Public Written Comments

Submitted to PCAST

February 28, 2013 to April 30, 2013

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From: Tom Gonzalez
Sent: Wednesday, March 20, 2013 3:51 PM
To: 'pcast@ostp.gov' [redacted]
Cc: Harold Johnson; David Henning; Darin Swartz
Subject: Lufkin PT visit to Washington - Request for Meeting with OSTP - PCAST

Dear Dr. Hartman and Dr. Ford

Greetings from Lufkin, Texas.

We are a global leader in the design and manufacture of high speed turbomachinery gearboxes and industrial low speed gearboxes. Lufkin Industries has been in operation since 1902 in our current location and has manufacturing facilities in Texas, New York, California, Alabama, Indianna and offices in over 40 countries.

We are key suppliers to many leading US OEM's such as Solar Turbines (Caterpillar), Dresser, Flowserve, GE Energy, GE Oil and Gas, Wier and International OEM's like Siemens, MAN, Mitsubishi, Hyundai, Atlas Copco, Alstom.

Among our design portfolio are hundreds of unique designs, with dozens more in our engineering department at any given time.

We would like the opportunity to introduce our company to the Executive Office of the President's PCAST/OSTP. We will be in Washington for meetings with the US Coast Guard on April 2-3 (DC) and April 4 (Baltimore). We have yet to firm our meeting times on the 2-3 so anytime on either day would be great.

We are including a brief summary on our division's projects and a portion of our slide show as an introduction.

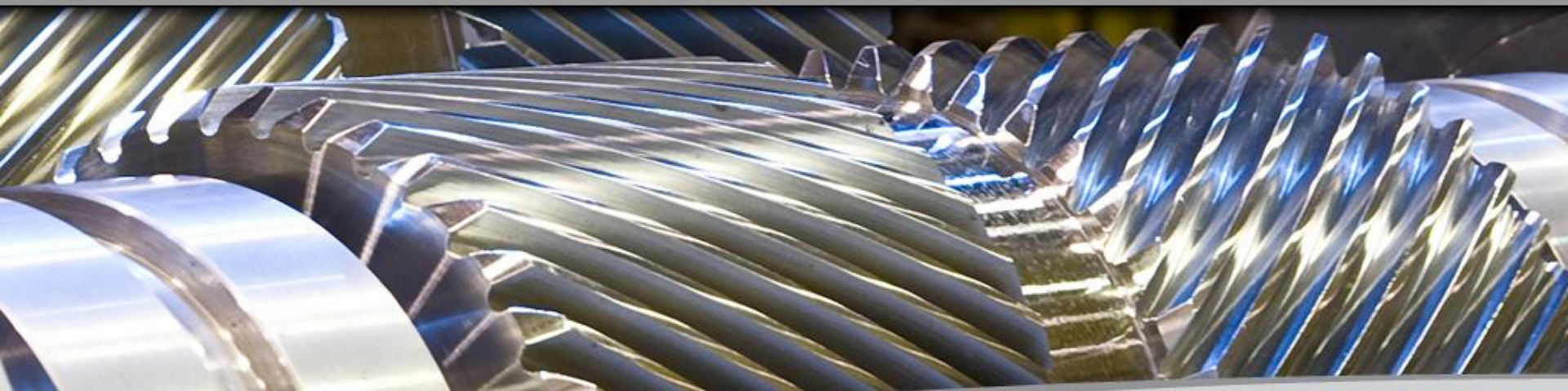
We appreciate your interest in our company and technology and look forward to meeting you in the near future.

Best regards,

Tomás A. González
Product Management
Power Transmission Division
Lufkin Industries, Inc.
[redacted]
LUFKIN

LUFKIN

The Energy Flows Through Us™



Office of Science and Techology Policy
Executive Office of the President of the United States

Lufkin Industries – Power Transmission Division Advanced Techology Strategic Development Plans

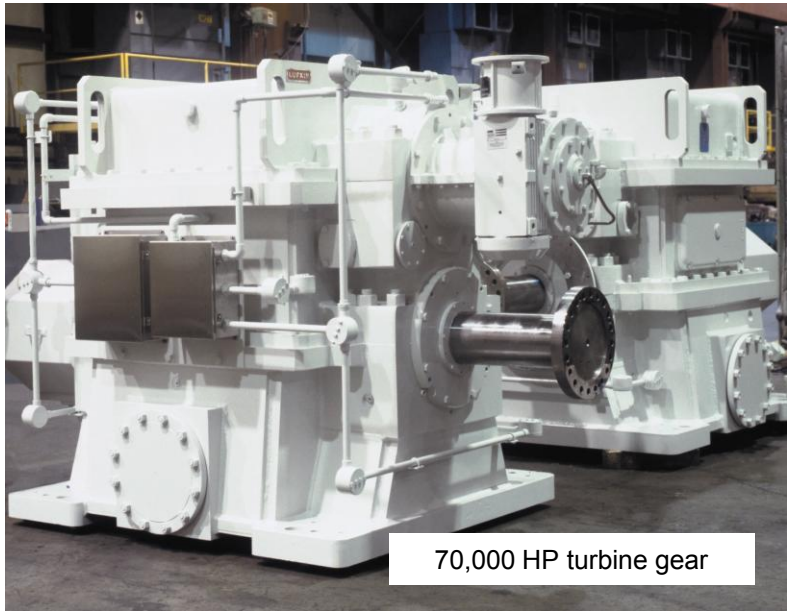
(INTRODUCTION TO SLIDE SHOW – SAMPLE SLIDES)

Power Technology Innovation

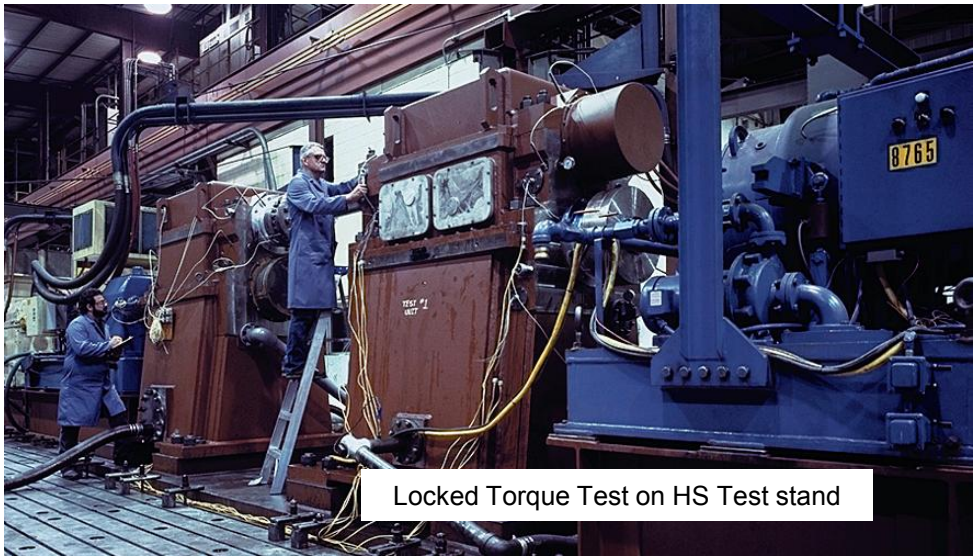
Lufkin Industries – Power Transmission Divison is an global industry leader in the design, manufacturing, testing, installation and support of industrial gearboxes for domestic and insternational markets in:

- Power Generation
- Hydrocarbon exploration, production, processing and transportation
- Industrial refining and processing equipment
- Compressors/Expanders
- Gas and Steam Turbines
- Pumps for industrial, flood control, mining, dredging, pipelines
- Steel industry
- Rubber and Plastics Industry
- Paper Industry
- Sugar Mills – Bio-Fuels
- Marine Transportation

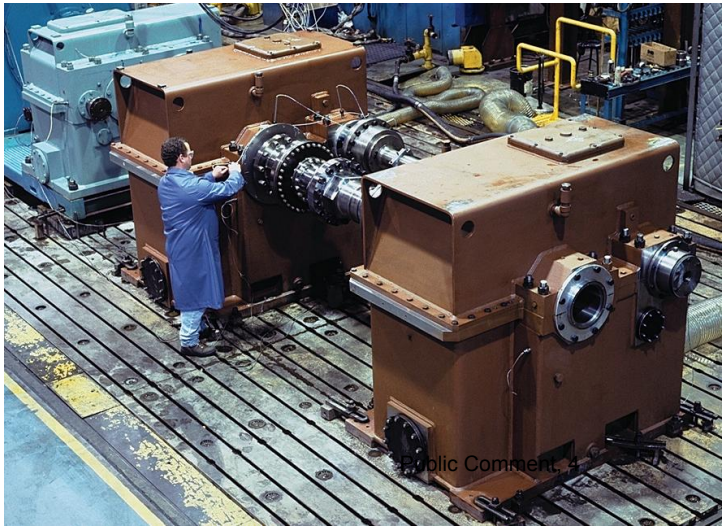
Power Generation



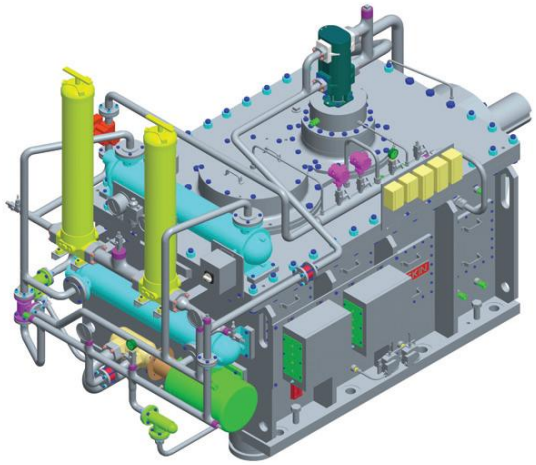
70,000 HP turbine gear

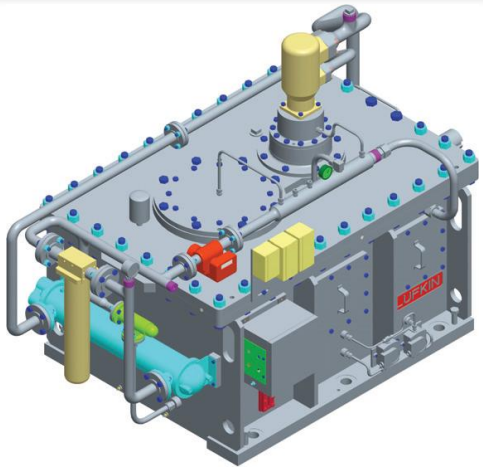


Locked Torque Test on HS Test stand



Flood Control Pump Drives





Custom gearboxes designed and built in Texas

New Orleans – West Closure Project
Eleven (11) Pumps each with capacity of 850,000 gallons per minute.

Summary of Benefits for US Economic Development

Lukin Power Transmission helps US and International customers meet the challenges of:

- Climate Change – carbon capture and sequestration compressors, injection well compressors, flood control infrastructure (New Orleans West Closure) .

- Sustainable Development – long lasting capital equipment with 20+ year service life, design for remanufacture and rebuild, energy efficiency.

- Clean energy -LNG technologies (liquefaction compressors, pipelines), bio-fuels, geo-thermal power, wind turbine repair and upgrade.

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Introduction – Lufkin PT

Lufkin Industries Power Transmission Divison (PT) is Texas based global leader in the design, manufacture, testing, installation, repair, remanufacturing and servicing of industrial gearboxes for a wide range of industrial applications.

Lufkin's technology for design and analysis, simulation, manufacture, inspection and testing of precision gears, bearings and gearbox allows us to be a key supplier to leading Original Equipment Manufacturers (OEM's) and end users.

Markets served include: power generation, oil & gas, refining, compressor/expanders, pumps, geo-thermal, carbon capture and sequestration (CCS), hydro thermal, offshore drilling (topdrives), steel production, rubber/plastic production, sugar/bio-fuels, flood control, wind/clean energy, geo-thermal, marine and test stands/R&D. As an industry leader Lufkin PT engineers participate in key global organizations such as ISO, AGMA, API and collaborate with academia on research as well as educational symposia (Turbomachinery Symposium). Please visit www.lufkin.com

This paper outlines the areas in which Lufkin's advanced manufacturing efforts are in line with the efforts of the Executive Office of the President – President's Council of Advisors on Science and Techology – Advanced Manufacturing Initiatives (PCAST AMI).

Abstact

Lufkin PT is engaged in several industrial and techological sectors that are part of the strategic national needs: energy, sustainability, international trade (exports), infrastructure to name a few. Advanced techologies include:

Advanced sensing, measurement, and process control (including high speed test stands)

Advanced material design and processing (proprietary steel chemistry and metallurgy)

Information technology for configuration, modeling, simulation, design, testing and PLM.

Sustainable manufacturing (repower, retrofit, remanufacturing)

Advanced manufacturing equipment (gear cutting, grinding, inspection, testing)

Advanced forming (near net forgings of ultra-clean steel and castings)

Lufkin PT gearboxes and components can be made with 100% locally sourced (US Made) materials.

Lufkin exports more than 50% of the unit manufactured. Lufkin is engaged in design collaborations with most of the leading global OEM's in the turbomachinery, energy, mining, infrastructure and oil& gas industries – thus making our Made in USA products a global leader.

There is significant alignment between the goals and mission of PCAST and Lufkin PT.

Goal: Expand Global Competiveness – Manufacture in the US

New Product Development

Lufkin PT's new product development activities are part of our day-to-day business activity. The average number of gearboxes of a given design and configuration manufactured at any given time is less than 2 units. Lufkin PT works closely with OEM's to make gearbox and power transmission systems tailored to specific applications. The Lufkin PT products are a critical component of the "package" manufacturedd by the OEM – be it a compressor, power generator, innovative enegy technology, geo thermal power, FPSO, pump, dredge, sugar mill, steel mill, rubber mill, ship or drawbridge. At any given moment our engineering department in engaged in the design of over 25-50 unique gearbox projects. We manufacture what is arguably a "key enabling technology" linking the prime movers (turbines, diesels, VSD's, electric motors) to the driven equipment. Some examples include:

Turbomachinery – is the group of machines that use rotating elements to tranfer energy between a rotor (impeller/pelton wheel/turbine) and a fluid (gas/water). There are open (windmills) and closed (compressors, hydro generators, gas turbines, steam turbines) turbomachines. An excellent resource on Turbomachinery is www.turbolab.tamu.edu Lufkin PT is a global leader in high speed gearbox design and manufacture for this important industrial sector. Some of the applications of turbomachinery that are in alignment with the PCAST initiatives include:

Sustainability – Lufkin PT has four remanufacturing facilities in the US (Lufkin, TX; Cullman, Al; Peru, IN; Bakersfield, CA) where gearboxes and machinery manufactured by most of the leading global OEM's is remanufactured to "like new – better than new" specifications. Over 75% of the equipment serviced/remanufactured is not manufactured by Lufkin. The processes developed in our Gear Repair division contribute to the recovery of residual value added in the "cores", conservation of energy and reduction of solid waste. Gear repair and remanufacturing is also a job creation activity whereby over 40% of the value added is direct labor. There are significant opportunities for growth and job creation in this important sector through the transfer of knowledge, technological improvements and process improvements.

Remediation – high energy processes and equipment play an important role in developing strategies to curb environmental impacts of industry and remediate past damage. The ongoing impact of industry can begin to be mitigated through the use of more emergy efficient processes and equipment, including closed loop systems that recapture waste heat or gas flows (using gas trubines), efficient gas compression/expansion, gas separation equipment, refrigeration, pumps, mixers, centrifuges and fans. Lufkin also makes gearboxes for high volume pumping systems, flood control, dredges, slurry pumps, pipelines. These applications are part of the critical industrial infrastructure of our nation.

Carbon Capture Sequestration (CCS) – Lufkin PT is working with several industrial partners in the design and testing of high volume compressor technology for CCS and gas injection systems.

Geo Thermal Energy uses highly engineered multi-stage gas driven machinery to convert the steam generated in deep injection wells into mechanical energy for compression or power generation. Lufkin is building equipment for this industry currently in field use.

Low Head Hydro is one of the market segments served by Lufkin through it's Lufkin France manufacturing facility. Lufkin could easily transfer this technology to the US for use in the

upgrade and refurbishment of existing or closed down hydroelectric facilities or new installations.

Flood Control – Lufkin PT designed and built the gearboxes for the pumping station at New Orlean's West Closure (Lake Pontchartrain) where eleven (11) pumps each rated for over 850,000 Gallons per minute are now in service. As climate change driven ocean level rises the investmetn in flood control infrastructure will be an important market and job creation opportunity.

Test Stands for Industrial and Military Applications – Lufkin PT has built test stands for high energy machines being tested at Wright Patterson Air Force Base (Dayton, OH), South West Research Institute SWRI (San Antonio, TX) and many other locations. The research and development of new energy sources or more energy efficient systems for current industrial techologies will be an important element in our national development strategy.

Wind energy – Lufkin PT Gear Repair has a dedicated remanufacturing facility for windmill gearboxes and main shafts in Lufkin, Texas.

Clean Energy – Lufkin gearboxes are key components in natural gas exploration, compression, transportation and liguefaction (LNG). Bio-fuels production from Sugar Cane is an important market for our line of TITAN sugar mill gearboxes (for crushing the cane).

Energy Conservation – ultra-efficient bearings built by Lufkin RMT for high speed applications in a wide range of turbomachinery. The specialized engineering involved in the design and manufacture of these key components is being updgraded on a continuous basis.

National Security – Lufkin PT built gearboxes for tanks dring WWII and for landing craft, we also supplied Marine Geaboxes for the US Coast Guard in the 60's and 70's many of which are still in service. We are currently bidding to supply the gearboxes for the new USCG Offshore Patrol Cutters and plan to expand our manufacturing and engineering capabilities to also serve the needs of the US Navy.

Advanced Technology Development Opportunities

The PCAST document: Report to the President – Capturing a Domestic Competitive Advantage in Advanced Manufacturing (July 2012), outlines the need to "identifying and nurturing the set of technologies that will have the greatest impact on the retention and future growth of manufacturing in the United States, enabiling differentiation and competitiveness for U.S. manufacturing from an end-to-end supply-chain perspective over a sustained period of time". (page 4)

To improve our competiveness in the global market and offer our customers more value (better designs, faster engineering and production) Lufkin is investing in human and capital resources. As outlined in the PCAST report a key constraint to growth is the lack of skilled labor, LPT collaborated with local universities (mainly Texas A&M) and local community colleges (Angelina College) to attract and train new engineers and improve the math/blueprint reading skills of its production technicians.

Gear manufacturing may not sound like a "high tech" industry but when you look at the work involved in the type of products Lufkin PT makes it becomes apparent that indeed it is a high tech process.

The analysis of each application and the design of the gear rotating elements to last 20+ years in full duty service is only the start of the process. Design of the bearings, lube systems, heat transfer and cooling, filtration and monitoring, instrumentation for temperature/pressure/vibration, installation criteria, coupling, seals and guards all play an important role in the final system design. Each unit is carefully designed to assure optimal energy efficiency, durability and customization to the application.

As an introduction to some areas of current and potential collaborative work with National Labs and Universities we cite:

Advanced sensing, measurement, and process control

Process sensing is critical in the production of precision gearing. Gear grinding machines have to be custom ordered to achieve the levels of quality ISO 4 or better that Lufkin makes on a day to day basis. The maintenance and calibration of equipment and training of operators is of vital importance. Tolerances of 0.0002" are common in our gearing. We propose to explore collaborative research opportnities with NIST and academia on the use of 3D scanning, imaging and inspection technology in this critical area of our manufacturing process.

Non-destructive testing and balancing of rotating elements are also critical sensing and measurement steps in our manufacturing process. The use of improved tools to enable higher levels of precision with reduced risk is a current area of research and improvement.

Quality processes such as dimensional verification, surface finish, electrical run-out, coating inspection require ever-more complex equipment, and trained operators. We are working on the use of portable coordinate measuring machines (CMM) and digital video scanners as part of our metrology/quality process.

Lufkin PT operates a high speed test stand with 2500HP and 1000HP motors which featuresand full instrumentation to monitotr acceleration, vibration, temperature and noise. Proposed enhancements to the test stand will include improved safety, rapid set-up and tear down fixtures, modular alignment and set up fixtures, improved digital sensing and data logging equipment.

Advanced material design and processing

Lufkin PT uses proprietary steel chemistry and metallurgy for its forgings, the ingots for the forgings are made exclusively by Ellwood Quality Steel (EQS) in New Castle, PA. LPT works closely with top US forging suppliers on the shaped forgings required for our products. Training of engineers and metallurgists, as well as the testing and inspection of materials and test samples is a priority area for improved competitivenss.

Processing of the machined parts for case hardening (carburizing) is a key technology in this industry. We do both in-house carburizing and work with leading external suppliers in Texas, PA and Chicago, Illinois for our carburizing. The equipment required for metallurgical analysis and material testing (tensile tests, fatigue testing) is one area where we will be looking to invest in to improve competitiveness.

Information technology

Lufkin PT uses advanced IT programs (Pro-E, Unigraphics) and ERP programs (SAP, Baan) and we have developed proprietary software for gearing design, application analysis, rotor dynamics and machine tool programming.

We are also investing in automated product configurator and design software, product life cycle management (PLM) software to equip and train the next generation of applications engineers/salesment and design teams.

Current development efforts include collecting and making available the extensive knowledge base of over 50 years of manufacturing and design experience is a challenge that can be met with new advance information technology tools – and technicians. Linking the manufacturing processes and shop floors with our engineering and quality department is another challenge for IT projects in our manufacturing operations (fully networked operations).

Sustainable manufacturing

Lufkin products are designed for service lives of 20 or more years, they are also designed for ease of service and remanufacture.

Opportunities for improvement in our gear repair operations include: knowledge management and data mining, improved inspection equipment (portable CMM, metals analysis), design of modular KITS for repower, retrofit and remanufacturing of equipent “in-situ” in the field.

Reverse engineering tools and better non-destructive testing equipment (vibration monitoring, thermal imaging, noise monitoring, x-ray).

Advanced manufacturing equipment

New and improved gear cutting, grinding and inspection equipment will be purchased to contiue our market leadership in the high speed gear market. We plan to improve our capabilities to return to the military marine gear markets which require larger (3 meter diameter +) high precision equipment. The naval gearing market is currently “owned” by the Germans, we intent to bring it back to the United States.

Advanced forming

LufkinPT is working with it’s suppliers on near net forgings of ultra-clean steel and precision castings. The investment in tooling and casting patterns for new products represents a significant capital investment and one which would benefit from incentives.

For futher information please contact:

Tomás González, Lufkin Power Transmission Division, Product Management

[redacted]

From: Clark Tibbs VHO-PVI-CTA [redacted]
Sent: Thursday, March 28, 2013 3:15 PM
To: DHS-AgDefense Jennifer Rinderknecht with Dr. Colby; DHS-AgDefense Dr. Michelle Colby DVM MS; Ford, Knatokie
Cc: FDA-CBER Marie Keller-Robbins - Dr. Midthun Asst.; FDA-CBER Lorrie McNeill for Dr. Midthun; FDA-CBER Dr. Karen Midthun; HHS-DR. NICOLE LURIE-BARDA via MARK MCKINNON; BARDA-HHS Dr. Nicole Lurie Asst Sec-Preparedness; BARDA - Jonathan Seals (HHS/ASPR/BARDA); BARDA-Dr. ROBIN ROBINSON; Dr. Eugene Davidson - CSO-PhageV [redacted] yril Gay-DVM-Ph.D.-Nat'lProgLeader-AnimalHealth; USDA-APHIS - Ms. Hallie Zimmers - Stakeholder Liaison; [redacted]
Subject: To DHS-S&T via PhageVax - Delivery of Genes & Proteins -Pg 9- Comparison of T4 Phage Head with -other- Platforms.pdf
Importance: High

28 March 2013
-
TO: Dr. Tara O'Toole, MD, MPH Ref: http://www.dhs.gov/tara-otoole
-and- Dr. Michelle Colby, DVM, MS via .....
Ms. Jennifer Rinderknecht, MPH - Support Contractor
Chemical and Biological Defense Division - Science & Technology Directorate - Department of Homeland Security (DHS)
[redacted]
-
CC: ... To other US Government officials who are involved in this globally important health matter.
-
..... as promised ..... as you all can see, the T4 Phage Head has very broad efficiencies. (see attachment)
-
The other attachment describes the current Coronavirus's "wide tissue susceptibility". And, H7 Flu is in Mexico killing chickens. ~ 1.5 Million+ so far killed by the Flu or culled by humans. Please remember the 2009 Novel H1N1 that jumped from swine to humans (in Mexico) ?
-
Compare even cell-culture Flu vaccine manufacture (in North Carolina) to the information on Page 9 of this document showing hundreds to thousands of doses of candidate vaccine within 3 hours with high yield [using only one (1) container]. Compare this to MVA that has drawbacks, as we all know.
-

PhageVax desires a simple CRADA to share the physical T4 Phage Head (via MTA) with one or more Government Labs to further develop the specific vaccines against Pandemic Influenza and Pandemic Coronavirus ... before it is too late.

-

If there is overlap of in-kind cooperation, then this is a good thing. We are not asking for the Tax-payer's money.

-

As you all examine this information, please call back to tell me what you do not understand about the T4 Phage Head properties ? [redacted]

-

Thank you and Regards .... we hope to hear from you soon.

-

-

Clark Tibbs, CEO
PhageVax, Inc. www.PhageVax.com
CAGE CODE: 4M4V6 www.sam.gov
Phone: [redacted]
[redacted] USA

Supporting Information

Tao et al. 10.1073/pnas.1300867110

SI Materials and Methods

Purification of *10*-amber*13*-amber *hoc*(highly antigenic outer capsid protein)-del*soc*(small outer capsid protein)-del Heads. The *10*-amber*13*-amber*hoc*-del*soc*-del mutant was constructed by standard genetic crosses. *Escherichia coli* P301 (sup-) cells (500 mL) infected with this mutant were lysed in 40 mL of Pi-Mg buffer (26 mM Na_2HPO_4/68 mM NaCl/22 mM KH_2PO_4/1 mM $MgSO_4$, pH 7.5) containing 10 μg/mL DNase I and chloroform (1 mL) and incubated at 37 °C for 30 min. The lysate was subjected to two low-speed (6,000 × *g* for 10 min) and high-speed (35,000 × *g* for 45 min) centrifugations, and the final heads pellet was resuspended in 200 μL of Tris·Mg buffer (10 mM Tris·HCl, pH 7.5/50 mM NaCl/5 mM $MgCl_2$) and purified by CsCl density gradient centrifugation. The major head band sedimented at about 1/3 from the bottom of a 5-mL gradient was extracted and dialyzed overnight against Tris·Mg buffer. The heads were further purified by DEAE-Sepharose chromatography (1). The peak heads fractions were concentrated and stored at −80 °C.

In Vitro DNA Packaging. In vitro DNA packaging assays were performed by the procedure described earlier (2). The reaction mixture contained purified Hoc⁻ Soc⁻ heads [or wild type (WT) heads where indicated] (~2×10^{10} particles), purified gp17 (~1.5 μM), and DNA (~300 ng) using a buffer containing 30 mM Tris·HCl (pH 7.5), 100 mM NaCl, 3 mM $MgCl_2$, and 1 mM ATP. The DNA was a linearized molecule produced by digestion with a restriction enzyme. Examples include the MluI-linearized 4.7-kb pEGFP-C1 plasmid containing eGFP expression cassette and the BamHI-linearized 6.2-kb psiCHECK2 plasmid containing luciferase expression cassette. In some experiments, either the PCR-amplified 2.3-kb DNA corresponding to the expression cassettes or the ~80-kb ligated plasmid concatemer were used as a packaging substrate. The packaging reactions were terminated by the addition of DNase I to digest the unpackaged DNA. The encapsidated and DNase I-resistant DNA was released by treatment with proteinase K and analyzed by agarose gel electrophoresis. The packaged DNA was quantified by Quantity One software (Bio-Rad). Each experiment included one to several negative controls that lacked one of the essential packaging components; heads, gp17, ATP, or DNA. Packaging efficiency is defined as the number of DNA molecules packaged per number of head particles used in the packaging reaction.

In Vitro Display on Hoc⁻ Soc⁻ T4 Heads. In vitro display of fusion proteins on T4 capsids was carried out as described (3, 4). After packaging luciferase and/or eGFP DNA as described above, the Hoc⁻Soc⁻ T4 heads (2–3 × 10^{10} particles) were incubated with Soc and/or Hoc fusion proteins in the same tube for 30 min at 4 °C for Soc fusions and 37 °C for Hoc fusions. For the display of DEC205 mAb, the Hoc- or Soc-fused GG domain and the mAb were simultaneously added to the reaction mixture. The ratio of fusion protein to the respective binding sites on the capsid was adjusted as indicated in the Fig. S3. The binding sites on the capsid are occupied by the fusion proteins, decorating the head with one protein or a combination of proteins included in the reaction mixture. The heads were spun down for 45 min at 34,000 × *g*, and the unbound proteins were removed by washing twice with 20 mM Tris·HCl (pH 8.0) and 100 mM NaCl. The head pellets were resuspended with Opti-MEM for transduction or with PBS (pH7.4) for SDS/PAGE analysis. The gels were stained with Coomassie blue R250 (Bio-Rad) and the protein bands were quantified by laser densitometry (PDSI, GE Healthcare). The density of Hoc, Soc, gp23*, and gp18 (major tail sheath protein; 70 kDa) bands were determined for each lane separately and the number of bound Hoc or Soc molecules per capsid was calculated using the known copy numbers of gp23* (930 copies per head) or gp18 (138 copies per phage). A saturation binding curve relating the number of bound Soc molecules per capsid (Y) and the concentration of unbound protein in the binding reaction (X) was not sigmoidal (e.g., Fig. S3*E*), indicating that there is no cooperativity between neighboring Hoc or Soc binding sites. The apparent K_d (association constant) and B_{max} (maximum copies of Soc bound per capsid) were determined using the equation $Y = B_{max}X/(K_d + X)$ as programmed in the GraphPad PRISM-4 software.

Gene Delivery by T4 Heads. Cells (2×10^5 cells per well for HEK293T and Hep3B, 1.5×10^5 cells per well for DC2.4) were seeded into a 24-well plate and incubated at 37 °C overnight in 5% (vol/vol) CO_2. The medium was then replaced with 500 μL of opti-MEM medium (Invitrogen) and 100 μL of engineered T4 heads (2×10^{10} heads for HEK293T or Hep3B cells or 1.5×10^{10} heads for DC2.4 cells or as indicated in figures) were directly added into each well. After incubation for 24 h, luciferase expression was quantified as per the standard protocol (Promega). No significant difference in the luciferase signal was observed when the medium was changed after only 2 h incubation. The cells were then washed with PBS and lysed by adding 160 μL per well of passive lysis buffer and shaking for 30 min at room temperature. A 20-μL aliquot of each lysate was transferred to a 96-well white plate and mixed with 100 μL of LARII buffer. The luminescence signal was detected by luminometer (Promega). In parallel, the same amount of cell lysate was analyzed for β-actin by Western blotting using β-actin antibody to confirm that the same number of cells was taken for each assay.

Protein Delivery by T4 Heads. β-galactosidase-Soc was incubated with Hoc⁻Soc⁻ T4 heads for 45 min at 4 °C. Hoc-T or Hoc-GG and DEC205 mAb were added to the same reaction tube and incubated for another 45 min at 4 °C. The heads were spun down for 45 min at 34,000 × *g*, and the unbound proteins were removed by washing twice with the buffer as described above. The head pellets were resuspended in opti-MEM for transduction or PBS for determination of β-galactosidase activity. For the latter, X-Gal was added to the tube and incubated for 10 min at room temperature. Delivery was carried out as described above and the β-galactosidase activity was visualized by microscopy at 3 or 24 h after adding heads by staining with X-Gal using the β-galactosidase staining kit (Sigma).

Single-Molecule Optical Tweezers DNA Packaging. Packaging by single packaging machines using optical tweezers packaging was performed according to the procedure described earlier (5, 6). Purified heads (4×10^9 particles) were mixed with purified gp17 (1 μM) and 125-bp "priming" DNA (0.44 μM) in the presence of 1 mM ATP-γ-S in a 10-μL reaction volume consisting of packaging buffer (50 mM Tris·HCl, pH 7.6/100 mM NaCl/5 mM $MgCl_2$). After incubation at 37 °C for 30 min, T4 phage antibody-coated polystyrene beads (1.5 μL) (0.79 μm in diameter, Spherotech) were added. The DNA beads were prepared by adding PCR-amplified 10-kb λ DNA biotinylated at one end to the Streptavidin coated polystyrene beads (0.86 μm in diameter, Spherotech) and incubating at 37 °C for 30 min. Measurements were taken using a calibrated dual-trap optical tweezers at 100 Hz in a "force–feedback" mode, where packaging was allowed to occur against a constant force of 5 pN. Tether formation and packaging was initiated by infusing 1 mM ATP into the flow cell. The contour length of DNA was

calculated from the measured force and extension between the beads using the worm-like chain model assuming a persistence length of 53 nm, a stretch modulus of 1,200 pN/nm, and distance per base pair of 0.34 nm. The velocity of DNA packaging was determined from a linear fit of the contour length of DNA over a sliding window of 0.1 s (10 data points).

Single-Molecule Fluorescence DNA Packaging. The single-molecule packaging of fluorescently labeled oligonucleotide was performed according to the basic procedure described earlier (1). The individual T4 packaging machines were immobilized through T4 phage antibody attached to the PEG surface-passivated coverslips. The unbound heads were washed off and ATP and 39 bp Cy5 DNA in the packaging buffer were flowed in [50 mM Tris·HCl buffer, pH 8.0/5% (wt/vol) PEG/5 mM $MgCl_2$/1 mM spermidine/1 mM putrescene/60 mM NaCl, and the oxygen scavenger system (0.8% dextrose/0.1 mg/mL glucose oxidase/0.02 mg/mL catalase, and 3 mM Trolox)]. Packaging of Cy5 DNA by single machines was imaged by a charged-coupled-device camera (iXon DV 887-BI; Andor Technology) at 100-ms time resolution.

Construction of Recombinant Plasmids. All of the cell penetration peptide (CPP) recombinant genes were amplified by two rounds of PCR. The first round of PCR was performed by fusing Hoc or Soc genes to a sequence containing the 12-aa linker and part of CPP (Fig. S3). The PCR products were used as a template for the second round of PCR using an end primer containing the rest of the CPP sequence and an appropriate restriction site. The resulting fragments containing restriction enzyme site-CPP-linker-Hoc/Soc-restriction enzyme site were purified by agarose gel electrophoresis, digested with appropriate restriction enzymes, and ligated with the gel-purified pET-28b vector DNA digested with the same restriction enzymes. Insertion of the Hoc-fused DNA fragment resulted in in-frame fusion with a 23-aa vector sequence containing a hexa-histidine sequence at the N terminus (Fig. S3). Insertion of the Soc-fused DNA fragment resulted in in-frame fusion with an 8-aa vector sequence containing a hexa-histidine sequence at the C terminus (Fig. S3). For the rest of recombinant constructions (GG domain, β-galactosidase; Figs. S6 and S8), Soc and Hoc were amplified individually with appropriate primers. The purified PCR products were digested with appropriate restriction enzymes. The three restriction-enzyme-digested fragments (for Hoc-GG: GG domain, pET-28b, and Hoc; for Soc-GG: GG domain, pET-28b, and Soc; for β-gal-Soc: β-galactosidase, pET-28b, and Soc) were directionally ligated to generate the appropriate fusion products. Insertion of the recombinant DNA resulted in in-frame fusion with a 23-aa vector sequence containing a hexa-histidine sequence at the N terminus and in the case of the Soc-fusions, a second hexa-histidine tag was also added to the C terminus of the recombinant. The ligated DNAs were transformed into *E. coli* XL10 Gold cells (Stratagene), miniprep plasmid DNAs were prepared from the transformants by alkaline lysis, and the sequence of each clone was confirmed by DNA sequencing (Retrogen). The recombinant DNAs were then transformed into the expression strain *E. coli* codon-plus BL21 (DE3) RIPL (Stratagene) for IPTG-induced overexpression of the recombinant proteins.

Purification of Recombinant Proteins. The recombinant proteins were purified according to the basic protocol described as follows. The BL21 (DE3) RIPL cells harboring the recombinant clones were induced with 1 mM IPTG for 2 h at 30 °C. The cells were harvested by centrifugation (4,000 × *g* for 15 min at 4 °C) and resuspended in 50 mL of HisTrap binding buffer (50 mM Tris·HCl, pH 8.0/20 mM imidazole/300 mM NaCl). The cells were lysed using French-press (Aminco) and the soluble fraction containing the His-tagged fusion protein was isolated by centrifugation at 34,000 × *g* for 20 min. The supernatant was loaded onto a HisTrap column (GE Healthcare) and washed with 50 mM imidazole containing buffer, and the protein was eluted with 20–500 mM linear imidazole gradient. The peak fractions were concentrated and purified by size exclusion chromatography using Hi-Load 16/60 Superdex-200 (prep-grade) gel filtration column (GE Healthcare) in a buffer containing 20 mM Tris·HCl (pH 8.0) and 100 mM NaCl. The peak fractions were concentrated and stored at −80 °C.

Purification of DEC205 monoclonal antibody (mAb). The hybridoma cell line HB-290 which produces rat IgG2a monoclonal antibody against DEC-205 was obtained from ATCC and grown at 37 °C in 5% (vol/vol) CO_2 with RPMI1640 supplemented with 10% (vol/vol) FBS. The mAb was purified from the supernatant by affinity chromatography on a Protein G column (GE Healthcare). The cell culture medium was centrifuged at low speed to remove the cell debris and the buffer composition of supernatant was adjusted to 20 mM sodium phosphate (pH 7) by passing through Tangential Flow Filtration System (Pall Corporation). The samples were then load onto the Protein G column and the mAbs were eluted with 0.1 M Glycine·HCl (pH 2.8). The mAb fractions were collected in tubes containing 80 μL of 1 M Tris·HCl (pH 9) to neutralize the eluate and preserve the function of mAb.

Adeno-Associated Virus (AAV) Vector Production. AAV serotype DJ (Cell Biolabs), engineered by DNA family shuffling to create a hybrid capsid from eight different native serotypes was used in current study (7). AAV-DJ vectors exhibit significantly higher infectivity rates compared with native serotypes across a broad range of tissue and cell types. The firefly luciferase gene was cloned into the AAV vector and the luciferase-AAV was produced by co-transfection into HEK293T cells using three-plasmids (Cell Biolabs). Seventy hours after transfection, AAV was collected and purified according to the manufacturer's instructions. The AAV titer was determined by QuickTiter AAV Quantitation kit (Cell Biolabs).

