Genomics Impact on Infectious Diseases

Jonathan Zenilman MD
Johns Hopkins University
March 2, 2012
Conflicts--None
Welcome to the Genomic Era

Alan E. Guttmacher, M.D., and Francis S. Collins, M.D., Ph.D.

To him who devotes his life to science, nothing can give more happiness than increasing the number of discoveries, but his cup of joy is full when the results of his studies immediately find practical applications.

— Louis Pasteur

announcement (available at http://www.genome.gov/11006929) that it had achieved the last of the project’s original goals, the complete sequencing of the human genome. The extent and pace of progress in genomics are suggested by the fact that this achievement occurred 11 days shy of the 50th anniversary of the publication of Watson and Crick’s
Bacterial Diagnosis until 2000

3% carbon dioxide environment for culture plates
Problems with Cultures

- Cultures take 24-48 hours to process
- Quantitative Cultures take longer
- Cultures are prone to overgrowth
- Are there molecular approaches?
Genomics Diagnostics--Principles

- Detect DNA
- Amplification via PCR—Impact on Sensitivity
- Bacterial DNA have unique 16S ribosome DNA elements
- March to libraries
- Link to detection system
- Specificity can be a problem
DNA Amplification Using Polymerase Chain Reaction

Reaction mixture contains target DNA sequence to be amplified, two primers (P1, P2) and heat-stable Taq polymerase.

Reaction mixture is heated to 95°C to denature target DNA. Subsequent cooling to 37°C allows primers to hybridize to complementary sequences in target DNA.

When heated to 72°C, Taq polymerase extends complementary strands from primers.

First synthesis cycle results in two copies of target DNA sequence.

Second synthesis cycle results in four copies of target DNA sequence.

Source: DNA Science; see Fig. 13.
Accelerated Progress

- Nucleic Acid Diagnostics Commercialized in 1990s (STDs, HIV viral load)
- Non cultivable pathogens identified
- 2001 attacks—Major investments
- Simultaneous HGP and Sequence projects
- ~2000 organisms fully sequenced
Current Trends

• Commercialization of Discovery
• Rapid Clinical Diagnostics
• Genomics as Clinical Management Tools
• Bacterial Population Genomics and Impact
• Host Genomics and Susceptibility
• Microbiome Projects
• Expert and Benchtop systems
A Novel Coronavirus Associated with Severe Acute Respiratory Syndrome

Thomas G. Ksiazek, D.V.M., Ph.D., Dean Erdman, Dr.P.H., Cynthia S. Goldsmith, M.S., Sherif R. Zaki, M.D., Ph.D., Teresa Peret, Ph.D., Shannon Emery, B.S., Suxiang Tong, Ph.D., Carlo Urbani, M.D.,* James A. Comer, Ph.D., M.P.H., Wilina Lim, M.D., Pierre E. Rollin, M.D., Scott F. Dowell, M.D., M.P.H., Ai-Ee Ling, M.D., Charles D. Humphrey, Ph.D., Wun-Ju Shieh, M.D., Ph.D., Jeannette Guarner, M.D., Christopher D. Paddock, M.D., M.P.H.T.M., Paul Rota, Ph.D., Barry Fields, Ph.D., Joseph DeRisi, Ph.D., Jyh-Yuan Yang, Ph.D., Nancy Cox, Ph.D., James M. Hughes, M.D., James W. LeDuc, Ph.D., William J. Bellini, Ph.D., Larry J. Anderson, M.D., and the SARS Working Group†

ABSTRACT
**Figure 3.** Estimated Maximum-Parsimony Tree Based on the Sequence Alignment of 405 Nucleotides of the Coronavirus Polymerase Gene Open Reading Frame 1b (Nucleotide Numbers 15173 to 15578 Based on Bovine Coronavirus Complete Genome Accession Number NC_003045) Comparing SARS Coronavirus with Other Human and Animal Coronaviruses.

