidence in referral centres that treat highly complex patients.

2.17 A Major Reduction in Hospital-Onset Staphylococcus aureus Bacteremia in Australia—12 Years of Progress: An Observational Study. Brett G. Mitchell et al. Clin. Infect. Dis. 2014; 59: 969–75

Background. Staphylococcus aureus bacteremia (SAB) is a serious cause of morbidity and mortality. This longitudinal study describes significant reductions in hospital-onset SAB (HO-SAB) in Australian hospitals over the past 12 years.

Methods. An observational cohort study design was used. Prospective surveillance of HO-SAB in 132 hospitals in Australia was undertaken. Aggregated data from all patients who acquired HO-SAB was collected (defined as 1 or more blood cultures positive for S. aureus taken from a patient who had been admitted to hospital for >48 hours). The primary outcome was the incidence of HO-SAB, including both methicillin-resistant (MRSA) and methicillin-susceptible (MSSA) S. aureus strains.

Results. A total of 2733 HO-SAB cases were identified over the study period, giving an aggregate incidence of 0.90 per 10 000 patient-days (PDs) (95% confidence interval [CI], .86–.93). There was a 63% decrease in the annual incidence, from 1.72 per 10 000 PDs in 2002 (95% CI, 1.50–1.97) to 0.64 per 10 000 PDs (95% CI, 0.53–0.76) in 2013. The mean reduction per year was 9.4% (95% CI, −8.1% to

−10.7%). Significant reductions in both HO-MRSA (from 0.77 to 0.18 per 10 000 PDs) and HO-MSSA (from 1.71 to 0.64 per 10 000 PDs) bacteremia were observed.

Conclusions. There was a major and significant reduction in incidence of HO-SAB caused by both MRSA and MSSA in Australian hospitals since 2002. This reduction coincided with a range of infection prevention and control activities implemented during this time. It suggests that national and local efforts to reduce the burden of health care associated infections have been very successful.

2.18 Dramatic increase in vancomycin-resistant enterococci in Germany. Petra Gastmeier et al. J. Antimicrob. Chemother. 2014; 69: 1660–1664

Objectives Among European countries, Germany has one of the highest proportions of vancomycin-resistant Enterococcus faecium bloodstream infections. The aim of this study was to investigate the development of vancomycin-resistant enterococci (VRE) in German hospitals and to consider the regional distribution of VRE in Germany.

Methods Data from three components of the German national nosocomial surveillance system (KISS) from the period 2007–12 were used for analysis: ICU-KISS data on nosocomial primary bloodstream infections and urinary tract infections from intensive care units (ICUs); OP-KISS data on surgical site infections from surgical departments; and Pathogen-KISS data concentrating on VRE cases (infections and colonizations) in ICUs. Trends over time were calculated and a map according to German federal states was prepared.

Results Data from up to 645 ICUs and 681 surgical departments for 2 year periods from 2007 to 2012 were analysed. The proportion of VRE increased significantly for surgical site infections (526%; $P<0.01$) and bloodstream infections (265%; $P<0.01$) and non-significantly for urinary tract infections (278%; $P=0.07$). A large subgroup of ICUs also reported VRE cases in the same period, with a significant increase of 282%. The mapping of federal states showed large variation in VRE proportions and incidence rates in a belt of states with significantly higher VRE proportions from west (North Rhine-Westphalia) to east (Saxony).

Conclusions The high overall VRE proportion in Germany is mainly due to the situation in four states. There is an urgent need to analyze the epidemiology of VRE in detail to develop appropriate infection control strategies.

2.19 Unexpected Challenges in Treating Multidrug-Resistant Gram-Negative Bacteria: Resistance to Ceftazidime-Avibactam in Archived Isolates of Pseudomonas aeruginosa. Marisa L. Winkler et al. Antimicrob. Agents Chemother. 2015; 59:1020-1029

Pseudomonas aeruginosa is a notoriously difficult-to-treat pathogen that is a common cause of severe nosocomial infections. Investigating a collection of β-lactam-resistant P. aeruginosa clinical isolates from a decade ago, we uncovered resistance to ceftazidime-avibactam, a novel β-lactam/β-lactamase inhibitor combination. The isolates were systematically analyzed through a variety of genetic, biochemical, genomic, and microbiological methods to understand how resistance manifests to a unique drug combination that is not yet clinically released. We discovered that avibactam was able to inactivate different AmpC β-lactamase enzymes and that blaPDC regulatory elements and penicillin-binding protein differences did not contribute in a major way to resistance. By using carefully selected combinations of antimicrobial agents, we deduced that the greatest barrier to ceftazidime-avibactam is membrane permeability and drug efflux. To overcome the constellation of resistance determinants, we show that a combination of antimicrobial agents (ceftazidime/avibactam/fosfomycin) targeting multiple cell wall synthetic pathways can restore susceptibility. In P. aeruginosa, efflux, as a general mechanism of resistance, may pose the greatest challenge to future antibiotic development. Our unexpected findings create concern that even the development of antimicrobial agents targeted for the treatment of multidrug-resistant bacteria may encounter clinically important resistance. Antibiotic therapy in the future must consider these factors.

2.20 Dominance of international 'high-risk clones' among metallo-β-lactamase-producing Pseudomonas aeruginosa in the UK. Laura L Wright et al. J. Antimicrob. Chemother. 2015; 70: 103–110

Objectives. Carbapenem-resistant isolates of Pseudomonas aeruginosa producing metallo-β-lactamases (MBLs) are increasingly reported worldwide and often belong to particular 'high-risk clones'. This study aimed to characterize a comprehensive collection of MBL-producing P. aeruginosa isolates referred to the UK national reference laboratory from multiple UK laboratories over a 10 year period.

Methods. Isolates were referred to the UK national reference laboratory between 2003 and 2012 for investigation of resistance mechanisms and/or outbreaks. MBL genes were detected by PCR. Typing was carried out by nine-locus variable-number tandem repeat (VNTR) analysis and MLST.

Results. MBL-producing P. aeruginosa isolates were referred from 267 source patients and 89 UK laboratories. The most common isolation sites were urine (24%), respiratory (18%), wounds (17%) and blood (13%). VIM-type MBLs predominated (91% of all MBLs found), but a few IMP- and NDM-type enzymes were also identified. Diverse VNTR types were seen, but 86% of isolates belonged to six major complexes. MLST of representative isolates from each complex showed that they corresponded to STs 111, 233, 235, 357, 654 and 773, respectively. Isolates belonging to these complexes were received from between 9 and 25 UK referring laboratories each.

Conclusions. The incidence of MBL-producing P. aeruginosa is increasing in the UK. The majority of these isolates belong to several 'high-risk clones', which have been previously reported internationally as host clones of MBLs.

2.21 Characterization of Plasmids in Extensively Drug-Resistant Acinetobacter Strains Isolated in India and Pakistan. Lim S. Jones et al. Antimicrob. Agents Chemother. 2015; 59:923-929

The blaNDM-1 gene is associated with extensive drug resistance in Gram-negative bacteria. This probably spread to Enterobacteriaceae from Acinetobacter spp., and we characterized plasmids associated with blaNDM-1 in Acinetobacter spp. to gain insight into their role in this dissemination. Four clinical NDM-1-producing Acinetobacter species strains from India and Pakistan were investigated. A plasmid harboring blaNDM-1, pNDM-40-1, was characterized by whole-genome sequencing of Acinetobacter bereziniae CHI-40-1 and comparison with related plasmids. The presence of similar plasmids in strains from Pakistan was sought by PCR and sequencing of amplicons. Conjugation frequency was tested and stability of pNDM-40-1 investigated by real-time PCR of isolates passaged with and without antimicrobial selection pressure. A. bereziniae and Acinetobacter haemolyticus strains contained plasmids similar to the pNDM-BJ01-like plasmids identified in Acinetobacter spp. in China. The backbone of pNDM-40-1 was almost identical to that of pNDM-BJ01-like plasmids, but the transposon harboring blaNDM-1, Tn125, contained two short deletions. Escherichia coli and Acinetobacter pittii transconjugants were readily obtained. Transconjugants retained pNDM-40-1 after a 14-day passage experiment, although stability was greater with meropenem selection. Fragments of pNDM-BJ01-like plasmid backbones are found near blaNDM-1 in some genetic contexts from Enterobacteriaceae, suggesting that cross-genus transfer has occurred. pNDM-BJ01-like plasmids have been described in isolates originating from a wide geographical region in southern Asia. In vitro data on plasmid transfer and stability suggest that these plasmids could have contributed to the spread of blaNDM-1 into Enterobacteriaceae.

2.22 Monotherapy versus combination therapy for sepsis due to multidrug-resistant Acinetobacter baumannii: analysis of a multicentre prospective cohort. L. E. López-Cortés et al. J. Antimicrob. Chemother: 69; 3119-3126

Background. Treatment of multidrug-resistant Acinetobacter baumannii (MDRAB) infection presents a challenge because of the scarcity of available options. Even though combination therapy (CT) is frequently used in clinical practice, data are needed to support its use instead of monotherapy (MT).

