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Literature

Year in Clinical Microbiology ECCMID 2018

Nature. 2018 Jan 18;553(7688):291-294. doi: 10.1038/nature25178. Epub 2018 Jan 3.

Dietary trehalose enhances virulence of epidemic Clostridium difficile.

Collins J[1], Robinson C[2], Danhof H[1], Knetsch CW[3], van Leeuwen HC[3], Lawley TD[4], Auchtung JM[1], Britton RA[1].

ABSTRACT: Clostridium difficile disease has recently increased to become a dominant nosocomial pathogen in North America and Europe, although little is known about what has driven this emergence. Here we show that two epidemic ribotypes (RT027 and RT078) have acquired unique mechanisms to metabolize low concentrations of the disaccharide trehalose. RT027 strains contain a single point mutation in the trehalose repressor that increases the sensitivity of this ribotype to trehalose by more than 500-fold. Furthermore, dietary trehalose increases the virulence of a RT027 strain in a mouse model of infection. RT078 strains acquired a cluster of four genes involved in trehalose metabolism, including a PTS permease that is both necessary and sufficient for growth on low concentrations of trehalose. We propose that the implementation of trehalose as a food additive into the human diet, shortly before the emergence of these two epidemic lineages, helped select for their emergence and contributed to hypervirulence.

Clin Microbiol Infect. 2018 Feb;24(2):97-104. doi: 10.1016/j.cmi.2017.06.017. Epub 2017 Jun 23.

Evaluation of vancomycin MIC creep in methicillin-resistant Staphylococcus aureus infections-a systematic review and meta-analysis.

Diaz R[1], Afreixo V[2], Ramalheira E[3], Rodrigues C[4], Gago B[5].

OBJECTIVES: Vancomycin is currently the primary option treatment for methicillin-resistant Staphylococcus aureus (MRSA). However, an increasing number of MRSA isolates with high MICs, within the susceptible range (vancomycin MIC creep), are being reported worldwide. Resorting to a meta-analysis approach, this study aims to assess the evidence of vancomycin MIC creep.

METHODS: We searched for studies in the PubMed database. The inclusion criteria for study eligibility included the possibility of retrieving the reported data values of vancomycin MIC and information concerning the applied MIC methodology.

RESULTS: The mean values of vancomycin MICs, of all 29 234 S. aureus isolates reported in the 55 studies included in the meta-analysis, were 1.23 mg/L (95% CI 1.13-1.33) and 1.20 mg/L (95% CI 1.13-1.28) determined by Etest and broth microdilution method, respectively. No significant differences were observed between these two methodologies. We found negative correlation between pooled mean/pooled proportion and time strata.

CONCLUSIONS: We have found no evidence of the MIC creep phenomenon.

Clin Microbiol Infect. 2018 Feb;24(2):118-124. doi: 10.1016/j.cmi.2017.08.025. Epub 2017 Sep 5.

To test or not to test? Laboratory support for the diagnosis of Lyme borreliosis: a position paper of ESGBOR, the ESCMID study group for Lyme borreliosis.

Dessau RB[1], van Dam AP[2], Fingerle V[3], Gray J[4], Hovius JW[5], Hunfeld KP[6], Jaulhac B[7], Kahl O[8], Kristoferitsch W[9], Lindgren PE[10], Markowicz M[11], Mavin S[12], Ornstein K[13], Rupprecht T[14], Stanek G[11], Strle F[15].

BACKGROUND: Lyme borreliosis (LB) is a tick-borne infection caused by Borrelia burgdorferi sensu lato. The most frequent clinical manifestations are erythema migrans and Lyme neuroborreliosis. Currently, a large volume of diagnostic testing for LB is reported, whereas the incidence of clinically relevant disease manifestations is low. This indicates overuse of diagnostic testing for LB with implications for patient care and cost-effective health management.

AIM: The recommendations provided in this review are intended to support both the clinical diagnosis and initiatives for a more rational use of laboratory testing in patients with clinically suspected LB.

SOURCES: This is a narrative review combining various aspects of the clinical and laboratory diagnosis with an educational purpose. The literature search was based on existing systematic reviews, national and international guidelines and supplemented with specific citations.

IMPLICATIONS: The main recommendations according to current European case definitions for LB are as follows. Typical erythema migrans should be diagnosed clinically and does not require laboratory testing. The diagnosis of Lyme neuroborreliosis requires laboratory investigation of the spinal fluid including intrathecal antibody production, and the remaining disease manifestations require testing for serum antibodies to B. burgdorferi. Testing individuals with non-specific subjective symptoms is not recommended, because of a low positive predictive value.

Clin Microbiol Rev. 2017 Nov 15;31(1). pii: e00029-17. doi: 10.1128/CMR.00029-17. Print 2018 Jan.

Candida auris: a Review of the Literature.

Jeffery-Smith A[1,2], Taori SK[3], Schelenz S[4], Jeffery K[5], Johnson EM[6], Borman A[6]; Candida auris Incident Management Team, Manuel R[6], Brown CS[1,7].

ABSTRACT: The emerging pathogen *Candida auris* has been associated with nosocomial outbreaks on five continents. Genetic analysis indicates the simultaneous emergence of separate clades of this organism in different geographical locations. Invasive infection and colonization have been detected predominantly in patients in high-dependency settings and have garnered attention due to variable antifungal resistance profiles and transmission within units instituting a range of infection prevention and control measures. Issues with the identification of *C. auris* using both phenotypic and molecular techniques have raised concerns about detecting the true scale of the problem. This review considers the literature available on *C. auris* and highlights the key unknowns, which will provide direction for further work in this field.

Emerg Infect Dis. 2018 Apr;24(4). doi: 10.3201/eid2404.171873.

Cooperative Recognition of Internationally Disseminated Ceftriaxone-Resistant Neisseria gonorrhoeae Strain.

Lahra MM, Martin I, Demczuk W, Jennison AV, Lee KI, Nakayama SI, Lefebvre B, Longtin J, Ward A, Mulvey MR, Wi T, Ohnishi M, Whiley D.