The three major coronavirus antigenic groups (I, II, and III), represented by the three branches, are illustrated in the diagram. Group I includes PEDV, TGEV, CCoV, and FIPV. Group II consists of MHV, Rat SDAV, HEV, and BCoV. Group III comprises Avian IBV and TCoV. The scale bar indicates 10 nucleotide mismatches (nt).
Nucleic Acid Amplification Tests

- NAAT tests are the dominant mode of gonococcal and chlamydia testing
- Can be used in genital and non-genital samples—e.g. urine, self-administered swabs—field applications
- Screening in field settings, schools, jails etc
- No transport issues
- Turnaround 24 hours
- Multiplex
- BUT—can’t detect resistance
Untreated Gonococcal and Chlamydial Infection in a Probability Sample of Adults

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William C. Miller, MD, PhD
James N. Gribble, ScD
James R. Chromy, PhD
Peter A. Leone, MD
Phillip C. Cooley, MS
Thomas C. Quinn, MD
Jonathan M. Zenilman, MD

Context  The prevalence and distribution of gonococcal and chlamydial infections in the general population are poorly understood. Development of nucleic acid amplification tests, such as the ligase chain reaction assay, provides new opportunities to estimate the prevalence of untreated infections in the population.

Objective  To estimate the overall prevalence of untreated gonococcal and chlamydial infections and to describe patterns of infection within specific demographic subgroups of the young adult population in Baltimore, Md.


Participants  A total of 728 adults aged 18 to 35 years completed the interview portion of the study, and 579 of these respondents also provided a urine specimen adequate for testing.

Main Outcome Measure  Prevalence of untreated infection, as measured by the proportion of participants testing positive for gonococcal and chlamydial infection by...
Table 2. Estimated Prevalence of Untreated Gonococcal and Chlamydial Infections by Race and Sex: 1997-1998 Baltimore STD and Behavior Survey*

<table>
<thead>
<tr>
<th></th>
<th>Black</th>
<th></th>
<th>Other Race</th>
<th></th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Women</td>
<td>Men</td>
<td>Women</td>
<td>Men</td>
<td></td>
</tr>
<tr>
<td>Unweighted sample size</td>
<td>193</td>
<td>126</td>
<td>142</td>
<td>118</td>
<td>579</td>
</tr>
<tr>
<td><em>Neisseria gonorrhoeae</em> and/or <em>Chlamydia trachomatis</em></td>
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<td></td>
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<tr>
<td>No. of cases (unweighted)†</td>
<td>21</td>
<td>14</td>
<td>8</td>
<td>6</td>
<td>49†</td>
</tr>
<tr>
<td>Prevalence (weighted), % (SE)§</td>
<td>15.0 (3.7)</td>
<td>6.4 (2.1)</td>
<td>1.3 (0.5)</td>
<td>2.8 (1.3)</td>
<td>7.9 (1.6)</td>
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<tr>
<td><em>N gonorrhoeae</em></td>
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<td></td>
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<tr>
<td>No. of cases (unweighted)†</td>
<td>12</td>
<td>10</td>
<td>8</td>
<td>3</td>
<td>33</td>
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<tr>
<td>Prevalence (weighted), % (SE)§</td>
<td>9.3 (3.3)</td>
<td>5.3 (2.0)</td>
<td>1.3 (0.5)</td>
<td>1.3 (0.9)</td>
<td>5.3 (1.4)</td>
</tr>
<tr>
<td><em>C trachomatis</em></td>
<td></td>
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<tr>
<td>No. of cases (unweighted)†</td>
<td>10</td>
<td>4</td>
<td>0</td>
<td>4</td>
<td>18</td>
</tr>
<tr>
<td>Prevalence (weighted), % (SE)§</td>
<td>6.4 (2.2)</td>
<td>1.1 (0.7)</td>
<td>0</td>
<td>2.4 (1.3)</td>
<td>3.0 (0.8)</td>
</tr>
</tbody>
</table>

*STD indicates sexually transmitted disease. Estimates are based on age-eligible respondents who provided urine specimens for *N gonorrhoeae* and *C trachomatis* testing (ligase chain reaction assay). The estimates are weighted to account for differing probabilities of selection and poststratification adjustments to match US Census marginals. The estimates presented differ slightly from preliminary estimates for sexually experienced subjects presented at the International Society for Sexually Transmitted Diseases Research meetings. In addition to the difference in population definition (all subjects vs subjects with sexual experience), subsequent comparison of laboratory records and the preliminary analysis file revealed 1 instance in which a subject who tested positive for gonococcal infection was mistakenly coded as positive for both pathogens in the preliminary analysis file.