Methods. A prospective observational study was conducted in 28 Spanish hospitals. Patients with sepsis caused by MDRAB, defined according to strict criteria, and who received active antibiotic treatment (according to in vitro susceptibility testing) for at least 48 h, were included. The main outcome variable

was all-cause 30 day mortality after initiation of targeted therapy. Multivariate analysis, including a propensity score (for receiving CT), was performed by Cox regression.

Results. One hundred and one patients were included in the analysis; 68 (67.3%) received MT and 33 (32.7%) received CT. Pneumonia was the most common infection (50.5%), 68.6% of cases being associated with mechanical ventilation. Colistin (67.6%) and carbapenems (14.7%) were the most common drugs used in MT; colistin plus tigecycline (27.3%) and carbapenem plus tigecycline (12.1%) were the most frequent combinations. Crude 30 day mortality was 23.5% and 24.2% for the MT and CT groups, respectively (RR=1.03; 95% CI 0.49–2.16; P=0.94). Multivariate analysis of 30 day survival showed no trend towards reduced 30 day mortality with CT (HR=1.35; 95% CI 0.53–3.44; P=0.53). Subgroup analysis showed similar results.

Conclusions. Our data do not support an association of CT with reduced mortality in MDRAB infections. More data for specific types of infection and combinations are needed.

2.23 Surveillance Cultures Growing Carbapenem-Resistant Acinetobacter baumannii Predict the Development of Clinical Infections: A Retrospective Cohort Study. Rachel Latibeaudiere et al. Clin. Infect. Dis. 2015; 60: 415–22

Background. We aimed to determine the effect of the presence of carbapenem-resistant Acinetobacter baumannii in accordance with surveillance cultures on the subsequent development of clinical infections by this organism.

Methods. This retrospective cohort study was conducted at a tertiary hospital from January 2010 to November 2011. We included all consecutive patients admitted to the trauma intensive care unit, who had weekly surveillance cultures performed (from rectum, and if intubated, respiratory secretions), and without evidence of A. baumannii infections prior to the collection of the first surveillance culture. Univariable and multivariable analyses were performed using log-binomial regression. Survival analyses were performed using Cox proportional hazards.

Results. Three hundred sixty-four patients were included, of whom 49 (13.5%) had carbapenem-resistant A. baumannii on surveillance cultures. Patients with positive surveillance cultures had 8.4 (95% confidence interval [CI], 5.6–12.7; P < 0.0001) times the risk of developing a subsequent A. baumannii infection compared with patients who remained negative on surveillance cultures. Multivariable analysis showed significant associations between clinical infection and both positive surveillance cultures (relative risk [RR], 5.9 [95% CI, 3.8–9.3]; P < 0.0001) and mechanical ventilation (RR, 4.3 [95% CI, 1.03–18.2]; P = 0.05). On survival analyses, the only variable associated with the development of clinical infections was the presence of positive surveillance cultures (hazard ratio, 16.3 [95% CI, 9.1–29.1]; P < 0.001).

Conclusions. Presence of carbapenem-resistant A. baumannii on surveillance cultures is strongly associated with subsequent development of carbapenem-resistant A. baumannii infections. Prevention efforts should be focused at limiting the acquisition of this organism during hospitalization.

2.24 Polymyxin Resistance Caused by mgrB Inactivation Is Not Associated with Significant Biological Cost in Klebsiella pneumoniae. Antonio Cannatelli et al. Antimicrob. Agents Chemother. 2015; 59:2898-2900

The inactivation of the mgrB gene, which encodes a negative-feedback regulator of the PhoPQ signaling system, was recently shown to be a common mutational mechanism responsible for acquired polymyxin resistance among carbapenemase-producing Klebsiella pneumoniae strains from clinical sources. In this work, we show that mgrB mutants can easily be selected in vitro from different K. pneumoniae lineages, and mgrB inactivation is not associated with a significant biological cost.

2.25 Incidence rates of carbapenemase-producing Enterobacteriaceae clinical isolates in France: a prospective nationwide study in 2011–12. Jérôme Robert et al. J. Antimicrob. Chemother. 2014; 69: 2706-2712

Objectives. To determine proportions and incidence rates of Enterobacteriaceae producing carbapenemase among those non-susceptible (NS) to carbapenems in France.

Methods. From November 2011 to April 2012, 71 laboratories recorded non-duplicate Enterobacteriaceae clinical isolates NS to at least one carbapenem and the total number of isolates of the different species. Carbapenem MICs were determined by broth microdilution and the β-lactamase content by DNA microarray.

Results. During the study period, the 71 laboratories identified 133244 Enterobacteriaceae isolates, of which 846 (0.63%) were NS to at least one carbapenem. Carbapenem-NS isolates accounted for 0.07% (61/90148) among Escherichia coli isolates, 1.1% (111/10436) among Klebsiella pneumoniae, 8.2% (492/5971) among Enterobacter cloacae and 4.0% (84/2104) among Enterobacter aerogenes. Among the 541 available carbapenem-NS isolates, 222 (including 63 randomly selected E. cloacae) were further analysed after confirmation of carbapenem non-susceptibility. None of the Enterobacter spp. isolates produced carbapenemase. Among the other species, 28 isolates produced carbapenemases (22 OXA-48, 4 KPC and 2 NDM), accounting for an estimated proportion of carbapenemase-producing isolates of 0.08% for all species, 0.01% for E. coli and 0.27% for K. pneumoniae. The incidence-density rate in the participating hospitals was 0.0041 per 1000 hospital-days and the incidence rate was 0.0027 per 100 admissions.

Conclusions. The incidence-density rate of carbapenemase-producing isolates per 1000 hospital-days was low and 30-fold lower than that of carbapenem-NS isolates (0.125) and almost 300-fold lower than that of ESBL-producing isolates (1.104) in 71 French hospitals.

2.26 Gentamicin therapy for sepsis due to carbapenem-resistant and colistin-resistant Klebsiella pneumoniae. Marcelino Gonzalez-Padilla et al. J. Antimicrob. Chemother. 2015; 70: 905–913

Objectives. Antimicrobial therapy for sepsis caused by carbapenem- and colistin-resistant Klebsiella pneumoniae is not well established. We hypothesized that the early use of gentamicin in cases due to susceptible organisms would decrease the crude mortality rate of this infection.

Methods. This retrospective cohort study examined 50 cases of sepsis caused by carbapenem-resistant K. pneumoniae occurring between June 2012 and February 2013 during an outbreak of K. pneumoniae ST512 producing KPC-3, SHV-11 and TEM-1. Survival curves categorized by the use of gentamicin were constructed using the Kaplan–Meier method and compared using the log-rank test. Eight multivariate models using Cox regression were designed to study the risk factors for mortality and test the hypothesis.

Results. The 30 day crude mortality rate was 38%. The use of targeted gentamicin was associated with reduced mortality (20.7% versus 61.9%, P=0.02). In all multivariate regression models, the use of gentamicin was independently associated with lower mortality until Day 30 (HR 0.17–0.29, P=0.03–0.002 depending on the model) after controlling for other potential confounding variables such as age, optimal treatment, renal function, severity of infection, underlying disease, use of tigecycline and previous hospitalization.

Conclusions: Gentamicin reduced the mortality from sepsis caused by this K. pneumoniae ST512 clone producing KPC-3, SHV-11 and TEM-1.

2.27 Performance of Vitek 2 for Antimicrobial Susceptibility Testing of Enterobacteriaceae with Vitek 2 (2009 FDA) and 2014 CLSI Breakpoints. April M. Bobenchik et al. J. Clin. Microbiol. 2015; 53: 816-823

Vitek 2 (bioMérieux Inc., Durham, NC) is a widely used commercial antimicrobial susceptibility test system. We compared the MIC results obtained using the Vitek 2 AST-GN69 and AST-XN06 cards to those obtained by CLSI broth microdilution (BMD) for 255 isolates of Enterobacteriaceae, including 25 isolates of carbapenem-resistant Enterobacteriaceae. In total, 25 antimicrobial agents were examined. For 10 agents, the MIC data were evaluated using two sets of breakpoints: (i) the Vitek 2 breakpoints, which utilized the 2009 FDA breakpoints at the time of the study and are equivalent to the 2009 CLSI M100-S19 breakpoints, and (ii) the 2014 CLSI M100-S24 breakpoints. There was an overall 98.7% essential agreement (EA). The categorical agreement was 95.5% (CA) using the Vitek 2 breakpoints and 95.7% using the CLSI breakpoints. There was 1 very major error (VME) (0.05%) observed using the Vitek 2 breakpoints (cefazolin) and 8 VMEs (0.5%) using the CLSI breakpoints (2 each for aztreonam, cefepime, and ceftriaxone, and 1 for cefazolin and ceftazidime). Fifteen major errors (MEs) (0.4%) were

noted using the Vitek 2 breakpoints and 8 (0.5%) using the CLSI breakpoints. Overall, the Vitek 2 performance was comparable to that of BMD for testing a limited number of Enterobacteriaceae commonly isolated by clinical laboratories. Ongoing studies are warranted to assess performance in isolates with emerging resistance.