ABSTRACT: Ceftriaxone remains a first-line treatment for patients infected by Neisseria gonorrhoeae in most settings. We investigated the possible spread of a ceftriaxone-resistant FC428 N. gonorrhoeae clone in Japan after recent isolation of similar strains in Denmark (GK124) and Canada (47707). We report 2 instances of the FC428 clone in Australia in heterosexual men traveling from Asia. Our bioinformatic analyses included core single-nucleotide variation phylogeny and in silico molecular typing; phylogenetic analysis showed close genetic relatedness among all 5 isolates. Results showed multilocus sequence type 1903; N. gonorrhoeae sequence typing for antimicrobial resistance (NG-STAR) 233; and harboring of mosaic penA allele encoding alterations A311V and T483S (penA-60.001), associated with ceftriaxone resistance. Our results provide further evidence of international transmission of ceftriaxone-resistant N. gonorrhoeae. We recommend increasing awareness of international spread of this drug-resistant strain, strengthening surveillance to include identifying treatment failures and contacts, and strengthening international sharing of data.

Emerg Infect Dis. 2017 May;23(5):830-832. doi: 10.3201/eid2305.170088.

Azithromycin Resistance and Decreased Ceftriaxone Susceptibility in Neisseria gonorrhoeae, Hawaii, USA.

Papp JR, Abrams AJ, Nash E, Katz AR, Kirkcaldy RD, O'Connor NP, O'Brien PS, Harauchi DH, Maningas EV, Soge OO, Kersh EN, Komeya A, Tomas JE, Wasserman GM, Kunimoto GY, Trees DL, Whelen AC.

ABSTRACT: During 2016, eight Neisseria gonorrhoeae isolates from 7 patients in Hawaii were resistant to azithromycin; 5 had decreased in vitro susceptibility to ceftriaxone. Genomic analysis demonstrated a distinct phylogenetic clade when compared with local contemporary strains. Continued evolution and widespread transmission of these strains might challenge the effectiveness of current therapeutic options.

Emerg Infect Dis. 2017 May;23(5):809-812. doi: 10.3201/eid2305.161745.

Increasing Macrolide and Fluoroquinolone Resistance in Mycoplasma genitalium.

Murray GL, Bradshaw CS, Bissessor M, Danielewski J, Garland SM, Jensen JS, Fairley CK, Tabrizi SN.

ABSTRACT: Escalating resistance to azithromycin and moxifloxacin is being reported for Mycoplasma genitalium in the Asia-Pacific region. Analyzing 140 infections, we found pretreatment fluoroquinolone-resistance mutations in parC (13.6%) and gyrA (5%). ParC S83 changes were associated with moxifloxacin failure. Combined macrolide/fluoroquinolone-resistance mutations were in 8.6% of specimens, for which recommended therapies would be ineffective.

Clin Microbiol Rev. 2018 Feb 28;31(2). pii: e00089-17. doi: 10.1128/CMR.00089-17. Print 2018 Apr.

Emerging Technologies for Molecular Diagnosis of Sepsis.

Sinha M[#1], Jupe J[#2], Mack H[1], Coleman TP[1,3], Lawrence SM[4,5,6,3], Fraley SI[7,6,3].

ABSTRACT: Rapid and accurate profiling of infection-causing pathogens remains a significant challenge in modern health care. Despite advances in molecular diagnostic techniques, blood culture analysis remains the gold standard for diagnosing sepsis. However, this method is too slow and cumbersome to significantly influence the initial management of patients. The swift initiation of precise and targeted antibiotic therapies depends on the ability of a sepsis diagnostic test to capture clinically relevant organisms along with antimicrobial resistance within 1 to 3 h. The administration of appropriate, narrow-spectrum antibiotics demands that such a test be extremely sensitive with a high negative predictive value. In addition, it should utilize small sample volumes and detect polymicrobial infections and contaminants. All of this must be accomplished with a platform that is easily integrated into the clinical workflow. In this review, we outline the limitations of routine blood culture testing and discuss how emerging sepsis technologies are converging on the characteristics of the ideal sepsis diagnostic test. We include seven molecular technologies that have been validated on clinical blood specimens or mock samples using human blood. In addition, we discuss advances in machine learning technologies that use electronic medical record data to provide contextual evaluation support for clinical decision-making.

Clin Microbiol Rev. 2017 Nov 15;31(1). pii: e00024-17. doi: 10.1128/CMR.00024-17. Print 2018 Jan.

Syndromic Panel-Based Testing in Clinical Microbiology.

Ramanan P[1], Bryson AL[1], Binnicker MJ[1], Pritt BS[1,2], Patel R[3,2].

ABSTRACT: The recent development of commercial panel-based molecular diagnostics for the rapid detection of pathogens in positive blood culture bottles, respiratory specimens, stool, and cerebrospinal fluid has resulted in a paradigm shift in clinical microbiology and clinical practice. This review focuses on U.S. Food and Drug Administration (FDA)-approved/cleared multiplex molecular panels with more than five targets designed to assist in the diagnosis of bloodstream, respiratory tract, gastrointestinal, or central nervous system infections. While these panel-based assays have the clear advantages of a rapid turnaround time and the detection of a large number of microorganisms and promise to improve health care, they present certain challenges, including cost and the definition of ideal test utilization strategies (i.e., optimal ordering) and test interpretation.

J Clin Microbiol. 2017 Aug;55(8):2313-2320. doi: 10.1128/JCM.00476-17. Epub 2017 May 24.

Point-of-Care Testing for Infectious Diseases: Past, Present, and Future.

Kozel TR[1], Burnham-Marusich AR[2].

ABSTRACT: Point-of-care (POC) diagnostics provide rapid actionable information for patient care at the time and site of an encounter with the health care system. The usual platform has been the lateral flow immunoassay. Recently, emerging molecular diagnostics have met requirements for speed, low cost, and ease of use for POC applications. A major driver for POC development is the ability to diagnose infectious diseases at sites with a limited infrastructure. The potential use in both wealthy and resource-limited settings has fueled an intense effort to build on existing technologies and to generate new technologies for the diagnosis of a broad spectrum of infectious diseases.