†Unweighted case counts are not appropriate for making inferences about the prevalence of infection in populations since they do not take account of the differing probabilities of selection of households and individuals.

‡Includes 2 cases that were positive for both infections.

§SEs were calculated from weighted data using statistical algorithms that take account of impact of complex sample design on variance estimates.
Diagnostic Progression of C Difficile

- Culture—Takes days and is non-specific
- Toxin assay—Stool filtrate in tissue culture
- ELISA assay—Sensitivity ~80%
- Current—PCR of toxinA/toxinB genes—potential 6 hour turnaround
BUT—what happens when you start using the new tests?
Figure 1  Percentage positive chlamydia tests per yearly quarter Royal Infirmary Edinburgh GUM dataset for 1992 to 2003, separated for males and females; date of change from culture to NAAT for rectal specimens indicated.
Comparison of the Idaho Technology FilmArray System to Real-Time PCR for Detection of Respiratory Pathogens in Children

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Division of Infectious Diseases, Department of Pediatrics, Clinical Virology Laboratory, Clinical Microbiology Laboratory, and Department of Pathology and Laboratory Medicine, Children’s Hospital of Philadelphia and University of Pennsylvania School of Medicine, Philadelphia, Pennsylvania, USA

The FilmArray Respiratory Panel (RP) multiplexed nucleic acid amplification test (Idaho Technology, Inc., Salt Lake City, UT) was compared to laboratory-developed real-time PCR assays for the detection of various respiratory viruses and certain bacterial pathogens. A total of 215 frozen archived pediatric respiratory specimens previously characterized as either negative or positive for one or more pathogens by real-time PCR were examined using the FilmArray RP system. Overall agreement between the FilmArray RP and corresponding real-time PCR assays for shared analytes was 98.6% (kappa = 0.92 [95% confidence interval (CI), 0.89 to 0.94]). The combined positive percent agreement was 89.4% (95% CI, 85.4 to 92.6); the negative percent agreement was 99.6% (95% CI, 99.2 to 99.8). The mean real-time PCR threshold cycle (C_T) value for specimens with discordant results was 36.46 ± 4.54. Detection of coinfections and correct identification of influenza A virus subtypes were comparable to those of real-time PCR when using the FilmArray RP. The greatest comparative difference in sensitivity was observed for adenovirus; only 11 of 24 (45.8%; 95% CI, 27.9 to 64.0) clinical specimens positive for adenovirus by real-time PCR were also positive by the FilmArray RP system.
FIG 1 Illustration of the FilmArray RP pouch and the steps involved in processing a specimen for testing using the FilmArray system.
Detecting Undetectable/Hard to detect organisms

- Bartonella and other fastidious bacteria (eg TB)
- HPV viruses
- T. pallidum and LGV in lesions
- Newly discovered organisms
20 patients with brain abscess
Cultures=22 strains; PCR=72 strains
27 species not previously seen in brain abscess
1 subject had 16 strains

JID 2009; 48:1169
Antimicrobial Resistance

• Genomics can rapidly detect antimicrobial resistance
• You need to know what you are looking for
• Can be used as rapid screens
• High utility in tracking outbreaks, identifying clones
Quinolone Resistance—Determining Region Mutations and *por* Type of *Neisseria gonorrhoeae* Isolates: Resistance Surveillance and Typing by Molecular Methodologies

Julie A. Giles,¹ Jason Falconio,² Jeffrey D. Yuenger,¹ Jonathan M. Zenilman,¹ Michael Dan,³ and Margaret C. Bash²

¹Division of Allergies and Infectious Disease, Johns Hopkins University School of Medicine, Baltimore, and ²Division of Bacterial, Parasitic and Allergenic Products, Center for Biologics Evaluation and Research, United States Food and Drug Administration, Bethesda, Maryland; ³Infectious Disease Unit, Edith Wolfson Hospital, Tel Aviv, Israel

Quinolone resistance is increasing rapidly in *Neisseria gonorrhoeae* and is a significant public health problem...
Table 3. Mutations in the quinolone resistance-determining regions (QRDRs) of *Neisseria gonorrhoeae* strains isolated in Israel from January 2000 through October 2001.