2.28 Impact of carbapenem heteroresistance among clinical isolates of invasive Escherichia coli in Chongqing, southwestern China. J. D. Sun et al. Clin. Microbiol. Infect. 2014 Dec 26; pii:S1198-743x

Although heteroresistance is common in a wide range of microorganisms, carbapenem heteroresistance among invasive Escherichia coli infections has not been reported. The objective of this study was to evaluate the clinical significance of carbapenem heteroresistance and to identify risk factors for its acquisition. A case–control study was conducted at a 3200-bed teaching hospital in Chongqing, southwestern China. Successive and non-duplicate nosocomial E. coli isolates (n = 332) were obtained from July 2011 to June 2013. Bloodstream isolates made up 50.6% of the strains collected. The rates of heteroresistance were 25.0% to imipenem, 17.2% to ertapenem, and 3.9% to meropenem. The population analysis profile revealed the presence of subpopulations with higher carbapenem resistance, showing MICs ranging from 2.0–128.0 mg/L. Male gender, invasive intervention, antibiotic use and bacterial extended spectrum β-lactamase (ESBL) production contributed to invasive infections by carbapenem-heteroresistant E. coli (CHEC). The production of ESBL was identified as the common independent risk factor for heteroresistance to both ertapenem and imipenem. Pulsed field gel electrophoresis revealed clonal diversity among the CHEC isolates. Most importantly, characterization of two successive E. coli strains isolated from the same patient indicated that carbapenem resistance evolved from heteroresistance. In conclusion, the high prevalence of heteroresistance to carbapenem among invasive E. coli merits great attention. Routine detection of ESBLs and the prudent use of imipenem and ertapenem are advocated. The early targeted intervention should be formulated to reduce CHEC infection and carbapenem resistance of E. coli.

2.29 Prevention of Colonization and Infection by Klebsiella pneumoniae Carbapenemase‐Producing Enterobacteriaceae in Long Term Acute Care Hospitals. Mary K. Hayden et al. Clin. Infect Dis. 2015; 60: 1153-61

Background: Klebsiella pneumoniae carbapenemase‐producing Enterobacteriaceae (KPC) are an increasing threat to healthcare institutions. Long‐term acute care hospitals (LTACHs) have especially high prevalence of KPC.

Methods: Using a stepped‐wedge design, we tested whether a bundled intervention (screening patients for KPC rectal colonization upon admission and every other week; contact isolation and geographic separation of KPC‐positive patients in ward cohorts or single rooms; bathing all patients daily with chlorhexidine gluconate; and healthcare worker education and adherence monitoring) would reduce colonization and infection due to KPC in four LTACHs with high endemic KPC prevalence. The study was conducted between February 1, 2010-June 30, 2013; 3,894 patients were enrolled during preintervention (16‐29 months) and 2,951 patients were enrolled during intervention (12‐19 months).

Results: KPC colonization prevalence was stable during pre‐intervention (average, 45.8%; 95% CI 42.1‐49.5%), declined early during intervention, then reached a plateau (34.3%; 95% CI 32.4%‐36.2%; p<0.001 for exponential decline). During intervention, KPC admission prevalence remained high (average, 20.6%, 95% CI 19.1%‐22.3%). The incidence‐rate of KPC colonization fell during intervention from 4 to 2 acquisitions/100 patient‐weeks (p=0.004 for linear decline). Compared to pre‐intervention, average rates of clinical outcomes declined during intervention: KPC in any clinical culture (3.7 to 2.5/1000 patient‐days, p=0.001), KPC bacteremia (0.9 to 0.4/1000 patient‐days, p=0.008), all‐cause bacteremia (11.2 to 7.6/1000 patient‐days, p=0.006) and blood culture contamination (4.9 to 2.3/1000 patient‐days,

p=0.03).

Conclusions: A bundled intervention was associated with clinically important and statistically significant reductions in KPC colonization, KPC infection, all‐cause bacteremia and blood culture contamination in a high‐risk LTACH population.

2.30 β-lactam and β-lactamase inhibitor combinations in the treatment of extended-spectrum β-lactamase producing Enterobacteriaceae: time for a reappraisal in the era of few antibiotic options? Patrick N A Harris et al. Lancet Infect. Dis. 2015 Published Online February 23, 2015

The spread of extended-spectrum β-lactamase (ESBL) genes in Enterobacteriaceae such as Escherichia coli or Klebsiella spp is a major challenge to modern medical practice. Carbapenems are the treatment of choice for serious infections caused by ESBL producers; however, carbapenem resistance has increased globally. ESBL producers might be susceptible to β-lactam-β-lactamase inhibitor (BLBLI) combination antibiotics such piperacillin–tazobactam or amoxicillin–clavulanate. These drugs are frequently avoided in serious infections caused by ESBL producers because of the inoculum effect in-vitro (especially for piperacillin–tazobactam), animal data suggesting inferior efficacy when compared with carbapenems, concerns about pharmacokinetic–pharmacodynamic drug target attainment with standard doses, and poor outcomes shown in some observational studies. Prospective cohort data and a meta-analysis suggest that BLBLIs are non-inferior to carbapenems in the treatment of bloodstream infections caused by ESBL producers. We examine why BLBLIs are perceived as inferior in the treatment of infection with ESBL producers, and discuss data that suggest these concerns might not be strongly supported by clinical evidence.

3.1 Mishra N, Pereira M, Rhodes RH, An P, Pipas JM, Jain K, Kapoor A, Briese T, Faust PL, Lipkin WI. Identification of a novel polyomavirus in a pancreatic transplant recipient with retinal blindness and vasculitic myopathy. J Infect Dis 2014;210:1595-9.

BACKGROUND: A 33 year-old pancreatic transplant recipient developed weakness, retinal blindness, and necrotic plaques on her face, scalp, and hands. METHODS: A muscle biopsy was analyzed by light and electron microscopy and high-throughput nucleic acid sequencing. RESULTS: The biopsy revealed microthrombosis and viral particles in swollen endothelial cell nuclei. High-throughput sequencing of nucleic acid revealed a novel polyomavirus. In situ hybridization confirmed the presence of the polyomavirus in endothelial cells at sites of myositis and cutaneous necrosis. CONCLUSIONS: New Jersey polyomavirus (NJPyV-2013) is a novel polyomavirus that may have tropism for vascular endothelial cells.

3.2 Brown JR, Morfopoulou S, Hubb J, Emmett WA, Ip W, Shah D, Brooks T, Paine SM, Anderson G, Virasami A, Tong CY, Clark DA, Plagnol V, Jacques TS, Qasim W, Hubank M, Breuer J. Astrovirus VA1/HMO-C: An increasingly recognized neurotropic pathogen in immunocompromised patients. Clin Infect Dis 2015;60:881-8.

BACKGROUND: An 18-month-old boy developed encephalopathy, for which extensive investigation failed to identify an etiology, 6 weeks after stem cell transplant. To exclude a potential infectious cause, we performed high-throughput RNA sequencing on brain biopsy. METHODS: RNA-Seq was performed on an Illumina Miseq, generating 20 million paired-end reads. Nonhost data were checked for similarity to known organisms using BLASTx. The full viral genome was sequenced by primer walking. RESULTS: We identified an astrovirus, HAstV-VA1/HMO-C-UK1(a), which was highly divergent from human astrovirus (HAstV 1-8) genotypes, but closely related to VA1/HMO-C astroviruses, including one recovered from a case of fatal encephalitis in an immunosuppressed child. The virus was detected in stool and serum, with highest levels in brain and cerebrospinal fluid (CSF). Immunohistochemistry of the brain biopsy showed positive neuronal staining. A survey of 680 stool and 349 CSF samples identified a related virus in the stool of another immunosuppressed child. CONCLUSIONS: The discovery of HAstV-VA1/HMO-C-UK1(a) as the cause of encephalitis in this case provides further evidence that VA1/HMO-C viruses, unlike HAstV

1-8, are neuropathic, particularly in immunocompromised patients, and should be considered in the differential diagnosis of encephalopathy. With a turnaround from sample receipt to result of <1 week, we confirm that RNA-Seq presents a valuable diagnostic tool in unexplained encephalitis.

3.3 Naccache SN, Peggs KS, Mattes FM, Phadke R, Garson JA, Grant P, Samayoa E, Federman S, Miller S, Lunn MP, Gant V, Chiu CY. Diagnosis of neuroinvasive astrovirus infection in an immunocompromised adult with encephalitis by unbiased next-generation sequencing. Clin Infect Dis 2015;60:919-23.

Metagenomic next-generation sequencing (NGS) was used to diagnose an unusual and fatal case of progressive encephalitis in an immunocompromised adult presenting at disease onset as bilateral hearing loss. The sequencing and confirmatory studies revealed neuroinvasive infection of the brain by an astrovirus belonging to a recently discovered VA/HMO clade.

3.4 Kosoy OI, Lambert AJ, Hawkinson DJ, Pastula DM, Goldsmith CS, Hunt DC, Staples JE. Novel Thogotovirus associated with febrile illness and death, United States, 2014. Emerg Infect Dis 2015;in press.