Indirect Immunofluorescence Microscopy. Cells (4×10^5 cells per well for HEK293T and 3×10^5 cells per well for DC2.4) were seeded into two-chamber slides and incubated at 37 °C overnight in 5% (vol/vol) CO_2 incubator. Twenty four hours after transduction, the cells were washed with PBS (pH7.4), and stained with X-Gal to check the β-galactosidase activity as described above. The cells were then further fixed with 4% (vol/vol) paraformaldehyde and permeabilized with 0.1% Surfact-Amps X-100 (Thermo). After incubation with goat anti-luciferase primary antibody (1:1,000 dilution, Promega) or mouse anti-GFP primary antibody (1:2,000 dilution, Invitrogen) for 1 h at 37 °C, the cells were probed with rhodamine-labeled rabbit anti-goat second antibody (1:1,000 dilution, KPL) or FITC-labeled rabbit anti-mouse second antibody (1:2,000 dilution, Jackson ImmunoResearch), respectively. The cells were imaged by an inverted AX10 Observer D1 microscope (Carl Zeiss), and images of cells that exhibited β-galactosidase activity, eGFP fluorescence, and rhodamine fluorescence were acquired sequentially and analyzed with Axiovision software.

ELISA. Each well of a 96-well plate was coated with 0.1 μg of protein diluted in coating buffer (0.05 M sodium carbonate-sodium bicarbonate, pH 9.6) overnight at 4 °C. The plates were then blocked with 3% (wt/vol) BSA in PBS (pH 7.4) for 1 h at 37 °C. Equal volumes of the serum samples were serially diluted with dilution buffer (PBS, pH 7.4, with 1% BSA) and added into each well. After 1-h incubation at 37 °C, plates were washed with washing buffer PBS-T (PBS with 0.1% Tween 20, pH 7.4). Sheep anti-mouse IgG-HRP (Invitrogen) diluted 1:2,000 in dilution buffer was used as second antibody. After incubation with second antibody for 1 h at 37 °C, TMB Microwell Peroxidase Substrate System (KPL) was used for color development and the

reaction was stopped by adding TMB BlueSTOP (KPL) solution. The OD values at a wavelength of 650 nm were read by ELISA reader (VERSA max, Molecular Devices).

Elispot. A total of 55 5- to 6-week-old Balb/cJ mice were vaccinated by the intramuscular route (i.m.) with phage T4 head particles containing F1-V DNA and or plrotein. At 21 d, spleens from three mice in each vaccinated group were harvested and splenocytes were isolated from each animal and pooled for each group. Splenocytes (10^6 per mL) were plated in triplicate and stimulated with 5 μg/mL of F1-V, recombinant mouse IFN-γ, or media, and incubated for 24 h at 37 °C. The mouse IFN-γ ELIspot kit (R&D Systems) was used to determine the relative number of IFN-γ-expressing cells in the single-cell spleen suspensions following the manufacturer's instructions. The Immunospot Series 1 Analyzer Elispot Reader (Cellular Technology), was used to quantify the number of spot forming cells per well.

Live Animal Imaging Series. About 2–5 × 10^{11} head particles were injected into each Balb/cJ mice by the intramuscular route (i.m.). After 6 h, 10 h, 16 h, 30 h, 50 h, 14 d, and 30 d postinjection, mice were injected intraperitoneally (i.p.) with 30 μg of RediJect D-Luciferin Ultra (Perkin-Elmer) and, after 5 min, mice were subjected to in vivo imaging using an IVIS 200 bioluminescent and fluorescence whole-body imaging workstation (Caliper) after lightly anesthetizing the animals under isofluorane. The bioluminescent scale is provided within the figures and it ranges from most intense (red) to least intense (violet) scaled based on radiance intensity.

Statistical Analyses. Results are expressed as mean ± SD. Statistical comparisons between two groups were evaluated by Student *t* test and corrected by ANOVA for multiple comparisons. A value of $P < 0.05$ was considered to indicate statistical significance.

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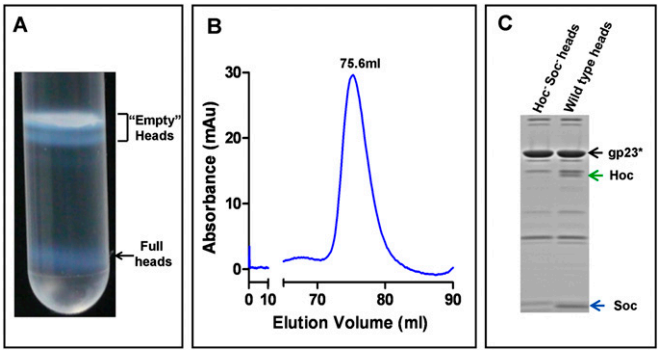

Fig. S1. Purification of Hoc⁻Soc⁻ heads. (*A*) Separation of Hoc⁻Soc⁻ heads by CsCl gradient centrifugation. The heads were prepared as described in *Materials and Methods* and passed through a step gradient of CsCl (0.26 mg/mL at the top layer and 0.93 mg/mL at the bottom layer) and centrifuged at 40,000 rpm using SW55Ti rotor for 60 min. The "empty" Hoc⁻Soc⁻heads (which retained ~8 kb DNA piece inside; see text) were extracted from the gradient and dialyzed. (*B*) The heads were further purified by binding to DEAE-Sepharose ion-exchange chromatography (AKTA prime, GE Healthcare). The bound heads were eluted with a NaCl gradient, and the heads were eluted at about 200 mM NaCl. The blue line shows the UV absorbance. (*C*) SDS/PAGE pattern of purified Hoc⁻Soc⁻ heads compared with the wild-type (WT) heads, which shows Hoc and Soc protein bands indicated by green and blue arrows, respectively. The position of the major capsid protein gp23* is shown by the black arrow.

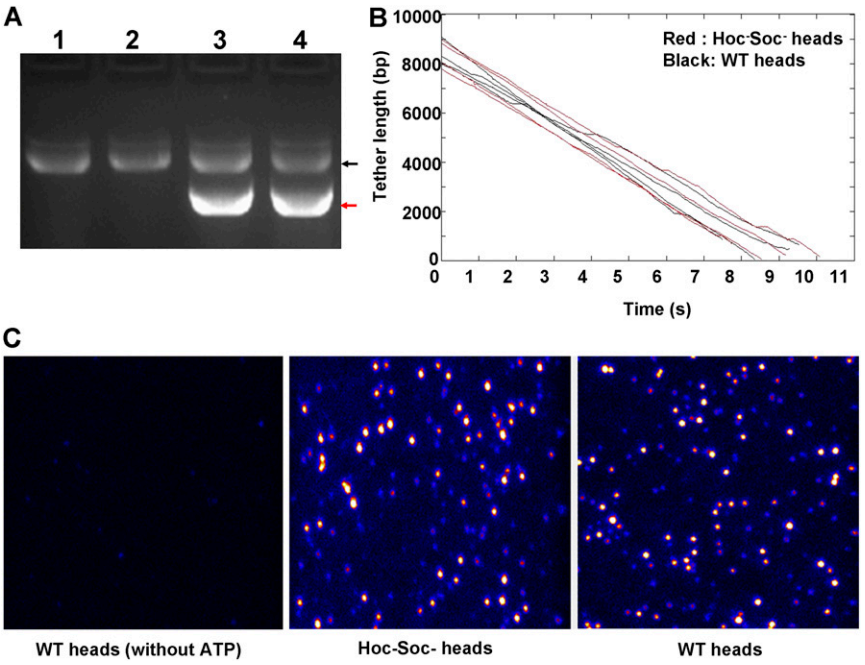

Fig. S2. Hoc^{-}Soc^{-}heads exhibited the same level of DNA packaging activity as the WT heads. (*A*) Bulk in vitro DNA packaging assays were performed according to the procedure described in *Materials and Methods*. The packaging reactions contained 2×10^{10} head particles, 1.5 μM gp17, and 300 ng of 4.3-kb MluI-linearized pEGFP-C1 plasmid DNA. Lanes: 1, no DNA; 2, no gp17; 3, WT heads; 4, Hoc^{-}Soc^{-}heads. Note that the heads retained an ~8-kb DNA, marked with a black arrow, which served as an internal control for the amount of heads in each lane. The red arrow shows the packaged DNA. (*B*) Single-molecule optical tweezers packaging assays were performed according to the procedure described in *Materials and Methods*. The DNA tether length decreases as the packaging machine reels DNA into the capsid with time. A representative set of traces using either Hoc^{-}Soc^{-}heads (red) or WT heads (black) are shown. The packaging velocities are in the range of 700–900 bp/s for both the Hoc^{-}Soc^{-} heads and WT heads. (*C*) Single-molecule packaging of fluorescently labeled 39-bp Cy5 oligonucleotide. See *Materials and Methods* for details.

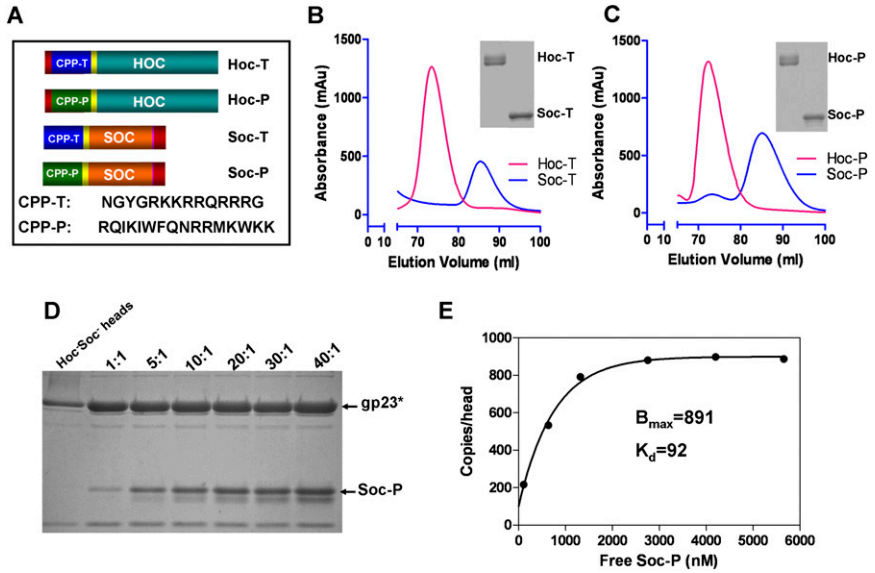

Fig. S3. Construction, purification, and display of recombinant CPPs. (*A*) Schematic of CPP recombinants. DNA corresponding to CPP-T (blue) and CPP-P (green) were fused to Hoc (cyan) and Soc (orange), respectively, via a 12-aa (GGSGGGSGGSGGS) linker (yellow). A hexa-histidine tag (red) was fused to the N terminus of Hoc or the C terminus of Soc. The sequences of CPP-T and CPP-P are shown under the schematics. (*B* and *C*) Purification of CPP recombinants. The recombinant proteins were overexpressed in *E. coli* RIPL cells and purified from the lysates by nickel affinity chromatography using HisTrap column (AKTA prime, GE Healthcare) followed by Hi-load 16/60 Superdex 200 gel filtration chromatography (AKTA FPLC, GE Healthcare). The blue and red profiles correspond to the elution of Soc and Hoc recombinants, respectively. The Hoc-T and Soc-T (*B*), and Hoc-P and Soc-P (*C*) proteins were collected and used for display on Hoc^- Soc^- heads. (*B Inset* and *C Inset*) SDS/PAGE of purified proteins. (*D*) Display of CPP on T4 heads. About 3×10^{10} Hoc^- Soc^- heads were incubated with increasing ratios of Soc-P molecules to Soc-binding sites on the capsid (1:1–40:1 as indicated on the top of the panel) and display was carried out as described in *Materials and Methods*. After washing off the unbound protein, the samples were electrophoresed on a 12% (wt/vol) SDS/PAGE. The positions of the major capsid protein gp23* and the Soc-P protein are marked with arrows. Similar results were obtained when the experiment was carried out with Hoc-P, Soc-T, and Hoc-T proteins. (*E*) The saturation binding curve for Soc-P. The Soc-P protein bands from *D* were quantified by laser densitometry (PDSI, GE Healthcare). The density volumes of Soc-P and gp23* (major capsid protein) bands were determined for each lane, and the number of Soc-P molecules per capsid was calculated using the known copy number of gp23* (930 copies per head). The apparent K_d (association constant) and B_{max} (maximum copies of Soc-P bound per capsid) were determined using the equation $Y = B_{max}X/(K_d + X)$ as programmed in the GraphPad PRISM-4 software (GraphPad Software). X refers to the concentration of free Soc-P, and Y refers to the copy number of bound Soc-P per capsid. The B_{max} and K_d values indicate that the capsid was saturated with Soc-P and Soc's affinity to the capsid was not significantly changed by fusion with CPPs.

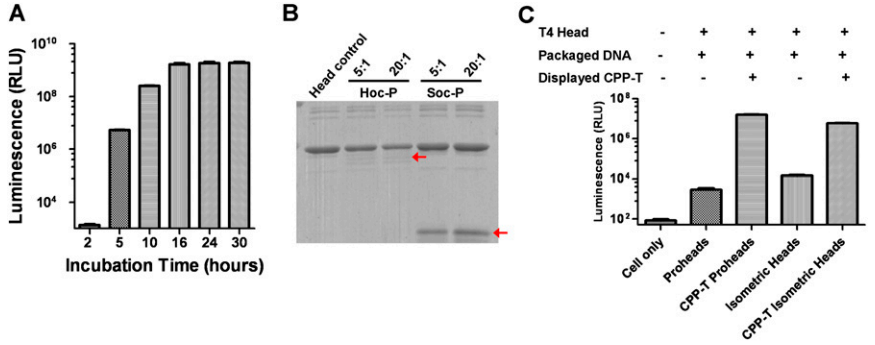

Fig. S4. (*A*) The time course of T4 gene delivery. At 2, 5, 10, 16, 24, and 30 h after addition of T4 heads packaged with luciferase gene to HEK293T cells, luciferase activity was determined as described in *Materials and Methods*. Luciferase activity could be detected at 2 h, dramatically increased by 5 h, and reached a maximum at 16 h. (*B*) Controlling the copy number of capsid-bound Hoc-P and Soc-P. The copy number of capsid-bound Hoc-P and Soc-P was controlled by changing the ratio of Hoc-P or Soc-P molecules to capsid binding sites, either 5:1 or 20:1, in the binding reaction. The copy number of Soc-P on the heads was ~580 per capsid in the 5:1 sample and ~870 per capsid (complete occupation) in the 20:1 sample. (*C*) Procapsids as well as isometric phage heads delivered DNA into mammalian cells. About 2×10^{10} T4 proheads (procapsids) or isometric heads produced from a petite mutant (*23*pt21-34c-*10*am*13*am-*hoc*del-*soc*del) were packaged with luciferase plasmid DNA and decorated with Hoc-T and/or Soc-T. These heads were used for transduction into HEK293T cells.

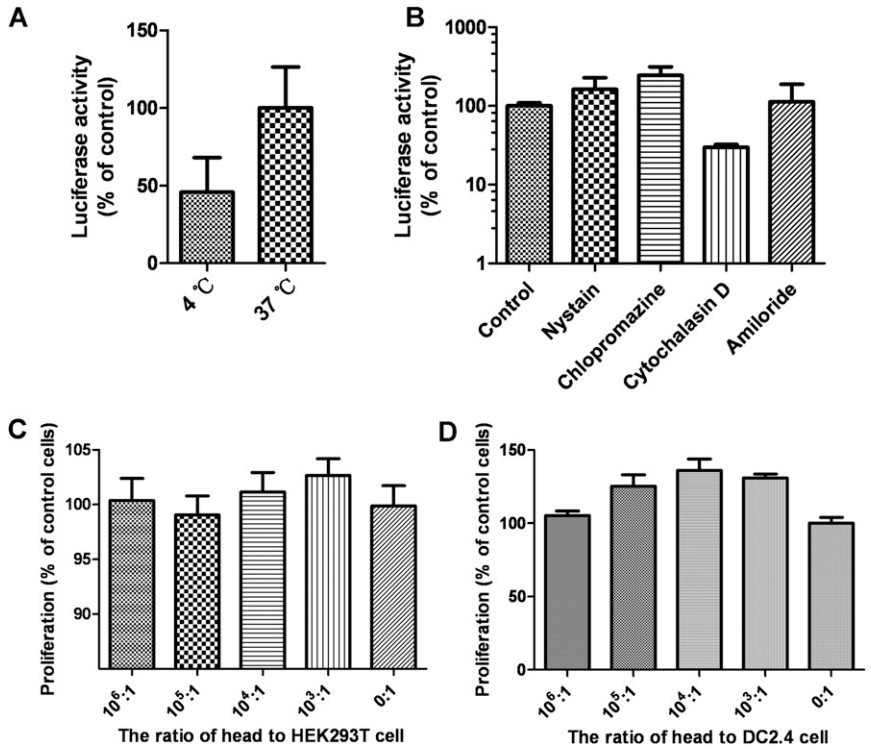

Fig. S5. Characteristics of T4 head-mediated gene delivery. (*A*) Effect of temperature. Transduction was done using the basic procedure described in *Materials and Methods* either at 4 °C or 37 °C for 2 h. Soc-T decorated Hoc$^-$ Soc$^-$ heads (2×10^{10} heads per assay) packaged with luciferase DNA inside were used for transduction. Luciferase signal was ~2-fold lower at 4 °C suggesting that the CPP-mediated transduction is energy dependent, consistent with endocytosis. (*B*) Effect of inhibitors. Thirty min before transduction, cells were washed and incubated with 500 μL of fresh Opti-MEM containing different inhibitors: nystain (inhibitor of caveolin-dependent endocytosis), chlorpromazine (inhibitor of clathrin-mediated endocytosis), cytochalasin D (actin polymerization inhibitor), amiloride (macropinocytosis inhibitor). Only cytochalasin D showed about 20% luciferase signal of control (without any inhibitors). None of the other compounds inhibited T4 mediated gene delivery suggesting that transduction by T4 heads might be through an endocytotic pathway that is independent of clathrin or caveolin but might require actin polymerization. (*C* and *D*) Proliferation of HEK293T (*C*) and DC2.4 (*D*) cells treated with different amounts of T4 heads. Twelve hours after seeding (~0.5 or 1×10^4 cells per 96-well for DC2.4 and HEK293T cells, respectively), different amounts of T4 heads were added to cells and incubated for another 24 h. Cell proliferation assays were carried out according to instructions in the manual of CellTiter-Glo Luminescent Cell Viability Assay (Promega).

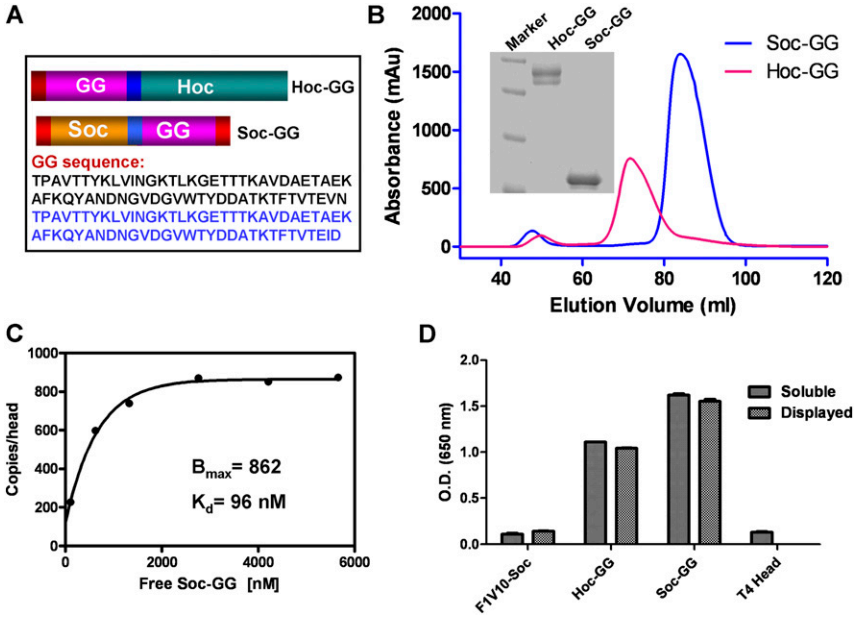

Fig. S6. Display of IgG molecules on Hoc$^-$Soc$^-$ heads through GG domain bridge. (*A*) Schematic of Hoc-GG and Soc-GG recombinants. The IgG-binding domain from the protein G (purple) was fused to Hoc (cyan) and Soc (orange), respectively, via a 2-aa (LE for Hoc fusion, GS for Soc fusion) linker (blue). Hexa-histidine tag (red) was attached to the N terminus of Hoc-fusions and to both N and C termini of Soc-fusions. The sequence of the duplicated GG domain is shown under the schematics. (*B*) The recombinant proteins were expressed in *E.coli* RIPL cells and purified from the supernatant by nickel affinity chromatography using HisTrap column followed by gel filtration using Hi-load 16/60 Superdex 200 column. Elution profiles of Hoc-GG and Soc-GG proteins from the Superdex column, shown in red and blue lines, respectively. The major peaks corresponding to Hoc-GG and Soc-GG were collected and used for display. (*Inset*) The purity of Hoc-GG and Soc-GG proteins as analyzed by SDS/PAGE. "Marker" lane shows the molecular weight (MW) standards. (*C*) Saturation curve of Soc-GG binding to Hoc$^-$Soc$^-$ heads. The apparent K_d (association constant) and B_{max} (maximum copies of Soc-GG bound per capsid) were determined as described in the legend for supplementary Fig. 3. The K_d and B_{max} values did not significantly change upon fusion of GG domain to Soc. (*D*) Display of IgG molecules on GG-heads was tested by ELISA. Hoc$^-$ Soc$^-$ T4 heads (2×10^{10} particles per well) were incubated with Hoc-GG or Soc-GG. After removing the unbound proteins, the Hoc-GG or Soc-GG decorated heads as well as soluble Soc-GG (0.8 μg per well) and soluble Hoc-GG (0.3 μg per well) were coated on a 96-well ELISA plate. T4 displayed *Y. pestis* F1-V10-Soc fusion was used as another control. After blocking with blocking buffer, HRP-labeled rat IgG antibodies were added to each well and incubated for 1 h at 37 °C. After removing the unbound IgG by washing three times with PBS-T, the amount of bound IgG was determined by adding the HRP substrate, TMB (3, 3′, 5, 5′-Tetramethylbenzidine), to the wells and the absorbance was recorded by an ELISA reader.

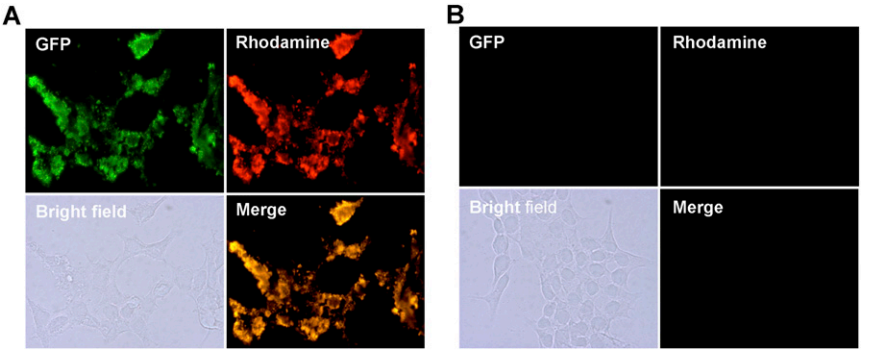

Fig. S7. T4 heads simultaneously deliver two genes into the same cell. About 2×10^{10} T4 heads packaged with luciferase and GFP plasmid DNAs and decorated with Hoc-T (*A*) or without Hoc-T (*B*) were used for transduction into HEK293T cells. Twenty-four h after transduction, cells were fixed and permeabilized. After 1 h incubation with blocking buffer (Sigma), the expression of GFP and luciferase was detected by staining the cells with goat anti-luciferase and mouse anti-GFP antibodies, respectively. Unbound antibody was washed off and the samples were incubated with rhodamine-labeled rabbit anti-goat second antibody, or FITC labeled rabbit anti-mouse second antibody. The cells were imaged using an inverted AX10 Observer D1 microscope (Carl Zeiss).

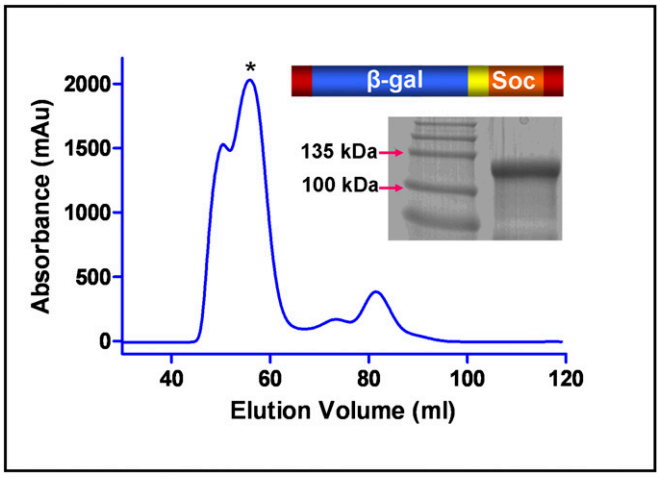

Fig. S8. Recombinant construction and purification of tetrameric β-galactosidase-Soc fusion protein. (*Inset*) A schematic of β-galactosidase–Soc fusion construct with a 4-aa (QASA) linker (yellow). Hexa-histidine tag (red) was attached to both the N and C termini of Soc. The recombinant protein was overexpressed in *E. coli* RIPL cells and purified from the supernatant by nickel affinity chromatography using HisTrap column followed by gel filtration using Hi-load 16/60 Superdex 200 gel filtration column. Purification profile of β-galactosidase-Soc shows that the protein eluted mainly as a 500-kDa tetramer (peak labeled with *), consistent with the fact that the native β-galactosidase oligomerizes into a tetramer. (*Inset*) The purity of β-galactosidase-Soc (second lane on right) as determined by SDS/PAGE. The first lane shows MW standards.

Table S1. Comparison of T4 head delivery with other delivery platforms

Vector/platform	Cargo	Capacity (max)	Targeting*	Tropism†	Safety concerns	Vector preparation	Source
T4 DNA packaging machine	Both DNAs and proteins	~170 kb DNA and 1,025 protein molecules per head	Yes, customized to specific cell-type	None	Nontoxic, all components are natural biomaterials	Pure system; defined in vitro assembly; takes 3 h; high yield	This study
Lipofectamine	DNA only (useful only in vitro)	Broad range	No	—	Synthetic material, cytotoxic	Commercially synthesized	1, 2
Adeno-associated viral vector	DNA only	4.5 kb	No‡	Yes (broad)	Oncogenesis	Needs packaging cell line; takes 3–4 d; poor yield.	3, 4
Retrovial/lentiviralvector	DNA only	8–10 kb	No‡	Yes (broad)	Oncogenesis	Needs packaging cell line; takes 3–4 d; poor yield	3, 4
Adenoviral vector	DNA only	28 kb	No‡	Yes (broad)	Oncogenesis	Needs packaging cell line; takes 2–3 wk; poor yield	3, 5
CPP-based protein delivery	Protein only	1	No	—	No	Recombinant fusion	6, 7
Gold nanoparticle- based protein delivery	Protein only	<100 per particle	No	—	Unknown	In vitro assembly	8

*Delivery to specific cells.

†Natural specificity of a virus to host cells as determined by the interaction of viral surface ligands with receptors on the host cell surface.

‡It is possible to retarget to specific cells in a limited way by attaching a short peptide ligand to the capsid protein, but the yields are low and the virus has to overcome its own broad cell tropism, which is very difficult.

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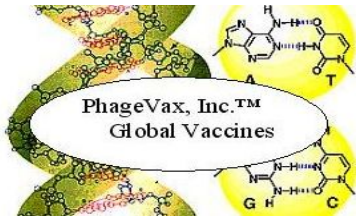

27 March 2013
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Ms. Zimmer ... as we discussed. See the Zoonotic connections, below. Thus the need for a vaccine !
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Clark Tibbs PhageVax, Inc. [redacted]
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FROM:
http://www.cidrap.umn.edu:80/cidrap/content/other/sars/news/mar2713corona.html

Novel coronavirus lab studies hint at wide tissue susceptibility

Lisa Schnirring Staff Writer

Mar 27, 2013 (CIDRAP News) – Experiments by Hong Kong researchers to gauge the susceptibility of several human and animal cell lines to novel coronavirus (NCoV) found signs that it can infect a broad range of tissues, which might shed light on the disease's **seemingly high mortality rate.**

Though many questions remain about the source of the new virus and how it spreads, health officials know that it can cause severe clinical illness in some patients, including severe pneumonia and renal failure, the group wrote. They added that until more is known about the disease, lab studies could help provide clues.

The tests they conducted with NCoV and the cell lines are surrogates of virus growth in the tissues. They measured viral load in the cultures, nucleoprotein expression, and cytopathic effect. They published their findings yesterday in an early online edition of the *Journal of Infectious Diseases (JID).*

The group used an autopsy NCoV virus sample obtained from the first known case-patient, a Saudi Arabian man who died from the disease in June. They used 27 cell lines from different tissues and organs in their susceptibility tests, 14 from humans and 13 from animals.

Tests suggested that NCoV can infect **human respiratory, kidney, and liver cells, as well as histiocytes**. The impact on neuronal cells and monocytes was much less.

Researchers suggested that the range of human tissues that are susceptible to infection appears to be **broader than all other human coronaviruses,** including the SARS (severe acute respiratory

syndrome) virus. The team said this finding might help explain the disease's pathogenesis and **high mortality.**

Human upper-airway cells did not seem to support the growth of the novel virus, which the investigators said isn't surprising, given that many of the lab-confirmed cases had severe acute pneumonia. However, more data on infected patients is needed to determine if the clinical pattern is similar to SARS, in which the virus was also shed in the upper airways, which played a role in human-to-human transmission.

More studies are needed to explore the histopathologic changes in the kidneys of patients infected with NCoV, the group wrote. They also noted that research is needed to determine the viral load in patients' urine specimens, because **acute renal failure** might be a mortality risk factor for NCoV, as it was for SARS.

Experiments on the animal cell lines suggested that NCoV can infect cells from **non-human primates, pigs, civets, and rabbits**, results that they said add to earlier findings showing the virus can infect bat cells. They wrote that their findings strengthen the suspicion that the virus **can jump interspecies barriers.**

"The presence of a receptor utilized by the virus which is common in **bats, primates, pigs, civets, rabbits, and humans** might imply **a broad species tropism,** which is unique among all the currently known human coronaviruses," the group wrote.

In an editorial in the same issue of *JID*, Kenneth McIntosh, MD, with the division of infectious Diseases at Boston Children's Hospital, wrote that aside from extensive genetic sequence data and a report on receptor usage in tissue culture, little has been published on the biology or pathogenicity of NCoV in humans or animals.

McIntosh noted that in the current study infectious virus wasn't measured in any of the cell lines and that evidence of viral replication was measured more indirectly, based on detection of viral RNA or viral nucleoprotein.

Though virologists have used in vitro tests to guide them in what cells to tests for in vitro growth and understanding more about pathogenesis, he wrote that there are several examples, including those involving coronaviruses, in which viruses didn't behave as expected in the organisms.

Filling knowledge gaps about NCoV based on in vitro tests is tempting, but difficult and risky, McIntosh wrote. However, he added that the Hong Kong group's work is useful for showing wide tissue tropism in the lab.

ECDC airs geographic considerations

In other developments, the European Centre for Disease Control and Prevention (ECDC) updated its epidemiologic assessment today, as four new cases have been reported since its last report on Feb 22.

It noted that three of the four cases were reported by Saudi Arabia's health ministry, with the last one reported on Mar 25 by Germany's Robert Koch Institute, the 73-year-old man from the United Arab Emirates who died in a Munich hospital yesterday.

The ECDC noted that six case-patients so far have been diagnosed and treated in Europe, three of whom were transferred from Arabian Peninsula countries where they were infected.

Though the number of NCoV infections increased over the past month, most cases continue to have links to the Arabian Peninsula, where contact tracing and epidemiologic investigations are under way, the group wrote.

Also, the ECDC issued a separate document that addresses the World Health Organization's (WHO's) NCoV surveillance update, which was published on Mar 18. Though the latest version from the WHO is more comprehensive, the ECDC took issue with one of the testing recommendations.

It noted that for testing, the WHO no long distinguishes between countries in which unexplained sporadic cases have occurred and those, such as in Europe, that have detected **imported cases** or infections linked to imported cases.

It said the November version of the WHO guidance specifically referenced countries in the Arabian Peninsula and their neighbors. However, the ECDC said it and the US Centers for Disease Control and Prevention (CDC) are retaining that recommendation. The CDC lists the countries considered in the Arabian Peninsula and neighboring areas as **Bahrain, Iraq, Iran, Israel, Jordan, Kuwait, Lebanon, Oman, Palestinian territories, Qatar, Saudi Arabia, Syria, the United Arab Emirates, and Yemen**.

"In ECDC's view, at present there is not a case for considering European countries epidemiologically the same as those countries in the Middle East where indigenous sporadic infections and clusters have been detected," the statement says. **Comment: This logic is questionable.**

The ECDC added that a strong case can be made for EU countries to continue using geography as one risk factor for testing patients for NCoV and for healthcare workers to remain vigilant when caring for patients with respiratory infections who are evacuated from Arabian Peninsula and neighboring countries.

Chan JF, Chan K, Choi GK, et al. Differential cell line susceptibility to the emerging novel human betacoronavirus 2c EMC/2012: implications on disease pathogenesis and clinical manifestation. J Infect Dis 2013 Mar 26 [Abstract]

McIntosh K. A new virulent human coronavirus: how much does tissue culture tropism tell us? (Editorial) J Infect Dis 2013 Mar 26 [Full text]

See also: Mar 27 ECDC epidemiological update Mar 27 ECDC public health development document

CDC novel coronavirus case definition

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Clark Tibbs, CEO
PhageVax, Inc. www.PhageVax.com
CAGE CODE: 4M4V6 www.sam.gov
[redacted]010
[redacted]USA
-

From: Chaim Scheff - Patent Attorney [redacted]
Sent: Friday, March 29, 2013 3:26 AM
To: pcast@ostp.gov
Subject: Looking for unified climate and biosphere databases to test simulate hypothesis

If planet Earth were an enterprise, then Global Warming would be (is) a symptom of bad management. Petro-technologies (waste product intense) are causing this dangerous climate shift. To save the Quality of Human Life on this planet, our urgent question is: "What should good management do?" Bad management lets market forces regulate environmental toxicity; until we retrograde shift into some high-tech Stone Age. Good management's paradigm is to modify the global biosphere to recycle more of the surplus atmospheric CO2. Lets look at the simple science, straightforward engineering, and down-home economics of this global eco-management procedure.

Science: In Nature, solar powered chlorophyll reactions bind atmospheric Carbon (Dioxide) to water – to build plant material (e.g. grasses, trees, algae). These chlorophyll-based plants absorb light around a few very narrow optical frequency bands – violet & blue (430, 453) and orange & red (644, 663). Exposing many of these plants to a "shot" of this light (during the night) will cause them to grow "as if" there had been an extra day. (Note: This is a well-known technique – used in Temperate/Sub-Arctic commercial green houses; to accelerate produce delivery schedules.)

Carefully altering the lunar spectrum is a rational paradigm for halting global warming. Taking sunlight that bounces off the lunar surface Earthward, and "improving" its spectrum will shift the biosphere's Carbon equilibrium. This provides petro-technology continuity and decreasing atmospheric CO2 levels – because artificially stimulating additional plant growth is actually binding surplus Carbon (Dioxide). Most plants will do this correctly, others will remain unchanged, and a few will develop imbalances. In the robust biosphere ecology, large scale shifting of ratios of plants in countless ecosystems will in turn respectively increase other species (e.g. molds, insects, birds, mammals, etc.). Classically, this is called Abundance.

Engineering: First, catalogue inert inorganic materials that return sunlight in the chlorophyll color bands; a reasonable task for an ordinary spectroscopic physicist. Next, eliminate materials that are unstable under the temperature, radiation, lunar surface contact, and vacuum conditions of the lunar surface; a reasonable task for an ordinary inorganic chemist. After that, eliminate materials that are difficult to make or to keep in a fine powdered form; a reasonable task for an ordinary chemical engineer. Of course, simply select the cheapest of the materials that qualify; a reasonable task for any prudent consumer.

Next, simulate the powder dispersal/deposition process – to understand how to apply it in small steps, so that the planet equilibrium is not overshot. Then, develop a delivery system for these materials; bringing the qualified materials to a location above the center of the visible lunar surface and dispersing them – probably in the form of a dry aerosol; perhaps from a lunar-tethered vehicle. Finally, even with super computers simulating this procedure, prudently introduce this phenomena in gradual steps over the course of 15-25 years – so that there is

enough time to monitor the effects on global weather, ecosystems, atmospheric CO2, agriculture, fish stocks, etc.

Economics: Needing to deliver only a few tons of powder to 20% cover the lunar surface with a coating of 20-micron dust surely costs less than any of the hot space-exploration projects of the last few decades. Like more ordinary space missions, this project requires the supervision of cost conscious space-science engineers and other systems-oriented mission specialists. While this is not something that has ever been done, neither exotic science nor not-yet-invented engineering are needed. Simply stated: "It's time to take another small step for mankind."

http://www.countercurrents.org/cc-scheff271005.htm

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EU Mobile: [redacted]

From: ROBERT CONIBEAR [redacted]
Sent: Saturday, April 06, 2013 10:58 PM
To: Ford, Knatokie
Subject: March 2013 Climate Change Report

Good report overall and may I offer suggestions that coincide with the Report and offer specific actions that when implemented will have immediate and increasing effect on reductions of GHG's and energy. Little or no additional government funding is necessary. The technology is in place so it is a matter of implementation, not study. Three areas are identified below followed by detailed reasoning.

- **Low emission vehicles including plug-in-hybrids: 80% less CO2 in ten years**

- **Building with structural insulated panels: 50% reduction energy and 60% less lumber**

- **Small nuclear reactors: replace coal/gas fired power generators.**

Hope this is of value.

Regards,

Robert A Conibear
[redacted]

PCAST REPORT OF March 2013, CLIMATE CHANGE 6April2013

Good report overall and may I offer suggestions that coincide with the Report and offer specific actions that when implemented will have immediate and increasing effect on reductions of GHG's and energy. Little or no additional government funding is necessary. The technology is in place so it is a matter of implementation, not study. Three areas are identified below followed by detailed reasoning.

- **Low emission vehicles including plug-in-hybrids: 80% less CO2 in ten years**
- **Building with structural insulated panels: 50% reduction energy and 60% less lumber**
- **Small nuclear reactors: replace coal/gas fired power generators.**

Low Emission Vehicles

Summary:

- Independence from foreign crude imports (except Canada).
- Incentives only for lowest carbon emission vehicles (grams/mile)
- Promote high volume purchases for competitive pricing
- Reduce sulfur content in vehicle fuels.
- No change in infrastructure.
- Discretionary spending change proportionally as fuel costs per vehicle decrease

The Report mentions Electric Vehicles (EV) and Hydrogen vehicles and no mention of Plug-in-Hybrids (PHEV). Rule of thumb is EV's use zero hydrocarbons, hybrids reduce hydrocarbon use by factor of 2 from present average vehicles and PHEV's reduce hydrocarbon use by a factor of 4 to 5.