J Clin Microbiol. 2017 Jun;55(6):1621-1628. doi: 10.1128/JCM.00211-17. Epub 2017 Mar 15.

Applications of Digital PCR for Clinical Microbiology.

Kuypers J[1], Jerome KR[2].

ABSTRACT: Digital PCR (dPCR) is an important new tool for use in the clinical microbiology laboratory. Its advantages over quantitative PCR (qPCR), including absolute quantification without a standard curve, improved precision, improved accuracy in the presence of inhibitors, and more accurate quantitation when amplification efficiency is low, make dPCR the assay of choice for several specimen testing applications. This minireview will discuss the advantages and disadvantages of dPCR compared to qPCR, its applications in clinical microbiology, and considerations for implementation of the method in a clinical laboratory.

SLAS Technol. 2017 Dec;22(6):585-608. doi: 10.1177/2472630317727519. Epub 2017 Aug 29.

Emerging Microtechnologies and Automated Systems for Rapid Bacterial Identification and Antibiotic Susceptibility Testing.

Li Y[1,2], Yang X[3], Zhao W[1,4].

ABSTRACT: Rapid bacterial identification (ID) and antibiotic susceptibility testing (AST) are in great demand due to the rise of drug-resistant bacteria. Conventional culture-based AST methods suffer from a long turnaround time. By necessity, physicians often have to treat patients empirically with antibiotics, which has led to an inappropriate use of antibiotics, an elevated mortality rate and healthcare costs, and antibiotic resistance. Recent advances in miniaturization and automation provide promising solutions for rapid bacterial ID/AST profiling, which will potentially make a significant impact in the clinical management of infectious diseases and antibiotic stewardship in the coming years. In this review, we summarize and analyze representative emerging micro- and nanotechnologies, as well as automated systems for bacterial ID/AST, including both phenotypic (e.g., microfluidic-based bacterial culture, and digital imaging of single cells) and molecular (e.g., multiplex PCR, hybridization probes, nanoparticles, synthetic biology tools, mass spectrometry, and sequencing technologies) methods. We also discuss representative point-of-care (POC) systems that integrate sample processing, fluid handling, and detection for rapid bacterial ID/AST. Finally, we highlight major remaining challenges and discuss potential future endeavors toward improving clinical outcomes with rapid bacterial ID/AST technologies.

Clin Microbiol Infect. 2017 Oct 24. pii: S1198-743X(17)30578-5. doi: 10.1016/j.cmi.2017.10.016. [Epub ahead of print]

Rapid detection of antibiotic resistance by MALDI-TOF mass spectrometry using a novel direct-on-target microdroplet growth assay.

Idelevich EA[1], Sparbier K[2], Kostrzewa M[2], Becker K[3].

OBJECTIVES: We aimed to develop a universal phenotypic method, which allows easy and rapid antimicrobial susceptibility testing independently of underlying resistance mechanisms.

METHODS: We established a novel direct-on-target microdroplet growth assay for the detection of antibiotic resistance within a few hours, which is based on matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS). The microorganisms were incubated with and without meropenem in nutrient broth as microdroplets directly on MALDI-TOF MS target. Subsequently, broth was separated from microbial cells by contacting the microdroplets with an absorptive material. The microorganisms grown in the presence of antibiotic were detected by MALDI-

TOF MS. A total of 24 Klebsiella pneumoniae and 24 Pseudomonas aeruginosa isolates were used to assess performance for detection of meropenem resistance. The microdroplet volumes investigated were 2, 4, 6, 8 and 10 µL.

RESULTS: The best performance was achieved using 6-µL microdroplets. Applying this volume, all growth controls were successfully detected (definition of valid test), and all isolates were correctly categorized as susceptible or non-susceptible after an 18-h incubation. For K. pneumoniae, rate of valid tests, sensitivity and specificity all reached 100% after a 4-h incubation of 6-µL microdroplets. Using the same microdroplet volume for P. aeruginosa, incubation for 5 h resulted in 83.3% of valid tests with 100% sensitivity and 100% specificity.

CONCLUSIONS: We demonstrated easy, rapid and accurate resistance detection using carbapenem-resistant Gram-negative bacteria as an example. Our technology is suitable for automatization and expandable to further applications, e.g. simultaneous testing of multiple antibiotics as well as resistance determination directly from clinical samples.

J Clin Microbiol. 2018 Mar 26;56(4). pii: e01329-17. doi: 10.1128/JCM.01329-17. Print 2018 Apr.

Multicenter Evaluation of the Accelerate PhenoTest BC Kit for Rapid Identification and Phenotypic Antimicrobial Susceptibility Testing Using Morphokinetic Cellular Analysis.

Pancholi P[#1], Carroll KC[#2], Buchan BW[3], Chan RC[4], Dhiman N[5], Ford B[6], Granato PA[7], Harrington AT[8], Hernandez DR[9], Humphries RM[10], Jindra MR[11], Ledeboer NA[3], Miller SA[10], Mochon AB[12], Morgan MA[4], Patel R[13], Schreckenberger PC[8], Stamper PD[14], Simner PJ[2], Tucci NE[7], Zimmerman C[14], Wolk DM[#9].

ABSTRACT: We describe results from a multicenter study evaluating the Accelerate Pheno system, a first of its kind diagnostic system that rapidly identifies common bloodstream pathogens from positive blood cultures within 90 min and determines bacterial phenotypic antimicrobial susceptibility testing (AST) results within ~7 h. A combination of fresh clinical and seeded blood cultures were tested, and results from the Accelerate Pheno system were compared to Vitek 2 results for identification (ID) and broth microdilution or disk diffusion for AST. The Accelerate Pheno system accurately identified 14 common bacterial pathogens and two *Candida* spp. with sensitivities ranging from 94.6 to 100%. Of fresh positive blood cultures, 89% received a monomicrobial call with a positive predictive value of 97.3%. Six common Gram-positive cocci were evaluated for ID. Five were tested against eight antibiotics, two resistance phenotypes (methicillin-resistant *Staphylococcus aureus* and *Staphylococcus* spp. [MRSA/MRS]), and inducible clindamycin resistance (MLSb). From the 4,142 AST results, the overall essential agreement (EA) and categorical agreement (CA) were 97.6% and 97.9%, respectively. Overall very major error (VME), major error (ME), and minor error (mE) rates were 1.0%, 0.7%, and 1.3%, respectively. Eight species of Gram-negative rods were evaluated against 15 antibiotics. From the 6,331 AST results, overall EA and CA were 95.4% and 94.3%, respectively. Overall VME, ME, and mE rates were 0.5%, 0.9%, and 4.8%, respectively. The Accelerate Pheno system has the unique ability to identify and provide phenotypic MIC and categorical AST results in a few hours directly from positive blood culture bottles and support accurate antimicrobial adjustment.