A previously healthy man from eastern Kansas, USA, sought medical care in late spring because of a history of tick bite, fever, and fatigue. The patient had thrombocytopenia and leukopenia and was given doxycycline for a presumed tickborne illness. His condition did not improve. Multiorgan failure developed, and he died 11 days after illness onset from cardiopulmonary arrest. Molecular and serologic testing results for known tickborne pathogens were negative. However, testing of a specimen for antibodies against Heartland virus by using plaque reduction neutralization indicated the presence of another virus. Next-generation sequencing and phylogenetic analysis identified the virus as a novel member of the genus Thogotovirus.

3.5 Vora NM, Li Y, Geleishvili M, Emerson GL, Khmaladze E, Maghlakelidze G, Navdarashvili A, Zakhashvili K, Kokhreidze M, Endeladze M, Mokverashvili G, Satheshkumar PS, Gallardo‐Romero N, Goldsmith CS, Metcalfe MG, Damon I, Maes EF, Reynolds MG, Morgan J, Carroll DS. Human infection with a zoonotic Orthopoxvirus in the country of Georgia. N Engl J Med 2015;372:1223-30.

During 2013, cutaneous lesions developed in two men in the country of Georgia after they were exposed to ill cows. The men had never received vaccination against smallpox. Tests of lesion material with the use of a quantitative real-time polymerase-chain-reaction assay for non–variola virus orthopoxviruses were positive, and DNA sequence analysis implicated a novel orthopoxvirus species. During the ensuing epidemiologic investigation, no additional human cases were identified. However, serologic evidence of exposure to an orthopoxvirus was detected in cows in the patients' herd and in captured rodents and shrews. A third case of human infection that occurred in 2010 was diagnosed retrospectively during testing of archived specimens that were originally submitted for tests to detect anthrax. Orthopoxvirus infection should be considered in persons in whom cutaneous lesions develop after contact with animals.

3.6 Gire SK, Goba A, Andersen KG, Sealfon RS, Park DJ, Kanneh L, Jalloh S, Momoh M, Fullah M, Dudas G, Wohl S, Moses LM, Yozwiak NL, Winnicki S, Matranga CB, Malboeuf CM, Qu J, Gladden AD, Schaffner SF, Yang X, Jiang PP, Nekoui M, Colubri A, Coomber MR, Fonnie M, Moigboi A, Gbakie M, Kamara FK, Tucker V, Konuwa E, Saffa S, Sellu J, Jalloh AA, Kovoma A, Koninga J, Mustapha I, Kargbo K, Foday M, Yillah M, Kanneh F, Robert W, Massally JL, Chapman SB, Bochicchio J, Murphy C, Nusbaum C, Young S, Birren BW, Grant DS, Scheiffelin JS, Lander ES, Happi C, Gevao SM, Gnirke A, Rambaut A, Garry RF, Khan SH, Sabeti PC. Genomic surveillance elucidates Ebola virus origin and transmission during the 2014 outbreak. Science 2014;345:1369-72.

In its largest outbreak, Ebola virus disease is spreading through Guinea, Liberia, Sierra Leone, and Nigeria. We sequenced 99 Ebola virus genomes from 78 patients in Sierra Leone to ~2000× coverage. We observed a rapid accumulation of interhost and intrahost genetic variation, allowing us to characterize patterns of viral transmission over the initial weeks of the epidemic. This West African variant likely diverged from central African lineages around 2004, crossed from Guinea to Sierra Leone in May 2014, and has exhibited sustained human-to-human transmission subsequently, with no evidence of additional zoonotic sources. Because many of the mutations alter protein sequences and other biologically meaningful targets, they should be monitored for impact on diagnostics, vaccines, and therapies critical to outbreak response.

3.7 Garamszegi S, Yen JY, Honko AN, Geisbert JB, Rubins KH, Geisbert TW, Xia Y, Hensley LE, Connor JH. Transcriptional correlates of disease outcome in anticoagulant-treated non-human primates infected with ebolavirus. PLoS Negl Trop Dis 2014;8:e3061.

Ebola virus (EBOV) infection in humans and non-human primates (NHPs) is highly lethal, and there is limited understanding of the mechanisms associated with pathogenesis and survival. Here, we describe a transcriptomic analysis of NHPs that survived lethal EBOV infection, compared to NHPs that did not survive. It has been previously demonstrated that anticoagulant therapeutics increase the survival rate in EBOV-infected NHPs, and that the characteristic transcriptional profile of immune response changes in anticoagulant-treated NHPs. In order to identify transcriptional signatures that correlate with survival following EBOV infection, we compared the mRNA expression profile in peripheral blood mononuclear cells from EBOV-infected NHPs that received anticoagulant treatment, to those that did not receive treatment. We identified a small set of 20 genes that are highly confident predictors and can accurately distinguish between surviving and non-surviving animals. In addition, we identified a larger predictive signature of 238 genes that correlated with disease outcome and treatment; this latter signature was associated with a variety of host responses, such as the inflammatory response, T cell death, and inhibition of viral replication. Notably, among survival-associated genes were subsets of genes that are transcriptionally regulated by (1) CCAAT/enhancer-binding protein alpha, (2) tumor protein 53, and (3) megakaryoblastic leukemia 1 and myocardin-like protein 2. These pathways merit further investigation as potential transcriptional signatures of host immune response to EBOV infection.

3.8 Dowall SD, Matthews DA, Garcia-Dorival I, Taylor I, Kenny J, Hertz-Fowler C, Hall N, Corbin-Lickfett K, Empig C, Schlunegger K, Barr JN, Carroll MW, Hewson R, Hiscox JA. Elucidating variations in the nucleotide sequence of Ebola virus associated with increasing pathogenicity. Genome Biol 2014;15:540.

BACKGROUND: Ebolaviruses causes a severe and often fatal hemorrhagic fever in humans, with some species such as Ebola virus having case fatality rates approaching 90%. Currently the worst Ebola virus outbreak since the disease was discovered is occurring in West Africa. Although thought to be a zoonotic infection, a concern is that with increasing numbers of humans being infected, Ebola virus variants could be selected which are better adapted for human-to-human transmission. RESULTS: To investigate whether genetic changes in Ebola virus become established in response to adaptation in a different host, a guinea pig model of infection was used. In this experimental system, guinea pigs were infected with Ebola virus (EBOV), which initially did not cause disease. To simulate transmission to uninfected individuals, the virus was serially passaged five times in naive animals. As the virus was passaged, virulence increased and clinical effects were observed in the guinea pig. An RNAseq and consensus mapping approach was then used to evaluate potential nucleotide changes in the Ebola virus genome at each passage. CONCLUSIONS: Upon passage in the guinea pig model, EBOV become more virulent, RNA editing and also coding changes in key proteins become established. The data suggest that the initial evolutionary trajectory of EBOV in a new host can lead to a gain in virulence. Given the circumstances of the sustained transmission of EBOV in the current outbreak in West Africa, increases in virulence may be associated with prolonged and uncontrolled epidemics of EBOV.

3.9 Hoenen T, Safronetz D, Groseth A, Wollenberg KR, Koita OA, Diarra B, Fall IS, Haidara FC, Diallo F, Sanogo M, Sarro YS, Kone A, Togo AC, Traore A, Kodio M, Dosseh A, Rosenke K, de Wit E, Feldmann F, Ebihara H, Munster VJ, Zoon KC, Feldmann H, Sow S. Mutation rate and genotype variation of Ebola virus from Mali case sequences. Science 2015;348:117-9.

The occurrence of Ebola virus (EBOV) in West Africa during 2013-2015 is unprecedented. Early reports suggested that in this outbreak EBOV is mutating twice as fast as previously observed, which indicates the potential for changes in transmissibility and virulence and could render current molecular diagnostics and countermeasures ineffective. We have determined additional full-length sequences from two clusters of imported EBOV infections into Mali, and we show that the nucleotide substitution rate (9.6 × 10(-4) substitutions per site per year) is consistent with rates observed in Central African outbreaks. In addition, overall variation among all genotypes observed remains low. Thus, our data indicate that EBOV is not undergoing rapid evolution in humans during the current outbreak. This finding has important implications for outbreak response and public health decisions and should alleviate several previously raised concerns.

3.10 D'arc M, Ayouba A, Esteban A, Learn GH, Boué V, Liegeois F, Etienne L, Tagg N, Leendertz FH, Boesch C, Madinda NF, Robbins MM, Gray M, Cournil A, Ooms M, Letko M, Simon VA, Sharp PM, Hahn BH, Delaporte E, Mpoudi Ngole E, Peeters M. Origin of the HIV-1 group O epidemic in western lowland gorillas. Proc Natl Acad Sci U S A 2015;112:E1343-52.