Decreasing fuel consumption by 80% will have a profound effect worldwide. World crude production presently is 88 million barrels per day then reduced to 20 million per day. In the USA fuel consumption presently near 9 million barrels per day reduced to 2 million barrels per day. That is equivalent to a crude oil reduction of 9 million barrels per day and **independence from foreign imports**. The amount of money spent daily on these fuels plus capital expense is staggering (in excess of $400 billion yearly (80% of 9 million barrels/day x 42 gallons/bbl x 365 day/year @$3.75 /gallon) and when converted to other discretionary spending (perhaps even saving) will have a profound effect on the US economy. Most importantly is mitigation of damage to the country and world due to increased carbon content in the atmosphere. Faster conversion is better—much better!

EV's are range limited and hydrogen fuel cells are not yet viable for road vehicles**. PHEV's address range and are available. Furthermore they with EV's would meet 80% decrease in carbon emissions goals**. EV's battery pack is expensive and materials for the pack are limited in supply. PHEV battery pack is about 60% the size of an EV. It then becomes only a question of 'how long' will it take to replace existing vehicles.

Examples of existing PHEV's are:

- Volvo V60, PHEV, Diesel, 4 door, sedan, 215 hp, 131mpg
- Volkswagen, Golf PHEV, gasoline, 150 hp, 156mpg
- Alt-e, PHEV retrofit for trucks, 80 to 200% fuel savings
- Ford and GM both have PHEV's but mileage is in 75mpg range—PHEV's not all equal!

PHEV's as well as EV's are at the early end of roll-out and as such carry a high price and low production volumes. Counter this with high volume purchases by combining orders across the country. We buy 15 million new cars each year, an order of 100,000 Volvo V60's would have a major impact on price and production. Their present production is 6000 PHEV's for 2013. Rebates for fuel efficient vehicles, now up to $7000 could be increased with a trust fund or replaced by the

fund. Trust Fund to be funded by adding to the fuel purchases prices and use the proceeds to exclusively increase rebates. The vehicle owners are compensated with their own investment to the fund when they replace it with a low emission vehicle.—a savings plan! A 50 cent per gallon increase (oil companies do this regularly) would add $4600 to rebate for 15 million new vehicles per year or greater rebates for lesser numbers of new vehicles purchased. **Combination of high volume and high rebates would propel switch to low emission vehicles.**

It is not necessary to change the type of vehicle normally used for instance if you need a SUV then you still drive a PHEV SUV; the drive changes not the vehicle. As importantly the **PHEV is a substitute for an electric generator for emergency power** or working in areas not normally supplied with power.

Diesel engines are more efficient than spark ignition (gasoline) engines therefore less carbon emissions per mile of travel. This is due to the higher carbon content per gallon and better mechanical efficiency. The difference is about 30%. or a **diesel powered auto will travel 30% further per gallon** and therefore lower grams of CO2 emitted per mile of travel. European vehicles are now over 50% diesels while North America is 3%. The reason North America is behind is due to the sulfur content of the fuel. New (now 15 years old) technology improved the fuel injection system and the efficiency of the engine but needed low sulfur content fuel. USA chose to limit the amount of sulfur in diesel fuel to 15 parts per million. New diesels from Europe are now coming into the USA market but with added costs for additional equipment to compensate for the high sulfur content. The Auto Companies in the USA are now lobbying to lower the sulfur content in gasoline as it clogs the catalytic converter and the government should agree **to change this specification contingent on lower sulfur content in diesel to the 6 ppm.**

Other advantages of diesel engines are that bio-oils are generally compatible in any mix ratio with hydrocarbon fuels. Bio-diesel is becoming available in higher quantities, in many locations and circumstances and they do not contain sulfur. Natural gas to liquids processes such as Fischer-Tropsch changes natural gas to sulfur free diesel as well as other products **Use of the F-T process would also greatly affect our independence from foreign imports of crude.**

As important, there is **no change in infrastructure to use these fuels---immediate utilization** with no added costs for vehicle owners as there will be for compressed natural gas vehicles or hydrogen vehicles.

Resistance to incorporate diesel into the mainstream is from the US auto companies and the hydrocarbon industry. Diesel engines have a much longer working life than spark ignition engines and diesel or gasoline engines in PHEV's would extend this life four to five times. Electric motor life is about 30 years.

Your report recommending cooperation between nations to move to reduce carbon emissions from vehicles is important. China have started a program to have 15 million PHEV's on the road by 2015. Europe is ahead of us in development of high efficiency vehicles. **Join forces and communize specifications to make the change come quickly.**

Structural Insulated Panels,(SIP's).

Encourage use of SIP's by promotion, increased lumber tariffs, decreased OSB tariffs, permitting, energy rebates, etc.
In 2012 over 880,000 new homes constructed in the USA but very few with SIP's. **Reductions in energy** had SIP's been used is 750 watts per home or 660,000 kilowatts total; a significant amount.

SIP's uses orientated strand board (OSB), engineered panels with insulation bonded between them and used for outside walls, roofs and floors. Go to Wikipedia for full info on SIP's. The method meets buildings codes as revised in 2012 and in fact should make SIP's the preferred building method based on tightness of the building and even more so were the building to meet the same standard after ten years. twenty thirty years or more.

Resistance to change by construction industry is an impediment as SIP's will move the framing costs to a shop with significant reductions in manpower. Walls, floors, roof and computer designed and cut then hauled to site and erected in two or three days with three/four men. Cost with SIP's presently is equal in some areas but five to ten percent greater in other areas but should decrease as competition increased.

The amount of lumber usage decreases significantly and translates into less forest removal. Furthermore promotion of engineered panels and forms would encourage a new industry as fibrous plants can be grown all across the USA and harvested yearly or more. **Keep our forests in store**. Do this worldwide.

Small Modular Reactors (SMR's)
Your report minimized nuclear power and it is integral to eliminating GHG's. SMR's located near the demand, expandable, base units, safer but a hard sell. Since design approvals are several years off it would **be prudent to promote the alternatives at every opportunity with emphasis on the Green community members**.

The larger challenge is and least emphasis is replacing the energy source that creates the highest CO2 emissions--- coal and gas fired power generators. 65% of the CO2 emissions is from these sources compared to 35% from hydrocarbon sources. Presently **the ONLY way to eliminate these CO2 emitters is through nuclear power**.

While the US government is already assisting introduction of SMR's with grants to a variety of parties the timeline is conservative. Other countries are also working in this aea and cooperation between all would speed introduction (see: http://www.world-nuclear.org/info/Nuclear-Fuel-Cycle/Power-Reactors/Small-Nuclear-Power-Reactors/#.UV9W_lc4Adw). Accelerator Driven Sub-critical Reactors (ADSR's) could well be an addition or alternative. Many countries are working on these also and since we have a world wide crisis it would be prudent as you recommend in your report to cooperate here also in work and vision and speed the process! (http://www.world-nuclear.org/info/Current-and-Future-Generation/Accelerator-driven-Nuclear-Energy/#.UV9cxFc4Adw)

From: Clark Tibbs VHO-PVI-CTA [redacted]
Sent: Friday, April 12, 2013 8:59 AM
To: HHS-OS Secretarys Operations Center; FDA-Margaret Hamburg-Commissioner; HHS-Sec. Kathleen Sebelius; Ford, Knatokie
Cc: FDA-CBER Marie Keller-Robbins - Dr. Midthun Asst.; FDA-CBER Lorrie McNeill for Dr. Midthun; FDA-CBER Dr. Karen Midthun; BARDA-HHS Dr. Nicole Lurie Asst Sec-Preparedness
Subject: To Sec. of HHS & FDA Commissioner :: CRE in CA & CO & RI, etc. :: Videos on 'PHAGES' :: VA Med-Centers must use "LAST RESORT" Antibiotics via PhageVax
Importance: High

2013-04-12

-

QUESTION: What do you plan to do about this dire healthcare situation ?

-

http://www.youtube.com/watch?v=d-v8uSG2ewk&feature=related << **The Nature of Things**

Above is an 8-Min Video about Bacteriophages

-

At this link it describes "Antivirals".

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Where is the protocol for Biologics that are "Antimicrobial" Emergency Investigational New 'Drugs' (EIND) ??
http://www.fda.gov/Drugs/DevelopmentApprovalProcess/HowDrugsareDevelopedandApproved/ApprovalApplications/InvestigationalNewDrugINDApplication/ucm090039.htm

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I hope to hear from you soon. Clark Tibbs [redacted]

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----- Original Message -----
From: Clark Tibbs [redacted]
Sent: Thursday, April 11, 2013 5:58 AM
Subject: Back to Dr. McKee-CMO at The Joint Commission & Others :: CRE in CA & CO & RI, etc. :: Videos on 'PHAGES' :: VA Med-Centers must use "LAST RESORT" Antibiotics via PhageVax

2013-04-11

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TO: ALL and to Ms. Knatokie Ford: [redacted] ..... Please publish this Globally Important Information so the President and the Congress and the US Tax-payers can become more aware of their healthcare options.

-

Why don't we take better "selective control" of bacteria (with lytic phages and other phage-derived products), **so we can concentrate more on various viruses** like the 100's of combinations of Influenza. For example H7 flu is in China and the H5 flu is in Egypt & Cambodia and the H7 flu is in Mexico and the SARS-like novel Coronavirus is in Europe and

the Middle East and the 4-types of Dengue is in the tropics and temporate areas. Dengue is coming our way via hungry mosquitoes, as you know. WNV may be very virulent this spring, summer and fall. Dallas area of Texas, remember ? Cancers, some caused by viruses ?

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SPECIAL REQUEST TO THE JOINT COMMISSION: Will you please contact the ~200 hospitals [documented below] (that operate under your accreditation) and inform said healthcare facilities that they must participate in a nationwide clinical trial to begin utilizing various "bacteriophages" for in vivo treatment of the gram-positive and gram-negative bacteria, in order to maintain their accreditation? I don't have to write again that there are few alternative, do I? The proof is in these Attachments and in the Videos and the words in the body of this electronic message to you. We hope to hear from you soon on your 'action-plan'.

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"Notes from the Field: Hospital Outbreak of Carbapenem-Resistant Klebsiella pneumoniae Producing New Delhi Metallo-Beta-Lactamase - **Denver, Colorado**, 2012" http://www.cdc.gov/MMWR/preview/mmwrhtml/mm6206a5.htm?s_cid=mm6206a5_w

Why did it take ~6 mons. to publish this ?

-

Our company has on hand reports from Brazil and Europe that ESBL-types & CRE-types, etc. of bacteria certainly do escape from Clinics and Hospitals and Long Term Care Facilities, into the general environment. **Question:** Is the same thing happening in the USA ? Has anyone checked ?

-

Few if any states test for these events in the USA. The ESBL-types can and do transform even harmless environmental bacteria into antibiotic-resistant types via plasmids and/or lysogenic bacteriophages, that become prophages and innovate bacteria and **make them more fit** with toxins and antimicrobial-resistance genes. Examples are the NDM-1 gene documented below. (further transformation of the NDM-1, now extends out to **NDM-8**). Please look it up.

-

On the majority of food-animal farms in the USA the continued use of **low-level, sub-therapeutic antibiotics** is posioning the soil and water via faeces and urine. Can you prove me wrong ? This contamination is taken-up by our plants, not just on the surface, but through and through.

-

We hope to hear from you soon with questions and comments. Let's work together while using Lytic Phages to control KPC and other CRE and C. diff and other ESBLs such as MRSA, extra-intestinal E. coli, ?

-

CBS VIDEO :: "Superbugs" becoming harder to fight - aired on Sunday, January 27, 2013
Antibiotic-resistant bacteria can kill patients, especially those too weak to fight back.
One expert believes that unless we combat these new organisms we are close to **returning to pre-antibiotic era** mortality rates.
http://www.cbsnews.com/video/watch/?id=50139815n 40% Kill-Rate ? ~ 8-mins of your time.

-

VIDEO from Canada :: 84 patients have died of C. difficile at Burnaby Hospital - Feb-Mar 2012
http://www.ctvbc.ctv.ca/servlet/an/local/CTVNews/20120229/bc_burnaby_hospital_c_difficile_120229/20120229?hub=BritishColumbiaHome

-
Would you like to work with PhageVax on a **Universal Flu Vaccine,** while using the **T4 Phage Head** (as you will see at the top of this Attachment to the US Army) ?
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-
And this* ProMedMail *publishing (below) is just the beginning (or a continuation of) of the public's loss of confidence in the US healthcare system because the individuals addressed in this message ... ignore the obvious.
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Published Date: **2013-04-10** 16:06:49
Subject: PRO/EDR> Enterobacteriaceae, drug resistant – USA (02): (CA) CRE
Archive Number: **20130410.1636420**

ENTEROBACTERIACEAE, DRUG RESISTANT - USA (02): (CALIFORNIA) CRE
****
A ProMED-mail post http://www.promedmail.org
ProMED-mail is a program of the International Society for Infectious Diseases http://www.isid.org

Date: 04/10/2013
Source: ABC 23 KERO **Bakersfield, California** [edited]
http://www.turnto23.com/news/local-news/potentially-deadly-drug-resistant-bacteria-found-in-patients-at-several-local-hospitals

The Center For Disease Control [CDC] said a new and deadly superbug **is sweeping the nation's hospitals**.

It has hit more than **200 hospitals** in the U.S. **in only 6 months** and last month [March 2013] appeared for the 1st time in Bakersfield at several hospitals.

Those hospitals discovered **carbapenem resistant enterobacteria** known as CRE. It's a drug-resistant bacterium that could kill someone and he or she wouldn't even know it. Kern Medical Center, San Joaquin Community Hospital and Mercy Hospital Downtown each discovered a case of CRE last month.

The [CDC] said CRE is bacteria from the human colon that poses a triple threat: It is resistant to almost all antibiotics, spreads its resistance to other bacteria and **can kill 50 percent of infected patients**. It's usually found in nursing homes and hospitals.

People at risk are those with compromised immune systems and patients undergoing treatment in hospitals, especially those using temporary medical devices like catheters or ventilators, which is prime breeding ground for CRE.

"They can appear almost anywhere and with lots of different symptoms," said KMC'S Chief of Infectious Diseases **Doctor Royce Johnson.** Johnson said CRE is typically stumbled upon during medical tests. "There is **no possibility you will know** you have a CRE until the organism is cultured and (doctors) tell you," said Johnson.

"They are very difficult to treat and [treatment] is done with high levels of antibiotics or combinations of antibiotics and removing temporary medical devices as soon as possible," said County Public Health Department Lab Director **Dr. Michael Lancaster.**

Hospitals handle CRE cases by isolating patients, washing hands frequently, having people wear gowns and masks and thoroughly cleaning everything. Doctors are reminding people not to take antibiotics unless they are prescribed, otherwise you could be putting yourself at risk for CRE.

[Byline: Christine Dinh]

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Communicated by: ProMED-mail from HealthMap alerts promed@promedmail.org

[The Enterobacteriaceae is a large family of **Gram-negative** bacteria that includes _Escherichia coli_, _Salmonella_, _Shigella_, _Yersinia pestis_, _Klebsiella_, _Proteus_, _Enterobacter_, _Serratia_, and _Citrobacter_, some of which live in the intestines of humans and other animals.

The following has been extracted from moderator ML's comments in ProMED-mail post Enterobacteriaceae, drug resistant - USA **20130306.1572873**:

Carbapenems are a class of beta-lactam antibiotics that includes ertapenem, doripenem, imipenem, and meropenem. Carbapenems historically have been antibiotics of last resort to treat many infections due to carbapenem-sensitive, but otherwise multidrug-resistant Gram negative bacilli, such as those that produce extended-spectrum beta-lactamases (ESBLs) or AmpC beta-lactamases, **which are enzymes that destroy** most beta-lactams, except for the carbapenems.

Resistance to carbapenems develops commonly because organisms acquire the ability to produce carbapenemases, enzymes that destroy the carbapenems. There are several distinct types of carbapenemase: KPCs; NDM (New Delhi metallo-beta-lactamase); IMP; VIM (Verona integron-encoded metallo-beta-lactamase); and OXA-types (oxacillin-hydrolyzing carbapenemases). KPCs are the most common carbapenemases in the U.S., but have also been reported worldwide.

Many of the carbapenemases are encoded on plasmids, pieces of DNA that can easily spread from one organism to another of the same or even different bacterial species. KPCs (such as, KPC-1, -2, -3, -4, etc.) were first found in _K. pneumoniae_ isolates (hence, the name) and were subsequently found in _Escherichia coli_, _Serratia marcescens_, _Klebsiella oxytoca_, _Citrobacter freundii_, _Enterobacter_ spp., _Pseudomonas aeruginosa_, and _Salmonella enterica_.

The plasmids frequently carry genes that confer resistance to multiple other classes of antibiotics, so that these organisms **have been susceptible only to toxic and otherwise suboptimal antibiotics.**

Carbapenem-resistant Enterobacteriaceae (CRE) can colonize the gastrointestinal systems of humans for prolonged periods and once introduced can spread through contamination of water sources, hands of health care personnel, and environmental surfaces (for example bed rails, IV poles, computer touchpads, and equipment used to monitor vital signs such as stethoscopes, blood pressure cuffs, etc.) within health care facilities (http://www.cdc.gov/mmwr/preview/mmwrhtml/mm6124a3.htm?s_cid=mm6124a3_e).

CRE are easy to transfer from patient to patient. Hand hygiene is key to prevent the spread of these organisms and any other multidrug resistant microorganism. Infection control interventions in healthcare facilities aimed at preventing transmission of these multi-drug resistant isolates include their rapid recognition by the microbiology laboratory when cultured from clinical specimens, placement of patients colonized or infected with these isolates on contact precautions, and in some circumstances, conducting point prevalence surveys or active-surveillance cultures of high-risk patients and their environment (CDC, Healthcare Infection Control Practices Advisory Committee. Management of multidrug-resistant organisms in healthcare settings, 2006. Atlanta, GA: US Department of Health and Human Services, CDC, Healthcare Infection Control Practices Advisory Committee; 2007. Available at http://www.cdc.gov/hicpac/pdf/guidelines/MDROGuideline2006.pdf).

Active case detection may be followed by cohorting staff and CRE patients (i.e., segregating CRE-colonized or CRE-infected patients and the health-care personnel who care for them from those without CRE and the health-care personnel who care for them) (http://www.cdc.gov/mmwr/preview/mmwrhtml/mm62e0305a1.htm?s_cid=mm62e0305a1_w).

Bakersfield is a city near the southern end of the San Joaquin Valley in Kern County, California and is about 110 miles (180 km) to the north of the city of Los Angeles (http://en.wikipedia.org/wiki/Bakersfield,_California). The city's population was 347,483 in the 2010 census.

A HealthMap/ProMED-mail map can be accessed at: http://healthmap.org/r/5-mA. - Mod.ML]

See Also

Enterobacteriaceae, drug resistant - USA 20130306.1572873 2012

NDM-carrying Enterobacteriaceae - USA (03): (CO) nosocomial 20121118.1414608
NDM-1 carrying Pseudomonas - France: (ex Serbia) 20121110.1402258
NDM-1 carrying Enterobacteriaceae - USA (02): feline 20120916.1294263
NDM-1 carrying Enterobacteriaceae - China (02): (HK) ex Guangdong 20120914.1291460
NDM-1 carrying Enterobacteriaceae - France: ex Cameroon 20120817.1249316
NDM-1 carrying **Vibrio cholerae** - India 20120801.1224333

NDM-1 carrying Enterobacteriaceae - USA: (RI) ex Viet Nam 20120621.1175799
NDM-1 carrying Enterobacteriaceae - China: (HK) ex Thailand 20120612.1165421
NDM carrying bacilli - Canada: (Alberta) **nosocomial**, fatal 20120520.1138608
NDM-1 carrying Acinetobacter - Czech Rep ex Egypt 20120219.1044883
NDM-1 carrying Enterobacteriaceae - Ireland: 1st rep, ex India 20120217.1044861
2011

NDM-1 carrying Enterobacteriaceae - India (03): comment 20111230.3708
NDM-1 carrying Enterobacteriaceae - Guatemala: 1st rep, PAHO 20111128.3472
NDM-1 carrying Enterobacteriaceae - Italy: link to India 20111127.3466
Gram negative bacilli, MDR - South Africa: NDM-1, nosocomial 20111018.3117
NDM-1 carrying Enterobacteriaceae - India (02): **nosocomial infections** 20111006.3009
NDM-1 carrying Enterobacteriaceae - India, China: govt. response 20110412.1156
NDM-1 carrying Enterobacteriaceae - India: (New Delhi) water supply 20110411.1145
2010

Gram negative bacilli, resistant, update (01): NDM-1, KPC 20101028.3908
NDM-1 carrying Enterobacteriaceae (04): Taiwan ex India 20101005.3604
NDM-1 carrying Enterobacteriaceae - **worldwide ex India, Pakistan** (02) 20100914.3325
NDM-1 carrying Enterobacteriaceae - worldwide ex India, Pakistan 20100817.2853
NDM-1 carrying Enterobacteriaceae - N America, UK ex India 20100815.2812
.....sb/ml/mpp
-
Clark Tibbs, CEO
PhageVax, Inc. www.PhageVax.com
CAGE CODE: 4M4V6 www.sam.gov
[redacted]
-
----- Original Message -----
From: Clark Tibbs VHO-PVI-CTA
To: [redacted]
Sent: Tuesday, **June 12, 2012** 1:02 PM
Subject: To Dr. McKee-CMO at The Joint Commission :: Videos on Bacteriophages :: 127 VA Med. Centers from 2005 to 2010 must use "LAST RESORT" Antibiotics. via VHO-Tibbs
-
June 12, 2012
-
TO:
Dr. Ana Pujols McKee, M.D. - Executive Vice President and Chief Medical Officer - The Joint Commission [redacted]
-
I talked with Heather and I would appreciate your review of this globally important Therapeutic Bacteriophage information and your feed-back, since you can impact benefitial (cost-cutting) change in the healthcare market sector, **save lives and reduce suffering**. Please see: http://aac.asm.org/content/45/3/649.full

-
Thank you, Clark Tibbs [redacted]
-
-
----- Original Message -----
From: Clark Tibbs VHO-PVI-CTA
To: [redacted]
Sent: Thursday, June 07, 2012 5:48 PM
Subject: To Joint Commission Personnel :: Videos on Bacteriophages :: 127 VA Med. Centers from 2005 to 2010 must use "LAST RESORT" Antibiotics. via VHO-Tibbs

TO:
Ms. Sharon Weidenbach [redacted]
-
..... for distribution to: Ms. Anne Marie Benedicto - Support Operations - Chief of Staff & Exec. Vice President - [redacted]
-and-
Ana Pujols McKee, M.D. - Executive Vice President and Chief Medical Officer
Ref: http://www.jointcommission.org/ana_pujols-mckee_md
-and-
Jerod M. Loeb, Ph.D. - Executive Vice President, Division of Healthcare Quality Evaluation
Ref: http://www.jointcommission.org/jerod_m_loeb_phd/
-
Hello Joint Commission Personnel:
-
My name is Clark Tibbs with VHO, Inc. I see that your organization accredits some of this nation's VA Medical Centers, as well as more than 19,000 other health care organizations and programs in the United States.
-
Your Mission Statement: To continuously improve health care for the public, in collaboration with other stakeholders, by evaluating health care organizations and inspiring them to excel in providing safe and effective care of the highest quality and value. Vision: All people always experience the safest, highest quality, best-value health care across all settings.
-
I, hereby, ask for your cooperation to enlighten yourselves and therefore help to instruct your subject healthcare organizations on the features and benefits of Bacteriophages (Phages).
-
Please use these Videos to help in your visualization of the threat and the **logical "Phage-solution".**
-

Your probable concern that superbugs can become resistant to any particular Bacteriophage cocktail is correct. This is why we offer a "Service Agreement" to monitor the resistance, identify the **Cocktail** of naturally occurring (and properly cleared for zero virulence & resistance genes) Lytic-only Phages **that will kill** the selected bacteria and continue the process over the months and years. Please see "**The Nature of Things"** video below. This 8-Min Video is

truncated by Dr. David Suzuki from an original full-length 1997 BBC Video. http://en.wikipedia.org/wiki/David_Suzuki We have the links to the full-length video, just ask.

-

(1) http://www.youtube.com/watch?v=d-v8uSG2ewk&feature=related << **The Nature of Things**

Above is an 8-Min Video about Bacteriophages

-

-

(2) VIDEO on the **NDM-1 Gene -- 10-Min. UK Video at:**
http://www.channel4.com/news/drug-resistant-superbug-threatens-uk-hospitals

-

"A recent study (Fall of 2011) in Pakistan has shown community carriage of the **NDM-1 Gene** at 13.8 per cent and a carriage rate of 27.1% in patients in the hospitals. If these calculations are applied to India, over 100 million carriers would emerge," Professor Walsh said.
FROM: http://www.thehindu.com/health/policy-and-issues/article2512814.ece

-

-

The chemical molecules to fight some bacteria are running out, only 1 or 2 left, in some situations.

-

Would the Joint Commission care to partner with VHO to establish Bacteriophages (Phages) as the logical alternative to Chemical Antibiotics ?

-

Is there anyone (or a team) at any VA Med. Center (or any US-based Hospital) who wants to set up Clinical Trials while using "Phages" ? Can you help to investigate ?

-

Bacteriophages ... can ordered via VHO, Inc. or PhageVax, Inc.

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BELOW IS FROM:
http://www.cidrap.umn.edu:80/cidrap/content/other/resistance/news/may1712newsscan.html

Increase seen in use of ***'Last Resort'*** antibiotics for resistant pathogens

Antibiotics that remain effective against multidrug-resistant (**MDR**) and cabapenem-resistant (**CR**) Gram-negative pathogens are being used more frequently in recent years as these pathogens increase in prevalence, a study from Salt Lake City found.

The researchers, writing in *Public Library of Sciences* (*PLoS*) *One* yesterday, retrospectively examined the use of the **polymyxins** and **tigecycline** in **127 Veterans Affairs (VA) medical centers** **across the country** from October 2005 through September 2010.

Use of the drugs overall was low, at 0.8 and 1.6 days of therapy per 1,000 patients-days, respectively. The frequency of their administration varied by geographic region but increased over the study period in all regions except the Northeast, where polymixin use stayed stable.

Use of the agents was uneven across facilities: 75% of all polymyxin use occurred at just eight centers, and 75% of all tigecycline use occurred at 26 centers. There were 1,081 **MDR** or **CR** isolates during 747 hospitalizations associated with polymyxin use (1.4/hospitalization), compared with MDR or CR isolates during 500 hospitalizations for tigecycline (1.3/hospitalization) ($P = 0.06$).

A PLoS press release states, "While this is the first study assessing use of these drugs in the United States on a large scale, **the trend is almost certainly not limited to the VA.**" Lead author Makoto Jones, MD, concluded in the press release that, to address this trend, "a clear strategy of infection control, **antibiotic development,** and antibiotic stewardship will be necessary."

May 16 *PLoS One* study May 16 PLoS press release

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NOTE: Any information from Nordmann is always very instructive. **PLEASE READ ALL OF THESE PAPERS !**

Nordmann P, Naas T, Poirel L (2011) Global spread of Carbapenemase-producing Enterobacteriaceae. Emerg Infect Dis 17: 1791–1798. Find this article online

Falagas ME, Karageorgopoulos DE, **Nordmann P** (2011) Therapeutic options for infections with Enterobacteriaceae producing carbapenem-hydrolyzing enzymes. Future Microbiol 6: 653–666. Find this article online

Magiorakos AP, Srinivasan A, Carey RB, Carmeli Y, Falagas ME, et al. (2012) Multidrug-resistant, extensively drug-resistant and **pandrug-resistant bacteria:** an international expert proposal for interim standard definitions for acquired resistance. Clin Microbiol Infect 18: 268–281. Find this article online

Bogdanovich T, Adams-Haduch JM, Tian GB, Nguyen MH, Kwak EJ, et al. (2011) **Colistin-resistant,** Klebsiella pneumoniae carbapenemase (KPC)-producing Klebsiella pneumoniae belonging to the international epidemic clone ST258. Clin Infect Dis 53: 373–376. Find this article online

Marchaim D, Chopra T, Pogue JM, Perez F, Hujer AM, et al. (2011) Outbreak of **colistin-resistant, carbapenem-resistant** Klebsiella pneumoniae in metropolitan **Detroit, Michigan**. Antimicrob Agents Chemother 55: 593–599. Find this article online

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IN CHINA (PRC): Bacteria that cannot be stopped by common drugs are proliferating around the world (*Science*, 18 July 2008, p. 356). But a health care system that encourages doctors to churn out prescriptions, intensive marketing by pharmaceutical companies, and heavy use of antibiotics in animal husbandry and fisheries make China a special case.

More than 60% of *Staphylococcus aureus* isolates from Chinese patients in surveyed hospitals in 2009 were methicillin-resistant—the dreaded MRSA—**up from 40% in 2000**.

The proportion of *Streptococcus pneumoniae* isolates resistant to macrolides, meanwhile, **now tops 70%**. Roughly the same share of *E. coli* isolates are resistant to quinolones—**the highest rate in the world**.

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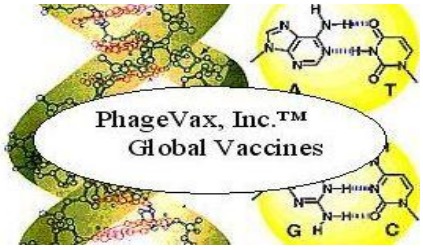
Clark Tibbs, CEO
PhageVax, Inc. www.PhageVax.com
CAGE CODE: 4M4V6 www.sam.gov
[redacted]



11 March 2013

TO:
Ms. Shannyn Scassero [redacted]
US Army Medical Research Acquisition Activity ATTN: MCMR-AAA, [redacted]

-

Regarding your RFI: A--Bacteriophage Medical Products Number: **W81XWH-13-RFI-BACT**
Agency: Department of the Army - Office: U.S. Army Medical Research Acquisition Activity
https://www.fbo.gov/index?s=opportunity&mode=form&id=b7259f48636a38e158d95c18005aae51&tab=core&_cview=0

-

Please note: Various "Lytic" Whole Bacteriophages (Phages) can be used as treatments for skin and soft tissue infections caused by Staphylococcus aureus and other various Phages can prevent and treat various bacterial diarrhea, as well as many more out-of-control bacterial infections and colonizations.

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Here is a **UNIVERSAL VACCINE PLATFORM** for your review via PhageVax, Inc.
(Please see PhageVax, Inc. contact information at the bottom of this document)

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T4 Bacteriophage Bound To A Substrate

(PhageVax's Dr. Eugene A. Davidson can assist the US Army with its learning-curve)
http://patft.uspto.gov/netacgi/nph-Parser?Sect1=PTO2&Sect2=HITOFF&p=1&u=%2Fnetahtml%2FPTO%2Fsearch-bool.html&r=1&f=G&l=50&co1=AND&d=PTXT&s1=8148130.PN.&OS=PN/8148130&RS=PN/8148130

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Rao VB[Author] - PubMed - NCBI = 104 Qty.
http://www.ncbi.nlm.nih.gov/pubmed?term=Rao%20VB%5BAuthor%5D&cauthor=true&cauthor_uid=23169641

-

OTHER THOUGHT-LEADERS IN THE "BACTERIOPHAGE" FIELD:

Adhya, Sankar: http://appft.uspto.gov/netacgi/nph-Parser?Sect1=PTO2&Sect2=HITOFF&p=1&u=%2Fnetahtml%2FPTO%2Fsearch-bool.html&r=0&f=S&l=50&TERM1=adhya%2C+sankar&FIELD1=IN&co1=AND&TERM2=&FIELD2=&d=PG01

-

Methods for Controlled Attachment of Bioactive Bacteriophages to Devices for the Purpose of Reducing Bacterial Colonization http://ibb.gatech.edu/sites/default/files/GTRC%205600.pdf

-

Biofilms: Microbial Life on Surfaces - Rodney M. Donlan
http://www.ncbi.nlm.nih.gov/pmc/articles/PMC2732559/

Bacteriophage having multiple host range
The present invention discloses compositions and methods for the prophylaxis and treatment of bacterial infections by the use of polyvalent bacteriophage having multiple host range.
US Patent 7,163,818 **Merril, et al**. January 16, 2007
http://patft.uspto.gov/netacgi/nph-Parser?Sect1=PTO2&Sect2=HITOFF&p=1&u=%2Fnetahtml%2FPTO%2Fsearch-bool.html&r=3&f=G&l=50&co1=AND&d=PTXT&s1=%22ADHYA,+Sankar%22.INNM.&OS=IN/"ADHYA,+Sankar"&RS=IN/"ADHYA,+Sankar"
-
Antibacterial therapy with bacteriophage genotypically modified to delay inactivation by the host defense system US Patent 5,688,501 - **Merril, et al**. November 18, 1997
The present invention is directed to bacteriophage therapy, using methods that enable the bacteriophage to delay inactivation by any and all parts of the host defense system (HDS) against foreign objects that would tend to reduce the numbers of bacteriophage and/or the efficiency of those phage at killing the host bacteria in an infection. Disclosed is a method of producing bacteriophage modified for anti-HDS purposes, one method being selection by serial passaging, and the other method being genetic engineering of a bacteriophage, so that the modified bacteriophage will remain active in the body for longer periods of time than the wild-type phage.
http://patft.uspto.gov:80/netacgi/nph-Parser?Sect2=PTO1&Sect2=HITOFF&p=1&u=%2Fnetahtml%2FPTO%2Fsearch-bool.html&r=1&f=G&l=50&d=PALL&RefSrch=yes&Query=PN%2F5688501
-
Triple acting antimicrobials that are refractory to resistance development
Multi-drug resistant superbugs are a persistent problem in modern health care. This invention provides an antimicrobial endolysin-Lysostaphin triple fusion protein, comprising (1) an endolysin CHAP endopeptidase domain, (2) an endolysin amidase domain, and (3) a Lysostaphin glycyl-glycine endopeptidase domain. The domains are derived from two proteins that show antimicrobial synergy when used in combination. The protein has specificity and exolytic activity for the peptidoglycan cell wall of untreated, live Staphylococcus aureus from many growth phases i.e. stationary, logarithmic and biofilm growth. The recombinant triple fusion protein comprising the three functional antimicrobial domains is designed to be refractory to resistance development.
http://appft.uspto.gov/netacgi/nph-Parser?Sect1=PTO2&Sect2=HITOFF&p=1&u=%2Fnetahtml%2FPTO%2Fsearch-bool.html&r=3&f=G&l=50&co1=AND&d=PG01&s1=%22Donovan,+David+M%22.IN.&OS=IN/"Donovan,+David+M"&RS=IN/"Donovan,+David+M"
-
Recombinant bacteriophage (Endo)lysins as antibacterials
http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3035150/
With the increasing worldwide prevalence of antibiotic resistant bacteria, bacteriophage endolysins (lysins) represent a very promising novel alternative class of antibacterial in the fight against infectious disease. Lysins are phage-encoded peptidoglycan hydrolases which, when applied exogenously (as purified recombinant proteins) to Gram-positive bacteria, bring about rapid lysis and death of the bacterial cell. A number of studies have recently demonstrated the strong potential of these enzymes in human and veterinary medicine to control and treat pathogens on mucosal surfaces and in systemic infections. They also have potential in diagnostics and detection, bio-defence, elimination of food pathogens and control of phytopathogens. This review discusses the extensive research on recombinant bacteriophage lysins in the context of antibacterials, and looks forward to future development and potential.
-

Fu W., Forster T., Mayer O., Curtin J.J., Lehman S.M., Donlan R.M. **Bacteriophage cocktail for the prevention of biofilm formation by Pseudomonas aeruginosa on catheters in an in vitro model system**. http://www.ncbi.nlm.nih.gov/pubmed/19822702
Antimicrob Agents Chemother. 2010 Jan;54(1):397-404.
Rodney M. Donlan, **Preventing biofilms of clinically relevant organisms using bacteriophage,** Trends in Microbiology, Volume 17, Issue 2, February 2009, Pages 66-72.
John J. Curtin, and Rodney M. Donlan**. Using Bacteriophages To Reduce Formation of Catheter-Associated Biofilms by Staphylococcus epidermidis.** Antimicrobial Agents and Chemotherapy, April 2006, p. 1268-1275, Vol. 50, No. 4.

-

ENGINEERED BACTERIOPHAGES AS ADJUVANTS FOR ANTIMICROBIAL AGENTS AND COMPOSITIONS AND METHODS OF USE THEREOF
http://appft1.uspto.gov/netacgi/nph-Parser?Sect1=PTO2&Sect2=HITOFF&p=1&u=%2Fnetahtml%2FPTO%2Fsearch-bool.html&r=1&f=G&l=50&co1=AND&d=PG01&s1=20100322903&OS=20100322903&RS=20100322903

PROBLEMS OR SOLUTIONS (You Choose)

CDC says 'nightmare bacteria' a growing threat – March 5th, 2013
"Our strongest antibiotics don't work, and patients are left with potentially untreatable infections," he said. Quote from Thomas Frieden, director of the Centers for Disease Control and Prevention.

http://www.washingtonpost.com/national/health-science/cdc-warns-of-rise-in-nightmare-bacteria/2013/03/05/5596b952-85cb-11e2-999e-5f8e0410cb9d_story.html

-

From the US Government: EHP – Phage Renaissance: New Hope against Antibiotic Resistance
http://ehp.niehs.nih.gov/121-a48/

-

You may want to use these Videos to help in visualization of the threat and the logical "Phage-solution".
http://www.youtube.com/watch?v=d-v8uSG2ewk&feature=related ... **The Nature of Things.**

-

Is www.IntraLytix.com (with its Lytic Bacteriophages) at least one of the solutions to pan-resistant bacteria ? Reminder that www.IntraLytix.com now has three (3) FDA & USDA Approved Products to control **Salmonella** + **E. coli O157:H7** + **Listeria** **Proctor & Gamble** Collaboration.

-

Phages for **Food Safety** (Video) ... http://www.youtube.com/watch?v=fFoo1OVa11E (4 MINS)

-

Intralytix Completes Regulatory Clearance for Phage-Based *E. coli* Food Safety Technology - ***June 14th, 2011*** http://www.intralytix.com/Intral_News_PR061411.htm

-

IMPORTANT VIDEO ... AmpC + ESBL + CRE + KPC + NDM-1 Gene + CTX Gene ... are on the march. I hope that you will have the time to watch this recent (10 MINS) UK Video at:
http://www.channel4.com/news/drug-resistant-superbug-threatens-uk-hospitals

-

"A recent study (Fall of 2011) in Pakistan has shown community carriage of the NDM-1 Gene at 13.8 per cent and a carriage rate of 27.1% in patients in the hospitals. If these calculations are applied to India, over 100 million carriers would emerge," Professor Tim Walsh said.
FROM: http://www.thehindu.com/health/policy-and-issues/article2512814.ece

-

FROM: http://www.xcdsystem.com/icid2012/56.086.html Session: Antibiotics Abstract No.:56.086
Title: Ignorance: a blessing or a curse-awareness among Indian physicians on NDM-1 and its impact on antibiotic selection

-

CBS VIDEO :: "Superbugs" becoming harder to fight - aired on Sunday, January 27, 2013
Antibiotic-resistant bacteria can kill patients, especially those too weak to fight back.
One expert believes that unless we combat these new organisms we are close to returning to pre-antibiotic era mortality rates. http://www.cbsnews.com/video/watch/?id=50139815n 40% Kill-Rate ?