Clin Microbiol Infect. 2018 Apr;24(4):335-341. doi: 10.1016/j.cmi.2017.10.013. Epub 2017 Oct 23.

Next-generation sequencing technologies and their application to the study and control of bacterial infections.

Besser J[1], Carleton HA[1], Gerner-Smidt P[2], Lindsey RL[1], Trees E[1].

BACKGROUND: With the efficiency and the decreasing cost of next-generation sequencing, the technology is being rapidly introduced into clinical and public health laboratory practice.

AIMS: The historical background and principles of first-, second- and third-generation sequencing are described, as are the characteristics of the most commonly used sequencing instruments.

SOURCES: Peer-reviewed literature, white papers and meeting reports.

CONTENT AND IMPLICATIONS: Next-generation sequencing is a technology that could potentially replace many traditional microbiological workflows, providing clinicians and public health specialists with more actionable information than hitherto achievable. Examples of the clinical and public health uses of the technology are provided. The challenge of comparability of different sequencing platforms is discussed. Finally, the future directions of the technology integrating it with laboratory management and public health surveillance systems, and moving it towards performing sequencing directly from the clinical specimen (metagenomics), could lead to yet another fundamental transformation of clinical diagnostics and public health surveillance.

Clin Microbiol Infect. 2018 Apr;24(4):355-360. doi: 10.1016/j.cmi.2017.11.001. Epub 2017 Nov 5.

Practical issues in implementing whole-genome-sequencing in routine diagnostic microbiology.

Rossen JWA[1], Friedrich AW[2], Moran-Gilad J[3]; ESCMID Study Group for Genomic and Molecular Diagnostics (ESGMD).

BACKGROUND: Next generation sequencing (NGS) is increasingly being used in clinical microbiology. Like every new technology adopted in microbiology, the integration of NGS into clinical and routine workflows must be carefully managed.

AIM: To review the practical aspects of implementing bacterial whole genome sequencing (WGS) in routine diagnostic laboratories.

SOURCES: Review of the literature and expert opinion.

CONTENT: In this review, we discuss when and how to integrate whole genome sequencing (WGS) in the routine workflow of the clinical laboratory. In addition, as the microbiology laboratories have to adhere to various national and international regulations and criteria for their accreditation, we deliberate on quality control issues for using WGS in microbiology, including the importance of proficiency testing. Furthermore, the current and future place of this technology in the diagnostic hierarchy of microbiology is described as well as the necessity of maintaining backwards compatibility with already established methods. Finally, we speculate on the question of whether WGS can entirely replace routine microbiology in the future and the tension between the fact that most sequencers are designed to process multiple samples in parallel whereas for optimal diagnosis a one-by-one processing of the samples is preferred. Special reference is made to the cost and turnaround time of WGS in diagnostic laboratories.

IMPLICATIONS: Further development is required to improve the workflow for WGS, in particular to shorten the turnaround time, reduce costs, and streamline downstream data analyses. Only when these processes reach maturity will reliance on WGS for routine patient management and infection control

management become feasible, enabling the transformation of clinical microbiology into a genome-based and personalized diagnostic field.

Clin Microbiol Infect. 2018 Apr;24(4):35 0-354. doi: 10.1016/j.cmi.2017.12.016. Epub 2018 Jan 5.

Whole genome sequencing options for bacterial strain typing and epidemiologic analysis based on single nucleotide polymorphism versus gene-by-gene-based approaches.

Schürch AC[1], Arredondo-Alonso S[1], Willems RJL[1], Goering RV[2].

BACKGROUND: Whole genome sequence (WGS)-based strain typing finds increasing use in the epidemiologic analysis of bacterial pathogens in both public health as well as more localized infection control settings.

AIMS: This minireview describes methodologic approaches that have been explored for WGS-based epidemiologic analysis and considers the challenges and pitfalls of data interpretation.

SOURCES: Personal collection of relevant publications.

CONTENT: When applying WGS to study the molecular epidemiology of bacterial pathogens, genomic variability between strains is translated into measures of distance by determining single nucleotide polymorphisms in core genome alignments or by indexing allelic variation in hundreds to thousands of core genes, assigning types to unique allelic profiles. Interpreting isolate relatedness from these distances is highly organism specific, and attempts to establish species-specific cutoffs are unlikely to be generally applicable. In cases where single nucleotide polymorphism or core gene typing do not provide the resolution necessary for accurate assessment of the epidemiology of bacterial pathogens, inclusion of accessory gene or plasmid sequences may provide the additional required discrimination.

IMPLICATIONS: As with all epidemiologic analysis, realizing the full potential of the revolutionary advances in WGS-based approaches requires understanding and dealing with issues related to the fundamental steps of data generation and interpretation.

Clin Microbiol Rev. 2017 Oct;30(4):1015-1063. doi: 10.1128/CMR.00016-17.

Whole-Genome Sequencing of Bacterial Pathogens: the Future of Nosocomial Outbreak Analysis.

Quainoo S[1], Coolen JPM[2], van Hijum SAFT[3,4], Huynen MA[3], Melchers WJG[2], van Schaik W[5], Wertheim HFL[2].