HIV-1, the cause of AIDS, is composed of four phylogenetic lineages, groups M, N, O, and P, each of which resulted from an independent cross-species transmission event of simian immunodeficiency viruses (SIVs) infecting African apes. Although groups M and N have been traced to geographically distinct chimpanzee communities in southern Cameroon, the reservoirs of groups O and P remain unknown. Here, we screened fecal samples from western lowland (n = 2,611), eastern lowland (n = 103), and mountain (n = 218) gorillas for gorilla SIV (SIVgor) antibodies and nucleic acids. Despite testing wild troops throughout southern Cameroon (n = 14), northern Gabon (n = 16), the Democratic Republic of Congo (n = 2), and Uganda (n = 1), SIVgor was identified at only four sites in southern Cameroon, with prevalences ranging from 0.8-22%. Amplification of partial and full-length SIVgor sequences revealed extensive genetic diversity, but all SIVgor strains were derived from a single lineage within the chimpanzee SIV (SIVcpz) radiation. Two fully sequenced gorilla viruses from southwestern Cameroon were very closely related to, and likely represent the source population of, HIV-1 group P. Most of the genome of a third SIVgor strain, from central Cameroon, was very closely related to HIV-1 group O, again pointing to gorillas as the immediate source. Functional analyses identified the cytidine deaminase APOBEC3G as a barrier for chimpanzee-to-gorilla, but not gorilla-to-human, virus transmission. These data indicate that HIV-1 group O, which spreads epidemically in west central Africa and is estimated to have infected around 100,000 people, originated by cross-species transmission from western lowland gorillas.

3.11 Faria NR, Rambaut A, Suchard MA, Baele G, Bedford T, Ward MJ, Tatem AJ, Sousa JD, Arinaminpathy N, Pépin J, Posada D, Peeters M, Pybus OG, Lemey P. The early spread and epidemic ignition of HIV-1 in human populations. Science 2014;346:56-61.

Thirty years after the discovery of HIV-1, the early transmission, dissemination, and establishment of the virus in human populations remain unclear. Using statistical approaches applied to HIV-1 sequence data from central Africa, we show that from the 1920s Kinshasa (in what is now the Democratic Republic of Congo) was the focus of early transmission and the source of pre-1960 pandemic viruses elsewhere. Location and dating estimates were validated using the earliest HIV-1 archival sample, also from Kinshasa. The epidemic histories of HIV-1 group M and nonpandemic group O were similar until ~1960, after which group M underwent an epidemiological transition and outpaced regional population growth. Our results reconstruct the early dynamics of HIV-1 and emphasize the role of social changes and transport networks in the establishment of this virus in human populations.

3.12 Wertheim JO, Leigh Brown AJ, Hepler NL, Mehta SR, Richman DD, Smith DM, Kosakovsky Pond SL. The global transmission network of HIV-1. J Infect Dis 2014;209:304-13.

Human immunodeficiency virus type 1 (HIV-1) is pandemic, but its contemporary global transmission network has not been characterized. A better understanding of the properties and dynamics of this network is essential for surveillance, prevention, and eventual eradication of HIV. Here, we apply a simple and computationally efficient network-based approach to all publicly available HIV polymerase sequences in the global database, revealing a contemporary picture of the spread of HIV-1 within and between countries. This approach automatically recovered well-characterized transmission clusters and extended other clusters thought to be contained within a single country across international borders. In addition, previously undescribed transmission clusters were discovered. Together, these clusters represent all known modes of HIV transmission. The extent of international linkage revealed by our comprehensive approach demonstrates the need to consider the global diversity of HIV, even when describing local epidemics. Finally, the speed of this method allows for near-real-time surveillance of the pandemic's progression.

3.13 Centers for Disease Control and Prevention and Association of Public Health Laboratories. Laboratory testing for the Diagnosis of HIV Infection: Updated Recommendat. Available at http://stacks.cdc.gov/view/cdc/23447. Publ. Jun 27, 2014.

3.14 Gökengin D, Geretti AM, Begovac J, Palfreeman J, Stevanovic M, Tarasenko O, Radcliffe K. 2014 European Guideline on HIV testing. Int J STD AIDS 2014;25:695-704.

Testing for HIV is one of the cornerstones in the fight against HIV spread. The 2014 European Guideline on HIV Testing provides advice on testing for HIV infection in individuals aged 16 years and older who present to sexually transmitted infection, genito-urinary or dermato-venereology clinics across Europe. It may also be applied in other clinical settings where HIV testing is required, particularly in primary care settings. The aim of the guideline is to provide practical guidance to clinicians and laboratories that within these settings undertake HIV testing, and to indicate standards for best practice.

3.15 Ritchie AV, Ushiro-Lumb I, Edemaga D, Joshi HA, De Ruiter A, Szumilin E, Jendrulek I, McGuire M, Goel N, Sharma PI, Allain JP, Lee HH. SAMBA HIV semiquantitative test, a new point-of-care viral-load-monitoring assay for resource-limited settings. J Clin Microbiol 2014;52:3377–83.

Routine viral-load (VL) testing of HIV-infected individuals on antiretroviral therapy (ART) is used to monitor treatment efficacy. However, due to logistical challenges, implementation of VL has been difficult in resource-limited settings. The aim of this study was to evaluate the performance of the SAMBA semi-Q (simple amplification-based assay semiquantitative test for HIV-1) in London, Malawi, and Uganda. The SAMBA semi-Q can distinguish between patients with VLs above and below 1,000 copies/ml. The SAMBA semi-Q was validated with diluted clinical samples and blinded plasma samples collected from HIV-1-positive individuals. SAMBA semi-Q results were compared with results from the Roche COBAS AmpliPrep/COBAS TaqMan HIV-1 test, v2.0. Testing of 96 2- to 10-fold dilutions of four samples containing HIV-1 subtype C as well as 488 samples from patients in the United Kingdom, Malawi, and Uganda yielded an overall accuracy for the SAMBA semi-Q of 99% (95% confidence interval [CI], 93.8 to 99.9%) and 96.9% (95% CI 94.9 to 98.3%), respectively, compared to to the Roche test. Analysis of VL data from patients in Malawi and Uganda showed that the SAMBA cutoff of 1,000 copies/ml appropriately distinguished treated from untreated individuals. Furthermore, analysis of the viral loads of 232 patients on ART in Malawi and Uganda revealed similar patterns for virological control, defined as either <1,000 copies/ml (SAMBA cutoff) or <5,000 copies/ml (WHO 2010 criterion; WHO, Antiretroviral Therapy for HIV Infection in Adults and Adolescents: Recommendations for a Public Health Approach, 2010). This study suggests that the SAMBA semi-Q has adequate concurrency with the gold standard measurements for viral load. This test can allow VL monitoring of patients on ART at the point of care in resource-limited settings.

3.16 Singleton J, Osborn JL, Lillis L, Hawkins K, Guelig D, Price W, Johns R, Ebels K, Boyle D, Weigl B, LaBarre P. Electricity-free amplification and detection for molecular point-of-care diagnosis of HIV-1. PLoS One 2014;9:e113693.

In resource-limited settings, the lack of decentralized molecular diagnostic testing and sparse access to centralized medical facilities can present a critical barrier to timely diagnosis, treatment, and subsequent control and elimination of infectious diseases. Isothermal nucleic acid amplification methods, including reverse transcription loop-mediated isothermal amplification (RT-LAMP), are well-suited for decentralized point-of-care molecular testing in minimal infrastructure laboratories since they significantly reduce the complexity of equipment and power requirements. Despite reduced complexity, however, there is still a need for a constant heat source to enable isothermal nucleic acid amplification. This requirement poses significant challenges for laboratories in developing countries where electricity is often unreliable or unavailable. To address this need, we previously developed a low-cost, electricity-free heater using an exothermic reaction thermally coupled with a phase change material. This heater achieved acceptable performance, but exhibited considerable variability. Furthermore, as an enabling technology, the heater was an incomplete diagnostic solution. Here we describe a more precise, affordable, and robust heater design with thermal standard deviation <0.5°C at operating temperature, a cost of approximately US$.06 per test for heater reaction materials, and an ambient temperature operating range from 16°C to 30°C. We also pair the heater with nucleic acid lateral flow (NALF)-detection for a visual readout. To further illustrate the utility of the electricity-free heater and NALF-detection platform, we demonstrate sensitive and repeatable detection of HIV-1 with a ß-actin positive internal amplification control from processed sample to result in less than 80 minutes. Together, these elements are building blocks for an electricity-free platform capable of isothermal amplification and detection of a variety of pathogens.

3.17 Pou C, Noguera-Julian M, Pérez-Álvarez S, García F, Delgado R, Dalmau D, Álvarez-Tejado M, Gonzalez D, Sayada C, Chueca N, Pulido F, Ibáñez L, Rodríguez C, Casadellà M, Santos JR, Ruiz L, Clotet B, Paredes R. Improved prediction of salvage antiretroviral therapy outcomes using ultrasensitive HIV-1 drug resistance testing. Clin Infect Dis 2014;59:578-88.