-

VIDEO from Canada :: 84 patients have died of **C. difficile** at Burnaby Hospital - Feb-Mar 2012
http://www.ctvbc.ctv.ca/servlet/an/local/CTVNews/20120229/bc_burnaby_hospital_c_difficile_120229/20120229?hub=BritishColumbiaHome

-

Notes from the Field: Hospital Outbreak of Carbapenem-Resistant Klebsiella pneumoniae Producing New Delhi Metallo-Beta-Lactamase - Denver, Colorado, Summer of 2012
FROM: http://www.cdc.gov/MMWR/preview/mmwrhtml/mm6206a5.htm?s_cid=mm6206a5_w

THESE REPORTS BELOW DOCUMENT THAT TRADITIONAL ANTIMICROBIALS ARE FAILING AND THAT "BACTERIOPHAGE-PRODUCTS" MUST BE USED NOW TO CONTROL GRAM-POSITIVE AND GRAM-NEGATIVE BACTERIA:

(1) FROM: http://www.plosone.org/article/info%3Adoi%2F10.1371%2Fjournal.pone.0036649
Increasing use of "Last Resort" Antimicrobials at various VISN's (Veterans Affairs Medical Centers) 2005-2010

(2) FROM: http://cid.oxfordjournals.org/content/early/2013/03/03/cid.cit055.abstract
Enterohemorrhagic *Escherichia coli* O26:H11/H⁻: A New Virulent Clone Emerges in Europe

(3) FROM: http://cid.oxfordjournals.org/content/42/5/692.abstract
The Epidemiology and Control of *Acinetobacter baumannii* in Health Care Facilities

(4) FROM: http://cid.oxfordjournals.org/content/46/Supplement_5/S378.abstract
Pneumonia Caused by Methicillin-Resistant Staphylococcus aureus (MRSA)

(5) FROM: http://cid.oxfordjournals.org/content/50/5/625.abstract
Diagnosis, Prevention & Treatment of Catheter-Associated Urinary Tract Infection (UTI) in Adults: 2009 Int'l Clinical Practice Guidelines from IDSA

(6) FROM: http://cid.oxfordjournals.org/content/54/12/e132.abstract
2012 IDSA Clinical Practice Guideline for the Diagnosis and Treatment of Diabetic Foot Infections

(7) FROM: http://cid.oxfordjournals.org/content/32/9/1249.extract
Guidelines for the Management of Intravascular Catheter-Related Infections

(8) FROM: http://cid.oxfordjournals.org/content/35/2/113.extract
Practice Guidelines for the Diagnosis & Management of Group A Streptococcal Pharyngitis – 2002

(9) FROM: http://cid.oxfordjournals.org/content/56/2/236.abstract
Continuous Infusion of Beta-Lactam Antibiotics in Severe Sepsis - A Multicenter Double-Blind, Randomized Controlled Trial

(10) FROM: http://www.nature.com/nature/journal/vaop/ncurrent/full/nature11450.html#/affil-auth
A metagenome-wide association study of gut microbiota in type 2 diabetes

The US Army writes:
(1) identify the bacteriophage(s) you believe are suitable for development of medical products;
(2) describe in general terms the manufacturing process for the bacteriophage(s) preparations;
(3) present a summary of the results of relevant nonclinical and clinical studies bearing on product safety and efficacy;
(4) state any interest you have as an inventor or owner of an invention, or holder of any other type of interest that permits you to exploit, or prevent others from exploiting, relevant technologies; and
(5) outline your concept for development of bacteriophages as products approved for marketing by the FDA. USAMRMC will evaluate your statement of interest and maintain it as trade secrets and/or confidential business information.

[Answer to all five (5) questions from PhageVax, Inc. (740-366-9013) … We can discuss the forward direction (while using various types of Bacteriophage-derived Products) with the US Army.]

-

COMPRESSED HIGHLIGHTS OF THE VACCINE: U.S. Patent 8,148,130 Inventors: Dr. Venigalla B. Rao and Dr. Carl Alving
The Promiscuous DNA Packaging Machine from Bacteriophage T4 Packaging System and T4 Bacteriophage-Bound Adjuvant.
Unique methods of packaging DNA in bacteriophages and making bacteriophage components to improve DNA vaccine formulations.
Key Features:
· Flexible and high density platform. Accommodates a variety of proteins, toxins, phage packaging proteins, and antigens.
· Enhanced immunological response through efficient delivery of antigen and through simultaneous stimulation of both antibody and cellular immunity. · Antigens could be assembled to be highly immunogenic without adjuvant.
· Accommodates 5 to 10 times more DNA than current packaging systems. Provides highly efficient delivery into a host cell.
· Could be used for large segments of foreign DNA, multiple genes, or multivalent DNA vaccines.

-

Improved methods of utilizing bacteriophages for immunization: The Catholic University of America (CUA) has developed a novel class of nanocapsid delivery vehicles that can transport DNA into targeted cells. The described invention is a mutant bacteriophage T4 nanoscale capsid shell consisting of a head into which the genome is packaged, and a targeting molecule on its surface that delivers the genome into targeted cells. When the nanoshell is packed with foreign DNA, it has the potential to deliver DNA vaccines against infectious disease, vaccines against cancer, and genes for gene therapy. The use of the highly stable nanoshell as packaging containers is a significant breakthrough from a technical standpoint and will have broad implications.

-

Increased DNA packaging ability and enhanced immunological response:
There are a number of advantages of the described mutant bacteriophage T4 packaging machine. The flexible packaging machine developed can indiscriminately translocate DNA molecules into a capsid receptacle and continue packaging until the capsid is full. The filled shells, by virtue of the energy (internal pressure) present in the tightly packed DNA, can more efficiently deliver the ‘‘genome’’ into a host cell.

-

The mutant bacteriophage T4 nanoscale capsid shell consists of an interior space to pack DNA and an outer surface to tether proteins. The shell is composed of three proteins and the outer surface has binding sites for 870 copies of Small Outer Capsid protein (Soc) and 155 copies of Highly antigenic Outer Capsid protein (Hoc). The efficient assembly of proteins on the capsid shell is an in vitro approach that distinguishes itself from the classic in vivo approach by allowing efficient and controlled display of large full-length proteins on the capsid surface through specific interactions between Hoc, or Soc, and capsid. A variety of proteins, anthrax toxins, phage packaging proteins, and HIV antigens of various size (up to 90 kDa), structure, and biological function, have been fused to Hoc, over-expressed in E. coli, purified and assembled on the phage particles in a native functional state, under defined binding conditions.

Clark Tibbs, CEO
PhageVax, Inc. www.PhageVax.com
CAGE CODE: 4M4V6 www.sam.gov

[redacted]

OPEN ACCESS Freely available online

PLoS one

Drugs of Last Resort? The Use of Polymyxins and Tigecycline at US Veterans Affairs Medical Centers, 2005–2010

Benedikt Huttner[1☯], Makoto Jones[1*☯], Michael A. Rubin[1], Melinda M. Neuhauser[2], Adi Gundlapalli[1], Matthew Samore[1]

1 VA Salt Lake City Health Care System and University of Utah, Salt Lake City, Utah, United States of America, **2** Department of Veterans Affairs Pharmacy Benefit Management Services, Hines, Illinois, United States of America

Abstract

Multidrug-resistant (MDR) and carbapenem-resistant (CR) Gram-negative pathogens are becoming increasingly prevalent around the globe. Polymyxins and tigecycline are among the few antibiotics available to treat infections with these bacteria but little is known about the frequency of their use. We therefore aimed to estimate the parenteral use of these two drugs in Veterans Affairs medical centers (VAMCs) and to describe the pathogens associated with their administration. For this purpose we retrospectively analyzed barcode medication administration data of parenteral administrations of polymyxins and tigecycline in 127 acute-care VAMCs between October 2005 and September 2010. Overall, polymyxin and tigecycline use were relatively low at 0.8 days of therapy (DOT)/1000 patient days (PD) and 1.6 DOT/1000PD, respectively. Use varied widely across facilities, but increased overall during the study period. Eight facilities accounted for three-quarters of all polymyxin use. The same statistic for tigecycline use was twenty-six VAMCs. There were 1,081 MDR or CR isolates during 747 hospitalizations associated with polymyxin use (1.4/hospitalization). For tigecycline these number were slightly lower: 671 MDR or CR isolates during 500 hospitalizations (1.3/hospitalization) ($p = 0.06$). An ecological correlation between the two antibiotics and combined CR and MDR Gram-negative isolates per 1000PD during the study period was also observed (Pearson's correlation coefficient $r = 0.55$ polymyxin, $r = 0.19$ tigecycline). In summary, while polymyxin and tigecycline use is low in most VAMCs, there has been an increase over the study period. Polymyxin use in particular is associated with the presence of MDR Gram-negative pathogens and may be useful as a surveillance measure in the future.

Citation: Huttner B, Jones M, Rubin MA, Neuhauser MM, Gundlapalli A, et al. (2012) Drugs of Last Resort? The Use of Polymyxins and Tigecycline at US Veterans Affairs Medical Centers, 2005–2010. PLoS ONE 7(5): e36649. doi:10.1371/journal.pone.0036649

Editor: Brad Spellberg, Los Angeles Biomedical Research Institute, United States of America

Received March 9, 2012; **Accepted** April 11, 2012; **Published** May 16, 2012

Funding: Funding for this work was provided by The Pew Charitable Trusts. The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript. This material is also the result of work supported with resources and the use of facilities at the George E. Wahlen Department of Veterans Affairs Medical Center, Salt Lake City, UT and funding support from the VA Informatics and Computing Infrastructure (VINCI - VA HSR HIR 08–204), the Consortium for Healthcare Informatics Research (CHIR - VA HSR HIR 08–374) and the Centers for Disease Control and Prevention1s Prevention Epicenters Program, (5U01CI000334 and 07FED706504). In addition, M.J. was supported by a career development award (CDA 10-030-02) and B.H. was supported in part by a fellowship grant from Geneva University Hospitals, Switzerland.

Competing Interests: The authors have declared that no competing interests exist.

☯ These authors contributed equally to this work.

Introduction

In recent years, several studies have reported encouraging trends for multidrug-resistant (MDR) Gram-positive pathogens. In one such study, a decrease in health care-associated methicillin-resistant *Staphylococcus aureus* (MRSA) infection rates was observed after the implementation of a MRSA bundle in all Veterans Affairs medical centers (VAMCs) in 2007. [1] Similar trends have been reported from other settings in the United States and from other countries, such as the United Kingdom and France. [2,3,4] Unfortunately, these advances have been partly offset by the fact that a series of resistance genes coding for broad-spectrum beta-lactamases–NDM-1, KPC, VIM, OXA-48, CTX-M-15, and AmpC, to name just a few–are increasingly found in Gram-negative bacteria across the globe. [5,6]

These genes coding for enzymes capable of conveying resistance to broad-spectrum cephalosporins and carbapenems are not only harbored by typical nosocomial pathogens, but also by pathogens associated with the community, such as *Escherichia coli*. [7] The presence of extended-spectrum beta-lactamase (ESBL) genes in the strain of *E. coli* that caused a recent outbreak of hemolytic-uremic syndrome in Germany is a reminder that antimicrobial resistance in Enterobacteriaceae is not a problem limited to hospitals. [8] In addition, multiple resistance genes are often clustered on plasmids, rendering their owners extensively or even pan-drug resistant. [9,10] Carbapenem resistance is of particular concern since few antibiotics are available to treat infections with carbapenem-resistant (CR) organisms. [11]

This emerging resistance has led to a focus on antibiotics from alternative classes. Tigecycline, an antibiotic structurally related to tetracyclines, was approved in the United States in 2005 and shows activity against MDR Gram-negative pathogens, with a notable gap in coverage of *Pseudomonas* species. [12] Recent randomized controlled trial data showing higher all-cause

mortality in tigecycline-treated patients has, however, dampened enthusiasm for this drug. [13,14,15] Increasing attention is being paid to the polymyxins, a class of antibiotics that had fallen out of favor with clinicians due to concerns about toxicity and uncertainty about optimal dosing strategies. [16,17,18] Despite the fact that the use of polymyxins or tigecycline probably indicates the presence or suspicion of problematic pathogens, there are no comprehensive data about the use of these antibiotics, nor which pathogens motivate their use. The availability of comprehensive antimicrobial prescribing and microbiology data from VAMCs offers a unique opportunity to fill this gap in understanding both an older and newer antimicrobial agent.

Methods

There are 152 VAMCs providing acute and long-term care across the United States. To capture inpatient data, we included records from acute medical, surgical, neurological, and intensive-care units in our antibiotic and microbiologic analyses. We further restricted the cohort to facilities with at least 10 operational acute-care beds during fiscal years (FY) (October, 1- September, 30) 2006 through 2010, and those with barcode medication administration (BCMA) data available over the entire study period.

Antibiotic Use Data

We used nationwide, individual-level data drawn from the VA Informatics and Computing Infrastructure (VINCI) for this study. BCMA technology was introduced VA-wide in 2000 to improve medication safety. [19] With each administered dose of medication, data regarding drug and route of administration are recorded electronically with a time stamp. To detect missing data, we assessed the completeness of the BCMA data set by comparing it with electronic orders. We deemed antibiotic BCMA data to be complete in a facility from the first month in which the difference between antibiotic use in BCMA data and standard electronic orders was less than 20% (based on at least one recorded order or BCMA entry) for five commonly used index antibiotics (ciprofloxacin, vancomycin, piperacillin/tazobactam, metronidazole, and ceftriaxone). We used this cutoff because some medication orders are written and cancelled before a single dose can be administered. In addition it should be noted that a bar code quality program to ensure 100 percent scan success rates has been implemented VA-wide in 2006. BCMA data were used to assess the use of parenteral polymyxins (polymyxin B and colistin/colistimethate, which we will refer to collectively as "colistin") and tigecycline. [9,10]

Antibiotic use was expressed in terms of "days of therapy" (DOT). One DOT was defined as the administration of a single antibiotic on a given day independent of the number of doses, the strength of the dose administered, or the route of administration. [20] DOT were denominated by patient days using a midnight census approach. As such, the discharge day was not counted in the numerator or the denominator. We also denominated antibiotic use by "hospitalizations" where at least one hospital inpatient day fell within the study period.

Microbiology Data

The methods used to extract microbiology data from the VA network have been previously described elsewhere with regard to MRSA. [21] We used analogous methods to extract microbiology data for Gram-negative pathogens. In brief, organisms and susceptibilities were extracted into a relational structure from semi-structured text documents using natural language processing (NLP). All inpatient microbiology data between the 7th day before and 1st day after the first day of polymyxin or tigecycline use were also manually reviewed. Only the first unique isolate of a species (defined by the susceptibility pattern) for each hospitalization was analyzed. Isolates without at least one reported susceptibility and cultures obtained in the outpatient setting were excluded. Multi-drug resistance was defined as "acquired" non-susceptibility to at least one agent in three or more antimicrobial categories according to the definitions proposed by a joint initiative by the European Centre for Disease Prevention and Control (ECDC) and the Centers for Disease Control and Prevention (CDC). [22] Carbapenem-resistance definitions were also pathogen-specific and drawn from the same proposal. We were not able to apply the proposed definitions for extensively drug-resistant (XDR) and pan-drug resistant (PDR) bacteria due to the lack of standardization of reported susceptibilities; however, we modified the definition of XDR to be resistance in all reported drug classes except polymyxins. Analyses of multi-drug resistance were restricted to Enterobacteriaceae, *Acinetobacter*, and *Pseudomonas* species due to the lack of comprehensive definitions for other Gram-negative organisms.

We used descriptive statistics to describe antibiotic use and microbiology data. All analyses were performed using STATA version 11 (StataCorp. 2009. College Station, TX).

Ethics Statement

This study was approved by the Research Review Committee of the VA Salt Lake City Health Care System and Institutional Review Board of the University of Utah. As the study involved retrospective review of existing medical record data with no patient contact, the IRB approved and granted waiver of informed consent to access medical records. All results are presented in aggregate to protect the privacy and confidentiality of study subjects.

Results

Of 152 VAMCs, 20 did not meet our acute-care services criteria, 1 was excluded for less than 10 acute-care beds, and 4 were excluded for incomplete BCMA data. The remaining 127 VAMCs logged 2.42 million unique hospitalizations on the included wards over the study period accounting for over 13.57 million patient days (table 1).

Table 1. Characteristics of the 127 hospitals October 2005 through September 2010.

Hospital Characteristics	Count (%)
Hospitals with ICUs	**121 (95.3)**
University affiliated	**115 (90.6)**
VA region	
1 West	24 (18.9%)
2 Central	36 (28.4%)
3 Southeast	40 (31.5%)
4 Northeast	27 (21.3%)
	Value [IQR]
Average daily occupied acute Care Beds	53.0 [26.3–81.1]
Average daily occupied ICU Beds	8.0 [3.4–13.9]

doi:10.1371/journal.pone.0036649.t001

Antibiotic Use

There were 11,535 days of therapy (DOT) of polymyxins (0.1% of overall DOT for systemic antibiotics) administered during 1,145 hospitalizations over the study period. Polymyxin B, which is part of the national VA formulary, accounted for 63.7% (7,343 DOT) of all IV polymyxin DOT (the remaining DOT were colistin). In comparison, there were 21,886 DOT of tigecycline during 3,125 hospitalizations.

Tigecycline DOT rose more steeply than polymyxin DOT during the five-year period of analysis (figure 1). From FY 2006, the first full fiscal year of tigecycline approval, to FY 2010, tigecycline use increased 4.2 fold. Polymyxin use peaked in FY 2009 then declined modestly in FY 2010.

The distribution of hospital polymyxin use across facilities was more skewed than that of tigecycline use (figure 2). The facility with the highest polymyxin use prescribed 53.9% of all polymyxin DOT. Only 8 (6%) facilities accounted for 75% of polymyxin use. In FY 2010, polymyxin DOT in the highest use facility were more than four times greater than the polymyxin DOT in second highest use facility (20.6 vs 4.9 DOT per 1000 PD). Overall, 54 hospitals used no polymyxins during the five year time frame. In contrast, the facility with the highest tigecyline use accounted for 8.8% of tigecycline DOT. Twenty-six facilities (20%) accounted for 75% of tigecycline DOT over the study period, while 22 hospitals did not use tigecycline at all.

Polymyxin and tigecycline use varied across different geographic regions. Yet, each region demonstrated an upward temporal trend in polymyxin and tigecycline utilization (with the exception of the Northeast where polymyxin prescribing was relatively stable). The comparison of geographic regions was dominated by the effect of an outlier effect. Excluding the highest use facility, overall polymyxin prescribing was 0.7 DOT per 1000 PD in the northeastern region compared to 0.3, 0.3, and 0.4 DOT per 1000 PD in the other three regions. Tigecycline use (again excluding the outlier facility) was also highest in the Northeast at 2.9 DOT per 1000 PD.

Polymyxins were administered for a median of 7 days (IQR 4–13, mean 10.1) and tigecycline for a median of 5 days (IQR 3–9, mean 7.0) per hospitalization. In comparison, the individual median number of days for any antibiotic therapy was 3 days (IQR 2–6) per hospitalization. 49.0% of all polymyxin DOT were administered in intensive care units, while this was the case for only 33.5% of tigecycline DOT.

While 86.8% of all antibiotic courses were started within the first 2 days of admission, polymyxins were started a median of 15 days after the day admission (IQR 4–31) and only 14.8% of polymyxin courses were started within the first 2 days. Tigecycline was started earlier at a median of 4 days after the day admission (IQR 1–12), and 37.1% of all courses were started within 2 days. Patients receiving polymyxins received a median of 12 days (IQR 3–26) of antibiotic therapy before the first dose of polymyxins, while patients receiving tigecycline had received a median of 3 days of antibiotics (IQR 0–10).

Polymyxins were administered mostly in combination with other antibiotics (IV polymyxins were administered alone on only 16.3% of days). On average, 1.6 (SD 1.1) other antibiotics were given concomitantly. Tigecycline was administered with polymyxins on 7.1% of polymyxins days. For comparison tigecycline was given with an average of 0.9 (SD 1.0) other antibiotics and tigecycline was given alone on 44.5% of all tigecycline days.

Microbiology

There were 1,274 unique Gram-negative isolates recovered between the 7th day before and the 2nd day after the first dose of IV polymyxin each hospitalization. Of these, 1,081 isolates fulfilled criteria for MDR or CR Enterobacteriaceae, *Pseudomonas,* or

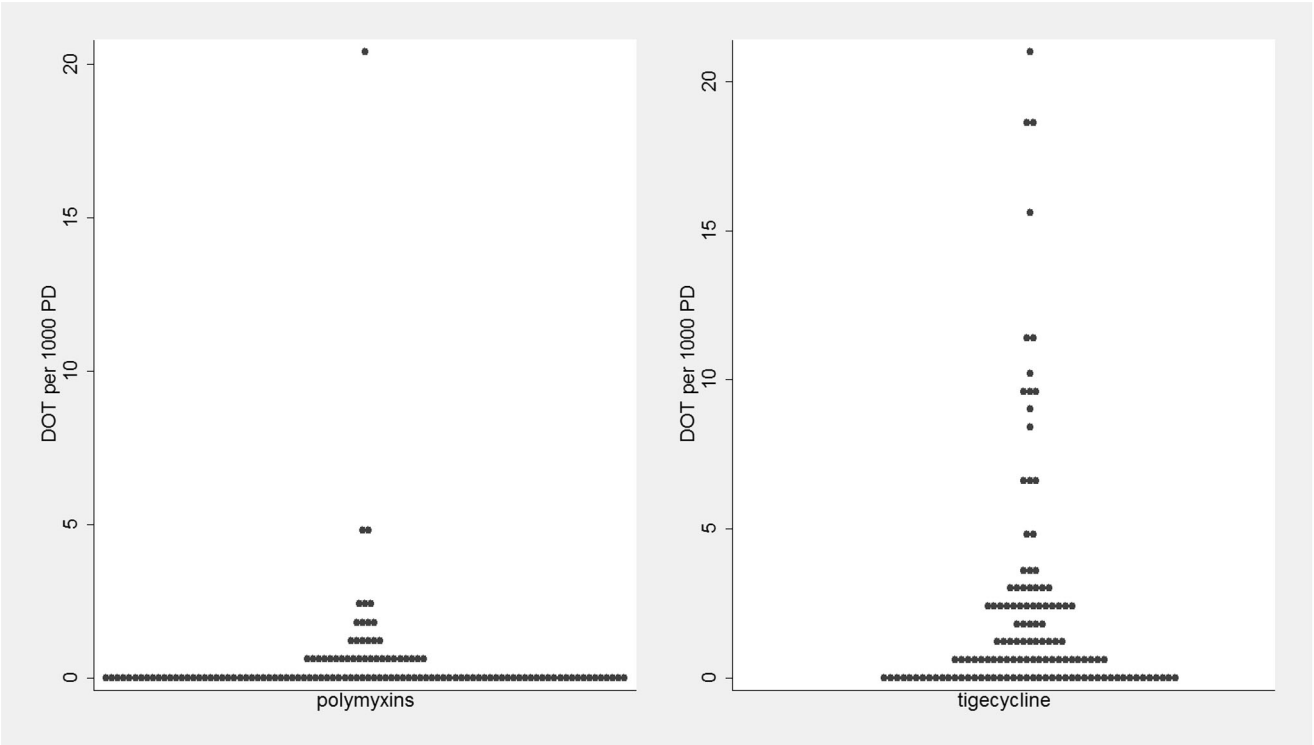


Figure 1. Trends in the use of polymyxin and tigecycline in days of therapy (DOT) per 1000 patient days (PD) by fiscal year.
doi:10.1371/journal.pone.0036649.g001

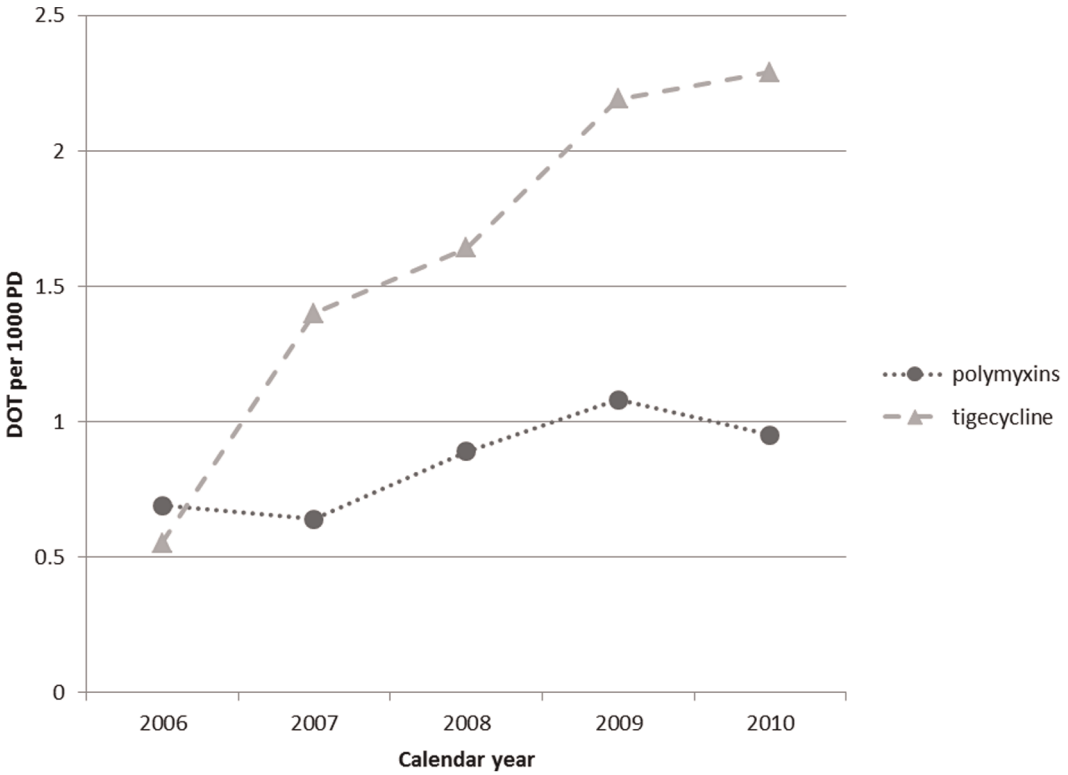


Figure 2. Dot plot of tigecycline and polymyxin use by facility in DOT per 1000 PD in fiscal year 2010. Each dot represents one facility (n = 127).
doi:10.1371/journal.pone.0036649.g002

Acinetobacter (figure 3). Among the rest there were 17 *Stenotrophomonas* isolates. The resistant isolates were found among 747 unique hospitalizations (65.2%) when polymyxins were used; no pathogen was identified in 350 hospitalizations (30.6%) (the remainder demonstrating pathogens not fulfilling MDR or CR criteria). *Pseudomonas aeruginosa* (361; 33.4%), *Klebsiella* spp. (344; 31.8%), *Acinetobacter* spp. (228; 21.1%), and *Escherichia coli* (49; 4.5%) together accounted for most resistant isolates. Enterobacteriaceae other than the organisms listed above made up an additional 9.2%. The most common culture site among resistant organisms was sputum (table 2).

Using the same definitions as for polymyxins, there were 1,280 unique Gram-negative isolates for tigecycline (174 of which were also found with concurrent polymyxin use). Of these, 671 met criteria for MDR or CR Enterobacteriaceae, *Pseudomonas*, or *Acinetobacter* (figure 3). There were 70 *Stenotrophomonas* isolates. All of the resistant isolates were found in 500 hospitalizations (16% of all hospitalizations when tigecycline was used). There were 2,281 hospitalizations without pathogens. The remainder were non-resistant isolates.

We also examined the ecological correlation between the two antibiotics and combined CR and MDR Gram-negative isolates per 1000 PD during the time period. Removing the extreme outlier facility, the Pearson correlation coefficient was 0.55 for polymyxin and 0.19 for tigecycline (figure 4).

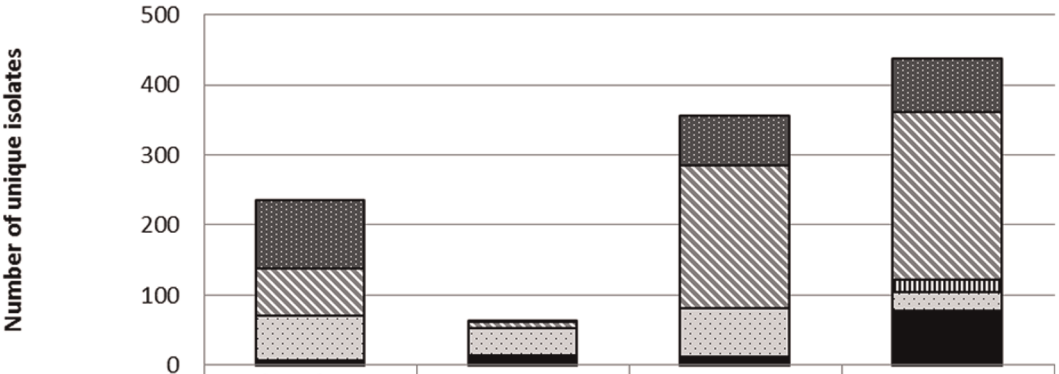
Discussion

In this explorative study, we found 1) an increase in the use of "last-resort" antibiotics over the study period, 2) important variation in the use of these antibiotics among facilities, and 3) as expected, the frequent presence of MDR and/or CR Gram-negative pathogens in patients treated with polymyxins.

We observed an increase in the use of polymyxins and tigecycline during the study period, although the overall use is still relatively low (0.3% of overall DOT for systemic antibiotics). There are no comprehensive data about the use of "last-resort" antibiotics in US facilities, so direct comparisons over similar geographic regions are difficult. Two recent studies describing outbreaks of CR *Klebsiella pneumoniae* (Detroit 2009, Pittsburgh 2010) reported their ICU colistin use: 20.7 and 30.8 defined daily doses (DDDs) per 1000 patient-days respectively. [23,24] Comparison with these data is difficult since they refer to outbreak settings. In addition caution has to be applied when comparing DDD to DOT, especially for drugs like polymyxins where there is significant uncertainty about the optimal dosing strategy. While the median antibiotic use density of polymyxin in VA ICUs was 2.1 DOT per 1,000 PD in ICUs that used at least one dose in 2010 there was one notable outlier at 57.3 DOT/1000 PD in FY 2010. In general, relatively few facilities, concentrated primarily in the Northeastern United States, contributed disproportionately to overall use.

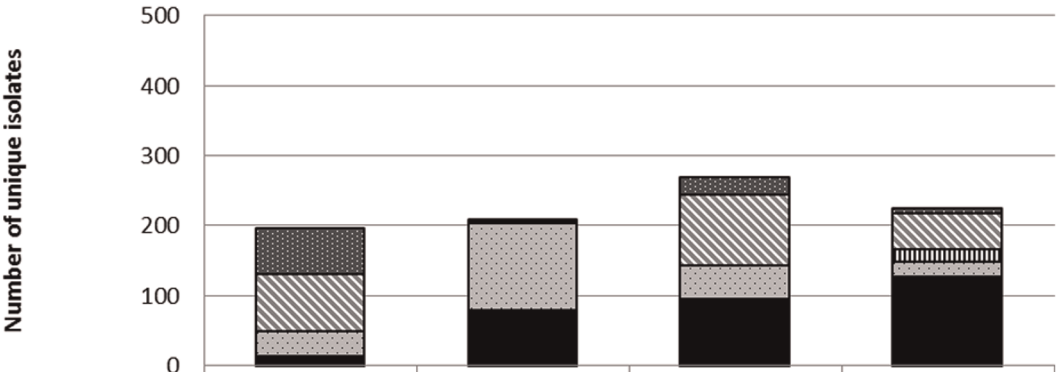
Tigecycline is frequently used in conjunction with polymyxins and is often considered a "last-resort" antibiotic in its own right, but it seems to be used very differently. Compared to polymyxins, it appears to have been used more frequently in empiric treatment regimens and more often as a single agent. This is not surprising since tigecycline has been marketed for a relatively broad-range of indications, including community-acquired pneumonia and Gram-positive pathogens. We are not aware of any publicly available data on tigecycline use with which to compare; it will be interesting to follow its use in the

a) **Microbiology associated with polymyxin use**



	Acinetobacter spp.	E. coli	Klebsiella spp.	P. aeruginosa
XDR	98	1	70	77
MDR & CR only	67	10	204	238
CR only	0	0	0	18
MDR only	63	38	70	27
not MDR or CR	8	15	12	78

b) **Microbiology associated with tigecycline use**



	Acinetobacter spp.	E. coli	Klebsiella spp.	P. aeruginosa
XDR	66	1	25	7
MDR & CR only	81	4	101	51
CR only	0	0	0	17
MDR only	36	124	47	23
not MDR or CR	14	80	96	127

Figure 3. Pathogens isolated. a) when polymyxins (1,145 hospitalizations) or b) tigecycline (3,125 hospitalizations) were used. Only *Acinetobacter spp.*, *E. coli*, *Klebsiella spp.* and *P. aeruginosa** are included **MDR:** multi-drug resistance (non-susceptibility to $>=3$ drug classes) **CR:** carbapenem resistance (non-susceptibility to carbapenems; ertapenem not considered for non-fermenters) **XDR:** non-susceptibility to all tested classes except to polymyxins (definitions of MDR, CR and XDR adapted from reference [22]) *In 206/225 cases where tigecycline was given and a Pseudomonas was isolated, the patient had at least one anti-pseudomonal spectrum agent (definition of anti-pseudomonal activity based on reference [22]).
doi:10.1371/journal.pone.0036649.g003

VA given recent concerns about its effectiveness in severe infections. [13,18].

The microbiology of Gram-negative isolates observed around the initiation of polymyxins demonstrated that this drug class was mainly used to treat resistant pathogens. There was a moderate

Table 2. Culture sample types by resistant (MDR, CR or XDR) pathogen.

POLYMYXINS				
Culture site	***Acinetobacter spp.* # isolates (%)**	***E. coli* # isolates (%)**	***Klebsiella spp.* # isolates (%)**	***P. aeruginosa* # isolates (%)**
Sputum	**122 (53.5%)**	**13 (26.5%)**	**138 (40.1%)**	**201 (55.7%)**
Urine	**17 (7.5%)**	**13 (26.5%)**	**84 (24.4%)**	**73 (20.2%)**
Blood	**55 (24.1%)**	**7 (14.3%)**	**74 (21.5%)**	**38 (10.5%)**
Other	**34 (14.9%)**	**16 (32.7%)**	**48 (14.0%)**	**49 (13.6%)**
TIGECYCLINE				
Culture site	***Acinetobacter spp.* # isolates (%)**	***E. coli* # isolates (%)**	***Klebsiella spp.* # isolates (%)**	***P. aeruginosa* # isolates (%)**
Sputum	**93 (50.8%)**	**29 (22.5%)**	**54 (31.2%)**	**49 (50.0%)**
Other	**39 (21.3%)**	**64 (49.6%)**	**38 (22.0%)**	**24 (24.5%)**
Blood	**35 (19.1%)**	**12 (9.3%)**	**32 (18.5%)**	**10 (10.2%)**
Urine	**16 (8.7%)**	**24 (18.6%)**	**49 (28.3%)**	**15 (15.3%)**

Only isolates recovered between the 7th day before and the 1st day after the first day of polymyxin or tigecycline use were included.
doi:10.1371/journal.pone.0036649.t002

correlation between polymyxin use in DOT and the number of drug resistant Gram-negative isolates over the study period. Although there is generally a two-way causal relationship between antibiotics and resistance, it seems unlikely that low-levels of polymyxin use greatly influence resistance to other antibiotic classes. We therefore surmise that the observed association between resistance and polymyxin use largely reflects the former driving the latter. Despite tigecycline being used more often than polymyxins, there were fewer resistant Gram-negative isolates associated with its use.

We were surprised to find that 30% of hospitalizations when polymyxins were used did not reveal any pathogens where susceptibility testing was performed. Possible explanations are that systemic polymyxins are often administered after long courses

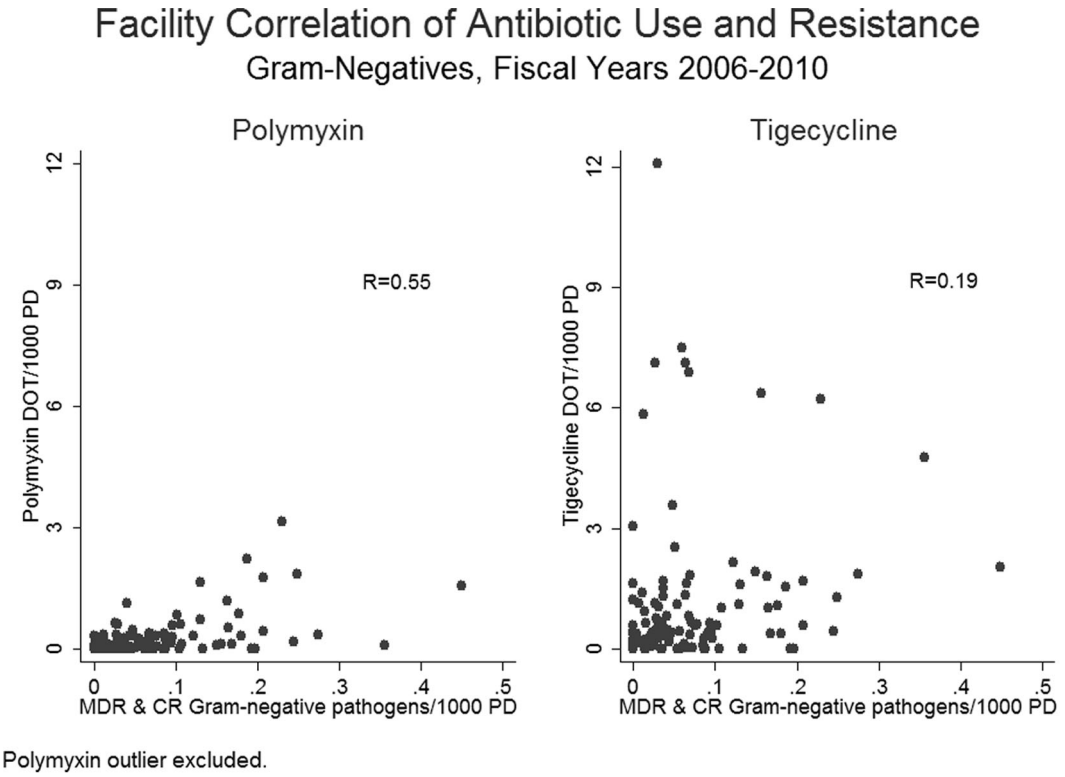


Figure 4. Correlation between polymyxin/tigecycline use in days of therapy (DOT) per 1000 patient days (PD) and MDR & CR Gram-negative pathogens per 1000 PD (aggregated over the entire study period). Extreme polymyxin use outlier excluded.
doi:10.1371/journal.pone.0036649.g004

of broad spectrum antibiotics, or that some polymyxin use represents either an empiric escalation in patients failing therapy or empiric coverage of pathogens isolated more than a week prior to polymyxin initiation or from outside the VA. Non-fermenters were the most frequent pathogens and most isolates of *P. aeruginosa* and *Acinetobacter* spp. were MDR and CR; however, *Klebsiella* species were nearly as common as *P. aeruginosa* and also demonstrated frequent carbapenem and extensive-drug resistance. Even though data on strain typing or carbapenem resistance mechanisms were not available, KPC enzymes are known to be present in the VA system. [25,26] Although tigecycline was sometimes used in association with *Pseudomonas* isolates, it should be noted that tigecycline is not active against this pathogen (idem for *Proteus* species) and in nearly all those case patient also received an anti-pseudomonal agent.