<table>
<thead>
<tr>
<th>Fluoroquinolone susceptibility</th>
<th>No. of isolates</th>
<th>MIC of ciprofloxacin, μg/mL</th>
<th>QRDR mutations, by gene and amino acid</th>
</tr>
</thead>
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<tr>
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<td></td>
<td></td>
<td>In gyrA&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>aa 91</td>
</tr>
<tr>
<td>CipR&lt;sup&gt;c&lt;/sup&gt;</td>
<td>39</td>
<td>2–16</td>
<td>TCC→TTC</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>4–8</td>
<td>TCC→TTC</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>8</td>
<td>TCC→TTC</td>
</tr>
<tr>
<td>Cipl&lt;sup&gt;d&lt;/sup&gt;</td>
<td>1</td>
<td>0.125</td>
<td>wt</td>
</tr>
<tr>
<td>CipS&lt;sup&gt;e&lt;/sup&gt;</td>
<td>37</td>
<td>0.002–0.016</td>
<td>wt</td>
</tr>
</tbody>
</table>

**NOTE.** Cipl, intermediatively resistant to ciprofloxacin; CipR, resistant to ciprofloxacin; CipS, susceptible to ciprofloxacin; wt, wild type.

<sup>a</sup> All isolates were wt at loci coding aa 92–94 of gyrA.

<sup>b</sup> All isolates were wt at loci coding aa 85 and 87–91 of parC.

<sup>c</sup> MIC of ciprofloxacin, ≥1 μg/mL.

<sup>d</sup> MIC of ciprofloxacin, ≥0.125 μg/mL.

<sup>e</sup> MIC of ciprofloxacin, <0.125 μg/mL.
Syphilis – *T pallidum* cannot be cultured – Genomics has facilitated understanding the epidemiology of resistance.
The Presence of the 23S rRNA Gene Mutation in *T. pallidum* Samples Collected from Sites in the United States and Ireland from 1912 through 2003.

<table>
<thead>
<tr>
<th>Geographic Site</th>
<th>Date Sample Collected</th>
<th>Samples with Mutation/Total Amplifiable Samples</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dublin</td>
<td>2002</td>
<td>15/17 (88)</td>
</tr>
<tr>
<td>Historical strains from multiple locations</td>
<td>1912–1987</td>
<td>1/18 (6)</td>
</tr>
</tbody>
</table>

Figure 3
*T. pallidum* strain types identified throughout the world. The strain type information, years of collection, and the frequency of each strain type from each location are based on information in references 126, 127, 129–131, 133–135, 149, and 150.
Sensitivitiy and Specificity $>98\%$ for both TB detection and Susceptibility

Rapid Turnaround (2 hours)
Figure 2. Assay Procedure for the MTB/RIF Test.

Two volumes of sample treatment reagent are added to each volume of sputum. The mixture is shaken, incubated at room temperature for 15 minutes, and shaken again. Next, a sample of 2 to 3 ml is transferred to the test cartridge, which is then loaded into the instrument. All subsequent steps occur automatically. The user is provided with a printable test result, such as “MTB detected; RIF resistance not detected.” PCR denotes polymerase chain reaction.
High-Level Cefixime- and Ceftriaxone-Resistant *Neisseria gonorrhoeae* in France: Novel *penA* Mosaic Allele in a Successful International Clone Causes Treatment Failure

Magnus Unemo, a Daniel Golparian, a Robert Nicholas, b Makoto Ohnishi, c Anne Gallay, d and Patrice Sednaoui a

WHO Collaborating Centre for Gonorrhoea and other STIs, Department of Laboratory Medicine, Microbiology, Örebro University Hospital, Örebro, Sweden; Department of Pharmacology, University of North Carolina, Chapel Hill, North Carolina, USA; National Institute of Infectious Diseases, Tokyo, Japan; Institut de Veille Sanitaire, Saint-Maurice, France; and Institut Alfred Fournier, Centre National de Référence des Gonocoques, Paris, France