BACKGROUND: The clinical relevance of ultrasensitive human immunodeficiency virus type 1 (HIV-1) genotypic resistance testing in antiretroviral treatment (ART)-experienced individuals remains unknown. METHODS: This was a retrospective, multicentre, cohort study in ART-experienced, HIV-1-infected adults who initiated salvage ART including, at least 1 ritonavir-boosted protease inhibitor, raltegravir or etravirine. Presalvage ART Sanger and 454 sequencing of plasma HIV-1 were used to generate separate genotypic sensitivity scores (GSS) using the HIVdb, ANRS, and REGA algorithms. Virological failure (VF) was defined as 2 consecutive HIV-1 RNA levels ≥200 copies/mL at least 12 weeks after salvage ART initiation, whereas subjects remained on the same ART. The ability of Sanger and 454-GSS to predict VF was assessed by receiver operating characteristic (ROC) curves and survival analyses. RESULTS: The study included 132 evaluable subjects; 28 (21%) developed VF. Using HIVdb, 454 predicted VF better than Sanger sequencing in the ROC curve analysis (area under the curve: 0.69 vs 0.60, Delong test P = .029). Time to VF was shorter for subjects with 454-GSS < 3 vs 454-GSS ≥ 3 (Log-rank P = .003) but not significantly different between Sanger-GSS < 3 and ≥3. Factors independently associated with increased risk of VF in multivariate Cox regression were a 454-GSS < 3 (HR = 4.6, 95 CI, [1.5, 14.0], P = .007), and the number of previous antiretrovirals received (HR = 1.2 per additional drug, 95 CI, [1.1, 1.3], P = .001). Equivalent findings were obtained with the ANRS and REGA algorithms. CONCLUSIONS: Ultrasensitive of ART-experienced subjects living with HIV-1.

3.18 Maldarelli F, Wu X, Su L, Simonetti FR, Shao W, Hill S, Spindler J, Ferris AL, Mellors JW, Kearney MF, Coffin JM, Hughes SH. Specific HIV integration sites are linked to clonal expansion and persistence of infected cells. Science 2014;345:179-83.

The persistence of HIV-infected cells in individuals on suppressive combination antiretroviral therapy (cART) presents a major barrier for curing HIV infections. HIV integrates its DNA into many sites in the host genome; we identified 2410 integration sites in peripheral blood lymphocytes of five infected individuals on cART. About 40% of the integrations were in clonally expanded cells. Approximately 50%

of the infected cells in one patient were from a single clone, and some clones persisted for many years. There were multiple independent integrations in several genes, including MKL2 and BACH2; many of these integrations were in clonally expanded cells. Our findings show that HIV integration sites can play a critical role in expansion and persistence of HIV-infected cells.

3.19 Marini B, Kertesz-Farkas A, Ali H, Lucic B, Lisek K, Manganaro L, Pongor S, Luzzati R, Recchia A, Mavilio F, Giacca M, Lusic M. Nuclear architecture dictates HIV-1 integration site selection. Nature 2015;doi10.1038/nature14226.

Long-standing evidence indicates that human immunodeficiency virus type 1 (HIV-1) preferentially integrates into a subset of transcriptionally active genes of the host cell genome. However, the reason why the virus selects only certain genes among all transcriptionally active regions in a target cell remains largely unknown. Here we show that HIV-1 integration occurs in the outer shell of the nucleus in close correspondence with the nuclear pore. This region contains a series of cellular genes, which are preferentially targeted by the virus, and characterized by the presence of active transcription chromatin marks before viral infection. In contrast, the virus strongly disfavours the heterochromatic regions in the nuclear lamin-associated domains and other transcriptionally active regions located centrally in the nucleus. Functional viral integrase and the presence of the cellular Nup153 and LEDGF/p75 integration cofactors are indispensable for the peripheral integration of the virus. Once integrated at the nuclear pore, the HIV-1 DNA makes contact with various nucleoporins; this association takes part in the transcriptional regulation of the viral genome. These results indicate that nuclear topography is an essential determinant of the HIV-1 life cycle.

3.20 Whitney JB, Hill AL, Sanisetty S, Penaloza-MacMaster P, Liu J, Shetty M, Parenteau L, Cabral C, Shields J, Blackmore S, Smith JY, Brinkman AL, Peter LE, Mathew SI, Smith KM, Borducchi EN, Rosenbloom DI, Lewis MG, Hattersley J, Li B, Hesselgesser J, Geleziunas R, Robb ML, Kim JH, Michael NL, Barouch DH. Rapid seeding of the viral reservoir prior to SIV viraemia in rhesus monkeys. Nature 2014;512:74-7.

The viral reservoir represents a critical challenge for human immunodeficiency virus type 1 (HIV-1) eradication strategies. However, it remains unclear when and where the viral reservoir is seeded during acute infection and the extent to which it is susceptible to early antiretroviral therapy (ART). Here we show that the viral reservoir is seeded rapidly after mucosal simian immunodeficiency virus (SIV) infection of rhesus monkeys and before systemic viraemia. We initiated suppressive ART in groups of monkeys on days 3, 7, 10 and 14 after intrarectal SIVMAC251 infection. Treatment with ART on day 3 blocked the emergence of viral RNA and proviral DNA in peripheral blood and also substantially reduced levels of proviral DNA in lymph nodes and gastrointestinal mucosa as compared with treatment at later time points. In addition, treatment on day 3 abrogated the induction of SIV-specific humoral and cellular immune responses. Nevertheless, after discontinuation of ART following 24 weeks of fully suppressive therapy, virus rebounded in all animals, although the monkeys that were treated on day 3 exhibited a delayed viral rebound as compared with those treated on days 7, 10 and 14. The time to viral rebound correlated with total viraemia during acute infection and with proviral DNA at the time of ART discontinuation. These data demonstrate that the viral reservoir is seeded rapidly after intrarectal SIV infection of rhesus monkeys, during the 'eclipse' phase, and before detectable viraemia. This strikingly early seeding of the refractory viral reservoir raises important new challenges for HIV-1 eradication strategies.

3.21 Yang D, Zuo C, Wang X, Meng X, Xue B, Liu N, Yu R, Qin Y, Gao Y, Wang Q, Hu J, Wang L, Zhou Z, Liu B, Tan D, Guan Y, Zhu H. Complete replication of hepatitis B virus and hepatitis C virus in a newly developed hepatoma cell line. Proc Natl Acad Sci U S A 2014;111:E1264-73.

The absence of a robust cell culture system for hepatitis B virus (HBV) and hepatitis C virus (HCV) infection has limited the analysis of the virus lifecycle and drug discovery. We have established a hepatoma cell line, HLCZ01, the first cell line, to the authors' knowledge, supporting the entire lifecycle of

both HBV and HCV. HBV surface antigen (HBsAg)-positive particles can be observed in the supernatant and the lumen of the endoplasmic reticulum of the cells via electron microscopy. Interestingly, HBV and HCV clinical isolates propagate in HLCZ01 cells. Both viruses replicate in the cells without evidence of overt interference. HBV and HCV entry are blocked by antibodies against HBsAg and human CD81, respectively, and the replication of HBV and HCV is inhibited by antivirals. HLCZ01 cells mount an innate immune response to virus infection. The cell line provides a powerful tool for exploring the mechanisms of virus entry and replication and the interaction between host and virus, facilitating the development of novel antiviral agents and vaccines.

3.22 Smith DB, Bukh J, Kuiken C, Muerhoff AS, Rice CM, Stapleton JT, Simmonds P. Expanded classification of hepatitis C virus into 7 genotypes and 67 subtypes: updated criteria and genotype assignment web resource. Hepatology 2014;59:318-27.

The 2005 consensus proposal for the classification of hepatitis C virus (HCV) presented an agreed and uniform nomenclature for HCV variants and the criteria for their assignment into genotypes and subtypes. Since its publication, the available dataset of HCV sequences has vastly expanded through advancement in nucleotide sequencing technologies and an increasing focus on the role of HCV genetic variation in disease and treatment outcomes. The current study represents a major update to the previous consensus HCV classification, incorporating additional sequence information derived from over 1,300 (near-) complete genome sequences of HCV available on public databases in May 2013. Analysis resolved several nomenclature conflicts between genotype designations and using consensus criteria created a classification of HCV into seven confirmed genotypes and 67 subtypes. There are 21 additional complete coding region sequences of unassigned subtype. The study additionally describes the development of a Web resource hosted by the International Committee for Taxonomy of Viruses (ICTV) that maintains and regularly updates tables of reference isolates, accession numbers, and annotated alignments (http://talk.ictvonline.org/links/hcv/hcv-classification.htm). The Flaviviridae Study Group urges those who need to check or propose new genotypes or subtypes of HCV to contact the Study Group in advance of publication to avoid nomenclature conflicts appearing in the literature. While the criteria for assigning genotypes and subtypes remain unchanged from previous consensus proposals, changes are proposed in the assignment of provisional subtypes, subtype numbering beyond "w," and the nomenclature of intergenotypic recombinant. CONCLUSION: This study represents an important reference point for the consensus classification of HCV variants that will be of value to researchers working in clinical and basic science fields.

3.23 Murphy DG, Sablon E, Chamberland J, Fournier E, Dandavino R, Tremblay CL. Hepatitis C virus genotype 7, a new genotype originating from central Africa. J Clin Microbiol 2015;53:967-72.