ABSTRACT: Outbreaks of multidrug-resistant bacteria present a frequent threat to vulnerable patient populations in hospitals around the world. Intensive care unit (ICU) patients are particularly susceptible to nosocomial infections due to indwelling devices such as intravascular catheters, drains, and intratracheal tubes for mechanical ventilation. The increased vulnerability of infected ICU patients demonstrates the importance of effective outbreak management protocols to be in place. Understanding the transmission of pathogens via genotyping methods is an important tool for outbreak management. Recently, whole-genome sequencing (WGS) of pathogens has become more accessible and affordable as a tool for genotyping. Analysis of the entire pathogen genome via WGS could provide unprecedented resolution in discriminating even highly related lineages of bacteria and revolutionize outbreak analysis in hospitals. Nevertheless, clinicians have long been hesitant to implement WGS in outbreak analyses due to the expensive and cumbersome nature of early sequencing platforms. Recent improvements in sequencing technologies and analysis tools have rapidly increased the output and analysis speed as well as reduced the overall costs of WGS. In this review, we assess the feasibility of WGS technologies and bioinformatics analysis tools for nosocomial outbreak analyses and provide a comparison to conventional outbreak analysis workflows. Moreover, we review advantages and

limitations of sequencing technologies and analysis tools and present a real-world example of the implementation of WGS for antimicrobial resistance analysis. We aimed to provide health care professionals with a guide to WGS outbreak analysis that highlights its benefits for hospitals and assists in the transition from conventional to WGS-based outbreak analysis.

Emerg Infect Dis. 2017 Sep;23(9):1441-1445. doi: 10.3201/eid2309.170416.

Bioinformatic Analyses of Whole-Genome Sequence Data in a Public Health Laboratory.

Oakeson KF, Wagner JM, Mendenhall M, Rohrwasser A, Atkinson-Dunn R.

ABSTRACT: The ability to generate high-quality sequence data in a public health laboratory enables the identification of pathogenic strains, the determination of relatedness among outbreak strains, and the analysis of genetic information regarding virulence and antimicrobial-resistance genes. However, the analysis of whole-genome sequence data depends on bioinformatic analysis tools and processes. Many public health laboratories do not have the bioinformatic capabilities to analyze the data generated from sequencing and therefore are unable to take full advantage of the power of whole-genome sequencing. The goal of this perspective is to provide a guide for laboratories to understand the bioinformatic analyses that are needed to interpret whole-genome sequence data and how these in silico analyses can be implemented in a public health laboratory setting easily, affordably, and, in some cases, without the need for intensive computing resources and infrastructure.

Clin Microbiol Infect. 2017 Aug;23(8):574.e1-574.e6. doi: 10.1016/j.cmi.2017.02.006. Epub 2017 Feb 10.

Untargeted next-generation sequencing-based first-line diagnosis of infection in immunocompromised adults: a multicentre, blinded, prospective study.

Parize P[1], Muth E[2], Richaud C[3], Gratigny M[2], Pilmis B[1], Lamamy A[2], Mainardi JL[3], Cheval J[2], de Visser L[2], Jagorel F[2], Ben Yahia L[2], Bamba G[2], Dubois M[2], Join-Lambert O[4], Leruez-Ville M[4], Nassif X[4], Lefort A[5], Lanternier F[1], Suarez F[6], Lortholary O[1], Lecuit M[7], Eloit M[8].

OBJECTIVE: Infections are the major cause of morbidity and mortality in immunocompromised patients. Improving microbiological diagnosis in these patients is of paramount clinical importance.

METHODS: We performed this multicentre, blinded, prospective, proof-of-concept study, to compare untargeted next-generation sequencing with conventional microbiological methods for first-line diagnosis of infection in 101 immunocompromised adults. Patients were followed for 30 days and their blood samples, and in some cases nasopharyngeal swabs and/or biological fluids, were analysed. At the end of the study, expert clinicians evaluated the results of both methods. The primary outcome measure was the detection rate of clinically relevant viruses and bacteria at inclusion.

RESULTS: Clinically relevant viruses and bacteria identified by untargeted next-generation sequencing and conventional methods were concordant for 72 of 101 patients in samples taken at inclusion (κ test=0.2, 95% CI 0.03-0.48). However, clinically relevant viruses and bacteria were detected in a significantly higher proportion of patients with untargeted next-generation sequencing than conventional methods at inclusion (36/101 (36%) vs. 11/101 (11%), respectively, p <0.001), and even when the latter were continued over 30 days (19/101 (19%), p 0.003). Untargeted next-generation sequencing had a high negative predictive value compared with conventional methods (64/65, 95% CI 0.95-1).

CONCLUSIONS: Untargeted next-generation sequencing has a high negative predictive value and detects more clinically relevant viruses and bacteria than conventional microbiological methods.

Untargeted next-generation sequencing is therefore a promising method for microbiological diagnosis in immunocompromised adults.

J Clin Microbiol. 2017 Aug;55(8):2334-2347. doi: 10.1128/JCM.00462-17. Epub 2017 May 10.

Molecular Diagnosis of Orthopedic-Device-Related Infection Directly from Sonication Fluid by Metagenomic Sequencing.

Street TL[1], Sanderson ND[2], Atkins BL[3,4], Brent AJ[2,3], Cole K[5,6], Foster D[2], McNally MA[3], Oakley S[4], Peto L[2], Taylor A[3], Peto TEA[2,7], Crook DW[2,7], Eyre DW[2,7].