Cephalosporin Susceptibility Among *Neisseria gonorrhoeae* Isolates — United States, 2000–2010

*Neisseria gonorrhoeae* is a major cause of pelvic inflammatory disease, ectopic pregnancy, and infertility, and it can facilitate human immunodeficiency virus (HIV) transmission (1). Emergence of gonococcal resistance to penicillin and tetracycline occurred during the 1970s and became widespread during the early 1980s. More recently, resistance to fluoroquinolones developed. Resistance was documented first in Asia, then emerged in the United States in Hawaii followed by other 2000–2010 were analyzed. Cefixime susceptibilities were not determined during 2007–2008 because cefixime was unavailable in the United States during that period. Decreased antibiotic susceptibility for cefixime or ceftriaxone is defined by the Clinical and Laboratory Standards Institute (CLSI) as MICs ≥0.5 μg/mL; criteria for cefixime and ceftriaxone resistance in *N. gonorrhoeae* have not been defined (6). Because few isolates exhibited decreased susceptibility and
FIGURE 2. Percentage of gonorrhea isolates with cefixime MICs $\geq 0.25 \mu g/mL$ and ceftriaxone MICs $\geq 0.125 \mu g/mL$, by sex of sex partner — Gonococcal Isolate Surveillance Project, United States, 2000–2010.

Abbreviations: MICs = minimum inhibitory concentrations; MSM = men who have sex with men; MSW = men who have sex exclusively with women.
Alignment of PBP 2 sequences from strains of Neisseria gonorrhoeae with different penA alleles.

L'inquiétante émergence de « superbactéries »

Mots clés : Résistance, Bactérie, Antibiotique, INDE, PAKISTAN, GRANDE-BRETAGNE

Par Tristan Vey

12/08/2010 | Mise à jour : 00:01 Réactions (199)

Photo d'une colonie de bactéries d'eau prise en 2008. Crédits photo : ASSOCIATED PRESS

De nombreux cas de patients infectés par une famille de bactéries très résistantes aux antibiotiques usuels ont été découverts en Grande-Bretagne. La propagation rapide et massive de cette souche, isolée pour la première fois en Inde en 2008, inquiète la communauté médicale.
Facilitating Epidemiology
Subclinical Herpes Viral Shedding – Old Model

• >90% of persons with genital HSV-2 shed virus asymptotically
• Present 1%-10% of asymptomatic days in persons who have recurrent herpes due to HSV-2
• Responsible for most transmission

### Viral Shedding Patterns in Women

#### Subject 1: HSV-2 seropositive

<table>
<thead>
<tr>
<th>Day</th>
<th>Cervix</th>
<th>Vulva</th>
<th>Perianal Lesion(s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td></td>
<td></td>
<td>+</td>
</tr>
<tr>
<td>2</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>3</td>
<td></td>
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<td>+</td>
</tr>
<tr>
<td>4</td>
<td></td>
<td></td>
<td>+ + +</td>
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</table>

#### Subject 2: HSV-2 seropositive

<table>
<thead>
<tr>
<th>Day</th>
<th>Cervix</th>
<th>Vulva</th>
<th>Perianal Lesion(s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td></td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>+</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td></td>
<td>+</td>
<td>+ + +</td>
</tr>
</tbody>
</table>

#### Subject 3: HSV-1 seropositive

<table>
<thead>
<tr>
<th>Day</th>
<th>Cervix</th>
<th>Vulva</th>
<th>Perianal Lesion(s)</th>
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<tbody>
<tr>
<td>1</td>
<td>+ + +</td>
<td>+ + +</td>
<td>+ + +</td>
</tr>
<tr>
<td>2</td>
<td>+ + +</td>
<td>+ + +</td>
<td>+ + +</td>
</tr>
<tr>
<td>3</td>
<td>+ + +</td>
<td>+</td>
<td></td>
</tr>
</tbody>
</table>

#### Subject 4: HSV-2 seropositive

<table>
<thead>
<tr>
<th>Day</th>
<th>Cervix</th>
<th>Vulva</th>
<th>Perianal Lesion(s)</th>
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</thead>
<tbody>
<tr>
<td>1</td>
<td>+ + +</td>
<td></td>
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<td>2</td>
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<td>3</td>
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</tbody>
</table>