We report a new hepatitis C virus (HCV) genotype identified in patients originating from the Democratic Republic of Congo. The prototype QC69 virus is shown to be a new lineage distinct from genotypes 1 to 6. Three additional patients were also found to be infected by a virus from this lineage, confirming its circulation in humans. We propose that these viruses be classified into HCV genotype 7.

3.24 Guelfo JR, Macias J, Neukam K, Di Lello FA, Mira JA, Merchante N, Mancebo M, Nuñez-Torres R, Pineda JA, Real LM. Reassessment of genotype 1 hepatitis C virus subtype misclassification by LiPA 2.0: implications for direct-acting antiviral treatment. J Clin Microbiol 2014;52:4027-9.

The accuracy of LiPA 2.0 for hepatitis C virus 1 (HCV-1) subtype classification was analyzed. LiPA 2.0 genotype results from 101 HCV-1-infected patients were compared to genotype findings determined by direct core sequencing. Eleven (11%) samples were misclassified. Given the influence of the HCV-1-subtype in the anti-HCV therapy response, an alternative classification method is warranted.

3.25 Svarovskaia ES, Dvory-Sobol H, Parkin N, Hebner C, Gontcharova V, Martin R, Ouyang W, Han B, Xu S, Ku K, Chiu S, Gane E, Jacobson IM, Nelson DR, Lawitz E, Wyles DL, Bekele N, Brainard D, Symonds WT, McHutchison JG, Miller MD, Mo H. Infrequent development of resistance in genotype 1-6 hepatitis C virus-infected subjects treated with sofosbuvir in phase 2 and 3 clinical trials. Clin Infect Dis 2014;59:1666-74.

BACKGROUND: Sofosbuvir is a chain-terminating nucleotide analogue inhibitor of the hepatitis C virus (HCV) NS5B RNA polymerase that is efficacious in subjects with HCV genotype 1-6 infection. Sofosbuvir resistance is primarily conferred by the S282T substitution in NS5B. METHODS: NS5B sequencing and susceptibility testing of HCV from subjects infected with genotypes 1-6 who participated in phase 2 and 3 sofosbuvir clinical trials was performed. RESULTS: No NS5B variants present at baseline among 1645 sofosbuvir-treated subjects were associated with treatment failure; sofosbuvir susceptibility was within 2-fold of reference. Among 282 subjects who did not achieve sustained virologic response, no novel sofosbuvir resistance-associated variants were identified, and the NS5B changes observed did not confer significant reductions in sofosbuvir susceptibility. In 1 subject with S282T observed at relapse 4 weeks after sofosbuvir monotherapy, the resistant variant (13.5-fold reduced sofosbuvir susceptibility, replication capacity <2% of control) became undetectable by deep sequencing 12 weeks after treatment. L159F and V321A were identified as treatment-emergent variants but did not confer resistance to sofosbuvir in the replicon system. CONCLUSIONS: These data demonstrate a uniform susceptibility of subject-derived HCV to sofosbuvir, and also show that selection of sofosbuvir-resistant HCV is exceedingly rare and is associated with a significant reduction in viral fitness.

3.26 Hewitt PE, Ijaz S, Brailsford SR, Brett R, Dicks S, Haywood B, Kennedy IT, Kitchen A, Patel P, Poh J, Russell K, Tettmar KI, Tossell J, Ushiro-Lumb I, Tedder RS. Hepatitis E virus in blood components: a prevalence and transmission study in southeast England. Lancet 2014;384:1766-73.

BACKGROUND: The prevalence of hepatitis E virus (HEV) genotype 3 infections in the English population (including blood donors) is unknown, but is probably widespread, and the virus has been detected in pooled plasma products. HEV-infected donors have been retrospectively identified through investigation of reported cases of possible transfusion-transmitted hepatitis E. The frequency of HEV transmission by transfusion and its outcome remains unknown. We report the prevalence of HEV RNA in blood donations, the transmission of the virus through a range of blood components, and describe the resulting morbidity in the recipients. METHODS: From Oct 8, 2012, to Sept 30, 2013, 225,000 blood donations that were collected in southeast England were screened retrospectively for HEV RNA. Donations containing HEV were characterised by use of serology and genomic phylogeny. Recipients, who received any blood components from these donations, were identified and the outcome of exposure was ascertained. FINDINGS: 79 donors were viraemic with genotype 3 HEV, giving an RNA prevalence of one in 2848. Most viraemic donors were seronegative at the time of donation. The 79 donations had been used to prepare 129 blood components, 62 of which had been transfused before identification of the infected donation. Follow-up of 43 recipients showed 18 (42%) had evidence of infection. Absence of detectable antibody and high viral load in the donation rendered infection more likely. Recipient immunosuppression delayed or prevented seroconversion and extended the duration of viraemia. Three recipients cleared longstanding infection after intervention with ribavirin or alteration in immunosuppressive therapy. Ten recipients developed prolonged or persistent infection. Transaminitis was common, but short-term morbidity was rare; only one recipient developed apparent but clinically mild post-transfusion hepatitis. INTERPRETATION: Our findings suggest that HEV genotype 3 infections are widespread in the English population and in blood donors. Transfusion-transmitted infections rarely caused acute morbidity, but in some immunosuppressed patients became persistent. Although at present blood donations are not screened, an agreed policy is needed for the identification of patients with persistent HEV infection, irrespective of origin, so that they can be offered antiviral therapy.

3.27 Wen GP, Tang ZM, Yang F, Zhang K, Ji WF, Cai W, Huang SJ, Wu T, Zhang J, Zheng ZZ, Xia NS. A valuable antigen detection method for diagnosis of acute hepatitis E. J Clin Microbiol 2015;53:782-8.

Hepatitis E virus (HEV) is a serious public health problem. The commonly used tests that are specific for current HEV infection diagnosis include the detection of anti-HEV IgM and HEV RNA. Here, we report an improved enzyme-linked immunosorbent assay (ELISA) method for HEV antigen detection with a linear range equivalent to 6.3 × 10(3) to 9.2 × 10(5) RNA copies per ml. The monoclonal antibody (MAb) 12F12, a high-ability MAb that binds HEV virus, was selected as the capture antibody from a panel of 95 MAbs. The positive period of HEV antigenemia in infected monkeys using this test was, on average, 3 weeks longer than previously reported and covered the majority of the acute phase. The positive detection rates of IgM, RNA, and new antigen from the first serum samples collected from 16 confirmed acute hepatitis E patients were 81% (13/16), 81% (13/16), and 100% (16/16), respectively. In three patients, the initial serum specimens that tested negative for IgM, despite the presence of symptoms of acute hepatitis and elevated alanine aminotransferase (ALT) levels, were positive for HEV antigen and HEV RNA. In contrast, the serum samples of the three RNA-negative patients were antigen positive (and IgM positive), possibly due to the degradation of HEV nucleic acids. Our results suggest that this new antigen detection method has acceptable concordance with RNA detection and could serve as an important tool for diagnosing acute hepatitis E.

3.28 Abravanel F, Lhomme S, Rostaing L, Kamar N, Izopet J. Protracted fecal shedding of HEV during ribavirin therapy predicts treatment relapse. Clin Infect Dis 2015;60:96-9.

Twenty-four solid-organ-transplant recipients with chronic hepatitis E virus (HEV) infections were given ribavirin therapy for 3 months. All the patients with protracted fecal HEV shedding during treatment suffered a relapse. Monitoring HEV fecal excretion could be used to determine the optimal duration of ribavirin therapy.

3.29 Ronco G, Dillner J, Elfström KM, Tunesi S, Snijders PJ, Arbyn M, Kitchener H, Segnan N, Gilham C, Giorgi-Rossi P, Berkhof J, Peto J, Meijer CJ; International HPV screening working group. Efficacy of HPV-based screening for prevention of invasive cervical cancer: follow-up of four European randomised controlled trials. Lancet 2014;383:524-32.