ABSTRACT: Culture of multiple periprosthetic tissue samples is the current gold standard for microbiological diagnosis of prosthetic joint infections (PJI). Additional diagnostic information may be obtained through culture of sonication fluid from explants. However, current techniques can have relatively low sensitivity, with prior antimicrobial therapy and infection by fastidious organisms influencing results. We assessed if metagenomic sequencing of total DNA extracts obtained direct from sonication fluid can provide an alternative rapid and sensitive tool for diagnosis of PJI. We compared metagenomic sequencing with standard aerobic and anaerobic culture in 97 sonication fluid samples from prosthetic joint and other orthopedic device infections. Reads from Illumina MiSeq sequencing were taxonomically classified using Kraken. Using 50 derivation samples, we determined optimal thresholds for the number and proportion of bacterial reads required to identify an infection and confirmed our findings in 47 independent validation samples. Compared to results from sonication fluid culture, the species-level sensitivity of metagenomic sequencing was 61/69 (88%; 95% confidence interval [CI], 77 to 94%; for derivation samples 35/38 [92%; 95% CI, 79 to 98%]; for validation samples, 26/31 [84%; 95% CI, 66 to 95%]), and genus-level sensitivity was 64/69 (93%; 95% CI, 84 to 98%). Species-level specificity, adjusting for plausible fastidious causes of infection, species found in concurrently obtained tissue samples, and prior antibiotics, was 85/97 (88%; 95% CI, 79 to 93%; for derivation samples, 43/50 [86%; 95% CI, 73 to 94%]; for validation samples, 42/47 [89%; 95% CI, 77 to 96%]). High levels of human DNA contamination were seen despite the use of laboratory methods to remove it. Rigorous laboratory good practice was required to minimize bacterial DNA contamination. We demonstrate that metagenomic sequencing can provide accurate diagnostic information in PJI. Our findings, combined with the increasing availability of portable, random-access sequencing technology, offer the potential to translate metagenomic sequencing into a rapid diagnostic tool in PJI.

J Hepatol. 2018 Apr 2. pii: S0168-8278(18)31963-9. doi: 10.1016/j.jhep.2018.03.021. [Epub ahead of print]

Quantitation of HBV cccDNA in anti-HBc-positive liver donors by droplet digital PCR: a new tool to detect occult infection.

Caviglia GP[1], Abate ML[2], Tandoi F[3], Ciancio A[2], Amoroso A[4], Salizzoni M[3], Saracco GM[2], Rizzetto M[2], Romagnoli R[3], Smedile A[2].

BACKGROUND & AIMS: The accurate diagnosis of occult HBV infection (OBI) requires the demonstration of HBV DNA in liver biopsies of HBsAg-negative subjects. However, in clinical practice a latent OBI is deduced by the finding of the antibody to the HB-core antigen (anti-HBc). We investigated the true prevalence of OBI and the molecular features of intrahepatic HBV in anti-HBc-positive subjects.

METHODS: The livers of 100 transplant donors (median age 68.2 years; 64 males, 36 females) positive for anti-HBc at standard serologic testing, were examined for total HBV DNA by nested-PCR and for the HBV covalently closed circular DNA (HBV cccDNA) with an in-house droplet digital PCR assay (ddPCR)

(Linearity: R^2 = 0.9998; lower limit of quantitation and detection of 2.4 and 0.8 copies/10^5 cells, respectively).

RESULTS: A true OBI status was found in 52% (52/100) of the subjects and cccDNA was found in 52% (27/52) of the OBI-positive, with a median 13 copies/10^5 cells (95% confidence interval 5-25). Using an assay specific for anti-HBc of IgG class, the median antibody level was significantly higher in HBV cccDNA-positive than negative donors (5.7 [3.6-9.7] vs. 17.0 [7.0-39.2] COI, p = 0.007). By multivariate analysis, an anti-HBc IgG value above a 4.4 cut-off index (COI) was associated with the finding of intrahepatic HBV cccDNA (OR = 8.516, p = 0.009); a lower value ruled out its presence with a negative predictive value of 94.6%.

CONCLUSIONS: With a new in-house ddPCR-based method, intrahepatic HBV cccDNA was detectable in quantifiable levels in about half of the OBI cases examined. The titer of anti-HBc IgG may be a useful surrogate to predict the risk of OBI reactivation in immunosuppressed patients.

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Letermovir Prophylaxis for Cytomegalovirus in Hematopoietic-Cell Transplantation.

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BACKGROUND: Cytomegalovirus (CMV) infection remains a common complication after allogeneic hematopoietic-cell transplantation. Letermovir is an antiviral drug that inhibits the CMV-terminase complex**.**

METHODS: In this phase 3, double-blind trial, we randomly assigned CMV-seropositive transplant recipients, 18 years of age or older, in a 2:1 ratio to receive letermovir or placebo, administered orally or intravenously, through week 14 after transplantation; randomization was stratified according to trial site and CMV disease risk. Letermovir was administered at a dose of 480 mg per day (or 240 mg per day in patients taking cyclosporine). Patients in whom clinically significant CMV infection (CMV disease or CMV viremia leading to preemptive treatment) developed discontinued the trial regimen and received anti-CMV treatment. The primary end point was the proportion of patients, among patients without detectable CMV DNA at randomization, who had clinically significant CMV infection through week 24 after transplantation. Patients who discontinued the trial or had missing end-point data at week 24 were imputed as having a primary end-point event. Patients were followed through week 48 after transplantation.

RESULTS: From June 2014 to March 2016, a total of 565 patients underwent randomization and received letermovir or placebo beginning a median of 9 days after transplantation. Among 495 patients with undetectable CMV DNA at randomization, fewer patients in the letermovir group than in the placebo group had clinically significant CMV infection or were imputed as having a primary end-point event by week 24 after transplantation (122 of 325 patients [37.5%] vs. 103 of 170 [60.6%], P<0.001). The frequency and severity of adverse events were similar in the two groups overall. Vomiting was reported in 18.5% of the patients who received letermovir and in 13.5% of those who received placebo; edema in 14.5% and 9.4%, respectively; and atrial fibrillation or flutter in 4.6% and 1.0%, respectively. The rates of myelotoxic and nephrotoxic events were similar in the letermovir group and the placebo group. All-cause mortality at week 48 after transplantation was 20.9% among letermovir recipients and 25.5% among placebo recipients**.**

CONCLUSIONS: Letermovir prophylaxis resulted in a significantly lower risk of clinically significant CMV infection than placebo. Adverse events with letermovir were mainly of low grade.

Int J Mol Sci. 2017 Jul 29;18(8). pii: E1654. doi: 10.3390/ijms18081654.

A Different Microbiome Gene Repertoire in the Airways of Cystic Fibrosis Patients with Severe Lung Disease.

Bacci G[1], Mengoni A[2], Fiscarelli E[3], Segata N[4], Taccetti G[5], Dolce D[6], Paganin P[7], Morelli P[8], Tuccio V[9], De Alessandri A[10], Lucidi V[11], Bevivino A[12].