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Genital Shedding of Herpes Simplex Virus Among Symptomatic and Asymptomatic Persons With HSV-2 Infection

Elizabeth Tronstein, MPH
Christine Johnston, MD, MPH
Meei-Li Huang, PhD
Stacy Selke, MA
Amalia Magaret, PhD
Terri Warren, ANP
Lawrence Corey, MD
Anna Wald, MD, MPH

Context Since herpes simplex virus type 2 (HSV-2) antibody tests have become commercially available, an increasing number of persons have learned that they have genital herpes through serologic testing. The course of natural history of HSV-2 in asymptomatic, seropositive persons is uncertain.

Objective To evaluate the virologic and clinical course of HSV genital shedding among individuals with symptomatic and asymptomatic HSV-2 infection.

Design, Setting, and Participants Cohort of 498 immunocompetent HSV-2-seropositive persons enrolled in prospective studies of genital HSV shedding at the University of Washington Virology Research Clinic, Seattle, and Westover Heights Clinic, Portland, Oregon, between March 1992 and April 2008. Each participant obtained daily self-collected swabs of genital secretions for at least 30 days.
Figure 1. Distribution of Genital Shedding Rate Among Asymptomatic and Symptomatic Infection Groups

Tronstein, E. et al. JAMA 2011;305:1441-1449

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<table>
<thead>
<tr>
<th>Condition</th>
<th>Asymptomatic Persons (n=519)</th>
<th>Symptomatic Persons (n=4753)</th>
<th>Asymptomatic Persons (n=434)</th>
<th>Symptomatic Persons (n=2708)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Overall Genital Shedding</td>
<td></td>
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<tr>
<td>Subclinical Genital Shedding</td>
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<tr>
<td>Genital Shedding</td>
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</table>

Tronstein, E. et al. JAMA 2011;305:1441-1449
Triple-Reassortant Swine Influenza A (H1) in Humans in the United States, 2005–2009

Epidemiologic Curve of Confirmed Cases of Human Infection with Swine-Origin Influenza A (H1N1) Virus with Known Date of Illness Onset in the United States (March 28–May 5, 2009).

Influenza Transmission to Humans

Avian virus

Avian virus

Avian virus

Reassortment in swine

Reassortment in humans

Human virus

Avian virus

Avian virus
Genetic Components of Triple-Reassortant Swine Influenza A (H1) Viruses Isolated from 11 Patients between December 2005 and February 2009 in the United States.

HIV
Where Genomics Guides
Epidemiological Investigation
Understanding Transmission
Interventions
Therapy
Female-to-male HIV transmission in HIV-discordant couples, by circumcision status in Rakai, Uganda

Gray et al. AIDS 2000
Interventions

• Detecting Acute HIV cases
• Circumcision
• Treatment to Prevent Transmission
ART, Serodiscordant Couples, and HIV Transmission: Study Results

- ART initiation substantially protected HIV-negative sexual partners from acquiring HIV infection
  - **Group 1**: Early treatment group—only 1 partner infected by the HIV-infected participant, with a 96% reduction in risk of HIV infection
  - **Group 2**: Late treatment group—27 partners infected by the HIV-positive participant
- The difference was highly statistically significant (P<0.0001)

Hepatitis C

- Genomics Guide Detection and Therapy
- Resistance is genomically defined (similar to HIV)
- Therapy strategies based on genomic testing
- Host susceptibility genomically defined
Nucleotide Changes, Result In Codon Changes That Can Confer Resistance To A Drug

Example: Codon 155 of the HCV Protease

Consensus “wild type” amino acid

Resistant variant amino acid
Lack Of Cross-Resistance Between Peg-IFN/RBV &/Or A Combination Of Antiviral Agents May Provide An Opportunity For Elimination Of Resistant Variants