There is evidence of increasing carbapenem resistance throughout the United States. Thirty-eight states have reported CR Enterobacteriaceae (CRE) cases to the CDC in 2010 and some data indicate a dramatic rise in CRE in recent years. Given the sharing of patients between VAMCs, academic, and community hospitals, it is unlikely that VA trends will be independent from national trends.

Our study has several limitations. Although BCMA likely represents one of the most accurate ways to capture patient-level medication administration data and compliance with BCMA standards is regularly monitored within the VA system, we cannot be certain that all delivered doses were captured, which would underestimate antibiotic use. Similarly, since carbapenem resistance was difficult to identify in Enterobacteriaceae before revision of the Clinical and Laboratory Standards Institute (CLSI) carbapenem susceptibility breakpoints in mid-2010, and many facilities did not routinely test ertapenem in Enterobacteriaceae, we are likely to have underestimated carbapenem resistance in this family. [27] In addition, antibiotic susceptibility reporting is not standardized among VAMCs and some facilities routinely suppress antibiotic susceptibilities for certain organisms, potentially leading to an underestimation of carbapenem resistance. Multidrug resistance could also be biased simply from which susceptibilities laboratories chose to report. On the other hand, providers likely order cultures more frequently when they suspect resistance, thereby increasing the sampling of both colonizing and infecting organisms and biasing resistance estimates upward. Since the molecular mechanism of carbapenem resistance was not routinely determined, we were also unable to analyze the molecular epidemiology of carbapenem resistance in the VA system. Our approach to analyze microbiology culture within the 7th day before and 1st day after the first day of polymyxin or tigecycline use has not been formally validated. Electronic algorithms sometimes use, however, similar temporal rules. [28] Finally, the VA patient population may not be representative of other US hospitals and the findings of this study may thus not be generalizable to the entire United States.

To our knowledge this is the first study examining the use of "last-resort" antibiotics and the microbiology associated with their use in a large health-care system with detailed patient-level electronic data over several years. The total amount of polymyxin and tigecycline use is still relatively low in most VAMCs, but there has been an increase in the use of these drugs during the study period. Not surprisingly, our analysis shows polymyxin use is associated with MDR Gram-negative pathogens. The absence of antibiotics that are as effective and safe as the beta-lactams to treat infections with multi-resistant Gram-negative pathogens is a particular cause for concern. The Infectious Disease Society of America (IDSA) 10×'20 initiative has called for the development of 10 new systemic antibacterial drugs by 2020 by facilitating the antibiotic approval pathway and creating incentives for drug manufacturers to develop new antibiotics. [29] This issue is also currently under review in the US congress (Generating Antibiotic Incentives Now (GAIN) Act). It remains to be seen if this goal can be met. In the meantime, strict infection control practice, combined with the judicious use of antibiotics in all settings (including the ambulatory care and veterinary sector), are our best weapons to address this threat. Electronic biosurveillance systems, more in-depth analyses about the characteristics and clinical outcomes of the patient group described in this study, and additional insight into the mechanisms of resistant pathogens transmission will be invaluable in this endeavor.

Acknowledgments

We would like to thank Patricia and Kevin Nechodom for support with program and data management. The views expressed in this article are those of the authors and do not necessarily reflect the views of the Antibiotics and Innovation Project, the Pew Health Group, The Pew Charitable Trusts, the Department of Veterans Affairs, the United States government or the University of Utah.

Author Contributions

Conceived and designed the experiments: BH MJ MR MN AG MS. Analyzed the data: MJ BH. Wrote the paper: BH MJ MR MN AG MS.

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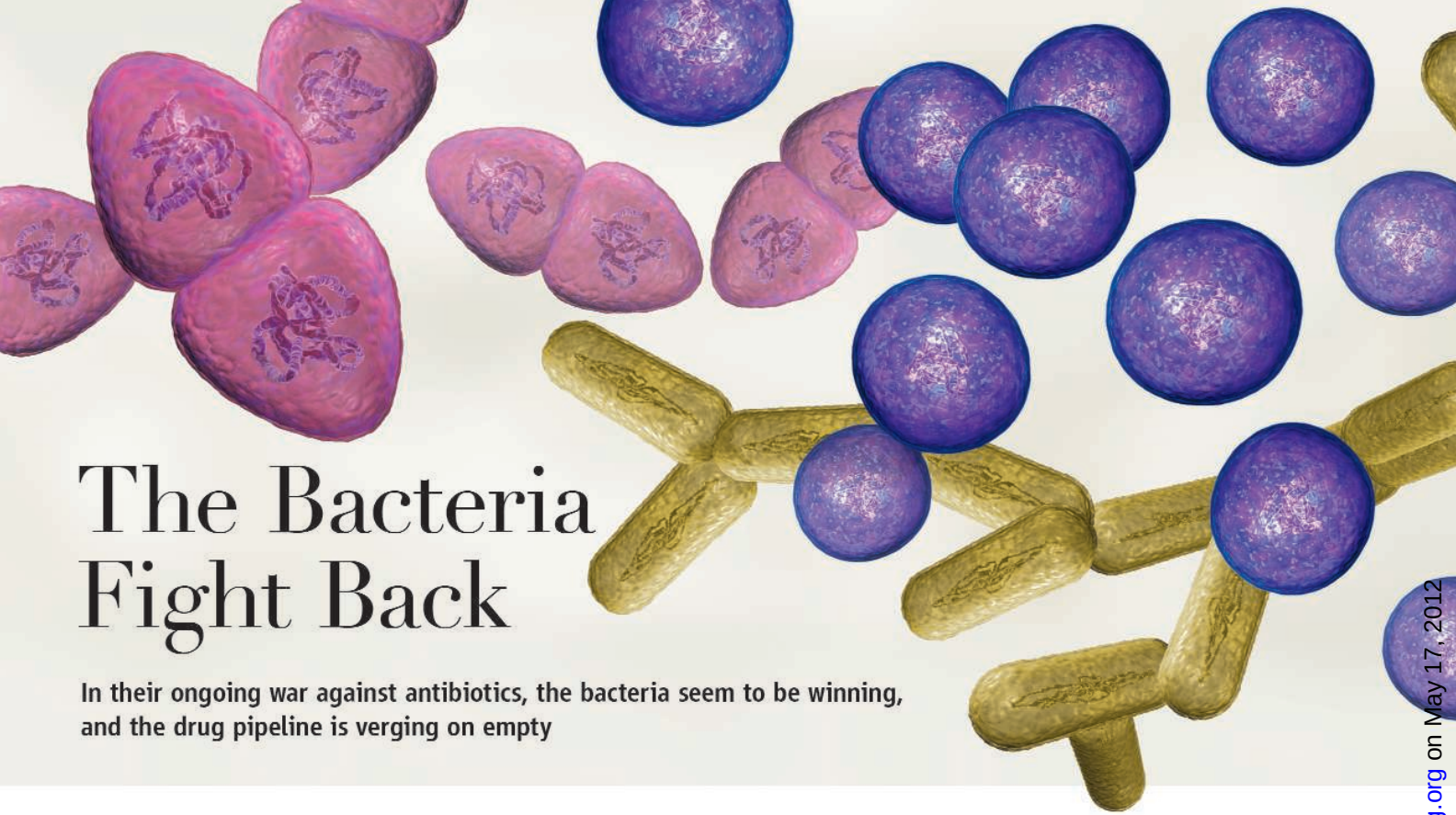

The Bacteria Fight Back

In their ongoing war against antibiotics, the bacteria seem to be winning, and the drug pipeline is verging on empty

MAYBE IT WAS JUST A BAD MONTH—AN unfortunate statistical fluctuation. Maybe not. As Vance Fowler, an infectious-disease specialist at Duke University Medical Center in Durham, North Carolina, tells it, the first case appeared in early spring 2008: a 13-year-old girl whose bout with the flu evolved into a life-and-death struggle, still ongoing, with necrotizing pneumonia and a particularly pernicious strain of bacteria known as methicillin-resistant *Staphylococcus aureus* (MRSA). Should the girl survive, her life will be "forever changed," says Fowler, from pulmonary disease caused by the death of the lung tissue. The next case, a week or so later, was a research technician from Fowler's laboratory, admitted to the hospital with a facial abscess that showed no signs of healing. Again, MRSA was the cause. A week or so after that, the victims were a husband and wife. "Both were admitted with life-threatening acute MRSA infections out of nowhere," he says. "Multiple surgeries. Life- and limb-threatening infections." Neither one worked in a hospital or a long-term care facility, the kind of environments in which such bacteria might commonly be found. Nor had they visited one recently. So how did they get it? "Bad luck, bad genes, a bad bug, or all three," says Fowler.

The last decade has seen the inexorable proliferation of a host of antibiotic-resistant bacteria, or bad bugs, not just MRSA but other insidious players as well, including *Acinetobacter baumannii*, *Enterococcus faecium*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, and *Enterobacter* species. The problem was predictable—"resistance happens," as Karen Bush, an anti-infectives researcher at Johnson and Johnson (J&J) in Raritan, New Jersey, puts it—but that doesn't make it any easier to deal with. In 2002, the U.S. Centers for Disease Control and Prevention (CDC) estimated that at least 90,000 deaths a year in the United States could be attributed to bacterial infections, more than half caused by bugs resistant to at least one commonly used antibiotic. Last October, CDC reported in the *Journal of the American Medical Association* that the number of serious infections caused by MRSA alone was close to 100,000 a year, with almost 19,000 related fatalities—a number, an accompanying editorial observed, that is larger than the U.S. death toll attributed to HIV/AIDS in the same year.

So far these outbreaks have been concentrated in hospitals, where the environment is particularly conducive to the acquisition and spread of drug-resistant bugs. But the big worry, for Fowler and others, is that they will spread to the wider community—a nightmare scenario, he says. MRSA is particularly worrisome, but so is another class of bacteria, called Gram-negative bacteria, that are even tougher to defeat. These include *A. baumannii*, which has plagued injured soldiers returning from Iraq. For these bacteria, the pipeline of new antibiotics is verging on empty. "What do you do when you're faced with an

Essential but not enough. Washing hands is one step, but ridding a hospital of resistant bacteria also requires identifying and isolating infected patients.

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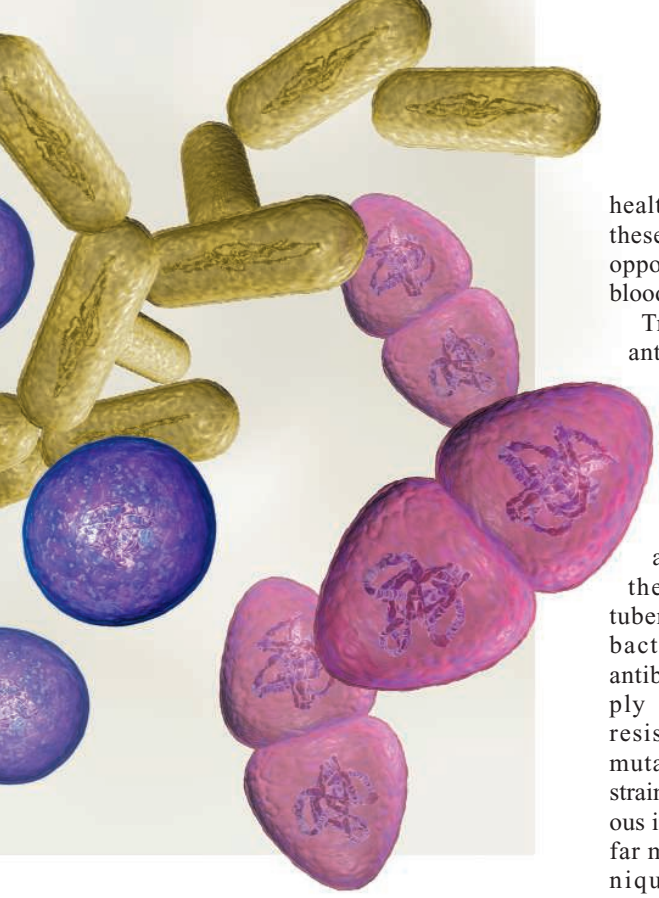
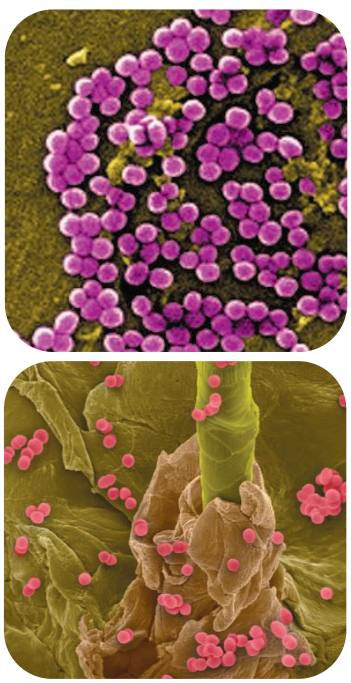


infection, with a very sick patient, and you get a lab report back and every single drug is listed as resistant?" asks Fred Tenover of CDC. "This is a major blooming public health crisis."

Right bug, wrong place

One of the many misconceptions about bacterial infections is that the bugs involved are not native to the human body or are particularly pernicious to begin with. Virtually all bacteria are capable of causing serious infections, at least in immunocompromised patients, although most do not. In hospitalized patients, many infections arise from the patient's own bacterial flora, flourishing where they're not supposed to be. Pneumonia, for instance, can be caused when bacteria from the mouth are aspirated to the lungs. Just as *Escherichia coli* is a normal inhabitant of the gut, *S. aureus* colonizes the skin and mucosal surfaces in the nose in 30% of the population. When it sets up shop somewhere else, *S. aureus* can cause a host of infections, including skin abscesses, necrotizing pneumonia, joint infections, and heart valve infections known as endocarditis. Similarly, *S. epidermidis* normally colonizes the human skin, but when it gets into the bloodstream, it can cause sepsis and endocarditis, as well as infections involving prosthetic devices such as pacemakers and artificial joints. The risk of acquiring one of these serious infections is highest in hospitals and health-care facilities simply because these environments offer the greatest opportunities for bacteria to enter the bloodstream or infect open wounds.

Treating a bacterial infection with antibiotics is the obvious first step. But in the 65 years since the first widespread use of penicillin during World War II, infectious-disease specialists have been treated to an ongoing tutorial in the many ways bacteria can acquire and spread resistance to these drugs. Unlike tuberculosis and other bacteria, in which antibiotic therapy simply selects for rare resistance-bestowing mutants, the bacterial strains that are so insidious in hospitals employ far more diverse techniques, says Louis Rice of Case Western Reserve University and the Louis Stokes Cleveland VA Medical Center in Ohio. *S. aureus* and *Enterococcus*, for instance, can acquire resistance by exchanging entire genes or multiple genes with other bacteria, either through plasmids—loops of DNA that are independent of the bacterial chromosomal DNA—or so-called gene cassettes or transposable elements that can be inserted directly into the chromosomal DNA.



Bad actors. Methicillin-resistant *S. aureus* (*above*) and vancomycin-resistant *Enterococcus*.

Penicillin and all penicillin-like antibiotics are ringlike molecular structures, known technically as β-lactams. They work by attacking a particular cell wall enzyme in the bacteria. The first strains of penicillin-resistant *S. aureus*, which arose within a few years of penicillin's introduction, were strains that have a survival advantage because they naturally produce an enzyme—penicillinase, one of a class of enzymes known as β-lactamases—that destroys the ring structure of penicillin. Within a decade, the effectiveness of penicillin against hospital-acquired staph infections was "virtually annulled," says microbiologist Alexander Tomasz of Rockefeller University in New York City, by "plasmid epidemics" that then spread the penicillinase gene through the entire species of *S. aureus*.

The pharmaceutical industry responded in the 1950s with a host of semisynthetic penicillins designed to be resistant to penicillinases. Methicillin, introduced in 1959, was believed to be the most effective. As Graham Ayliffe, a veteran hospital infection expert at the University of Birmingham in the U.K., recalled, "this was [supposed to be] the end of the resistant staphylococcus." Within 2 years, however, hospitals in Europe were identifying strains of *S. aureus* that were resistant to methicillin: the first MRSA strains.

Researchers later realized that these strains had taken a different route to acquiring resistance. Rather than generating new or different β-lactamases, which could attack the antibiotic directly, they had acquired a new gene entirely, called *mec*A, that coded for a variant of the antibiotic's target: the penicillin-binding protein. When the antibiotics attack the original penicillin-binding protein, explains Tomasz, this "surrogate" binding protein "takes over the task of cell wall synthesis" and works to keep the antibiotic at bay. The *mec*A gene itself, says Tomasz, appears to derive from a common bacterium on the skin of domestic and wild animals known as *S. sciuri*. How *S. aureus* came to acquire the gene is a mystery, but since it did, it passes it on by exchanging entire gene cassettes with the *mec*A gene on them.

Breaking out

Through the 1970s and 1980s, MRSA remained little more than a nuisance bug, although occasional hospital outbreaks would have to be reined in with strict isolation and control programs. In the mid-1980s, typically only 1% to 5% of all *S. aureus* isolates were methicillin-resistant, says Henry Chambers, an infectious-disease specialist at the University of California, San Francisco. Around that time, *S. aureus* began to acquire genes that confer resistance to other common antibiotics,

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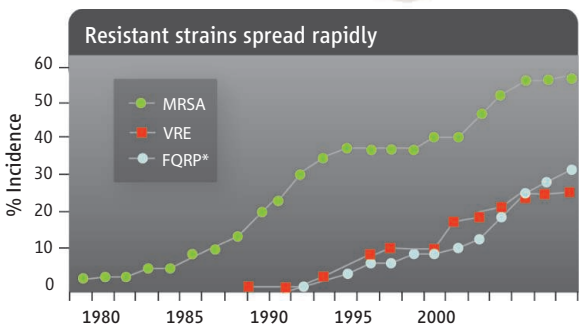
apparently from methicillin-resistant *S. epidermidis* and carried on the same mobile cassettes as *mec*A. The result was a bug that was both far more difficult to treat and, as Chambers says, "pretty adaptive to surviving in hospitals." Today, 60% to 70% of all *S. aureus* strains found in hospitals are multidrug-resistant MRSA.

Worries intensified when MRSA appeared a decade ago as a community-acquired infection rather than one exclusive to health-care settings. In 1999, CDC reported on four deaths in Minnesota and North Dakota, all children, all caused by MRSA infections that could not be traced back to hospitalizations by either the patients or family members. Somehow the *mec*A gene had emerged in *S. aureus* strains outside hospitals or health-care facilities. "This was a real biological success story," says CDC's Tenover. "And it all happened off our radar screens." MRSA isolates then began to appear in a range of unexpected community settings: children in day-care centers, army recruits, athletes in contact sports, native Americans living on reservations, prison populations, intravenous drug users, and among men who have sex with men.



*Fluoroquinoline-resistant *Pseudomonas aeruginosa*

The possibility that these MRSA strains were simply hospital strains that had migrated out into the community was refuted by analysis of the gene cassettes carrying the resistance. In hospital strains, these cassettes are relatively large and carry multiple resistance-bestowing genes, explains Tomasz. The "oddball" cassettes carrying the *mec*A gene in the early community-acquired isolates were small and contained only the one gene. In the last few years, however, MRSA strains in the community have begun to acquire multidrug resistance, suggesting that they've been intermingling with the hospital strains.

A half-dozen community-acquired MRSA clones have now spread around the world as their prevalence in the community has continued to increase; in San Francisco, for instance, up to 50% of all *S. aureus* isolates outside health-care settings are now methicillin-resistant. "These methicillin-resistant strains seem to be replacing the susceptible strains of *S. aureus* in the general population," says Mark Enright, an infectious-disease specialist at Imperial College London (ICL), "which means people are carrying strains of MRSA in their nose in the community. Now when they get infections, ones that were formerly treatable are going to be replaced with difficult-to-treat infections."

The public health anxiety increased still further in 2002 with the detection of isolates of MRSA that were fully resistant to the antibiotic vancomycin, traditionally considered the last resort for treating resistant staphylococcal infections. These *S. aureus* isolates seem to have acquired a gene for vancomycin resistance—*van*A—from enterococci, and specifically *E. faecalis*, which are part of the natural flora of the intestinal tract and can cause serious infections in hospitalized patients. When the enterococci developed resistance to common antibiotics in the 1980s, physicians had responded by using vancomycin to treat them. Vancomycin-resistant *Enterococcus* (VRE) was first reported in 1986, and the *van*A gene soon spread throughout the species. Because enterococci readily exchange genetic information with other bacterial species, says Tenover, he and other experts assumed that it would soon pass *van*A and vancomycin resistance to MRSA. "Everybody was waiting for the shoe to drop," says Tenover. In 2002 it did, when the Michigan Department of Community Health reported the first isolate of MRSA that had *van*A-mediated resistance to vancomycin. The patient was a 40-year-old diabetic who had recently been given an extended course of vancomycin for a foot ulcer.

Fortunately, vancomycin-resistant MRSA—now known as VRSA—has not developed into the nightmare researchers feared. Only nine isolates have been detected worldwide in 6 years, seven from the same region in Michigan, which suggests that *S. aureus*, unlike *Enterococcus*,

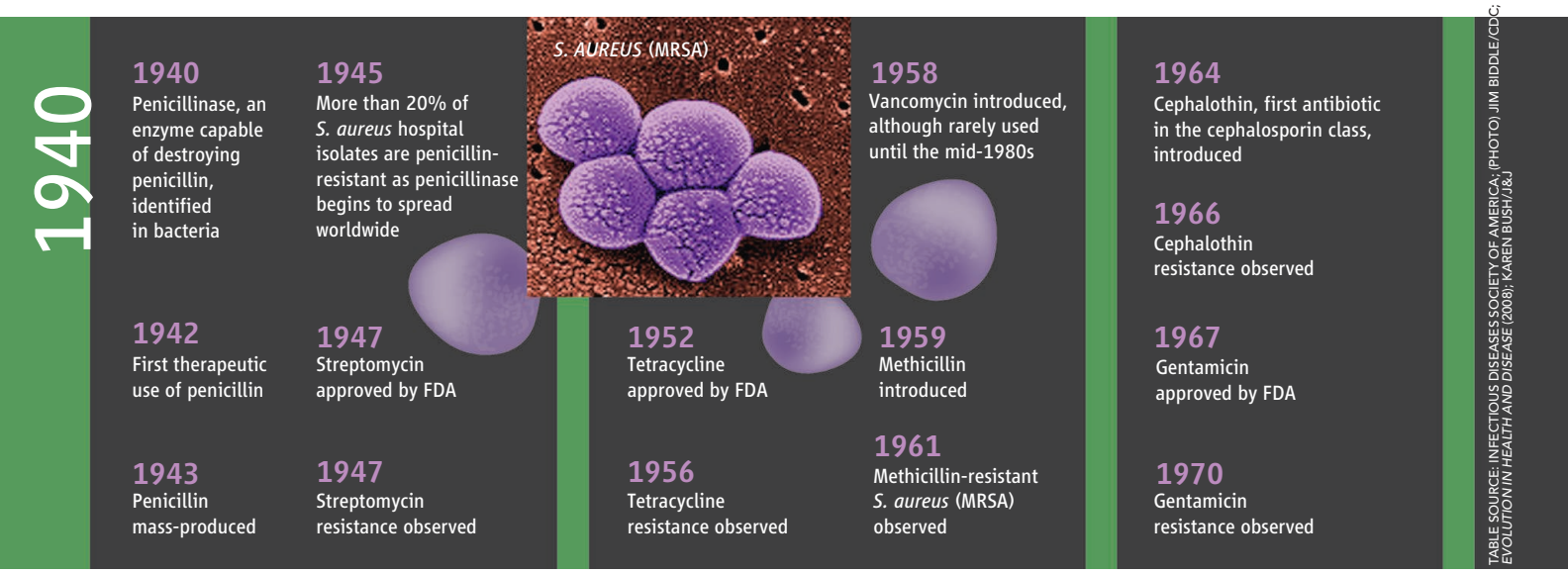


TABLE SOURCE: INFECTIOUS DISEASES SOCIETY OF AMERICA; (PHOTO) JIM BIDDLE/CDC; (TIMELINE SOURCE) C. T. BERGSTROM AND M. FELDGARDEN, *THE ECOLOGY AND EVOLUTION OF ANTIBIOTIC-RESISTANT BACTERIA*, IN S. STEARNS AND J. KOELLA, EDS., *EVOLUTION IN HEALTH AND DISEASE* (2008); KAREN BUSH/J&J

loses the ability to compete in the broader environment when it takes on the *van*A gene. That only one of these infections was life-threatening suggests that the vancomycin-resistant bug also loses its virulence.

A paltry pipeline

Although MRSA and other Gram-positive bacteria remain a major threat, a half-dozen new antibiotics have either just been approved or are in the pipeline that should work well against them—at least until the bugs evolve more resistance. This is not the case, however, for Gram-negative bacteria, such as *P. aeruginosa*, *A. baumannii*, and *K. pneumoniae*. These bacteria have both an inner and outer cell membrane, as opposed to the single cell membrane of Gram-positive bugs like MRSA. (The name comes from how these bacteria stain on a Gram stain test.) The pipeline for antibiotics against Gram-negative bacteria, says Bush of J&J, is limited to development programs in a few small companies; only one drug has made it through phase I clinical trials.

Prompted by the emergence of MRSA and VRE in the late 1980s, pharmaceutical companies focused their attention on Gram-positive bugs. Meanwhile, many Gram-negative bugs became resistant to virtually every known antibiotic, or at least every antibiotic that isn't toxic. "These organisms may well start to spread into the community," says Tenover, "and then we really will be in trouble. We have drugs to fall back on for *Staphylococcus*. But when you say, 'Where's the next anti-*Pseudomonas* drug?' I have to scratch my head."

One reason for the dearth of drug candidates is that Gram-negative bacteria are simply harder to kill. First, they have the extra cell membrane the drug has to penetrate. Then they have other defense mechanisms that Gram-positives lack, such as the ability to activate pumps or close down protein channels in the membranes that let these antibiotics in. "They can have three or four mechanisms working at once," says Case Western's Rice. "Even if you develop a new drug entirely, these bacteria may be just as likely to be resistant to new drugs as old ones. It's just really hard."

The problem has been exacerbated by the gradual exodus of pharmaceutical companies from antibiotic development—a trend that began in the 1980s and has accelerated since 2000, in large part because the market is iffy and the chances of success are slim. Of the 15 major pharmaceutical companies that once had flourishing antibiotic discovery programs, eight have left the field entirely, and two others have reduced their efforts significantly. That leaves only five—GlaxoSmithKline, Novartis, AstraZeneca, Merck, and Pfizer—that still have antibiotic discovery efforts commensurate with the size of the problem.

Estimated cases of hospital-acquired infections*

Antibiotic-Resistant Bacteria	Estimated Cases
Methicillin/*S. aureus*	102,000
Methicillin/CNS	130,000
Vancomycin/enterococci	26,000
Ceftazidime/*P. aeruginosa*	12,000
Ampicillin/*E. coli*	65,000
Imipenem/*P. aeruginosa*	16,000
Ceftazidime/*K. pneumoniae*	11,000

* Selected resistant bacteria, U.S., 2002

Even though the market for antibiotics is in the neighborhood of $25 billion a year, says Steve Projan, vice president of biological technologies at Wyeth Research in Cambridge, Massachusetts, other drugs, such as antidepressants or antihypertensives, offer a greater bang for the buck because they are often taken for years or decades rather than just a 7- to 14-day course. Resistance only compounds the problem: A drug that takes a decade to develop might be useful clinically for only a handful of years.

What's more, the better the antibiotic, the less health experts want to see it used to avoid the development of resistance. "It's probably the only area of medicine where a drug company can invest all this money to develop a drug, come up with a good one, and then the so-called thought leaders in the field, like myself, will tell people not to use it," says Rice. "We say it's such a good drug that we should save it."

As a result, virtually all the new antibiotics and all those in the pipeline for Gram-positive bacteria are second-generation drugs, that is, incremental improvements on existing classes. The one conspicuous exception—daptomycin, developed for *S. aureus* by the late Frank Tally at Cubist in Lexington, Massachusetts—was first identified 20 years ago by Eli Lilly and Co. and then shelved because it had toxicity problems at high doses.

To the surprise of many, the recent sequencing of more than 650 bacterial genomes has been a "dismal failure" when

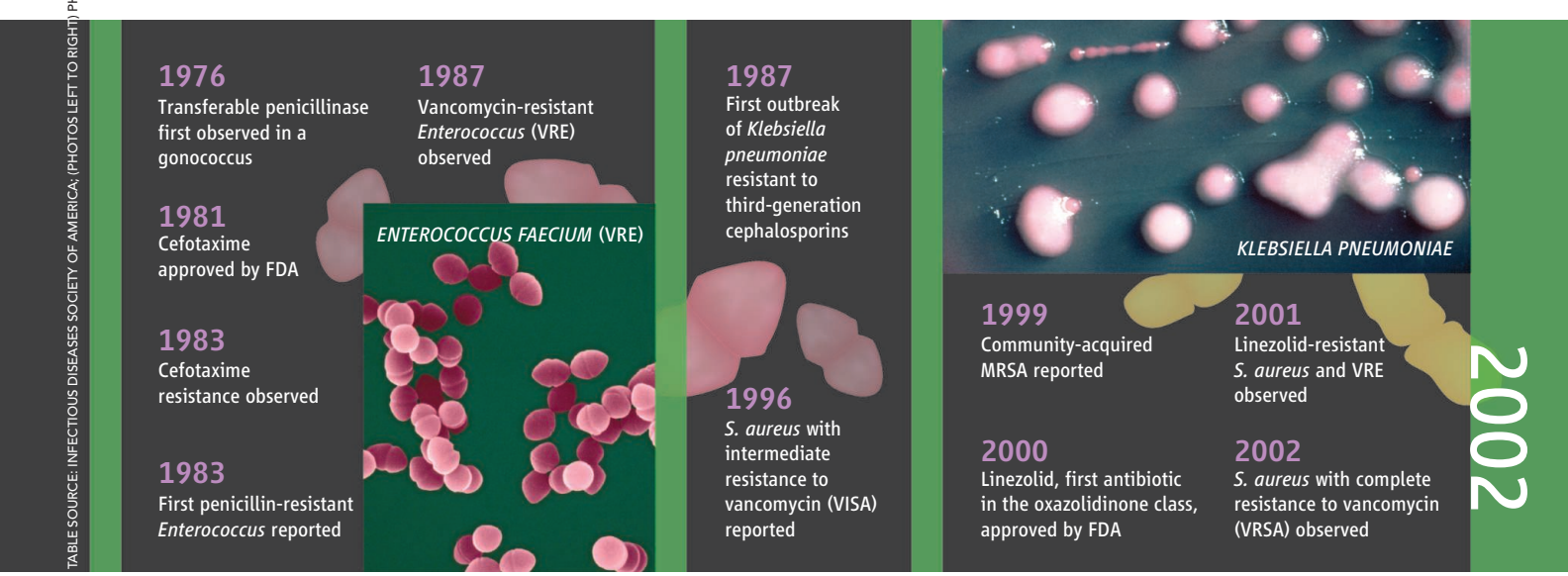


TABLE SOURCE: INFECTIOUS DISEASES SOCIETY OF AMERICA; (PHOTOS LEFT TO RIGHT) PHOTOTAKE INC./ALAMY; CDC; (ILLUSTRATIONS) N. KEVITIYAGALA/*SCIENCE*

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it comes to drug development, says ICL's Enright. Although genome sequences were expected to yield a "treasure trove of new targets for entirely new classes of antibiotics," as David Pompliano and colleagues at GlaxoSmithKline in Collegeville, Pennsylvania, recently wrote, this simply hasn't panned out. At GlaxoSmithKline, Pompliano and his colleagues spent 7 years and more than $70 million evaluating more than 300 "canonical" bacterial genes that they thought were essential to the viability of the bacteria. The result was just five leads, a success rate, they estimated, that was four- to fivefold lower than for other areas of therapeutics.

Genomics is simply not a good paradigm for discovering new antibiotics, suggests Projan. The genetic approach assumes that a candidate drug can knock out a single gene in the bacterium to render it unfit for survival. But the drugs don't knock out a gene's activity entirely, he says; instead they modulate activity. "As we found out in oncology," says Projan, "sometimes leaving even 5% activity is enough for the tumor to grow. The same thing is true for bacteria." Projan

Collateral Damage: The Rise of Resistant *C. difficile*

In April 2002, Mark Miller, an infectious-disease specialist and microbiologist working at Jewish General Hospital in Montreal, Canada, began to suspect that he had an outbreak on his hands. He was used to dealing with the bacteria *Clostridium difficile*, which can cause severe diarrhea in debilitated patients and had been a common problem in hospitals for more than 30 years. But now the number of cases had started to climb, as did their severity. "One of the first indications that we knew we had a problem," says Miller, "was when one of the colorectal surgeons called me and said, 'I just took out my second colon in a month on a *C. difficile* patient.' When we started looking at the numbers, they were absolutely horrendous." At the peak of the outbreak, says Miller, there were 50 new cases of *C. difficile* diarrhea every month in their 600-bed hospital. "Of those, about one in six was dying or going for a colectomy. That's kind of staggering."

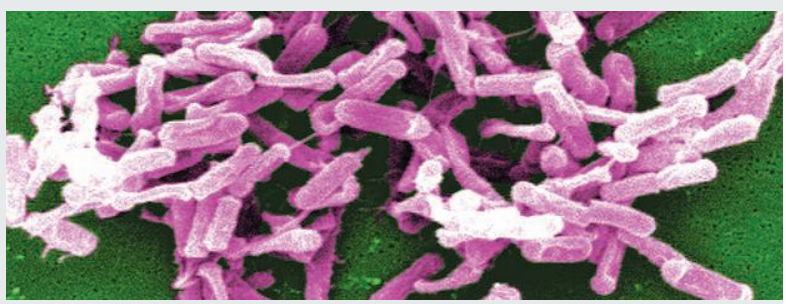
Resistance to antibiotics makes for bacteria that are harder to kill, but it can also bestow on a bacterial strain the advantage it needs to spread through the hospital environment and perhaps around the world—a kind of collateral damage in the escalating war between man and microbes. *C. difficile* is an unfortunate case in point. The bacterium has currently been linked to at least 5000 deaths a year in the United States; at the height of the Quebec epidemic it caused more than 7000 serious infections and 1200 deaths in a single year. In many hospitals, *C. difficile* constitutes a greater risk to patients than methicillin-resistant *Staphylococcus aureus* or any other bacteria.

The symptoms of a *C. difficile* infection range from mild diarrhea to severe colitis, and the elderly bear the brunt of the disease. One in four patients will have a recurrence or multiple recurrences. "It's a horrible problem," says Dale Gerding, an infectious-disease specialist at Hines Veterans Administration Hospital and Loyola University in Chicago, Illinois. Patients have to be treated almost constantly with oral vancomycin to prevent recurrences, he says.

C. difficile diarrhea first appeared in the medical literature in the 1970s, mistaken for a side effect of the antibiotic clindamycin. In 1978, physicians realized that the diarrhea was induced by toxins from clindamycin-resistant *C. difficile*, which had colonized the victim's colon after their normal gut flora had been decimated by the clindamycin treatment. *C. difficile* has remained a common hospital infection ever since because the bacteria produce heat-resistant spores that are exceedingly difficult to kill. "They're very resistant to detergents and cleaning agents," says Gerding. "Really, the only thing that destroys them is bleach or hydrogen peroxide."

Through the 1990s, however, *C. difficile* wasn't considered a major threat because the bacteria were susceptible to two antibiotics, vancomycin and metronidazole, the latter of which is inexpensive. As many as 40% of all hospitalized patients are colonized with *C. difficile*, but most tolerate it without symptoms. A series of hospital outbreaks in six U.S. states, beginning around 2000 and capped by the severe Quebec outbreak in a dozen hospitals, suggested that a new, hypervirulent strain of *C. difficile* was circulating.

Since then, the same offending strain has been identified in hospitals in 38 states and has also been linked to outbreaks in Western Europe. What sets it apart from its predecessors, say Gerding and Miller, is its high resistance to the newer fluoroquinolone antibiotics, such as levofloxacin and moxifloxacin. These antibiotics began to be used widely in the late 1980s, and usage has increased steadily ever since. Why this strain induces more severe disease—with a death rate among those infected of 10% compared with 1% percent in the 1980s—is still a mystery, but one possibility, says Gerding, is a mutation that enables the strain to produce more toxin.



The battle escalates. A hypervirulent strain of *C. difficile*, resistant to two of the newer, last-resort antibiotics, has triggered outbreaks across the United States and in Western Europe.

Although Quebec hospitals have reduced the incidence of *C. difficile* infections by two-thirds since the height of the outbreak, through very tight isolation and control and rigorous "housekeeping," says Miller, they have yet to get back to the levels preoutbreak. "*C. difficile* in health-care facilities and hospitals is a very unforgiving organism," he says. It exploits "any lapse in isolation, in housekeeping, in hygiene—whatever it is—and it comes back with a vengeance." **–G.T.**

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and others suggest that the only route to a new antibiotic—short of pure luck—will be through more fundamental research on the basic biology of the bacteria.

Cutting back

Barring the discovery of miracle antibiotics to which bacteria cannot evolve resistance—a "laughable" notion, says one expert—the only foreseeable route to curbing antibiotic resistance will be to rein in the use of antibiotics. One obvious way is to lower the risk of acquiring resistant bugs in the hospital. Countries that have mandated rigorous infection control in hospitals, such as Denmark, the Netherlands, and Finland, have been able to keep MRSA infection rates low. These infection-control procedures, however, go far beyond physicians and nurses wearing gloves and protective masks and washing their hands before and after patient contacts, essential as those are. These nations employ a technique known as "active detection and isolation," or "search and destroy," as it's called in the Netherlands. Patients considered at high risk of carrying MRSA and other antibiotic-resistant bugs are cultured when they're admitted to the hospital, and periodic cultures are taken of all patients, particularly those in high-risk wards. The greater the prevalence of pathogens and risk factors, the more frequent this surveillance. Patients who are infected or are carriers are isolated. Healthcare workers who are colonized with resistant bacteria can be "decolonized," using skin washes and nasal ointments.

Whether U.S. hospitals should be required to implement active detection and isolation is a long-running controversy. Some specialists—led by University of Virginia epidemiologist Barry Farr, an expert on controlling VRE and MRSA—have argued that it's the only proven method to control hospital MRSA infections. Others have questioned the technique's cost-effectiveness and viability, particularly when rates of MRSA in the community are beginning to rival those in many hospitals.