BACKGROUND: In four randomised trials, human papillomavirus (HPV)-based screening for cervical cancer was compared with cytology-based cervical screening, and precursors of cancer were the endpoint in every trial. However, direct estimates are missing of the relative efficacy of HPV-based versus cytology-based screening for prevention of invasive cancer in women who undergo regular screening, of modifiers (eg, age) of this relative efficacy, and of the duration of protection. We did a follow-up study of the four randomised trials to investigate these outcomes. METHODS: 176,464 women aged 20-64 years were randomly assigned to HPV-based (experimental arm) or cytology-based (control arm) screening in Sweden (Swedescreen), the Netherlands (POBASCAM), England (ARTISTIC), and Italy (NTCC). We followed up these women for a median of 6•5 years (1,214,415 person-years) and identified 107 invasive cervical carcinomas by linkage with screening, pathology, and cancer registries, by masked review of histological specimens, or from reports. Cumulative and study-adjusted rate ratios (experimental vs control) were calculated for incidence of invasive cervical carcinoma. FINDINGS: The rate ratio for invasive cervical carcinoma among all women from recruitment to end of follow-up was 0•60 (95% CI 0•40-0•89), with no heterogeneity between studies (p=0•52). Detection of invasive cervical carcinoma was similar between screening methods during the first 2•5 years of follow-up (0•79, 0•46-1•36) but was significantly lower in the experimental arm thereafter (0•45, 0•25-0•81). In women with a negative screening test at entry, the rate ratio was 0•30 (0•15-0•60). The cumulative incidence of invasive cervical carcinoma in women with negative entry tests was 4•6 per 10(5) (1•1-12•1) and 8•7 per 10(5) (3•3-18•6) at 3•5 and 5•5 years, respectively, in the experimental arm, and 15•4 per 10(5) (7•9-27•0) and 36•0 per 10(5) (23•2-53•5), respectively, in the control arm. Rate ratios did not differ by cancer stage, but were lower for adenocarcinoma (0•31, 0•14-0•69) than for squamous-cell carcinoma (0•78, 0•49-1•25). The rate ratio was lowest in women aged 30-34 years (0•36, 0•14-0•94). INTERPRETATION: HPV-based screening provides 60-70% greater protection against invasive cervical carcinomas compared with cytology. Data of large-scale randomised trials support initiation of HPV-based screening from age 30 years and extension of screening intervals to at least 5 years.

3.30 Gage JC, Schiffman M, Katki HA, Castle PE, Fetterman B, Wentzensen N, Poitras NE, Lorey T, Cheung LC, Kinney WK. Reassurance against future risk of precancer and cancer conferred by a negative human papillomavirus test. J Natl Cancer Inst 2014;106:dju153.

Primary human papillomavirus (HPV) testing (without concurrent Pap tests) every 3 years is under consideration in the United States as an alternative to the two recommended cervical cancer screening strategies: primary Pap testing every 3 years, or concurrent Pap and HPV testing ("cotesting") every 5 years. Using logistic regression and Weibull survival models, we estimated and compared risks of cancer and cervical intraepithelial neoplasia grade 3 or worse (CIN3+) for the three strategies among 1011092 women aged 30 to 64 years testing HPV-negative and/or Pap-negative in routine screening at Kaiser Permanente Northern California since 2003. All statistical tests were two sided. Three-year risks following an HPV-negative result were lower than 3-year risks following a Pap-negative result (CIN3+ = 0.069% vs 0.19%, P < .0001; Cancer = 0.011% vs 0.020%, P < .0001) and 5-year risks following an HPV-negative/Pap-negative cotest (CIN3+ = 0.069% vs 0.11%, P < .0001; Cancer = 0.011% vs 0.014%, P = .21). These findings suggest that primary HPV testing merits consideration as another alternative for cervical screening.

3.31 Huh WK, Ault KA, Chelmow D, Davey DD, Goulart RA, Garcia FA, Kinney WK, Massad LS, Mayeaux EJ, Saslow D, Schiffman M, Wentzensen N, Lawson HW, Einstein MH. Use of primary high-risk human papillomavirus testing for cervical cancer screening: interim clinical guidance. Gynecol Oncol 2015;136:178-82.

In 2011, the American Cancer Society, the American Society for Colposcopy and Cervical Pathology, and the American Society for Clinical Pathology updated screening guidelines for the early detection of cervical cancer and its precursors. Recommended screening strategies were cytology and cotesting (cytology in combination with hrHPV testing). These guidelines also addressed the use of hrHPV testing alone as a primary screening approach, which was not recommended for use at that time. There is now a growing body of evidence for screening with primary hrHPV testing, including a prospective US-based registration study. Thirteen experts including representatives from the Society of Gynecologic Oncology, American Society for Colposcopy and Cervical Pathology, American College of Obstetricians and Gynecologists, American Cancer Society, American Society of Cytopathology, College of American Pathologists, and the American Society for Clinical Pathology, convened to provide interim guidance for primary hrHPV screening. This guidance panel was specifically triggered by an application to the FDA for a currently marketed HPV test to be labeled for the additional indication of primary cervical cancer screening. Guidance was based on literature review and review of data from the FDA registration study, supplemented by expert opinion. This document aims to provide information for healthcare providers who are interested in primary hrHPV testing and an overview of the potential advantages and disadvantages of this strategy for screening as well as to highlight areas in need of further investigation.

3.32 Hu Z, Zhu D, Wang W, Li W, Jia W, Zeng X, Ding W, Yu L, Wang X, Wang L, Shen H, Zhang C, Liu H, Liu X, Zhao Y, Fang X, Li S, Chen W, Tang T, Fu A, Wang Z, Chen G, Gao Q, Li S, Xi L, Wang C, Liao S, Ma X, Wu P, Li K, Wang S, Zhou J, Wang J, Xu X, Wang H, Ma D. Genome-wide profiling of HPV integration in cervical cancer identifies clustered genomic hot spots and a potential microhomology-mediated integration mechanism. Nat Genet 2015;47:158-63.

Human papillomavirus (HPV) integration is a key genetic event in cervical carcinogenesis. By conducting whole-genome sequencing and high-throughput viral integration detection, we identified 3,667 HPV integration breakpoints in 26 cervical intraepithelial neoplasias, 104 cervical carcinomas and five cell lines. Beyond recalculating frequencies for the previously reported frequent integration sites POU5F1B (9.7%), FHIT (8.7%), KLF12 (7.8%), KLF5 (6.8%), LRP1B (5.8%) and LEPREL1 (4.9%), we discovered new hot spots HMGA2 (7.8%), DLG2 (4.9%) and SEMA3D (4.9%). Protein expression from FHIT and LRP1B was downregulated when HPV integrated in their introns. Protein expression from MYC and HMGA2 was elevated when HPV integrated into flanking regions. Moreover, microhomologous sequence between the human and HPV genomes was significantly enriched near integration breakpoints, indicating that fusion

between viral and human DNA may have occurred by microhomology-mediated DNA repair pathways. Our data provide insights into HPV integration-driven cervical carcinogenesis.

3.33 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. A novel host-proteome signature for distinguishing between acute bacterial and viral infections. PLoS One 2015;10:e0120012.

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.

3.34 Wilen CB, Monaco CL, Hoppe-Bauer J, Jackups R Jr, Bucelli RC, Burnham CA. Criteria for reducing unnecessary testing for herpes simplex virus, varicella-zoster virus, cytomegalovirus, and enterovirus in cerebrospinal fluid samples from adults. J Clin Microbiol 2015;53:887-95.

Excessive utilization of laboratory diagnostic testing leads to increased health care costs. We evaluated criteria to reduce unnecessary nucleic acid amplification testing (NAAT) for viral pathogens in cerebrospinal fluid (CSF) samples from adults. This is a single-center split retrospective observational study with a screening cohort from 2008 to 2012 and a validation cohort from 2013. Adults with available results for herpes simplex virus 1/2 (HSV-1/2), varicella-zoster virus (VZV), cytomegalovirus (CMV), or enterovirus (EV) NAAT with CSF samples between 2008 and 2013 were included (n = 10,917). During this study, 1.3% (n = 140) of viral NAAT studies yielded positive results. The acceptance criteria of >10 nucleated cells/μl in the CSF of immunocompetent subjects would have reduced HSV-1/2, VZV, CMV, and EV testing by 63%, 50%, 44%, and 51%, respectively, from 2008 to 2012. When these criteria were applied to the 2013 validation data set, 54% of HSV-1/2, 57% of VZV, 35% of CMV, and 56% of EV tests would have been cancelled. No clinically significant positive tests would have been cancelled in 2013 with this approach. The introduction of a computerized order entry set was associated with increased test requests, suggesting that computerized order sets may contribute to unnecessary testing. Acceptance criteria of >10 nucleated cells/μl in the CSF of immunocompetent adults for viral CSF NAAT assays would increase clinical specificity and preserve sensitivity, resulting in significant cost savings. Implementation of these acceptance criteria led to a 46% reduction in testing during a limited follow-up period.

3.35 Ladner JT, Beitzel B, Chain PS, Davenport MG, Donaldson EF, Frieman M, Kugelman JR, Kuhn JH, O'Rear J, Sabeti PC, Wentworth DE, Wiley MR, Yu GY; Threat Characterization Consortium, Sozhamannan S, Bradburne C, Palacios G. Standards for sequencing viral genomes in the era of high-throughput sequencing. mBIO 2014;5:e01360-14.

Thanks to high-throughput sequencing technologies, genome sequencing has become a common component in nearly all aspects of viral research; thus, we are experiencing an explosion in both the number of available genome sequences and the number of institutions producing such data. However, there are currently no common standards used to convey the quality, and therefore utility, of these various genome sequences. Here, we propose five "standard" categories that encompass all stages of viral genome finishing, and we define them using simple criteria that are agnostic to the technology used for sequencing. We also provide genome finishing recommendations for various downstream applications, keeping in mind the cost-benefit trade-offs associated with different levels of finishing. Our goal is to define a common vocabulary that will allow comparison of genome quality across different research groups, sequencing platforms, and assembly techniques.