<table>
<thead>
<tr>
<th>Target</th>
<th>Variant</th>
<th>NS3 Linear</th>
<th>NS3 Macrocyclic</th>
<th>NS5A inhibitor</th>
<th>NS5B nucleoside</th>
<th>NS5B Palm</th>
<th>NS5B Thumb</th>
<th>NS5B Finger</th>
<th>IFN</th>
<th>RBV</th>
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<tr>
<td>NS3 Protease</td>
<td>V36M</td>
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S = Susceptible
(< 4 fold shift in HCV replicon EC50)

R = Resistant
(>4 fold increase in EC50)
Patients With NS3 Inhibitor-Resistant Variants Can Respond To Peg-IFN/RBV

<table>
<thead>
<tr>
<th>Target</th>
<th>Variant</th>
<th>NS3 Linear</th>
<th>NS3 Macroyclic</th>
<th>NS5A inhibitor</th>
<th>NS5B nucleoside</th>
<th>NS5B Palm</th>
<th>NS5B Thumb</th>
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Patient with selected NS3 R155K variant achieved SVR with Peg-IFN/RBV

Initiation of Peg-IFN/RBV regimen

SVR

HCV RNA LOD (10 IU/mL)

HCV RNA (IU/mL)

Weeks

3/10/2010 FCHR-HCV DRAG www.hivforum.org

Unpublished data, example from TVR PROVE2 study
Figure 1. SNPs in IFN-λ gene cluster associated with HCV control. The IFN-λ gene cluster is shown in the top panel, indicating its position on chromosome 19. In the second panel, the positions of the relevant SNPs corresponding to the text and published data are indicated in relation to the IFN-λ gene cluster. IL28B is upstream and in reverse orientation compared with IL28A. The third panel depicts the genomic structure of IL28B, including its 5 exons, intervening introns, and flanking putative regulatory regions. Vertical lines denote the position of individual SNPs that are associated with HCV treatment response and are connected by hatched lines to the adjoining table. The only SNP that is in a coding region encodes.
These results indicate the involvement of the... This distribution of alleles could account for the high

**Figure 4.** Allele frequencies of the SNP rs12979860 among different ethnic populations. Thomas et al genotyped the rs12979860 SNP in 2371 subjects from 51 distinct populations. The frequency map shows the proportional prevalence of the C (associated with HCV clearance) and T (associated with persistence) alleles. People in East and Southeast Asia have the lowest frequency of the alleles associated with HCV persistence, people in Europe have intermediate incidence, and people in sub-Saharan Africa have the highest frequency. Adapted with permission from Macmillan Publishers Ltd: Nature, Thomas et al, © 2009.
The Human Microbiota

- Our adult bodies harbor ~10 times more microbial cells than human cells – a significant number of these species have not been successfully grown in culture.

- The “human genome” is an amalgam of human genes and the genes of our microbial partners.

- Our microbial partners carry out many metabolic reactions that are not encoded in the human genome and are necessary for health.

- A number of studies have suggested that various disease states are associated with microbial community disturbance.

- Without understanding the interactions between our human and microbial genomes, it is impossible to obtain a complete picture of our biology.
The NIH Human Microbiome Project

- Determining whether individuals share a core human microbiome
- Understanding whether changes in the human microbiome can be correlated with changes in human health
- Developing the new technological and bioinformatic tools needed to support these goals

NIH Human Microbiome Project is only one of several international efforts
Critical questions

- How do we acquire and maintain our microbial communities?

- How resilient is our microbiome in response to stress?

- Can we use this information to devise ways to intentionally manipulate our microbiome (probiotics, immunization) to promote health and/or to prevent or treat various diseases?

- How do genotype, environmental exposures, and physiological status affect microbiome composition?

KEY CONCEPT—MICROBIOLOGICAL COMMUNITY
16S rRNA Gene

- Highly Conserved Regions (99% Identity)
- Conserved Regions (93-95% Identity)
- Hypervariable Regions
Use of High-Throughput DNA Pyrosequencing for Pathogen Discovery

DNA-covered microbeads

Clinical specimen sequenced by chemiluminescence

Nucleic acid sequences obtained

Viral genome cloned

Virus propagated in tissue culture

Infection confirmed through serologic and molecular assays

Bacterial Vaginosis

- Ecologic disturbance of vaginal flora
- Not an STD
- Dx based on clinical criteria or gram stain
Vaginal Gram stain with fusobacillary forms
BV is characterized by 100-1000 fold increase in pathogenic bacteria. Lactobacilli concentrations decrease substantially.
Chronic Wounds Impact