Vaccines against antibiotic-resistant bacteria would also go a long way to reining in resistance, but only one such vaccine candidate, against *S. aureus*, has ever made it through phase III clinical trials: StaphVAX, licensed by Nabi Biopharmaceuticals. Although patients who received the vaccine had significantly lower rates of *S. aureus* infections at 40 weeks compared to controls, this apparent protection was lost at 54 weeks. A follow-up trial also failed to demonstrate efficacy. Several more vaccines are in development, including a new-generation StaphVAX. Even temporary protection could be useful, argue some experts, either for health-care workers, who could be vaccinated regularly, or for patients who are about to be hospitalized to undergo a procedure.

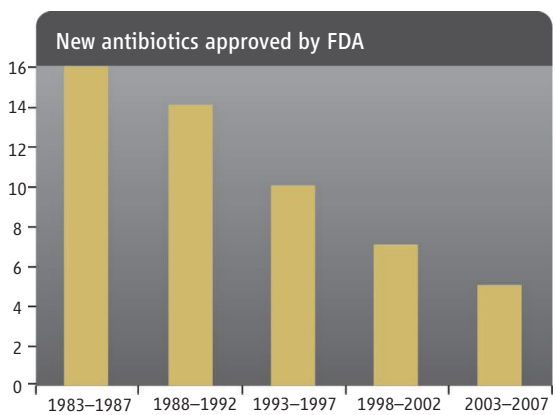
Ultimately, physicians will have to be persuaded to reduce their use of antibiotics, although that will be a hard sell. One step, for instance, would be to persuade physicians outside hospitals to treat only those patients who are truly infected. A 2001 study from the University of Colorado Health Sciences Center estimated that 55% of all antibiotics prescribed in the United States for upper respiratory infections were unnecessary. This is what Rice describes as the "get-a-little-sniffle-get-a-little-Levaquin" problem. "The patients want it," he says, and "the doctor wants to get the patient out of the office, and the quickest way to do it is to write a prescription." But the societal problem of antibiotic resistance should outweigh whatever personal peace of mind comes from the indiscriminate use of antibiotics, says Tenover (see sidebar on p. 360).

Similarly, many times physicians prescribe combination "broad-spectrum" antibiotics when a single "narrow-spectrum" antibiotic would do the trick. Understandably, says Rice, physicians are unwilling to wait to treat serious infections until the bug is cultured and they learn to which antibiotics it's still susceptible. But once the crisis is over, usually 1 to 3 days after starting therapy, physicians could switch their patients to the appropriate narrow-spectrum antibiotic.

What the field desperately needs, these experts say, are randomized, controlled trials to establish how long antibiotic therapy should be prescribed for different infections. The data are scarce, and misconceptions abound. The ubiquitous advice in the field—from physicians, patients, and even CDC—is that patients should continue the full course of antibiotics even after they feel better. Because antibiotics tend to have few side effects, physicians consider a longer course to be a no-lose proposition.

But from the perspective of preventing antibiotic resistance, says Rice, "this is totally wrong-headed." In patients with healthy immune systems, he explains, most antibiotics merely stun the bacteria sufficiently to make it easier for the host immune system to do its job. "You can take tetracycline until the cows come home," Rice says, and "all it does is stop most bacteria from growing. It doesn't kill them." Extending the course of the antibiotics unnecessarily increases the likelihood that the patient's normal flora will be inhibited to the point that bacteria resistant to the antibiotic will fill the void.

The few existing studies on the necessary length of therapy have suggested that it is often surprisingly short. Urinary tract infections in young women can be treated with 1- to 3-day courses of antibiotics. The Infectious Diseases Society of America recommends a 3-day treatment for traveler's diarrhea, while acknowledging that 1 day appears to be equally effective. Studies from the 1940s suggested that the "vast majority" of patients with pneumonia get better after 2 or 3 days, says Rice: "Somewhere along the line, that morphed into 7 days, 10 days, 21 days, with no real reason other than making the doctor more comfortable." In May, the U.S. National Institute of Allergy and Infectious Diseases responded to the expert demand and put out a request for proposals for clinical studies that would determine the optimal use of antibiotics, including the optimal duration of therapy. "I think most physicians would respond to compelling data from a well-done trial," says Rice.



SOURCE: INFECTIOUS DISEASES SOCIETY OF AMERICA

One beneficial side effect to curbing antibiotic use is that it may serve to rehabilitate those antibiotics that have lost their effectiveness. "Many of these were wonderful new drugs just 20 years ago, able to treat a wide variety of bugs, both inside and outside the hospital," says Rice. "Now we're at a point where some of them are next to useless, because they've been used for everything."

–GARY TAUBES

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Bacteriophages φMR299-2 and φNH-4 Can Eliminate *Pseudomonas aeruginosa* in the Murine Lung and on Cystic Fibrosis Lung Airway Cells

Debebe Alemayehu, Pat G. Casey, Olivia McAuliffe, et al. 2012. Bacteriophages φMR299-2 and φNH-4 Can Eliminate *Pseudomonas aeruginosa* in the Murine Lung and on Cystic Fibrosis Lung Airway Cells . mBio 3(2): . doi:10.1128/mBio.00029-12.

RESEARCH ARTICLE

Bacteriophages ϕMR299-2 and ϕNH-4 Can Eliminate *Pseudomonas aeruginosa* in the Murine Lung and on Cystic Fibrosis Lung Airway Cells

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ABSTRACT ***Pseudomonas aeruginosa*** **is a common cause of infection in the lungs of patients with cystic fibrosis (CF). In addition, biofilm formation and antibiotic resistance of *Pseudomonas* are major problems that can complicate antibiotic therapy. We evaluated the efficacy of using bacteriophages to kill the pathogen in both biofilms and in the murine lung. We isolated and characterized two phages from a local wastewater treatment plant, a myovirus (ϕNH-4) and a podovirus (ϕMR299-2). Both phages were active against clinical isolates of *P. aeruginosa*. Together, the two phages killed all 9 clinical isolate strains tested, including both mucoid and nonmucoid strains. An equal mixture of the two phages was effective in killing *P. aeruginosa* NH57388A (mucoid) and *P. aeruginosa* MR299 (nonmucoid) strains when growing as a biofilm on a cystic fibrosis bronchial epithelial CFBE41o- cell line. Phage titers increased almost 100-fold over a 24-h period, confirming replication of the phage. Furthermore, the phage mix was also effective in killing the pathogen in murine lungs containing 1×10^7 to 2×10^7 *P. aeruginosa*. *Pseudomonas* was effectively cleared (reduced by a magnitude of at least 3 to 4 log units) from murine lungs in 6 h. Our study demonstrates the efficacy of these two phages in killing clinical *Pseudomonas* isolates in the murine lung or as a biofilm on a pulmonary cell line and supports the growing interest in using phage therapy for the control and treatment of multidrug-resistant *Pseudomonas* lung infections in CF patients.**

IMPORTANCE **Given the rise in antibiotic resistance, nonantibiotic therapies are required for the treatment of infection. This is particularly true for the treatment of *Pseudomonas* infection in patients with cystic fibrosis. We have identified two bacterial viruses (bacteriophages) that can kill *Pseudomonas* growing on human lung cells and in an animal model of lung infection. The use of bacteriophages is particularly appropriate because the killing agent can replicate on the target cell, generating fresh copies of the bacteriophage. Thus, in the presence of a target, the killing agent multiplies. By using two bacteriophages we can reduce the risk of resistant colonies developing at the site of infection. Bacteriophage therapy is an exciting field, and this study represents an important demonstration of efficacy in validated infection models.**

Received 2 February 2012 **Accepted** 6 February 2012 **Published** 6 March 2012

Citation Alemayehu D, et al. 2012. Bacteriophages ϕMR299-2 and ϕNH-4 can eliminate *Pseudomonas aeruginosa* in the murine lung and on cystic fibrosis lung airway cells. mBio 3(2):e00029-12. doi:10.1128/mBio.00029-12.

Editor Keith Klugman, Emory University

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Cystic fibrosis (CF) is an inherited genetic disorder that chronically affects the lungs and digestive system (pancreas and intestine) of children and adults worldwide. CF also affects the mucus and glands of the liver, sinuses, and sex organs causing progressive disability due to multiorgan system failure. CF results from mutations in the transmembrane conductance regulator gene (1–3). The defective enzyme leads to the production of unusually thick and sticky mucus and high levels of chloride containing secretions into ducts and body cavities. These secretions clog the lungs, leading to life-threatening infections (4, 5).

The lungs of CF patients are often colonized at infancy or in early childhood with *Pseudomonas aeruginosa* that may damage the epithelial surface, resulting in altered airway physiology and impairment of mucocillary clearance. This chronic infection is one of the main causes of lung function decline and mortality in CF patients (6, 7). Indeed, 80 to 95% of patients with CF succumb to respiratory failure brought about by chronic bacterial infection and concomitant airway inflammation (7). *P. aeruginosa* is particularly persistent in the lungs due to its aerobic nature and its ability to form biofilms in the lungs of CF patients. Another significant factor is the inherent resistance of *P. aeruginosa* to many antibiotics due to membrane impermeability (8–12). Other acquired mechanisms of resistance include production of β-lactamases and carbapenemases (13) and multidrug efflux pumps (14). It has also been noted that the most prevalent and severe chronic lung infections in CF patients are caused by mucoid *P. aeruginosa* strains (4, 11, 15).

Reports have shown that organisms in biofilms are able to tol-

erate 10- to 1,000-fold-higher levels of antibiotics than planktonic bacteria (16, 17), and this can mean that the antibiotic concentration needed to eradicate a biofilm is higher than the peak serum concentration (18), rendering it ineffective. The continued emergence and reemergence of biofilm-forming *Pseudomonas* resistant to one or more antibiotics pose a continuous challenge in the treatment of lung infections in CF patients. As a result, there is a need for alternative, nonantibiotic approaches such as phage therapy (19, 20). Although phage therapy has been practiced in Eastern European countries for decades, it has been neglected by the Western world for many years. However, there is now a growing interest in the use of phage therapy for the control and treatment of multidrug-resistant bacterial infections in general, and for *Pseudomonas* lung infections in CF patients in particular. Studies on phage efficacy in clearing of biofilms formed on abiotic surfaces (catheters and microtiter plates) by *P. aeruginosa* confirmed that phage can reduce the bacterial load in these biofilms by 50 to 99% (21, 22). A recent *in vitro* study showed that *Pseudomonas* phage PT-6 was able to reduce the viscosity of alginate polymers extracted from *P. aeruginosa* by almost 65%, a mechanism used by phages to attack the exopolysaccharide matrix of mucoid *Pseudomonas* to gain access to the host cell (23). Recent reports show the promising role phage therapy could play in the treatment of acute lung infections in an *in vivo* murine lung model with *P. aeruginosa* (24, 25) and *Burkholderia cenocepacia* (26). The effectiveness of phage therapy in rescuing larvae (an invertebrate infection model) infected with *Burkholderia cepacia* complex from death was reported by Seed and Dennis (27).

Currently, there is little information concerning the effect of bacteriophage on biofilms growing on a lung tissue model. In this report, we describe how a mixture of two newly isolated phages can kill and clear *lux*-tagged *Pseudomonas* from the lungs of infected mice and in biofilms growing on the surface of a cystic fibrosis bronchial epithelial (CFBE41o-) monolayer. In this study, we used bioluminescence imaging—a powerful tool for studying bacterial infections in small-animal models, since it allows accurate real-time *in vivo* temporal and spatial tracking of tagged bacteria in living animals (25, 28). Monitoring the light emitted by tagged *Pseudomonas* cells *in vivo* was a valuable tool in verifying the effectiveness of the phage mix to kill the pathogen. *Pseudomonas* cell numbers were reduced by a magnitude of 3 to 4 log units when the phage mix was tested in both *in vivo* and *in vitro* systems.

RESULTS

Isolation of phage from a sewage treatment plant. The overall aim of this study was to assess the potential of bacteriophage therapy to treat *Pseudomonas* lung infections. We initially isolated two phages, ϕNH-4 and ϕMR299-2, from sewage obtained from a water treatment plant. Both phages were demonstrated to be virulent to *P. aeruginosa*. Scanning electron microscopy revealed that phage ϕMR299-2 virions have isometric capsids of 40 to 60 nm in diameter and very short tails measuring 10 to 20 nm (Fig. 1A). Morphologically, phage ϕMR299-2 shows similarity to *Pseudomonas* ϕPap3 (29), a podovirus that has an isometric capsid and short tail. We have assigned ϕMR299-2 to type species coliphage T7 and to the family *Podoviridae* (Report of the International Committee on Taxonomy of Viruses [30, 31]). Phage ϕNH-4 possessed an isometric capsid of 50 to 60 nm in diameter and a contractile nonflexible tail with cross striations (noncontracted tail length of 150 nm and contracted tail length of 85 nm; tail diameter

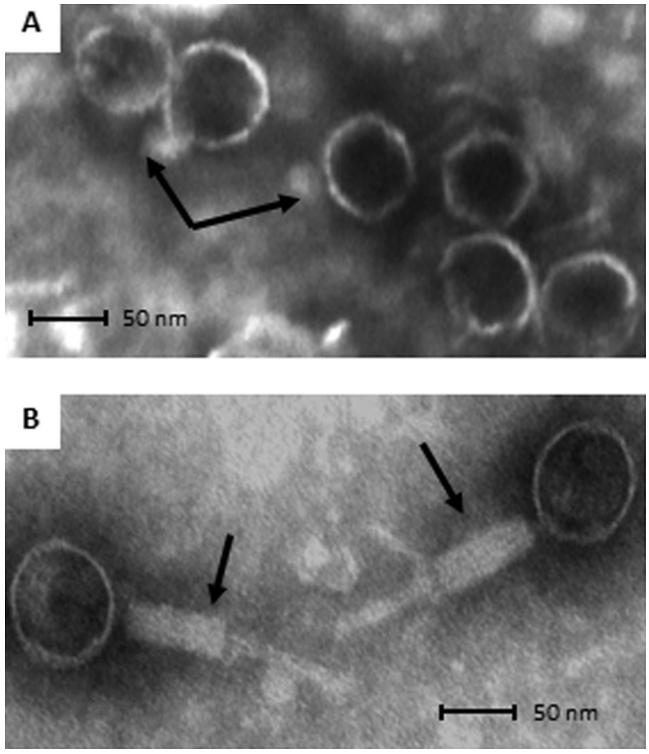

FIG 1 Scanning electron microscopy images of phage ϕ229-2, a podophage (A) and phage ϕNH-4, a myophage (B), stained with 0.2% phosphotungstic acid. The arrows point to the short tail (10 to 20 nm long) of the podophage in panel A and to the contracted tail sheath of the myovirus in panel B.

of 20 nm when contracted). In addition, a putative DNA injecting structure of 70 nm in length (narrower than the contracted tail sheath) and tail fibers were observed (Fig. 1B). Morphologically, ϕNH-4 shows similarity to ϕPB1, ϕLBL3, and ϕSN that are classified into the T4 morphological group of the *Myoviridae* (32). On the basis of structural characteristics obtained from the microscopic analysis, we assign ϕNH-4 as a member of the *Myoviridae* family according to the International Committee on Taxonomy of Viruses (30, 31).

An equal ratio of ϕNH-4 and ϕMR299-2 was used in all experiments to assess their ability to kill *Pseudomonas*. The host range of ϕNH-4 and ϕMR299-2 was determined by exposing different CF *Pseudomonas* isolates to each individual phage using a plaque assay. Of the ten *Pseudomonas* isolates tested, eight were sensitive to both phages, while all were sensitive to at least one phage (Table 1). When the ϕ299-2 and ϕNH-4 phages were added to *Pseudomonas* in LB broth either separately or in combination, the individual phage resulted in reduction of 3 to 4 log units while the combination of the two resulted in a reduction of about 4.5 log units (see Table S1 in the supplemental material).

Phage genome overview. Before phage can be used in human therapy, it is important to assess any potential risk associated with the phage genomes such as the presence of genetic determinants for toxins or other virulence factors or for the capability to integrate into the host genome. The complete sequences of phages ϕ299-2 and ϕNH-4 were determined using 454 pyrosequencing. Phage ϕ299-2 consists of a double-stranded DNA (dsDNA) molecule of 44,789 bp with a GC content of 52%, significantly lower

TABLE 1 Strains and plasmids used in this study

Strain or plasmid	Description[a]	Source[b] or reference	Sensitivity[c] of strain to the following phage:	
			*ϕ*NH-4	*ϕ*MR299-2
Pseudomonas aeruginosa strains				
MR299	Human CF sputum isolate	CUH	+++	+++
MR299::p16S*lux*	*lux*-tagged MR299	This study	+++	+++
NH57388A	Stable mucoid CF mouse sputum isolate	20	+++	++
NH57388A::p16S*lux*	*lux*-tagged NH57388A	This study	+++	++
MR300	Human CF sputum isolate	CUH	++	−
MR325	Human CF sputum isolate	CUH	−	+
MR326	Human CF sputum isolate	CUH	+++	++
MR327	Human CF sputum isolate	CUH	++	++
MR330	Human CF sputum isolate	CUH	+++	++
MR331	Human CF sputum isolate	CUH	++	+
CH001	Human CF sputum isolate	AH	+++	+++
POA1	UCC culture collection	UCC	+++	++
Plasmid p16S*lux*	*lux*-tagged plasmid vector	19		

[a] UCC, University College Cork.
[b] CUH, Cork University Hospital (Cork, Ireland); AH, Alimentary Health Ltd. (Cork, Ireland).
[c] Symbols: +++, very strong lysis; ++, strong lysis; +, moderate lysis; −, no lysis.

than the 66.6% of *P. aeruginosa*. Both the genome size and GC content of this phage are similar to the closely related *Pseudomonas* phage *ϕ*PaP3 (29). A total of 68 open reading frames (ORFs) are predicted, 21 of which have a leftward orientation and 47 of which are transcribed to the right (Fig. 2). Three tRNA genes ($tRNA^{Asn}$, $tRNA^{Asp}$, and $tRNA^{Pro}$) were also identified in phage *ϕ*299-2, clustered at the 5' end of the genome. While *ϕ*299-2 is a lytic phage, comparative genomic analysis revealed that its closest homolog is the temperate *Pseudomonas* phage, *ϕ*PaP3 (29), isolated from hospital sewage. Interestingly, a recent study calls into question the temperate nature of *ϕ*PaP3, since no site-specific recombinase is encoded up- or downstream of the *ϕ*PaP3 *attP* site, and immunity or reactivation of the integrated *ϕ*PaP3 DNA was not demonstrated (33). Of the 68 predicted protein products of *ϕ*299-2, 56 display the highest amino acid identity to proteins from *ϕ*PaP3 (see Table S3 in the supplemental material). Among these are proteins involved in particle formation (ORF03, ORF04, ORF06, and ORF07), genome replication (ORF29, ORF33/40, encoding DNA polymerase I subunits and ORF41, a putative primase/helicase), a putative lysozyme-like endolysin (ORF02) and 47 proteins of hypothetical function. A conserved genomic organization is also evident on comparing *ϕ*299-2 to *ϕ*PaP3. The remaining 12 ORFs show significant identity to proteins from the lytic *Pseudomonas* phage LUZ24 (33). According to Ceyssens et al., *ϕ*PaP3 and *ϕ*LUZ24 represent a new genus within the *Podoviridae* family (33). Considering the close relationship between *ϕ*299-2 and *ϕ*PaP3 and *ϕ*LUZ24, it is likely that *ϕ*299-2 also belongs to this genus.

Phage *ϕ*NH-4 is a member of the widespread and conserved PB1-like viruses, with a genome of 66,116 bp and a GC content of 55.5%, also significantly lower than that of its host. A total of 94 ORFs were identified in the sequence; the predicted protein products of 56 of these ORFs have the highest amino acid identity to *ϕ*LMA2 (ST4), isolated from a river in Maastricht, Holland, in 2007 (32). Other *ϕ*NH-4 proteins show significant identity to predicted proteins from PB1-like viruses such as *ϕ*14-1, *ϕ*SN, *ϕ*LBL3,

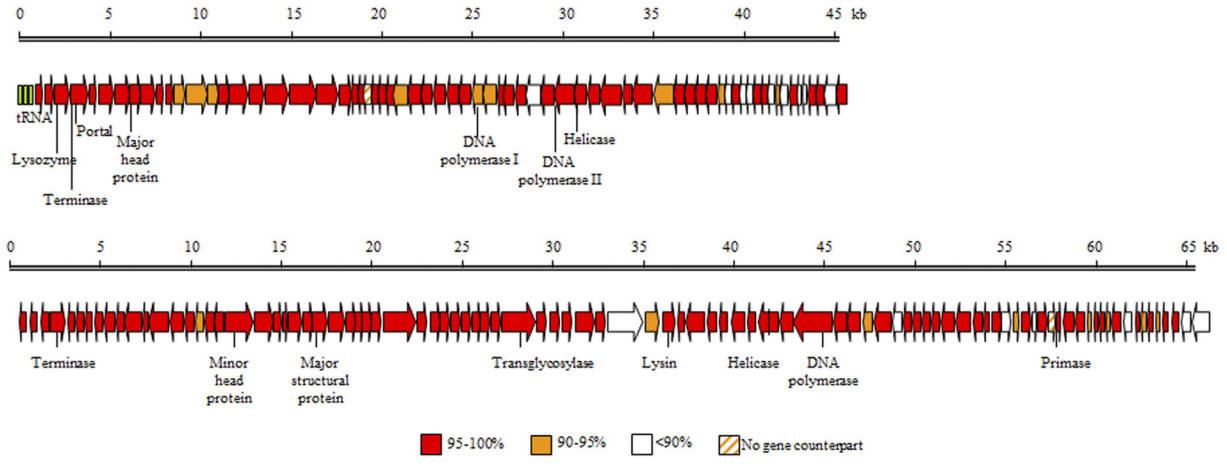

FIG 2 Genome organization of phage *ϕ*MR299-2 (top) and *ϕ*NH-4 (bottom). The predicted open reading frames are indicated by the thick arrows, which are shaded to show the level of protein identity to the corresponding regions of the closest *P. aeruginosa* (phage PaP3 for *ϕ*MR299-2) or phage LMA2 (for *ϕ*NH-4).

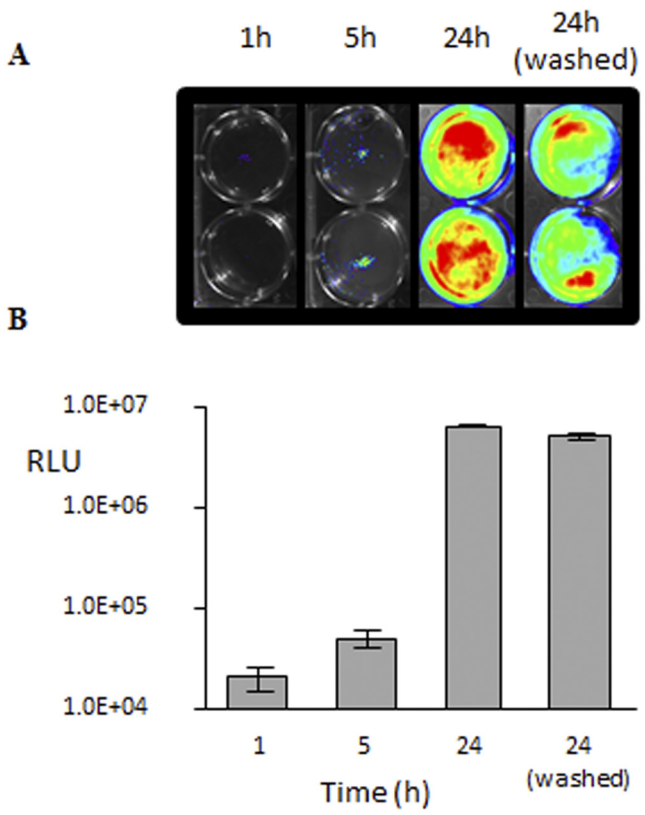

FIG 3 (A) Growth of *lux*-tagged *Pseudomonas* biofilms on the surface of the CFBE410- cell monolayer. Light was measured 1, 5, and 24 h (before and after the monolayer was washed with MEM). (B) Readings from 6 wells are shown. Values are shown as means ± standard deviations (SD) (error bars).

ϕJG024, and ϕPB1 itself (32). As is the case with other PB1-like viruses, the genes in ϕNH-4 are arranged in a compact manner and appear to be organized into at least 7 transcriptional blocks, alternating on both strands (Fig. 2). Based primarily on sequence similarity to ORFs in the genomes of phages LMA2 and JG024, genomic regions encoding phage particle formation (ORF19 to -47) and phage DNA replication (ORF55 to -70) could be identified. ORF48 encodes a putative endolysin with 100% identity to the endolysin of phage ϕLMA2 and belongs to a lysozyme-like superfamily.

Biofilm growth by *lux*-tagged *Pseudomonas* on CFBE41o- cell monolayer. We studied the ability of phage to penetrate and attack *Pseudomonas* cells growing as biofilms on the surface of a layer of human lung epithelial cells. CFBE41o- cells (CFBE stands for cystic fibrosis bronchial epithelial) were grown in standard 6-well tissue culture plates, and a confluent culture with a tight junction between cells was achieved after 8 to 10 days of incubation (12). The confluent culture was inoculated with *lux*-tagged *Pseudomonas* strains NH57388A::p16S*lux* or MR299::p16S*lux*, and growth was monitored in this static system for 24 h (Fig. 3A). The addition of arginine in the minimal essential medium (MEM) enhanced the formation of biofilms and helped preserve the integrity of the CFBE41o- cells, as suggested by Anderson et al. (12). The amount of luminescence recorded for the growing biofilms increased by 2 log units during the 24-h incubation (Fig. 3B). Washing the biofilm monolayer culture twice with MEM removed all planktonic *Pseudomonas* cells. The absence of motile cells and the presence of only adhered clusters of microcolonies of various sizes scattered across the epithelial cell monolayer were confirmed by phase-contrast microscopy (see Fig. S2 in the supplemental material). The amount of bioluminescence recorded after removing planktonic cells was only 25% lower than that obtained before washing (Fig. 3A), which confirmed that the majority of *Pseudomonas* cells were adhered to the epithelial monolayer. We determined the number of CFU after washing the monolayer at 2.6×10^7 to 3.8×10^7 CFU/well and 4.2 to 5.4×10^7 CFU/ well (well area of 9.5 cm^2) for strains NH57388A and MR299, respectively.

Calcofluor white, a fluorescence enhancer that binds to the $\beta(1–3)$ and $\beta(1–4)$ polysaccharide linkages found in biofilm matrices produced by exopolysaccharide-producing organisms (12, 34, 35) was used to stain biofilms. The staining revealed that the *Pseudomonas* cells were contained within a polysaccharide matrix (Fig. 4A and B). The structures formed by the two *Pseudomonas* strains NH57388A and MR299 measured on average 20 to 30 μm by 30 to 40 μm in diameter. The presence of abundant numbers of *Pseudomonas* cells packed in polysaccharide matrices and attached to the cell line led us to conclude that the microcolony structures formed on the monolayer fit the definition of biofilms.

Clearing of biofilms by phage. We examined the ability of the mixture of phages to clear 24-h-old biofilms of *P. aeruginosa* NH57388A or MR299. Calcofluor white staining revealed a change to open and weak matrices in the presence of the ϕNH-4 and ϕMR299-2 phages, indicating considerable destruction to the biofilm structure (Fig. 4C and D). At the same time, phage titers increased almost 2 log units during the 24-h period, confirming significant phage replication (Fig. 4E). The amount of luminescence recorded for both *Pseudomonas* biofilms also decreased by 2 log units over a 24-h period (Fig. 5). In contrast, the light level remained high and unchanged in the control biofilms (with no phage added) over the same period. Direct plating results also confirm that the significant reduction in light was a direct result of the destruction of the *Pseudomonas* cells by the phage. The numbers of CFU estimated for the biofilms before phage added were 2.6×10^7 to 3.8×10^7 CFU/well and 4.2×10^7 to 5.4×10^7 CFU/ well (well area of 9.5 cm^2) for strains NH57388A and MR299, respectively, and the amount of phage added was 0.5×10^8 to 1.0×10^8/well (multiplicity of infection [MOI] of 2 to 5). During the 24-h incubation, the number of *Pseudomonas* cells in the biofilms was reduced by 3 to 4 log units in the presence of phage.

Clearing of *Pseudomonas* from murine lungs. The ability of phage to kill *Pseudomonas in situ* in the lungs of infected 8-week-old female BALB/c mice ($n = 16$) was also examined (Fig. 6). In this regard, *lux* tagging of *Pseudomonas* cells was very useful in monitoring the fate of these cells in the lungs of infected mice. The presence of *Pseudomonas* was evident in the lungs of both test and control mice 2 h after infection. The amount of light recorded in the control mice (without phage) increased 3-fold and reached its maximum level after 6 h. The amount of light recorded in mice treated with phage decreased significantly during the same period.

DISCUSSION

There has been increased interest in phage therapy as a means of combating bacterial lung infections. However, there is little information about the efficacy of bacteriophage in clearing *Pseudomonas* growing on CF lung tissue or in an animal model. It has been well documented that the CF lung environment causes normally

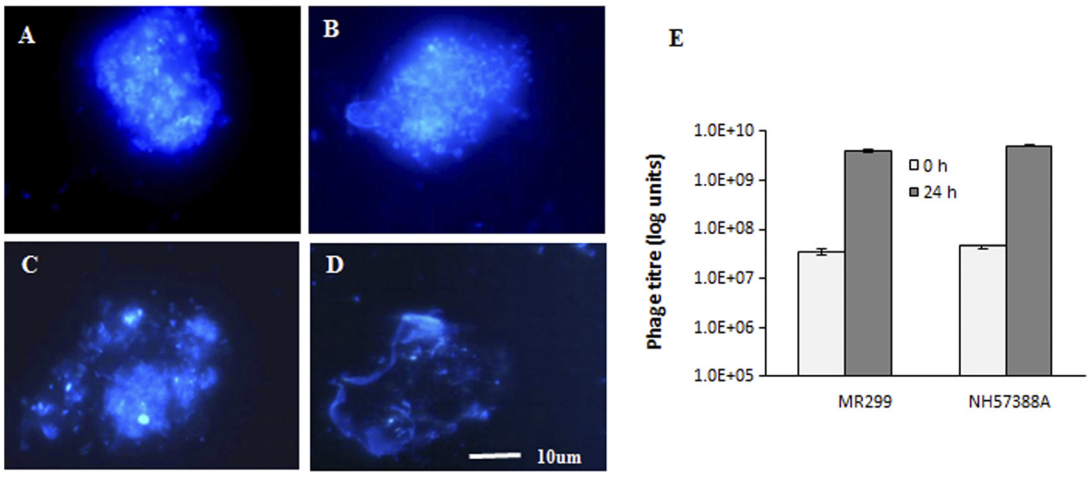

FIG 4 Fluorescent image of 24-h-old culture of *P. aeruginosa* cells grown on a CFBE41o- cell monolayer after Calcofluor white (fluorescent enhancer) staining. Staining confirms that *P. aeruginosa* NH57388A (A) and MR299 (B) are embedded in an exopolysaccharide structure prior to phage exposure. After 24-h incubation in the presence of mixed phages, staining indicates open and weak matrices with reduced numbers of cells for both NH57388A (C) and MR299 (D). An increase in phage titer was observed over the 24-h incubation period for both MR299 and NH57388A strains (E).

motile, planktonic *P. aeruginosa* to form mucoid biofilms (36–38). Our data show that 1 h after the addition of *lux*-tagged *Pseudomonas* cells to a CF epithelial monolayer, only a very low level of localized light was detected. The signal then increased over 100-fold in 24 h (Fig. 3). Washing the 24-h-old biofilm with MEM medium resulted in 25% reduction in the amount of light, indicating that 75% (2.6×10^7 to 5.4×10^7 CFU/well) of the *Pseudomonas* adhered as microcolonies over the entire surface of the CF cell monolayer. It is well documented in the literature that microcolony dispersal in *Pseudomonas* is a feature of biofilm maturation (39–42).

To be effective, phage must be able to penetrate the biofilm exopolysaccharide. This may account for the fact that it took more time (22 to 24 h) for phage (at similar MOI) to clear *Pseudomonas* growing on CFBE41o- cells than the 5 to 6 h required to clear recently introduced planktonic cells from the lungs of infected mice. We observed proliferation of *Pseudomonas* cells in the lungs of mice in the absence of phage, whereas phage treatment pre-

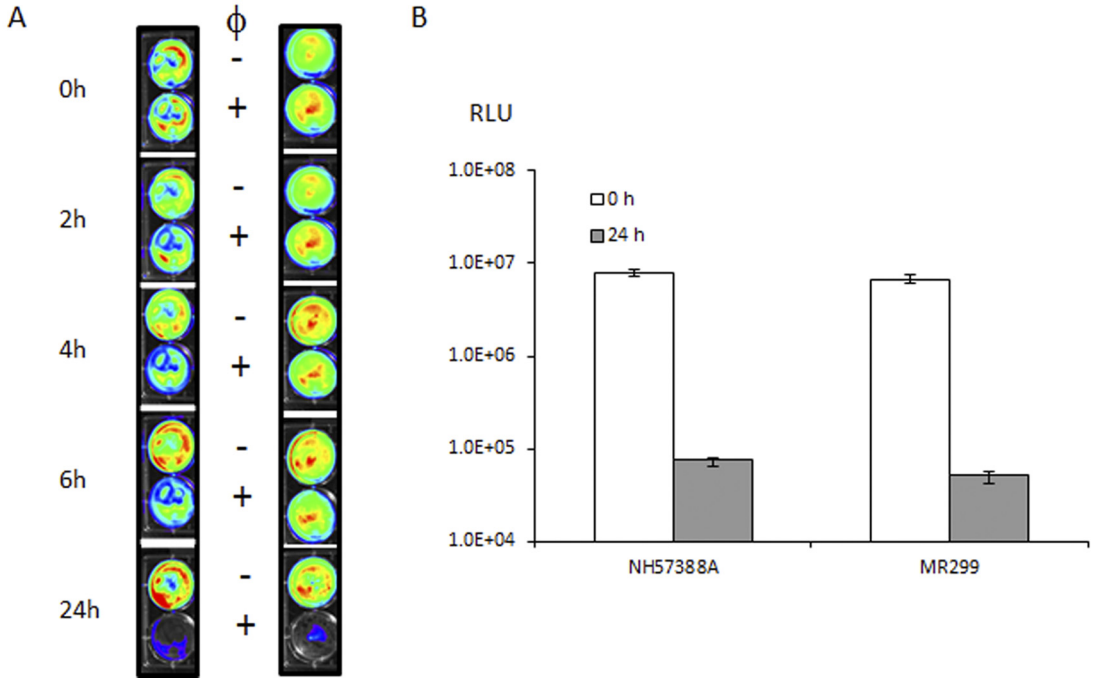

FIG 5 (A) Light emitted from nonmucoid *P. aeruginosa* MR299 strain and mucoid NH57388A strain grown on a CFBE410- cell monolayer for 24 h in the presence (+) and absence (−) of phage mix. (B) The RLU values are mean ± SD readings from 3 wells.

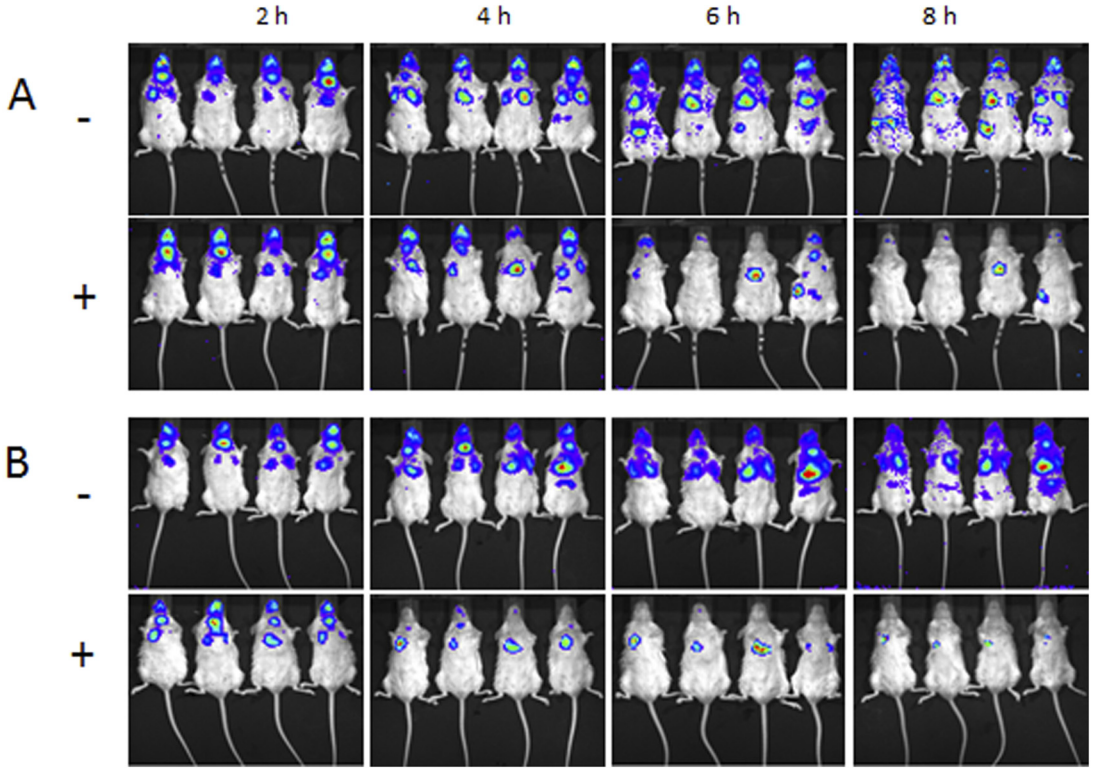

FIG 6 Mice ($n = 8$) were infected with nonmucoid *P. aeruginosa* MR299 (A) and mucoid NH57388A (mucoid strain) (B). Test mice (+) were treated with the phage mix (ϕMR299-2 and ϕNH-4B). Phage was given 2 h after the mice were infected with *Pseudomonas*. Control mice (−) did not receive the phage mix.

vented growth and reduced the bacterial load to a nondetectable level during the 6-h period. A recent study reported that the amount of light measured in mice infected with a *lux*-tagged *Pseudomonas* PAK strain also decreased over 6 h when phage PAK-P1 was administered 2 h after infection (24, 25). The amount of light increased in the control mice and in the mice that received a delayed phage treatment. The authors concluded that administering phage 2 h after infection was critical to resolving infection in the mouse model system and suggested that the efficacy of phage needed to be tested against biofilms.

One advantage of using phage to control bacterial infection is that they can replicate at the infection site (24). In addition to being a very effective way of clearing *Pseudomonas*, the use of a phage mix has another advantage over the use of a single phage in that it reduces the likelihood of a phage resistance population emerging. Different phages often use different bacterial receptors and therefore will require independent mutations to generate resistance to each phage (43). Consequently, unless mutants with generalized resistance mechanism evolve, a mix of different phages for which there is no cross-resistance should be able to prevent multiple phage resistance and provide indefinite control of bacterial population.