ABSTRACT: In recent years, next-generation sequencing (NGS) was employed to decipher the structure and composition of the microbiota of the airways in cystic fibrosis (CF) patients. However, little is still known about the overall gene functions harbored by the resident microbial populations and which specific genes are associated with various stages of CF lung disease. In the present study, we aimed to identify the microbial gene repertoire of CF microbiota in twelve patients with severe and normal/mild lung disease by performing sputum shotgun metagenome sequencing. The abundance of metabolic pathways encoded by microbes inhabiting CF airways was reconstructed from the metagenome. We identified a set of metabolic pathways differently distributed in patients with different pulmonary function; namely, pathways related to bacterial chemotaxis and flagellar assembly, as well as genes encoding efflux-mediated antibiotic resistance mechanisms and virulence-related genes. The results indicated that the microbiome of CF patients with low pulmonary function is enriched in virulence-related genes and in genes encoding efflux-mediated antibiotic resistance mechanisms. Overall, the microbiome of severely affected adults with CF seems to encode different mechanisms for the facilitation of microbial colonization and persistence in the lung, consistent with the characteristics of multidrug-resistant microbial communities that are commonly observed in patients with severe lung disease.

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Serum HBV pgRNA as a clinical marker for cccDNA activity.

Giersch K[1], Allweiss L[1], Volz T[1], Dandri M[2], Lütgehetmann M[3].

Letter to Editor

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In Vivo Excision of HIV-1 Provirus by saCas9 and Multiplex Single-Guide RNAs in Animal Models.

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ABSTRACT: CRISPR-associated protein 9 (Cas9)-mediated genome editing provides a promising cure for HIV-1/AIDS; however, gene delivery efficiency in vivo remains an obstacle to overcome. Here, we demonstrate the feasibility and efficiency of excising the HIV-1 provirus in three different animal models using an all-in-one adeno-associated virus (AAV) vector to deliver multiplex single-guide RNAs (sgRNAs) plus Staphylococcus aureus Cas9 (saCas9). The quadruplex sgRNAs/saCas9 vector outperformed the duplex vector in excising the integrated HIV-1 genome in cultured neural stem/progenitor cells from HIV-1 Tg26 transgenic mice. Intravenously injected quadruplex sgRNAs/saCas9 AAV-DJ/8 excised HIV-1 proviral DNA and significantly reduced viral RNA expression in several organs/tissues of Tg26 mice. In EcoHIV acutely infected mice, intravenously injected quadruplex sgRNAs/saCas9 AAV-DJ/8 reduced systemic EcoHIV infection, as determined by live bioluminescence imaging. Additionally, this quadruplex vector induced efficient proviral excision, as determined by PCR genotyping in the liver, lungs, brain, and spleen. Finally, in humanized bone marrow/liver/thymus (BLT) mice with chronic HIV-1 infection, successful proviral excision was detected by PCR genotyping in the

spleen, lungs, heart, colon, and brain after a single intravenous injection of quadruplex sgRNAs/saCas9 AAV-DJ/8. In conclusion, in vivo excision of HIV-1 proviral DNA by sgRNAs/saCas9 in solid tissues/organs can be achieved via AAV delivery, a significant step toward human clinical trials.

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Patients with familial adenomatous polyposis harbor colonic biofilms containing tumorigenic bacteria.

Dejea CM[1,2], Fathi P[1,2,3], Craig JM[4], Boleij A[1,5], Taddese R[5], Geis AL[1,2], Wu X[1,3], DeStefano Shields CE[1,2], Hechenbleikner EM[6], Huso DL[7], Anders RA[8], Giardiello FM[2,3], Wick EC[6], Wang H[1,2], Wu S[1,3], Pardoll DM[1,2], Housseau F[1,2], Sears CL[9,2,3].

ABSTRACT: Individuals with sporadic colorectal cancer (CRC) frequently harbor abnormalities in the composition of the gut microbiome; however, the microbiota associated with precancerous lesions in hereditary CRC remains largely unknown. We studied colonic mucosa of patients with familial adenomatous polyposis (FAP), who develop benign precursor lesions (polyps) early in life. We identified patchy bacterial biofilms composed predominately of *Escherichia coli* and *Bacteroides fragilis* Genes for colibactin (*clbB*) and *Bacteroides fragilis* toxin (*bft*), encoding secreted oncotoxins, were highly enriched in FAP patients' colonic mucosa compared to healthy individuals. Tumor-prone mice cocolonized with *E. coli* (expressing colibactin), and enterotoxigenic *B. fragilis* showed increased interleukin-17 in the colon and DNA damage in colonic epithelium with faster tumor onset and greater mortality, compared to mice with either bacterial strain alone. These data suggest an unexpected link between early neoplasia of the colon and tumorigenic bacteria.

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Gut microbiome modulates response to anti-PD-1 immunotherapy in melanoma patients.

Gopalakrishnan V[1,2], Spencer CN[2,3], Nezi L[3], Reuben A[1], Andrews MC[1], Karpinets TV[3], Prieto PA[1], Vicente D[1], Hoffman K[4], Wei SC[5], Cogdill AP[1,5], Zhao L[3], Hudgens CW[6], Hutchinson DS[7], Manzo T[3], Petaccia de Macedo M[6], Cotechini T[8], Kumar T[3], Chen WS[9], Reddy SM[10], Szczepaniak Sloane R[1], Galloway-Pena J[11], Jiang H[1], Chen PL[9], Shpall EJ[12], Rezvani K[12], Alousi AM[12], Chemaly RF[11], Shelburne S[3,11], Vence LM[5], Okhuysen PC[11], Jensen VB[13], Swennes AG[7], McAllister F[14], Marcelo Riquelme Sanchez E[14], Zhang Y[14], Le Chatelier E[15], Zitvogel L[16], Pons N[15], Austin-Breneman JL[1], Haydu LE[1], Burton EM[1], Gardner JM[1], Sirmans E[17], Hu J[18], Lazar AJ[6,9], Tsujikawa T[8], Diab A[17], Tawbi H[17], Glitza IC[17], Hwu WJ[17], Patel SP[17], Woodman SE[17], Amaria RN[17], Davies MA[17], Gershenwald JE[1], Hwu P[17], Lee JE[1], Zhang J[3], Coussens LM[8], Cooper ZA[1,3], Futreal PA[3], Daniel CR[4,2], Ajami NJ[7], Petrosino JF[7], Tetzlaff MT[6,9], Sharma P[5,19], Allison JP[5], Jenq RR[3], Wargo JA[20,3].