- Wounds have direct medical cost impact ($25 Billion)
- Wounds have substantial indirect cost benefit
  - Self image
  - Economic impact on family members
  - Disability
- There is little evidence basis for care that is provided
- Most research is dressing directed
- Little pathogenesis research is being done—OPPORTUNITY!!
Infection in Chronic Wounds—Current state of art

Definitions of infection in Chronic Wounds
Colonization versus Infection
  Clinical—erythema, advancing border, purulent drainage, “you know it when you see it”

– Quantitative Culture– >100,000 CFU/Gm of tissue
Problems with Cultures

- Cultures take 24-48 hours to process
- Quantitative Cultures take longer
- Cultures are prone to overgrowth
- Are there molecular approaches?
JHU Wound Research Program objectives

- Describe prevalence of bacterial species in chronic wounds in a tertiary wound care clinic.
- Assess microbial burden by: qualitative culture, quantitative culture, and bacterial DNA (real-time polymerase chain reaction (RT-PCR))
- Compare microbial populations found at 2 different locations within a single chronic wound (standardize methodology).
- Preliminary investigation of DNA footprints of microbes in wound tissue
Prevalence of bacterial species by quant culture

- **MRSA (44.8%)**, *Pseudomonas aeruginosa (27.6%)*, Group B Streptococcus (27.6%).
Prevalence of bacterial species

- 97% of wounds cultured had at least one organism, 60% three or more
- MRSA (44.8%), *Pseudomonas aeruginosa* (27.6%), Group B Streptococcus (27.6%).
- 19/22 samples positive for MRSA had $\geq 10^5$ CFU/g organisms.
- 11/14 of negative qualitative results were positive on quantitative microbiology (78.6%, 95%CI 49.2%-95.3%).
Preliminary 16S DNA clone libraries suggest that wounds contain many more species of organisms than recovered by culture.

- Bacteroides ureolyticus
- Alcaligenes sp.
- Janthinobacterium sp.
- Stenotrophomonas sp.
- Pseudomonas fluorescens AE1
- Pseudomonas poae
- Morganella morganii
- Geobacillus sp.
- Yersenia aldovae KB8
- Porphyromonas uenonis
- Streptococcus agalactiae
- Uncultured Clostridium clone
- Actinobaculum massiliae
- Mobiluncus mulieris
- Staphylococcus aureus USA300

- 16S-based phylogenetic tree from a single chronic wound.
- Organisms labeled in red were not recovered by quantitative microbiology.
Independent Rarefaction Analysis of Unique Species from All Wounds

Number of Unique OTUs vs. Number of clones observed.
Community Analysis of Chronic Wound Bacteria Using 16S rRNA Gene-Based Pyrosequencing: Impact of Diabetes and Antibiotics on Chronic Wound Microbiota

Lance B. Price¹ *, Cindy M. Liu¹,², Johan H. Melendez³, Yelena M. Frankel³, David Engelthaler¹, Maliha Aziz¹, Jolene Bowers¹, Rogan Rattray¹, Jacques Ravel⁴, Chris Kingsley¹, Paul S. Keim¹,², Gerald S. Lazarus³, Jonathan M. Zenilman³
Conclusions from Metagenomics

- Microbial Diversity was significantly lower in those patients treated with antimicrobials
- High proportion of anaerobes and non cultivables
- Pyrosequencing validated RT/PCR and culture results—when latter were positive; i.e. was more sensitive
- Genomics data suggest that anaerobes are critically important and this may represent synergistic infections
- This is DNA only—Need to do RNA transcriptome and Host
Conclusions

• Genomics has impacted
  – Discovery of new pathogens
  – Detection
  – Understanding the epidemiology
  – Guiding therapy and interventions
  – Understanding resistance
  – Understanding host susceptibility
Conclusions

• Genomic Methods are rapidly replacing traditional microbiology

• “Cellphone Paradigm” in appropriate settings
Conclusions

• The Microbiome is an ecological concept that is leading to new understanding of infectious diseases based on “microbial community” concepts