In conclusion, this study demonstrates that *Pseudomonas* growing on a CF airway tissue monolayer could be killed by phage. Since chronic lung infections in CF patients are associated with *Pseudomonas* biofilms rather than planktonic cells, we anticipate that biofilm clearing from lungs of CF patients by bacteriophage might take place in a similar manner to that observed with the exopolysaccharide-producing microcolonies growing on an epithelial cell monolayer. Moreover, we subsequently demonstrate that the same phage mix was effective in killing the pathogen in the lungs of infected mice. Our study reinforces the growing interest in using phage therapy as a means of attacking multidrug-resistant CF infections.

MATERIALS AND METHODS

Bacterial strains and culture conditions. *Pseudomonas* strains isolated from CF patients and used in this study are shown in Table 1. LB medium was used throughout this study to culture *Pseudomonas* strains. A double-strength LB medium was made for phage isolation by doubling the weight of dry ingredients required to prepare single-strength LB broth. Cultures were grown at 37°C under aerobic conditions and shaking at 180 rpm. Solid media and soft agar overlays contained 1.5% and 0.7% agar (BD Difco, Oxford, United Kingdom), respectively.

Transformation of *Pseudomonas* with p16*lux* plasmid. *P. aeruginosa* strains NH57388A (mucoid) and MR299 (nonmucoid) were transformed with p16S*lux* plasmid (44) by the method of Shen et al. (45). In brief, *Pseudomonas* strains were grown in LB medium at 37°C until an optical density (OD) at 600 nm of 0.8 is reached. To facilitate electroporation, *Pseudomonas* exopolysaccharide was digested by adding alginate-lyase (catalog no. A1603; Sigma, Japan) to a final concentration of 2 U ml^{-1}. The cell enzyme mixture was incubated at 37°C for 30 min. The cells were centrifuged at 10,000 × *g* (4°C) for 10 min. The resultant pellet was washed twice with chilled electroporation buffer (containing 300 mM glucose, 5 mm $CaCl_2$, and 25 mM HEPES in distilled water [pH 7.0]) and resuspended in 0.1 ml of buffer (1×10^9 to 1×10^{10} CFU/ml). These electrocompetent cells were mixed with 10 μl of p16S*lux*-tagged plasmid DNA (10 μg) and incubated on ice for 10 to 15 min. The mixture was immediately transferred to a chilled electroporation cuvette (0.2-cm electrode gap Gene-pulser cuvette; Bio-Rad, Hercules, CA) and subjected to a

single voltage shock by applying a pulse with an ECM 630 BTX Harvard precision pulse apparatus (Holliston, MA) at the following settings: capacitor, 25 μF; resistor, 200 Ω; and voltage, 2.5 kV. Immediately after the electric shock, 900 μl of chilled SOC medium (catalog no. S1797; Sigma-Aldrich) was added to the mixture and incubated on ice for 10 min, followed by incubation at 30°C for 2 to 3 h. The cells were concentrated by centrifugation at 10,000 $\times$ *g* (room temperature), and transformants were obtained by plating cells on LB agar containing erythromycin (800 μg/ml) and incubating at a permissive temperature (30°C) for 24 to 48 h. Ery^r colonies were checked for light emission using a VivoVision IVIS100 imaging system (Xenogen, Alameda, CA), luminescence was measured in relative light units (RLU) (in photons second^{-1}), and the presence of p16S*lux* was confirmed by miniprep and restriction analyses. A standard curve was generated to determine the relationship between light measurements and CFU (see Fig. S3 and Table S2 in the supplemental material).

Isolation of phage from sewage. *Pseudomonas* phages were isolated from fresh sewage obtained from a local sewage treatment plant as described previously (46) with some modifications. Sewage samples were centrifuged at 3,200 $\times$ *g* value (Heraeus Labofuge 400 centrifuge; Thermo Fisher Scientific, Inc., MA) for 12 min, and the supernatant was filtered using a 0.45-μm-pore-size filter (Sarstedt, Actiengeselischaft and Co., Germany). The sewage filtrate (5 ml) was mixed with an equal volume of double-strength LB broth, supplemented with 10 mM $CaCl_2$, and inoculated with a mixture of ten CF *P. aeruginosa* cultures (50 μl each of 1 $\times$ 10^7 to 2 $\times$ 10^7/ml). The samples were incubated overnight aerobically (37°C) with slow shaking (20 to 30 rpm). Following overnight incubation, the cultures were centrifuged at 3,200 $\times$ *g* value for 12 min to remove bacterial cells and debris, and the supernatant was filtered through a sterile 0.45-μm-pore-size filter. A double-layer LB agar plate containing a lawn of individual host culture supplemented with 10 mM $CaCl_2$ was prepared, and 10 μl of cell-free filtrate containing phage was applied to the plate. The plates were examined for the presence of plaques after incubating aerobically for 18 to 24 h at 37°C.

Plaque purification and bacteriophage titers. Phages were purified by successive single plaque isolation and propagation. In general, a single plaque was picked from a plate using a sterile capillary tube and added to a mid-log-phase *Pseudomonas* culture (10^8 CFU/ml) supplemented with 10 mM $CaCl_2$. The culture mixture and phage mixture were incubated at 37°C overnight. The lysate was filtered through a 0.45-μm-pore-size sterile filter, serial dilutions were made, and plaques were allowed to form on a lawn of the same host culture. Single plaques were purified through 3 successive rounds of plaquing and repeated three additional times after which purified phages were obtained. Phage titer was determined as the number of PFU/ml by plaque assay as previously described (47).

DNA extraction and restriction digestion analysis. High-titer-purified phage suspension was prepared by concentration of phage particles from 400 ml cell lysate in LB medium to a final volume of 1 ml in sterile ice-cold ammonium acetate (0.1 M, pH 7.2) as described before (46), and DNA was extracted from the high-titer-purified phage as previously described (46, 48, 49). Phage DNA was digested with restriction endonuclease EcoRI (New England Biolabs, MA) according to the supplier's recommendation, and digested samples were analyzed by gel electrophoresis using agarose gel (0.7%) containing ethidium bromide (SF1).

Genome sequencing and annotation. The genomes of phages ϕNH-4 and ϕ299-2 were sequenced by Beckman Coulter Genomics (Sanger sequencing services; Beckman Coulter, Takeley, United Kingdom) on a 454 GS-FLX sequencer, and sequences were assembled into contigs using the Newbler program (Roche Applied Sciences). The quality of the sequence was assessed using Hawkeye (Amos) (50). To confirm phage genome structure, primers were designed at contig ends for PCR amplification using Platinum PCR SuperMix (Invitrogen) followed by direct sequencing of the PCR products, and full phage genome assembly was performed using the Phred-Phrap-Consed package (51, 52). ORFs were predicted using GLIMMER 3.02 (53). The resulting gene models were fed into GAMOLA (54) for annotation. Complementary annotation was provided using the RAST annotation server (55). Data were manually curated using Artemis version 11 (56) where additional programs were then used, including BLASTp (57), GATU (58) and RBS finder (53). Comparative genomics with reference phages (ϕPaP3 and ϕLMA2) were analyzed using the Artemis comparison tool (ACT) (7) and the Mauve alignment tool (59).

Electron microscopy and phage characterization. Electron microscopy image of phages was obtained by applying a drop of high-titer phage suspension (1 $\times$ 10^9 to 2 $\times$ 10^9 PFU/ml) deposited on carbon-coated copper grids, negatively stained with 2% (wt/vol) potassium phosphotungstate (pH 7.2) and examined with Zeiss Supra 40VP scanning electron microscope (Carl Zeiss SMT Ltd., Cambridge, United Kingdom) fitted with a scanning transmission electron microscope detector (STEM) operating in bright-field mode at an accelerating voltage of 25 kV (Moorepark National Food Imaging Centre, TEAGASC Food Research Centre [TFRC], and Advanced Microscope Research Facility, University College Cork, Cork, Ireland).

Cell culture and *Pseudomonas* biofilm formation on the surface of a monolayer of human bronchial epithelial cells (CFBE41o- cells). Cystic fibrosis bronchial epithelial (CFBE41o-) cell (60, 61) cultures were grown by the method of Anderson et al. (12). In general, CFBE41o- cells were seeded in sterile 6-well, flat-bottom tissue culture plates (Sarstedt, Newton, NC) at a concentration of 10^6 cells/well and maintained in minimal essential medium (MEM) containing 10% fetal bovine serum, 2 mM l-glutamate, 100 μg/ml penicillin, and 100 μg/ml streptomycin (all from Invitrogen GIBCO, United Kingdom). The cells were grown at 37°C and 5% CO_2 using a Jouan IGO150 cell life incubator (Jouan, St. Herblain, France) for 8 to 10 days until cells form a confluent monolayer and tight junctions. The medium (MEM) was changed every 2 or 3 days until confluent growth was achieved.

For biofilm formation, *lux*-tagged *P. aeruginosa* cells were grown on the confluent CFBE41o- cell monolayer using a coculture model system (12). Once the monolayer growth was achieved (between 8 and 10 days), the medium was replaced with 1.5 ml fresh MEM (without fetal bovine serum, penicillin, and streptomycin), and *lux*-tagged *Pseudomonas* cells were inoculated (1 $\times$ 10^7 to 2 $\times$ 10^7 CFU/well). The plates were incubated at 37°C and 5% CO_2 for 1 h. The medium containing the unattached (planktonic) *Pseudomonas* cells was then removed using a sterile serological pipette and replaced with fresh MEM supplemented with 0.4% arginine and incubated for 24 h. Planktonic *Pseudomonas* cells were removed, and the biofilm culture was washed twice using MEM supplemented with 0.4% arginine. The integrity of the epithelial cell monolayer and the presence of growing *Pseudomonas* microcolonies were assessed by phase-contrast microscopy (Olympus IX50 inverted system microscope; Olympus Co., Tokyo, Japan). Luminescence from biofilms was monitored by Vivo Vision IVIS100 imaging system (Xenogen, Alameda, CA). Biofilm CFU was estimated by the method of Wirtanen et al. (62) with some modifications. A 24-h biofilm growing on an epithelial cell monolayer was washed twice with MEM and then scraped off using a tissue culture scrapper and transferred to a 2-ml Eppendorf tube containing 1 ml phosphate buffer. The tube was then vortexed thoroughly for 1 to 2 min to release the cells. After this, the samples were then serially diluted, and a plate count was made on a LB plate incubated at 37°C overnight.

Applying phages to biofilms and lung infections in mice. Fifty microliters of the phage mixture (1 $\times$ 10^9 to 2 $\times$ 10^9 PFU/ml of defined phage [containing ϕNH-4 and ϕMR299-2] in a 1:1 mixture) was applied to wells containing 24-h-old biofilms on a CFBE41o- cell monolayer, and the plates were incubated at 37°C and 5% CO_2 for 24 h. Biofilm clearing was monitored by measuring light and images taken for times indicated, using the IVIS100 imaging system. The lungs of 6- to 8-week-old conventional female BALB/c mice (n = 16) were infected with *Pseudomonas* by the method of Riedel et al. (28). The mice were infected intranasally with 50 μl *lux*-tagged *Pseudomonas* NH57388A or MR299 in phosphate buffer (2 $\times$ 10^8 to 5 $\times$ 10^8 CFU/ml). Two hours following infection, 50 μl of a phage mix suspension in phosphate buffer containing *Pseudomonas*

phages ϕNH-4 and ϕMR299-2 (2×10^9 to 5×10^9 PFU/ml for a multiplicity of infection [MOI] of 10) were given intranasally. Fifty microliters of phosphate buffer was given to the control groups. Animals were anesthetized with isoflurane, light was monitored, and images were taken using the IVIS 100 system at the time points indicated. Animals were kept in an animal colony, and all experiments were approved by the animal ethics committee of University College Cork.

Biofilm staining. *Pseudomonas* biofilms were stained with the fluorescent enhancer Calcofluor white (fluorescent brightener 28, catalog no. F3543; Sigma Aldrich, China). In brief, the monolayers of CFBE41o- cells containing biofilms were removed from wells using a sterile cell scraper (Sarstedt, Actiengeselischaft and Co., Germany) and mixed with a drop of Calcofluor white (0.1%) on a microscope slide. A coverslip was applied to the slide; the edges were sealed with paraffin oil, and the microscope slide was incubated for 1 h (37°C). Stained biofilm preparations were assessed by using an Olympus BX51 fluorescence microscope fitted with a U-RFL-T fluorescent power supply unit, and images were taken with a DP50 integrated camera (all from Olympus Optical Co., Japan).

Nucleotide sequence accession numbers. The completed phage genome sequences of phages NH-4 and MR299-2 were deposited in GenBank database and assigned accession numbers JN254800 and JN254801, respectively.

ACKNOWLEDGMENTS

The Alimentary Pharmabiotic Centre is a research center funded by Science Foundation Ireland (SFI), through the Irish Government's National Development Plan. This work was supported by SFI grants 02/CEB124 and 07/CE/B1368.

We thank Dieter C. Gruenert (California Pacific Medical Center, University of California, San Francisco, CA) for providing CFBE41o- cells and Nadine Hoffmann (Institute of Medical Microbiology and Immunology, University of Copenhagen, Copenhagen, Denmark) for providing the mucoid *P. aeruginosa* NH57388A strain. We also thank Mark Auty (Moorepark Imaging Centre, TFRC) and Don O'Leary (Advanced Microscope Research Facility, University College Cork) for electron microscopy (EM) imaging.

SUPPLEMENTAL MATERIAL

Supplemental material for this article may be found at http://mbio.asm.org/lookup/suppl/doi:10.1128/mBio.00029-12/-/DCSupplemental.

Figure S1, PDF file, 0.1 MB.
Figure S2, PDF file, 0.1 MB.
Figure S3, PDF file, 0.1 MB.
Table S1, DOC file, 0.1 MB.
Table S2, DOC file, 2.9 MB.
Table S3, DOC file, 0.1 MB.
Table S4, DOC file, 0.1 MB.

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From: Randy Schmidt [redacted]
Sent: Saturday, April 13, 2013 6:18 PM
To: Ford, Knatokie
Subject: Keshe "Plasma Reactor" Technology

Dear President Obama,

Please release Keshe "Plasma Reactor" technology for public and private production. Currently, by presidential decree, this technology is ILLEGAL except for the benefit of WESTERN ARMS MANUFACTURERS! WTF!?!?!

So we know that you know of this technology. The U.S.A. and the world needs Keshe technology Now to address critical issues like JOBS, climate change, energy, health issues, pollution, transportation and more! Plasma Reactors are safe and simple to produce. New scientific breakthroughs in plasma physics are easy to understand.

Go to http://www.keshefoundation.org and learn what is now possible with this new understanding of sub-atomic structure and gravity!

Keshe Foundation Home
http://keshefoundation.org

A New Horizon
http://keshefoundation.org/new-horizons/a-new-horizon/70-a-new-horizon-en.html

Teachings
http://keshefoundation.org/new-horizons/teachings.html

KNOWLEDGE TRANSFER
http://keshefoundation.org/applications/licensing/112-licensing-en.html

Book one: The Universal Order of Creation of Matters by M.T. Keshe
http://www.youtube.com/watch?feature=player_embedded&v=wXGe3A7WTZ4

More and more people are hearing about and learning of this new technology. Soon there will be no denying it. Release it NOW before everyone knows that you've been denying the public of this critical technology. Mr. M. T. Keshe has released this technology to the world for Free as of Sept. 21st, 2012. We the people DEMAND you release this technology immediately creating manufacturing jobs nationwide replicating and producing plasma reactors so we can get out from the control of big oil, coal, fracking and dangerous nuclear reactors. We simply don't need them anymore. Big oil has taken enough from the people and the economy. There time is over. It's time for a BIG Change.

Be the President known throughout history as "the President who changed the World for the Better in a Big Way!"

PCAST's leadership: From Big Data to Learning Systems? The Next NSF Director: National Implications of the NSF Rapid Learning Health System conference:

From: "Lloyd Etheredge" <lloyd.etheredge@policyscience.net>
Date: Mon, April 15, 2013 7:38 pm
To: "Dr. John Holdren - Science Adviser to President Obama and Co-Chair, PCAST" [redacted]

Cc: "Dr. Rosina Bierbaum - PCAST" [redacted]

Dear Co-Chairs Holdren, Lander, Savitz, and Press and PCAST Members:

Concerning the selection of the best person to be the new NSF Director: I enclose a copy, for your review, of one of the two keynote presentations to the recent, remarkable, NSF-organized conference in Washington on rapid learning health systems.

The broader, exciting challenge emerging from the conference was the opportunity to move, boldly, from Big Data to the design and implementation of Learning Systems in many areas of scientific and public concern. "How Rapidly Can We Learn?" was the concluding question. It is relevant to identifying the best person to be the new NSF Director (also, the selection of a new Deputy Director for Economic Growth and Innovation).

Just to mention: the second keynote address was by a computer scientist, who applied the kind of analysis that Karl Deutsch began in The Nerves of Government. Imagine that our current health/government/societal systems are computer programs that are designed for rapid learning, and that are slow and keep giving us unreliable answers.

with best wishes for your work,
Lloyd Etheredge

Dr. Lloyd S. Etheredge - Director, Government Learning Project
Policy Sciences Center Inc.
[redacted]net
(email)

[The Policy Sciences Center, Inc. is a public foundation that develops and integrates knowledge and practice to advance human dignity. It was founded by Harold Lasswell, Myres McDougal, and their associates in 1948 in New Haven, CT. Further information about the Policy Sciences Center and its projects, Society, and journal is available at www.policysciences.org.]

A Rapid-Learning Health System

A Behavioral Science-Economics-Public Policy Perspective

Lynn Etheredge

NSF Learning Health System Workshop

April 11, 2013

Perspectives

- #1. A learning society
- #2. Paradigms
- #3. Revolutionary technologies
- #4. Economics of information
- #5. Systems studies
- #6. Innovation & diffusion

#1. A Learning Society

- The generation and application of new, useful knowledge has been the key driver of economic growth & progress, since at least the Industrial Revolution
- Many elements of modern societies influence learning, e.g. federal R&D support, research universities, high-tech industries, education, venture capital & competitive economic system, science & science-based professions, engineering, IT, Internet/WWW, intellectual property & patents, tax policies
- There are many unresolved research questions, and large variations among countries and across sectors, in policies and practices
- Overall, US performance is mixed, particularly in health, education, economic growth where government has a large role. Learning how to create better learning systems from Big Data would have many benefits

Healthcare Needs Rapid Learning

- Learning problems contribute to many areas of poor performance
  - High and rising costs, wide variations in practice, poor quality, knowledge errors (Vioxx, food pyramid, hormone replacement therapy), long lags in adopting better practices, large gaps in clinical evidence base (comparative effectiveness, pregnant women, children, seniors with multiple chronic illnesses), falling research productivity, reliance on long, expensive RCT methods, out-of-date diagnostic taxonomy & wide heterogeneity of response to therapies, slow 40-year progress in war on cancer, federal budget deficits, lack of information on questions of importance to patients, emergency preparedness

- NRC report proposes new national learning system to revolutionize research, clinical care, and public health. (Francis Collins: 20-30M patient learning network). Latest issue of *Science* on cancer genetics data learning sees "a medical renaissance" and "a medical enlightenment".

2. Paradigms

- Most scientific research occurs within intellectual paradigms that shape – and focus (limit) - scientific disciplines, research questions, institutions and careers – and ideas about how to most quickly and reliably advance scientific knowledge. Scientific revolutions are fueled by new paradigms. The adoption of new intellectual paradigms is slow, resisted, and profoundly psychological & sociological

- The medical research paradigm-system differs from (lags?) other areas of modern science
  - Medical research emphasizes RCTs. Highly productive sciences combine experiments, observational data, and predictive models. The goal is the predictive model (E=MC squared). Predictive models for biology and medicine are under-developed.
  - The rate of scientific advances varies greatly among areas. The fastest advances come from using experiments to definitively test multiple theories. Biological sciences and research are theory poor; many RCTs produce limited information.
  - Medical research has been a "data poor" environment. Most research projects require timely, expensive collection of unique datasets.
  - Emphasis on R01s (individual researcher-initiated projects) vs national laboratories (DOE), research networks, focused projects (DARPA)

Paradigms

- The genetics revolution, IT-EHRs, the problems of a slow-learning health system, and leaders are starting to influence government agency thinking about new research paradigms, resources, and methods
  - Kaiser-VA-Geisinger-HMORN; FDA mini-Sentinel, National Center for Accelerating Translational Science, HCS Collaboratory, bio-banks, many new databases, registries, and research networks, PCORI and CER, BD2K

- Is it possible to learn much faster? What new ideas, research questions, and methods would be needed? *Once all influences on health are "in the computer" the rate of scientific progress shifts to how smart we are about using computers*

#3. Revolutionary Technologies

- The largest benefits come from revolutionary technologies, e.g. steam engine, electricity, telephone, automobiles, personal computers, Internet/WWW, microscope, DNA sequencing. They are high impact, disruptive – and hard to predict.

- Health sector learning now has the opportunities and challenges of five revolutions at the same time:
  - IT & computers – electronic health records, research registries, decision-support
  - Genetics – precision diagnostics, targeted therapies and prevention
  - WWW/Internet – ability to share information, learning networks, MOOCs
  - Consumer movement – informed patients
  - Smart technologies and Apps

- This will need new visions, new ideas, open minds, creative thinking – and a lot of learning!

A New Learning System

- A key concept is *in silico* research to complement *in vitro* and *in vivo*. The potential to design and pre-populate large computerized databases, with millions of patient records that can provide useful research in minutes or days vs 7+ years. e.g. mini-Sentinel (125m patients/24 hours); TASTE CER study in organized learning systems (90% cost reduction)

- Major changes in RL system:
  - number and type of patients studied, kinds & amounts of data (<6% in RCTs & selective→tens of millions, clinically rich, longitudinal data, representative populations in real-world settings)
  - who does research & where (small academic community→organized delivery systems, crowd sourcing), RO1's→national laboratories & DARPA initiatives; physician specialty societies
  - number of researchers and others able to use data, how research is done & results adopted
  - number of studies, speed, expense, more and different questions
  - log-on to world's evidence base, with smart software support !

#4. Economics of Information

- Many kinds of information are not a standard (physically limited, controllable) economic good. Most economists would agree that basic science research and other kinds of scientific data are "public goods" to be government-supported

- The "economics of the commons" for data-sharing is very strong (10 institutions contribute 100 patient records for a 1,000 patient registry, each gain 900 records (9:1 return)).

- The federal government must have a key role in data policy & data resources for a learning health care system (NIH, FDA, CDC, CMS, ONC-EHRs)

#5. Systems Studies

- Systems theory is one of the most widely used and powerful intellectual perspectives for natural and social sciences
- Recent developments study living organisms (and social institutions) as complex, adaptive, evolving systems - often with surprising emergent properties - that exist within eco-systems of other complex, adaptive, evolving systems
- An important aspect of the discussions about a learning healthcare system is that they go beyond "big data" to envision a "learning system". We need to build a new research field, with public-private collaboration, for how to design & evolve learning systems, for a learning society
- The "use cases" are structured for systems thinking – what would we want a learning system to do?, what are its elements?, relationships?, processes?, performance metrics? what are the key research questions?

#6. Innovation & Diffusion

Innovation & Diffusion

- Learning systems deal with human beings and human institutions, with their own ideas, psychologies, capabilities and limitations, resources, environments, incentives and agendas

- Can we replace the classic "S" curve with a "J" curve, i.e. "go viral" with new learning, using the Internet/WWW, social media and other new tools?

- The $10 B CMS Innovation Center adds potential "demand pull" to learning through supply-wide diffusion. Aligning learning, doing good and doing well may be a promising strategy.

Conclusion

"How much faster can we learn?" is now, and in exciting, new ways, a question to which new answers can evolve in healthcare and elsewhere.

PCAST: Urgent implications of the IMF Conference re Macro Economic lessons and science-based public policy

From: "Lloyd Etheredge" [redacted]
Date: Mon, April 22, 2013 2:10 pm
To: "Dr. John Holdren - Science Adviser to President Obama and Co-Chair, PCAST" <[redacted]ore)
Cc: "Dr. Rosina Bierbaum - PCAST" [redacted] (more)
Priority: Normal

Dear Co-Chairs Holdren, Lander, Savitz, and Press and PCAST Members

I enclose a copy of a letter to the National Science Board and a summary (by the columnist Robert Samuelson) from the recent, invitation only, IMF conference on Macro Economics in Washington.

The the scientific impasse: My perception is that there are urgent implications, that you may want to know about, for upgrading NSF and other government programs and designing a rapid learning system.

My letter also encloses a supporting Op Ed piece by the economist Jeffrey Sachs. He has reached a similar conclusion to the IMF lesson-drawing summit.

Yours truly,
Lloyd Etheredge

Dr. Lloyd S. Etheredge - Director, Government Learning Project
Policy Sciences Center Inc.
[redacted]
URL: www.policyscience.net
[redacted]
(email)

[The Policy Sciences Center, Inc. is a public foundation that develops and integrates knowledge and practice to advance human dignity. It was founded by Harold Lasswell, Myres McDougal, and their associates in 1948 in New Haven, CT. Further information about the Policy Sciences Center and its projects, Society, and journal is available at www.policysciences.org.]

THE POLICY SCIENCES CENTER, INC.

Project Director: DR. LLOYD ETHEREDGE
[redacted]

April 22, 2013

Dr. Dan Arvizu, Chair
National Science Board
c/0 National Science Foundation
[redacted]

Dear Dr. Arvizu and National Science Board Members:

I enclose a summary by Robert Samuelson ("The End of Macro Magic," The Washington Post, April 21, 2013) of the recent, invitation-only, International Monetary Fund conference, Rethinking Macro Policy II: First Steps and Early Lessons that was held in Washington on April 16-17.[1] There is an urgent need for the National Science Board to make an independent audit of the performance and needs of its SBE Directorate and Economics program in light of these conclusions.

The conceptual models, data systems, and coefficients that America and the world must rely upon for Macro Economic policy have been losing their grip on reality. As a benchmark: I suggest that NSF's scientific leadership for new data systems and R&D to support rapid learning should be at the same level as if equivalent, emergency problems arose in the scientific models of physical processes and coefficients that humankind relies upon.

In light of the economic, societal, political (and global) impact of eroding scientific performance, the National Science Board should move very quickly.

Public Candor, NSF Budget Rebalancing, New Leadership

The internal decision processes of NSF, and the planning and budget advice received by the National Science Board, appear to be severely biased and unreliable concerning the performance shortfalls and the planning, data capture, and funding needs of social scientists who want to address these mysteries and growing challenges by fast discovery.[2] The National Science Board, and especially its newer members, may wish to order an independent institutional review of the silence, about these growing shortfalls in scientific performance, in NSF's many annual reports and its five-year (and longer) strategic plans. [The scientific assessments at the IMF conference partly reflect the actions of American social scientists who have worked around NSF processes, silences, and its absent scientific warnings and strategic plans.]

Transformational Thinking

I also enclose a copy of a recent piece(December, 2012) by the economist Jeffrey Sachs. He has arrived at a similar conclusion that transformative rethinking about macro-economic models,

behavioral assumptions, and data systems is needed. He also believes that a continuing R&D program with rapid learning cycles may be needed to guide our thinking about a changing reality. [I also enclose a prescient warning in December 2002 by Robert Reischauer concerning a premature error of comfortably assuming the reliability of simple conceptual models and economic data systems developed by NSF's early Economics research.] NASA's COBE satellite was designed to secure data concerning more than 100 competing theories related to the origins of the universe: equivalent NSF strategic investments in an era of Big Data would be timely and could be equally as productive.[3]

Many people will respond to reporters, with self-assurance, to offer (orthodox) diagnoses and psychological ideas (that can seem intuitively plausible) about how to fix Economics. We do not know whether marginal, minimal change, improvements in theory are needed or a bold restructuring of how to think about a complex (sometimes) adaptive global system that is itself composed on diverse, complex, and (sometimes) adaptive systems. Thus: scientists obviously need new, exploratory, multi-disciplinary data systems and the formal scientific methods that NSF supports, to evaluate these "sound bite" ideas and range of speculations.

The world needs the institutional guidance and leadership of the National Science Board and NSF to brings the best of our scientific capacities to bear on these urgent and unexpected problems affecting the well-being and future of humankind.

Yours truly,

Lloyd Etheredge

Dr. Lloyd S. Etheredge, Director
Government Learning Project

cc: National Science Board, PCAST Members

Enclosures:
- Jeffrey Sachs, "We Must Look Beyond Keynes to Fix Our Problems," Financial Times, December 17, 2012.
- Robert Reischauer, Letter to LSE, December 23, 2002

1. Webcasts are online: http://www.imf.org/external/np/seminars/eng/2013/macro2/

2. A new position of a NSF Deputy Director for Innovation and Economic Growth may help to fill NSF's alarming gaps. The current Assistant Director (SBE) who is a former historian cannot provide the intellectual self-assurance and level of advocacy and planning for rapid learning that a new NSF Director and the National Science Board require to respond to these alarming and unexpected erosions.

3. Many actors share a mutual interest in reliable macro economic models. Some of the new R&D data systems may require prototyping and funding as public goods. Strategic investment also can include R&D datasets that merge information gathered in the private sector.

The End of Macro Magic

By Robert J. Samuelson

Published: April 21, 2013. Washington Post

The International Monetary Fund recently held a conference that should concern most people despite its arcane subject — "Rethinking Macro Policy II." Macroeconomics is the study of the entire economy, as opposed to the examination of individual markets ("microeconomics"). The question is how much "macro" policies can produce and protect prosperity. Before the 2008-09 financial crisis, there was great confidence that they could. Now, with 38 million unemployed in Europe and the United States — and recoveries that are feeble or nonexistent — macroeconomics is in disarray and disrepute.

Among economists, there is no consensus on policies. Is "austerity" (government spending cuts and tax increases) self-defeating or the unavoidable response to high budget deficits and debt? Can central banks such as the Federal Reserve or the European Central Bank engineer recovery by holding short-term interest rates near zero and by buying massive amounts of bonds (so-called "quantitative easing")? Or will these policies foster financial speculation, instability and inflation? The public is confused, because economists are divided.

Perhaps the anti-economist backlash has gone too far, as George Akerlof, a Nobel Prize-winning economist, argued. The world, he said, avoided a second Great Depression. "We economists have not done a good job explaining that our macro policies worked," he said. Those policies included: the Fed's support for panic-stricken financial markets; economic "stimulus" packages; the Troubled Assets Relief Program (TARP); the auto bailout; "stress tests" for banks; international cooperation to augment demand.

Fair point. Still, the subsequent record is disheartening. The economic models that didn't predict the crisis have also repeatedly overstated the recovery. The tendency is to blame errors on one-time events — say, in 2011, the Japanese tsunami, the Greek bailout and the divisive congressional debate over the debt ceiling. But the larger cause seems to be the models themselves, which reflect spending patterns and behavior by households and businesses since World War II.

"The events [stemming from] the financial crisis were outside the experience of the models and the people running the models," Nigel Gault said in an interview. (Gault, the former chief U.S.

economist for the consulting firm IHS, was not at the conference.) The severity of the financial crisis and Great Recession changed behavior. Models based on the past don't do well in the present. Many models assumed that lower interest rates would spur more borrowing. But this wouldn't happen if lenders — reacting to steep losses — tightened credit standards and potential borrowers — already with large loans — were leery of assuming more debt. Which is what occurred.

"We really don't understand what's happening in advanced economies," Lorenzo Bini Smaghi, a former member of the ECB's executive board, told the conference. "Monetary policy [policies affecting interest rates and credit conditions] has not been as effective as we thought." Poor economic forecasts confirm this. In April 2012, the IMF predicted that the euro zone (the 17 countries using the euro) would expand by 0.9 percent in 2013; the latest IMF forecast, issued last week, has the euro zone shrinking by 0.3 percent in 2013. For the global economy, the growth forecast for 2013 dropped from 4.1 percent to 3.3 percent over the same period.

Since late 2007, the Fed has pumped more than $2 trillion into the U.S. economy by buying bonds. Economist Allan Meltzer asked: "Why is there such a weak response to such an enormous amount of stimulus, especially monetary stimulus?" The answer, he said, is that the obstacles to faster economic growth are not mainly monetary. Instead, they lie mostly with business decisions to invest and hire; these, he argued, are discouraged by the Obama administration's policies to raise taxes or, through Obamacare's mandate to buy health insurance for workers, to increase the cost of hiring.

There were said to be other "structural" barriers to recovery: the pressure on banks and households to reduce high debt; rigid European labor markets; the need to restore global competitiveness for countries with large trade deficits. But these adjustments and the accompanying policies are often slow-acting and politically controversial.

The irony is rich. With hindsight, excessive faith in macroeconomic policy stoked the financial crisis. Deft shifts in interest rates by central banks seemed to neutralize major economic threats (from the 1987 stock crash to the burst "tech bubble" of 2000). Prolonged prosperity promoted a false sense of security. People — bankers, households, regulators — tolerated more risk and more debt, believing they were insulated from deep slumps.

But now a cycle of overconfidence has given way to a cycle of under-confidence. The trust in macroeconomic magic has shattered. This saps optimism and promotes spending restraint.

Scholarly disagreements multiply. Last week, a feud erupted over a paper on government debt by economists Kenneth Rogoff and Carmen Reinhart. The larger lesson is: We have moved into an era of less economic understanding and control.

December 17, 2012 6:46 pm. Financial Times

We must look beyond Keynes to fix our problems

By Jeffrey Sachs
[A different kind of growth path is required, says Jeffrey Sachs]

For more than 30 years, from the mid-1970s to 2008, Keynesian demand management was in intellectual eclipse. Yet it returned with the financial crisis to dominate the thinking of the Obama administration and much of the UK Labour party. It is time to reconsider the revival.

The rebound of Keynesianism, led in the US by Lawrence Summers, the former Treasury secretary, Paul Krugman, the economist-columnist, and the US Federal Reserve chairman Ben Bernanke, came with the belief that short-term fiscal and monetary expansion was needed to offset the collapse of the housing market.

The US policy choice has been four years of structural (cyclically adjusted) budget deficits of general government of 7 per cent of gross domestic product or more; interest rates near zero; another call by the White House for stimulus in 2013; and the Fed's new policy to keep rates near zero until unemployment returns to 6.5 per cent. Since 2010, no European country has followed the US's fiscal lead. However, the European Central Bank and Bank of England are not far behind the Fed on the monetary front.

We can't know how successful (or otherwise) these policies have been because of the lack of convincing counterfactuals. But we should have serious doubts. The promised jobs recovery has not arrived. Growth has remained sluggish. The US debt-GDP ratio has almost doubled from about 36 per cent in 2007 to 72 per cent this year.The crisis in southern Europe is often claimed by Keynesians to be the consequence of fiscal austerity, yet its primary cause is the countries' and eurozone's unresolved banking crises. And the UK's slowdown has more to do with the eurozone crisis, declining North Sea oil and the inevitable contraction of the banking sector, than multiyear moves towards budget balance.

There are three more reasons to doubt the Keynesian view. First, the fiscal expansion has been mostly in the form of temporary tax cuts and transfer payments. Much of these were probably saved, not spent.

Second, the zero interest rate policy has a risk not acknowledged by the Fed: the creation of another bubble. The Fed has failed to appreciate that the 2008 bubble was partly caused by its own easy liquidity policies in the preceding six years. Friedrich Hayek was prescient: a surge of excessive liquidity can misdirect investments that lead to boom followed by bust.

Third, our real challenge was not a great depression, as the Keynesians argued, but deep structural change. Keynesians persuaded Washington it was stimulus or bust. This was questionable. There was indeed a brief depression risk in late 2008 and early 2009, but it resulted from the panic after the abrupt and maladroit closure of Lehman Brothers.

There is no going back to the pre-crisis economy, with or without stimulus. Unlike the Keynesian model that assumes a stable growth path hit by temporary shocks, our real challenge is that the growth path itself needs to be very different from even the recent past.

The American labour market is not recovering as Keynesians hoped. Indeed, most high-income economies continue to shed low-skilled jobs, either to automation or to offshoring. And while US employment is rising for those with college degrees, it is falling for those with no more than a high school education.

The infrastructure sector is a second case in point. Other than a much-hyped boom in gas fracking, investments in infrastructure are mostly paralysed. Every country needs to move to a low-carbon energy system. What is the US plan? There isn't one. What is the plan for modernised transport? There isn't one. What is the plan for protecting the coastlines from more frequent and costly flooding? There isn't one.

Trillions of dollars of public and private investments are held up for lack of a strategy. The Keynesian approach is ill-suited to this kind of sustained economic management, which needs to be on a timescale of 10-20 years, involving co-operation between public and private investments, and national and local governments.

Our world is not amenable to mechanistic rules, whether they are Keynesian multipliers, or ratios of budget cuts to tax increases. The UK, for example, needs increased infrastructure and education investments, backed by taxes and public tariffs. Therefore, spending cuts should not form the bulk of deficit reduction as George Osborne, UK chancellor, desires. Economics needs to focus on the government's role not over a year or business cycle, but over an "investment cycle".

When the world is changing rapidly and consequentially, as it is today, it is misguided to expect a "general theory". As Hayek once recommended to Keynes, we instead need a tract for our times; one that responds to the new challenges posed by globalisation, climate change and information technology.

The writer is director of the Earth Institute at Columbia University

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December 18, 2012 7:25 pm

To understand Christmas, go to the pub By John Kay

With gift-giving as with finance, it takes an eclectic approach to understand human behaviour
Why do we exchange gifts? I once enjoyed a heated debate with a group of anthropologists. After discussing what we might learn from each other we adjourned to the pub, where the debate continued. We bought rounds of drinks. But why?

For the anthropologists, the custom of standing a round represented ritual gift exchange. They drew an analogy with Native American potlatch festivals, where tribes would gather to eat, sing, dance and confer lavish presents – sometimes treasured or essential possessions – on each other. The economists preferred a more hard-nosed explanation. Buying drinks in rounds rather than individually was a means of reducing transaction costs. The number of dealings between the customers and the bar was reduced, and the need for small change diminished.

> I proposed an empirical test between the competing hypotheses. Did you feel successful or unsuccessful if you had bought more drinks than had been bought for you?

Unfortunately, the result was inconclusive. The anthropologists believed their generosity enhanced their status. The economists sought to maximise the difference between the number of drinks they had consumed and the number they had bought. They computed appropriate strategies for finite games and even for extended evenings of indeterminate length. The lesson is that if you want a good time at a bar, go with an anthropologist rather than an economist.

So it is a relief that Christmas sounds more like a potlatch than a mathematical economist's multi-period equilibrium. The purpose of the festival is plainly not transaction-cost minimisation. Although commercial

interests obviously profit from Christmas, the economic function of the event is not apparent. Indeed, from time to time economists point out the inefficiency of customary gift exchange: the gifts we receive are often less valuable to us than those we would have bought ourselves with the money the donor devoted to their purchase. Canadian missionaries made the same observation. Concerned that such festivals seriously damaged the economic welfare of the tribes, they successfully lobbied the government to criminalise potlatches.