ABSTRACT: Preclinical mouse models suggest that the gut microbiome modulates tumor response to checkpoint blockade immunotherapy; however, this has not been well-characterized in human cancer patients. Here we examined the oral and gut microbiome of melanoma patients undergoing anti-programmed cell death 1 protein (PD-1) immunotherapy (n = 112). Significant differences were observed in the diversity and composition of the patient gut microbiome of responders versus nonresponders. Analysis of patient fecal microbiome samples (n = 43, 30 responders, 13 nonresponders) showed significantly higher alpha diversity ($P < 0.01$) and relative abundance of bacteria of the Ruminococcaceae family ($P < 0.01$) in responding patients. Metagenomic studies revealed functional differences in gut bacteria in responders, including enrichment of anabolic pathways. Immune profiling suggested enhanced systemic and antitumor immunity in responding patients with a favorable gut microbiome as well as in germ-free mice receiving fecal transplants from

responding patients. Together, these data have important implications for the treatment of melanoma patients with immune checkpoint inhibitors.

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Gut microbiome influences efficacy of PD-1-based immunotherapy against epithelial tumors.

Routy B[1,2,3], Le Chatelier E[4], Derosa L[1,2,3], Duong CPM[1,2,5], Alou MT[1,2,3], Daillère R[1,2,3], Fluckiger A[1,2,5], Messaoudene M[1,2], Rauber C[1,2,3], Roberti MP[1,2,5], Fidelle M[1,3,5], Flament C[1,2,5], Poirier-Colame V[1,2,5], Opolon P[6], Klein C[7], Iribarren K[8,9,10,11,12], Mondragón L[8,9,10,11,12], Jacquelot N[1,2,3], Qu B[1,2,3], Ferrere G[1,2,3], Clémenson C[1,13], Mezquita L[1,14], Masip JR[1,14], Naltet C[15], Brosseau S[15], Kaderbhai C[16], Richard C[16], Rizvi H[17], Levenez F[4], Galleron N[4], Quinquis B[4], Pons N[4], Ryffel B[18], Minard-Colin V[1,19], Gonin P[1,20], Soria JC[1,14], Deutsch E[1,13], Loriot Y[1,3,14], Ghiringhelli F[16], Zalcman G[15], Goldwasser F[9,21,22], Escudier B[1,14,23], Hellmann MD[24,25], Eggermont A[1,2,14], Raoult D[26], Albiges L[1,3,14], Kroemer G[27,9,10,11,12,28,29], Zitvogel L[30,2,3,5].

ABSTRACT: Immune checkpoint inhibitors (ICIs) targeting the PD-1/PD-L1 axis induce sustained clinical responses in a sizable minority of cancer patients. We found that primary resistance to ICIs can be attributed to abnormal gut microbiome composition. Antibiotics inhibited the clinical benefit of ICIs in patients with advanced cancer. Fecal microbiota transplantation (FMT) from cancer patients who responded to ICIs into germ-free or antibiotic-treated mice ameliorated the antitumor effects of PD-1 blockade, whereas FMT from nonresponding patients failed to do so. Metagenomics of patient stool samples at diagnosis revealed correlations between clinical responses to ICIs and the relative abundance of *Akkermansia muciniphila* Oral supplementation with *A. muciniphila* after FMT with nonresponder feces restored the efficacy of PD-1 blockade in an interleukin-12-dependent manner by increasing the recruitment of $CCR9^{+}CXCR3^{+}CD4^{+}$ T lymphocytes into mouse tumor beds.

Nature. 2018 Jan 3;553(7686):77-81. doi: 10.1038/nature25140.

Sooty mangabey genome sequence provides insight into AIDS resistance in a natural SIV host.

Palesch D[1], Bosinger SE[1,2], Tharp GK[1], Vanderford TH[1], Paiardini M[1,2], Chahroudi A[1,3], Johnson ZP[1], Kirchhoff F[4], Hahn BH[5], Norgren RB[6], Patel NB[1], Sodora DL[7], Dawoud RA[1], Stewart CB[8], Seepo SM[8], Harris RA[9,10], Liu Y[9], Raveendran M[9,10], Han Y[9], English A[9], Thomas GWC[11], Hahn MW[11], Pipes L[12], Mason CE[12], Muzny DM[9,10], Gibbs RA[9,10], Sauter D[4], Worley K[9,10], Rogers J[9,10], Silvestri G[1,2].

ABSTRACT: In contrast to infections with human immunodeficiency virus (HIV) in humans and simian immunodeficiency virus (SIV) in macaques, SIV infection of a natural host, sooty mangabeys (Cercocebus atys), is non-pathogenic despite high viraemia. Here we sequenced and assembled the genome of a captive sooty mangabey. We conducted genome-wide comparative analyses of transcript assemblies from C. atys and AIDS-susceptible species, such as humans and macaques, to identify candidates for host genetic factors that influence susceptibility. We identified several immune-related genes in the genome of C. atys that show substantial sequence divergence from macaques or humans. One of these sequence divergences, a C-terminal frameshift in the toll-like receptor-4 (TLR4) gene of C. atys, is associated with a blunted in vitro response to TLR-4 ligands. In addition, we found a major structural change in exons 3-4 of the immune-regulatory protein intercellular adhesion molecule 2 (ICAM-2); expression of this variant leads to reduced cell surface expression of ICAM-2. These data provide a resource for comparative genomic studies of HIV and/or SIV pathogenesis and may help to elucidate the mechanisms by which SIV-infected sooty mangabeys avoid AIDS.