A narrow focus is characteristic of scientific method but gets in the way of understanding social phenomena. That was my error when I sought the “true” explanation in the pub. The custom of the round has both economic and social advantages, and it is likely that both help to account for its prevalence and persistence. The earnest missionaries and misanthropic economists who want to shut festivals down because they damage the economy have missed the point that the prospective enjoyment of such events is the reason we engage in economic activity in the first place.

The economists who argue that the rationale of the family is found in cost savings have a point. Two together can live more cheaply than two separately, if not as cheaply as one. But anyone who thinks the quest for scale economies is the primary explanation of the human desire for family life is strangely deficient in observational capacity, as well as common sense.

The “economics of the family” is a prime example of an economic imperialism that seeks to account for all behaviour through a distorted concept of rationality, an extreme example of economists’ notorious physics envy. Some models developed in physics demonstrate a combination of simplicity and wide explanatory power so remarkable that it makes no sense to think about the world in any other way.

But such powerful explanations are rarely available in other natural sciences, and almost never in social sciences. Even the visit to the bar is governed by a complex and tacit collection of social conventions. How do you know that you have bought the beer but only rented the glass?

So if you want to understand, say, the 2007-08 financial crisis, your approach must be eclectic. You need to work through standard economic models of financial markets because without them you cannot appreciate how many market participants – and most regulators – think. But you also need the perspectives of journalists, historians and psychologists. And, of course, you need the anthropological insight that accounts for the peculiarity of human institutions, whether you are dealing with the pub, potlatch or trading floor.

johnkay@johnkay.com

PCAST: The IMF Summit v. NSF's failed planning. An emergency need to design a rapid learning system

From: "Lloyd Etheredge" <[redacted]>
Date: Tue, April 23, 2013 1:55 pm
To: "Dr. John Holdren - Science Adviser to President Obama and Co-Chair, PCAST" [redacted]
Cc: "Dr. Rosina Bierbaum - PCAST" [redacted]

Dear PCAST Co-Chairs and Colleagues:

It is urgent for PCAST to rethink the US government's poorly designed system for rapid learning R&D in Economics. We need to develop and evaluate new, eclectic paradigms for Macro Economic behavior and policy in the US and the international economic system.

- Concerning my recent letter (with a summary of the IMF summit about the crisis, and scientific impasse, in current economic orthodoxy and data systems), I enclose further documentation of the breakdown of NSF's strategic planning. The enclosed excerpts (from NSF's FY2014 funding request, submitted earlier this month) imply that NSF has distanced itself from the crisis. Members of the National Science Board, and NSF's senior leadership, may be unaware of the crisis: there do not appear to be any leading research economists involved in preparing and judging these priorities and plans.

It is unclear why Acting Director Marrett and Assistant Director-SBE Guttman omit discussion of the deeply alarming scientific failures within NSF's purview. There are no emergency budget requests and strategic plans for the large increase in R&D data systems that is an obvious scientific strategy for data capture and fast discovery research. If this was NIH, and we were dealing with an emerging phenomenon like HIV/AIDS, my expectation is that the NIH Director would be shocked by the silence: I hope that PCAST will use the same benchmark.

The SBE Directorate, without the appointment of a Deputy NSF Director for Economic Growth and Innovation, may have eroded to the point where it is not suited to provide bold, self-assured leadership that the nation, and humankind, need to engage this scientific emergency. In an era of Big Data, perhaps computer scientists, with technologies for Machine Learning, can help to integrate across numerous public and private data systems and achieve theoretical discoveries that are not inhibited by disciplinary blinders. Or bureaucrats and fools.

One of the challenges for PCAST and the Obama Administration is to design and activate an emergency learning system to respond to one of the worst scientific failures in NSF's history. [Before moving to its new designs for Rapid Learning systems NIH, as you may know, conducted research that showed a 6-7 year lag between an initial announcement of a funding cycle and the ultimate publication of useful and verified results.] Rapid learning systems are what economists call a "public good": they will require much better leadership.

Yours truly,

Lloyd etheredge
Dr. Lloyd S. Etheredge - Director, Government Learning Project
Policy Sciences Center Inc.
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[The Policy Sciences Center, Inc. is a public foundation that develops and integrates knowledge and practice to advance human dignity. It was founded by Harold Lasswell, Myres McDougal, and their associates in 1948 in New Haven, CT. Further information about the Policy Sciences Center and its projects, Society, and journal is available at www.policysciences.org.]

NSF FY 2014 Budget Request to Congress

The National Science Foundation Act of 1950 (Public Law 81-507) sets forth our mission: ***To promote the progress of science; to advance the national health, prosperity, and welfare; and to secure the national defense.***

*The National Science Foundation Strategic Plan for FY 2011 – 2016, "Empowering the Nation Through Discovery and Innovation," defines our vision: "****a nation that capitalizes on new concepts in science and engineering and provides global leadership in advancing research and education.****"*

The National Science Foundation (NSF) is the only federal agency dedicated to the support of basic research and education across all fields of science and engineering. For over 60 years, NSF has had a profound impact on our Nation's innovation ecosystem by funding transformative research that has explored – and extended – the frontiers of scientific knowledge, promoted new industries, and addressed societal challenges.

In an era of fiscal austerity and focus on return on investment for the U.S. taxpayer, the strategic investments in NSF's FY 2014 portfolio sustain national economic growth, create new high technology jobs, support the transition to a clean energy economy, and train and develop the Nation's globally competitive science and engineering (S&E) workforce.

NSF's FY 2014 Budget Request is $7.626 billion, an increase of $592.69 million (8.4 percent) over the 2012 Enacted level. This request reflects a rigorous prioritization of activities across the Foundation. Even as the overall budget grows, the Agency Operations and Award Management account increases only $4.89 million (1.6 percent) as administrative costs are kept constrained. Approximately $37 million in lower priority education and research programs are terminated, reduced, or consolidated.

NSF Funding by Account
(Dollars in Millions)

	FY 2012 Enacted	FY 2014 Request	FY 2014 Request Change Over FY 2012 Enacted Amount	Percent
Research & Related Activities	$5,689.00	$6,212.29	$523.29	9.2%
Education & Human Resources	829.00	880.29	51.29	6.2%
Major Research Equipment & Facilities Construction	197.06	210.12	13.07	6.6%
Agency Operations & Award Management	299.40	304.29	4.89	1.6%
National Science Board	4.44	4.47	0.03	0.7%
Office of Inspector General	14.20	14.32	0.12	0.8%
Total, NSF	**$7,033.10**	**$7,625.78**	**$592.69**	**8.4%**

Totals may not add due to rounding.

FY 2014 Priorities

Funding for FY 2014 Priorities
(Dollars in Millions)

Investment Priority	FY 2012 Enacted	FY 2014 Request	FY 2014 Request Change Over FY 2012 Enacted Amount	Percent
Cyber-Enabled Materials, Manufacturing and Smart Systems (CEMMSS)	$141.65	$300.42	$158.77	112.1%
Cyberinfrastructure Framework for 21st Century Science, Engineering, and Education (CIF21)	78.00	155.47	77.47	99.3%
NSF Innovation Corps (I-Corps)	7.50	24.85	17.35	231.3%
Integrated NSF Support Promoting Interdisciplinary Research & Education (INSPIRE)	20.35	63.00	42.65	209.6%
Science, Engineering, and Education for Sustainability (SEES)	157.00	222.79	65.79	41.9%
Secure and Trustworthy Cyberspace (SaTC)	111.75	110.25	-1.50	-1.3%

Investments may have funding overlap and thus should not be summed.

The investments that form this Budget Request flow from the goals established in the agency's strategic plan: Transform the Frontiers, Innovate for Society, and Perform as a Model Organization. In FY 2014, key NSF investments in all fields of science and engineering strive to create new knowledge, stimulate discovery, address complex societal problems, and promote national prosperity.

In keeping with NSF's mission of advancing basic research in science, engineering, and education, this Request ensures the health of fundamental science and engineering across all disciplines, primarily through merit reviewed awards to researchers at colleges and universities throughout the country. There are six areas where core research is encouraged to enable scientists to address problems that require integration across more than one discipline. These priority investments, which encompass roughly 11 percent of the FY 2014 Request, focus on areas where progress in basic research is vital to addressing key national challenges, such as spurring innovation in manufacturing, improving data storage and analysis (e.g., Big Data), securing critical infrastructure, and promoting innovation and economic growth generally. Priorities include:

- **Cyber-enabled Materials, Manufacturing, and Smart Systems (CEMMSS**) ($300.42 million) will transform static systems, processes, and edifices into adaptive, pervasive "smart" systems with embedded computational intelligence that can sense, adapt, and react. Through CEMMSS, NSF participates in the Administration's Materials Genome Initiative (MGI), the National Robotics Initiative (NRI), and the Advanced Manufacturing Partnership. These investments fund research in areas of national importance, such as cyber-physical systems and advanced robotics research, materials processing and manufacturing, and advanced semiconductor and optical device design. These efforts are integral to the Administration's overall emphasis on strengthening advanced manufacturing.

- **Cyberinfrastructure Framework for 21st Century Science, Engineering, and Education (CIF21)** ($155.47 million) aims to expand investment in the Big Data/National Data Infrastructure program, a joint solicitation with the National Institutes of Health (NIH). NSF, as the lead agency, strives to coordinate development of new knowledge, tools, practices, and infrastructure that will enable breakthrough discoveries in science, engineering, medicine, commerce, education, and national security.

- **NSF Innovation Corps (I-Corps)** ($24.85 million) continues to build a national innovation ecosystem by improving NSF-funded researchers' access to resources that can assist in bridging the gap between discoveries and downstream technological applications, including commercialization of new technologies, products, and processes. In FY 2014, NSF will continue investment in Innovation Teams, and will expand support for I-Corps Nodes and I-Corps Sites.

- **Integrated NSF Support Promoting Interdisciplinary Research and Education (INSPIRE)** ($63.0 million) investment will continue to strengthen NSF's support of interdisciplinary, potentially transformative research by complementing existing efforts with a suite of highly innovative Foundation-wide activities and funding opportunities.

- **Science, Engineering, and Education for Sustainability (SEES)** ($222.79 million) addresses the need to develop a sustainable world where human needs are met equitably without harm to the environment and without sacrificing the ability of future generations to meet their needs. SEES uses a systems-based approach to understanding, predicting, and reacting to change in the linked natural, social, and built environment and addresses challenges in environmental and energy research and education. In FY 2014, NSF focuses on enhancing the Water Sustainability and Climate, Cyber-SEES, Hazards, and Sustainable Chemistry, Engineering and Materials (SusChEM) programs.

- The **Secure and Trustworthy Cyberspace (SaTC)** ($110.25 million) investment aligns NSF's cybersecurity investments with the four thrusts outlined in the national cybersecurity strategy, *Trustworthy Cyberspace: Strategic Plan for the Federal Cybersecurity Research and Development Program*. SaTC seeks to protect the Nation's information technology infrastructure from a wide range of threats that challenge its security, reliability, availability, and overall trustworthiness.

Additional Priorities and Highlights

- NSF aims to increase the operational efficiency of **U.S. activities in the Antarctic** ($22.0 million) by implementing the recommendations of the U.S. Antarctic Program Blue Ribbon Panel (BRP) report, *More and Better Science in Antarctica through Increased Logistical Effectiveness*. Emphases include safety and health improvements, investments with positive net present value, and facilities renewal at McMurdo and Palmer stations. Additionally, NSF aims to plan and execute more effective observational approaches to the Antarctic science community, as outlined in the 2011 National Research Council report, *Future Science Opportunities in Antarctica and the Southern Ocean*.

- In FY 2014, NSF introduces three activities to improve program effectiveness and efficiency by:

    - Ensuring **Public Access** ($2.50 million) to NSF research. This initiative reflects the Administration and NSF priority to make government more open and accessible by improving public access to NSF-funded research. In FY 2014, NSF establishes a policy framework that will build on and refine existing technology to track research products, allow investigators and awardees to make their products known and available, and allow the general public, researchers, and policy makers to locate and make use of those products. This effort includes establishing a

publicly-accessible repository for publications, leveraging existing federal infrastructure to the maximum extent possible.

- Establishing an **Evaluation Capability** ($5.50 million) to improve NSF's ability to inform policy decisions and improve the impact of research grant investments. In FY 2014, NSF will build a central evaluation expertise and support capability to promote rigor, transparency, and independence of evaluations. The centralized capability will coordinate the evaluation of NSF-wide activities, expand data collection, and ensure that the results of evaluation are used to improve NSF programs.

- Improving the operational execution of the **Merit Review Process** ($4.09 million), an essential step to address the extraordinary pressures the Foundation faces due to a growing number of proposals and intense competition for NSF funding. The FY 2014 Request will support a multi-year effort to improve major aspects of this process, including use of virtual meeting technologies for merit review; technological support for the management of reviewers and reviews; increased automation of the preliminary processing of proposals; and demand management.

- **Clean Energy** ($372.45 million): NSF's clean energy investments include research related to sustainability science and engineering, such as the conversion, storage, and distribution of diverse power sources (including smart grids), and the science and engineering of energy materials, energy use, and energy efficiency.

- **Research at the Interface of Biological, Mathematical and Physical Sciences, and Engineering (BioMaPS)** ($50.67 million) is a collaboration among the Directorates for Biological Sciences, Mathematical and Physical Sciences, and Engineering, that seeks to discover fundamental knowledge at the intersections of these established disciplines. This activity will produce critical knowledge needed to catalyze the development of new technologies essential to the Nation's prosperity and economic competitiveness and will advance emerging areas of the bioeconomy, as described in the Administration's *National Bioeconomy Blueprint*.

- The **Cognitive Science and Neuroscience** investment ($13.85 million) supports a focused, cross-foundation activity with three multi-year goals: to advance understanding of adaptation to the ever-changing world; to determine the mechanisms underlying decision-making and problem-solving in a dynamic environment; and to break the neural code by elucidating how the brain represents the world around us. This builds on ongoing NSF-wide support (approximately $70 million per year) for fundamental research relevant to cognitive science and neuroscience. NSF's funding in FY 2014 will also contribute to the Administration's multi-agency research initiative designed to revolutionize understanding of the human brain. FY 2014 activities include workshops held to identify specific gaps in our current understanding of these issues and intractable technology problems that prevent scientific breakthroughs. These will allow development of a framework for future efforts in the Administration's initiative.

- **The Faculty Early Career Development program (CAREER)** ($223.73 million) develops the future STEM workforce through support of young faculty who are dedicated to integrating research with teaching and learning. In FY 2014, NSF will support approximately 500 new awards. The CAREER portfolio includes projects that range across all fields of science and engineering supported by the Foundation, including high priority fields such as clean energy, climate change, STEM education, and cybersecurity. Within CAREER, NSF will support more fully utilizing the talents of individuals in all sectors of the American population by promoting Career-Life Balance, including

supplemental funding requests to employ research technicians or the equivalent for up to three months to sustain research when principal investigators are on family leave.

Science, Technology, Engineering, and Mathematics (STEM) Education

NSF maintains a strong commitment to advancing science and engineering education at all levels and to strengthening the Nation's workforce in STEM. The Administration is proposing a government-wide reorganization of STEM education programs to support a cohesive national STEM strategy. As part of this reorganization, in FY 2014 NSF presents a comprehensive agency-wide program to address undergraduate education and expands its leadership role in graduate education.

- The **National Graduate Research Fellowship program (NGRF)** ($325.14 million) builds on and expands the longstanding NSF Graduate Research Fellowship program (GRF) to incorporate features and opportunities that allow fellows to gain specialized experiences and training in key STEM areas. Through this expanded program, an increase of approximately 700 fellows is expected, bringing the total estimated number of new fellows awarded in FY 2014 to 2,700.

- The **NSF Research Traineeships (NRT)** program ($55.07 million) is the Foundation's investment in traineeships that focus on strategically identified research areas, mutually leveraging NSF's traineeship and research investments. NRT will build on NSF's previous investments – particularly the Integrative Graduate Education and Research Traineeship (IGERT) program – to encourage effectual innovation and design of graduate programs to support opportunities within specific disciplines.

- **Catalyzing Advances in Undergraduate STEM Education (CAUSE)** ($123.08 million) is a comprehensive agency-wide program for FY 2014 that aims to maximize the impact of NSF's considerable ongoing investments in STEM undergraduate education. CAUSE aims to improve STEM learning and learning environments; broaden participation in STEM and increase institutional capacity; and build the STEM workforce of tomorrow.

- Funding for the **Research Experiences for Undergraduates (REU) Sites and Supplements** ($79.18 million total) is increased $13.19 million over the FY 2012 Enacted. This additional funding will support enhanced research experiences for students in their first two years of college, as recommended by the President's Council of Advisors on Science and Technology (PCAST) in their report, *Engage to Excel: Producing One Million Additional College Graduates with Degrees in Science, Technology, Engineering, and Mathematics*.

Major Research Equipment and Facilities Construction

In FY 2014, NSF requests funding to continue construction of four projects: the Advanced Laser Interferometer Gravitational-Wave Observatory (AdvLIGO), the Advanced Technology Solar Telescope (ATST), the National Ecological Observatory Network (NEON), and the Ocean Observatories Initiative (OOI).

Funds are also requested to begin construction of the Large Synoptic Survey Telescope (LSST), a partnership with the Department of Energy (DOE). LSST was ranked as the number one priority for a large ground-based astronomical facility in the National Academies' most recent *Decadal Survey of Astronomy and Astrophysics* (August 2010).

- **Advanced Laser Interferometer Gravitational-Wave Observatory (AdvLIGO).** A planned upgrade of the existing Laser Interferometer Gravitational-Wave Observatory (LIGO), AdvLIGO will be ten times more sensitive, powerful enough to approach the ground-based limit of gravitational-wave detection.
- **Advanced Technology Solar Telescope (ATST).** ATST will enable study of the sun's magnetic fields, which is crucial to our understanding of the types of solar variability and activity that affect Earth's civil life and may impact its climate.
- **Large Synoptic Survey Telescope (LSST).** LSST will produce an unprecedented wide-field astronomical survey of our universe, including the deepest, widest-field sky image ever. The LSST survey will change every field of astronomical study, from the inner solar system to the large scale structure of the universe.
- **National Ecological Observatory Network (NEON).** NEON will consist of geographically distributed field and lab infrastructure networked via cybertechnology into an integrated research platform for regional to continental scale ecological research.
- **Ocean Observatories Initiatives (OOI).** OOI will enable continuous, interactive access to the ocean via multiple types of sensors linked by cutting-edge cyberinfrastructure, which will produce never-before-seen views of the ocean's depths.

MREFC Account Funding, by Project
(Dollars in Millions)

	FY 2012 Enacted	FY 2014 Request
Advanced Laser Interferometer Gravitational-Wave Observatory (AdvLIGO)	$20.96	$14.92
Atacama Large Millimeter Array (ALMA)	3.00	-
Advanced Technology Solar Telescope (ATST)	10.00	42.00
Large Synoptic Survey Telescope (LSST)	-	27.50
National Ecological Observatory Network (NEON)	60.30	98.20
Ocean Observatories Initiative (OOI)	102.80	27.50
Total, MREFC	**$197.06**	**$210.12**

Totals may not add due to rounding.

Model Organization

To "Perform as a Model Organization" is an internally focused strategic goal that emphasizes the agency's desired outcome of attaining excellence in all aspects of its operations. Model Organization underpins NSF programmatic activities and encompasses all the agency's management activities. It also includes support for the activities of the Office of Inspector General (OIG) and the National Science Board (NSB), which are provided in separate appropriations.

iTRAK

NSF will continue to modernize its financial management systems through the implementation of iTRAK. iTRAK will transition NSF from its legacy financial system to a fully integrated financial management solution. In FY 2014, the total request for iTRAK is $2.60 million.

Promoting Efficient Spending

Efforts are underway in multiple accounts to reduce administrative costs through efficiencies in response to the Administration's Promoting Efficient Spending initiative (Executive Order 13589) and *Promoting Efficient Spending to Support Agency Operations* (OMB M-12-12). Travel costs across NSF will be held at no more than $27.67 million in FY 2014, an amount $5.60 million below FY 2010 levels. This is accomplished through strategic efficiencies that achieve savings while preserving the travel necessary for mission-critical oversight and management responsibilities. In addition, NSF will also employ strategic sourcing of administrative support contracts, specifically for printing and wireless devices.

Model Organization by Appropriations Account

(Dollars in Millions)

	FY 2012 Enacted	FY 2014 Request	FY 2014 Request Change Over FY 2012 Enacted Amount	Percent
Agency Operations and Award Management	$299.40	$304.29	$4.89	1.6%
Office of Inspector General	14.20	14.32	0.12	0.8%
National Science Board	4.44	4.47	0.03	0.7%
Program Support:				
Research & Related Activities	94.12	108.20	14.08	15.0%
Education and Human Resources	15.39	16.57	1.18	7.7%
Subtotal, Program Support	109.51	124.77	15.26	13.9%
Total	**$427.55**	**$447.85**	**$20.30**	**4.7%**

Performance and Evaluation

NSF embraces the use of goals to drive performance improvements. In FY 2014, NSF has set ten performance goals so that NSF can strategically monitor and oversee progress being made on the Foundation's most important activities: priority program investments, research infrastructure investments and key management initiatives. NSF's goals are:

- **Ensure that Key Program Investments are on track:** Meet critical targets for several key program investments: CEMMSS, CIF21, I-Corps, INSPIRE, SaTC, and SEES. Progress will be monitored using a set of common milestones and indicators.

- **Ensure that Infrastructure Investments are on track:** Ensure program integrity and responsible stewardship of major research facilities at varying stages of their lifecycle. This involves construction project monitoring, response to advisory reports, and deployment of the first implementation of the NSF Public Access system.

- **Use Evidence to Guide Management Decisions:** The Foundation will use evidence-based reviews to guide management investments.

- **Improve Undergraduate Education:** The Foundation will establish an NSF-wide undergraduate STEM education program that is evidence-based and evidence-building.

- **Enhance National Graduate Research Fellowships:** NSF will enhance the Graduate Research Fellowship program to provide a wider range of career development opportunities.

- **Promote Career-Life Balance Policies and Practices:** NSF aims to promote policies and practices that support more fully utilizing the talents of individuals in all sectors of the American population, principally women, underrepresented minorities, and persons with disabilities.

- **Foster an Environment of Diversity and Inclusion:** The Foundation seeks to foster an environment of diversity and inclusion while ensuring compliance with the agency's civil rights programs.

- **Modernize Financial System:** iTRAK is the Foundation-wide effort to transition NSF from its legacy financial support system to a fully integrated financial management shared services solution to ensure continuous improvement and achieve high levels of customer service.

- **Make Timely Award Decisions:** NSF aims to inform applicants whether their proposals have been declined or recommended for funding within 182 days, or six months of deadline, target, or receipt date, whichever is later.

- **Enable Increased Use of Virtual Merit Review:** NSF seeks to incorporate technological innovations into the merit review process by expanding the use of virtual merit review panels.

Please refer to performance.gov for information on NSF's agency Priority Goals and NSF's contributions to the federal Cross-Agency Priority (CAP) goals.

Cuts, Consolidations, and Savings

NSF's FY 2014 Request follows a thorough examination of programs and investments across NSF to determine where the potential exists for more innovative investments. In addition to last year's proposals, this Request includes six terminations; two reductions; and one consolidations, totaling $36.86 million below FY 2012 Enacted level.

Nanoscale Science & Engineering Centers (NSECs) (-$18.61 million)**:** six NSEC centers are terminated due to center graduations and a transition to the Nanosystems Engineering Research Centers (NERCs) program. NSF will continue to support five continuing NSECs in FY 2014.

Two programs are eliminated within the Directorate for Mathematical and Physical Sciences (MPS). **CCAT** (formerly the Cerro Chajnantor Atacama Telescope) **Design and Development** (-$1.50 million total) concludes in FY 2013. Future NSF contributions to construction and/or operations will depend on a successful proposal to a competed midscale activities program. The **International Materials Institutes (IMI)** (-$1.58 million total) were concluded after an internal evaluation of program achievements found that despite the success of individual projects, the collective effort has not made the intended impact.

Virtual Organizations (-$5.0 million total) has achieved its programmatic goals to support scientific research to advance understanding of the effectiveness of virtual organizations and how they can enable and enhance science and engineering research and education. The transition to supporting application of virtual organizations to science and engineering communities is now underway in multiple programs within the Directorate for Computer and Information Science and Engineering.

The **Sensors and Sensing Systems (SSS)** program (-$3.0 million) is reduced because there are other programs both within NSF and at other agencies that principal investigators can apply to for support. The program will be refined to have a narrower and more targeted focus.

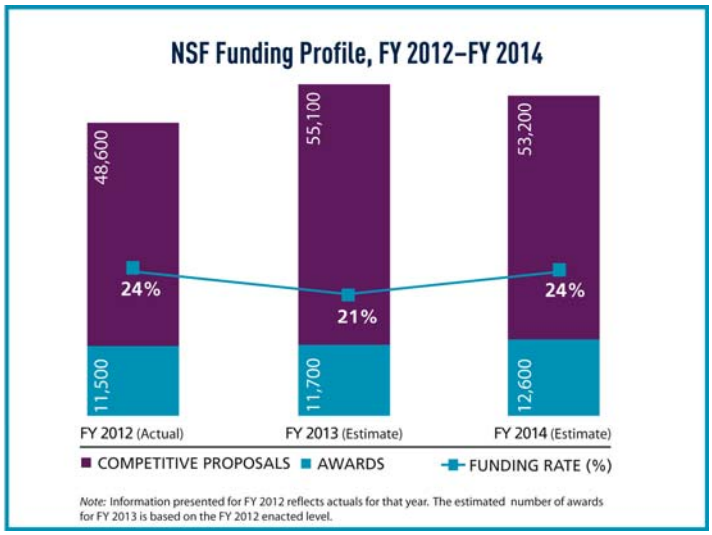
University Radio Observatories (UROs) (-$1.80 million) is being superseded scientifically by NSF's Atacama Large Millimeter/submillimeter Array (ALMA). It is expected that UROs will be eligible to compete for future funding in a broader midscale activities program.

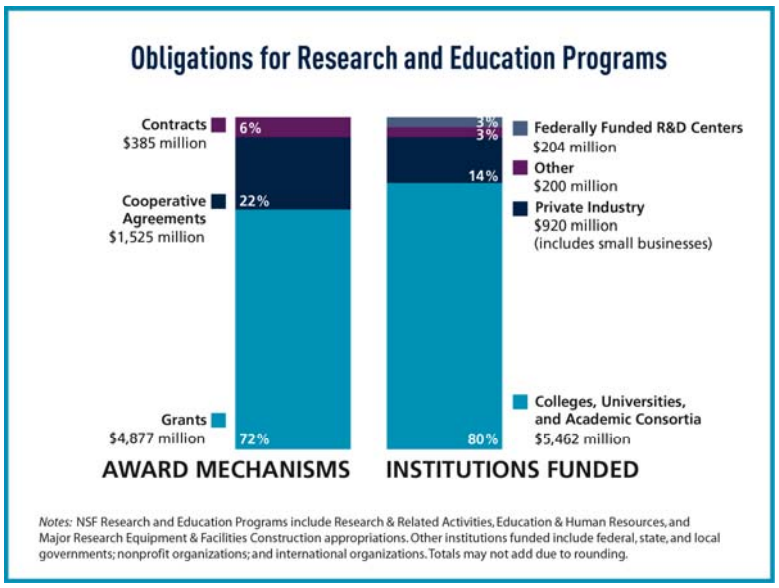
The Directorate for Education and Human Resources (EHR) will shepherd two major realignments to the current NSF STEM Education portfolio in order to use existing resources more effectively through a streamlined and consolidated approach. The new **Catalyzing Advances in Undergraduate STEM Education (CAUSE)** program includes undergraduate programs in EHR as well as Research and Related Activities (R&RA) directorates. NSF will take a leadership role in the coordination of government-wide graduate STEM education programs while developing national fellowship and traineeship programs.

As part of NSF's realignment of its STEM Education portfolio, two programs are terminated within the Directorate for Geosciences (GEO). The goals of the **Geoscience Teacher Training (GEO-Teach)** (-$2.0 million) program continue to be served through other STEM education initiatives at NSF. The **Centers for Ocean Science Education Excellence (COSEE)** (-$3.37 million) is terminated as the program has fulfilled its original goals. GEO will turn its attention to new educational initiatives through CAUSE.

NSF by the Numbers

NSF by The Numbers: In FY 2014 NSF expects to evaluate over 53,000 proposals through a competitive merit review process and make over 12,000 new awards. This will require over 260,000 proposal reviews, engaging on the order of 40,000 to 50,000 members of the science and engineering community participating as panelists and proposal reviewers. In a given year, NSF awards reach nearly 1,900 colleges, universities, and other public and private institutions in 50 states, the District of Columbia, and Puerto Rico. In FY 2014, NSF support is expected to reach approximately 276,000 researchers, postdoctoral fellows, trainees, teachers, and students.





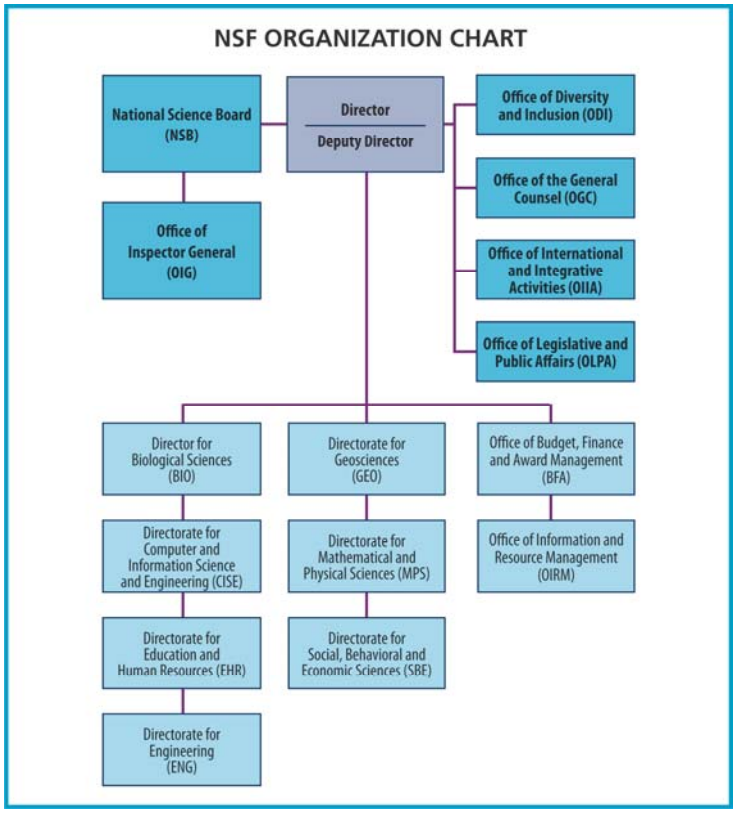
The chart on the left shows the distribution of NSF's obligations by institution type and funding mechanism. While the data are based on FY 2012, the relative shares should provide a good indication of the FY 2014 distribution. As shown on the graph, 94 percent of NSF's FY 2012 projects were funded using grants or cooperative agreements. Grants can be funded either as standard awards, in which funding for the full duration of the project is provided in a single fiscal year, or as continuing awards, in which funding for a multi-year project is provided in increments. Cooperative agreements are used when the project requires substantial agency involvement during the project performance period (e.g., research centers, multi-user facilities, etc.). Contracts are used to acquire products, services, and studies (e.g., program evaluations) required primarily for NSF or other government use.

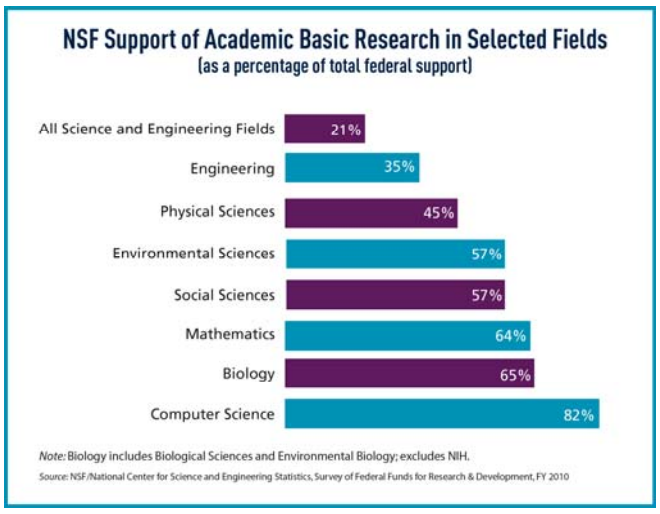
Most NSF awards are to academic institutions. Nonprofit organizations include state and local governments and international organizations. For-profit businesses include private and small businesses. Federal agencies and laboratories include funding for Federally Funded Research & Development Centers.

Organization and Role in the Federal Research Enterprise

NSF's comprehensive and flexible support of meritorious projects with broad societal impacts enables the Foundation to identify and foster both fundamental and transformative discoveries within and among fields of inquiry. NSF has the latitude to support emerging fields, high-risk ideas, interdisciplinary collaborations, and research that pushes – and even transforms – the very frontiers of knowledge. In these ways, NSF's discoveries inspire the American public – and the world.

NSF's organization represents the major science and engineering fields, including: biological sciences; computer and information science and engineering; engineering; geosciences; mathematical and physical sciences; and social, behavioral, and economic sciences. NSF also carries out specific responsibilities for education and human resources, cyberinfrastructure, integrative activities, international science and engineering, and polar programs. The 25-member National Science Board sets the overall policies of the Foundation.





NSF's annual budget represents 21 percent of the total federal budget for basic research conducted at U.S. colleges and universities, and this share increases to 58 percent when medical research supported by the National Institutes of Health is excluded. In many fields NSF is the primary source of federal academic support.

Highlights

Artificial Leaf Offers New Approach to Energy Production

Researchers at the Powering the Planet Center for Chemical Innovation at Caltech have created an artificial leaf. Just as a natural leaf converts sunlight into water, oxygen and sugar, this stand-alone device captures solar energy and splits water into hydrogen and oxygen gas. The artificial leaf converts sunlight into chemical fuel using a silicon photovoltaic cell and relatively inexpensive catalysts – materials that jump-start chemical reactions. To compete with cheap fossil fuels, novel materials are needed to generate fuels from solar energy. The materials must be inexpensive and abundant and their production simple and low-cost. Through a sustainable distribution infrastructure, the artificial leaf could become a viable energy source for both developed and developing countries. This finding was cited by Time magazine as an innovation of the year for 2011.

Credit: Dan Nocera, Massachusetts Institute of Technology

Detecting Explosives With Carbon-based Materials

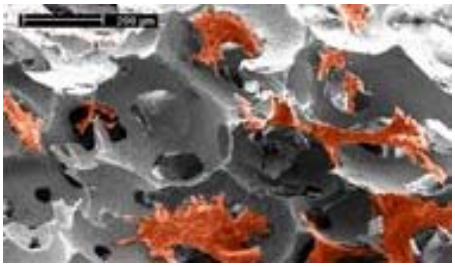
Researchers have created a novel carbon-based framework that produces materials for detecting explosive devices. Jeffrey Moore and his team at the University of Illinois, Urbana-Champaign, developed new methods to produce functionally useful materials based on carbon-rich nanostructures while reducing the generation of wasteful byproducts. The new methods allow for efficient, large-scale production and higher yields than previous methods.

Credit: Dorothy Loudermilk and Jeffrey Moore

This new environmentally friendly approach to explosive sensing materials has major implications for homeland security as well as combat soldiers who are targets of improvised explosive devices. These new materials are now being incorporated into field portable explosives detection devices.

Building Better Bone With Ceramics

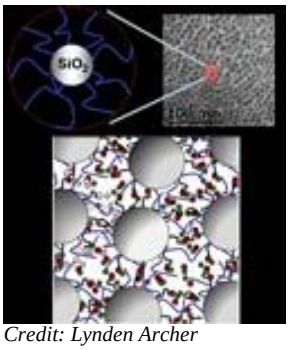
Researchers at the University of Florida have developed new ceramic foams that act as scaffolds for bone repair. These foams could mean an end to the use of metal plates as bone substitutes. Bioceramic foams are lightweight, porous, and possess a large surface area; porosity allows biofluids and arteries to flow through a ceramic implant, while high surface area allows more bone regeneration to occur. In experiments, the researchers demonstrated how cells spread across the foam struts, attach to inner foam pores and spread along foam contours. All of these steps are essential for bone regeneration and fracture healing.



Credit: Juan C. Nino, University of Florida

Highlights

Longer Life Lithium Batteries



Credit: Lynden Archer
Cornell University

Researchers from Cornell University have created a hybrid material that is particularly suitable for use as a solid electrical conductor or electrolyte in high-energy lithium batteries. Lynden Archer and his colleagues designed and refined new materials, composed of hard silica nanoparticles and a soft lithium-conducting polymer. The materials are stable, and have low flammability and volatility under battery operating conditions. Rechargeable lithium batteries are commonly used in consumer electronics and increasingly are finding applications in electric vehicles and defense. The new material will prolong the life of these batteries and allow them to provide higher powers than current technologies. A new start-up company – NOHMs Technologies – in Ithaca, N.Y., will manufacture and commercialize lithium batteries based on the new material.

Diagnosing Hidden Brain Trauma on the Field

The frontal cortex – the brain area directly behind the forehead – is vulnerable to damage. Scientists at the University of Texas Health Science Center in Houston, led by Anne Sereno and Saumil Patel, have discovered that people with impairments of the frontal cortex produce slower and more error-prone voluntary eye movements, but their reflexive eye and finger movements are unaffected. To evaluate whether even very mild injury to the frontal cortex has similar effects, the scientists developed a simple, tablet-based tool. The research team included a high-school student in the tool's development and tested the device with a local high-school women's soccer team. Results showed that frequent heading of balls – striking the ball with the forehead – disrupted and slowed voluntary movements in players tested right after soccer practice. These findings suggest that even mild injury to the frontal cortex can produce immediate, though short-lived, cognitive and behavioral changes that can affect one's ability to attend and respond to information or learn new information. This simple, tablet-based tool may be extremely useful for diagnosing deficits and evaluating treatment in mild traumatic brain injury at the time of injury, as well as for later follow-up care.

Credit: Anne Sereno,
University of Texas
Health Science Center

Imaging Groundwater Aquifers

Credit: Rosemary Knight, Stanford University

Most of the Earth's liquid fresh water exists as groundwater, a resource that lies beneath the Earth's surface. Conventional approaches to discover groundwater involve drilling and pumping wells, which are expensive and time-consuming. A Grant Opportunities for Academic Liaison with Industry (GOALI) project, involving researchers at Stanford University, the U.S. Geological Survey (USGS), and several industrial partners, used an alternative approach – surface nuclear magnetic resonance – which permits remote sampling and imaging of groundwater in less time, reducing monitoring costs. The approach also has applications in understanding the relationship between plant transpiration and groundwater.

Highlights

Understanding Urban Flooding

Credit: Parks & People Foundation

Although researchers agree that urban development affects flooding, in the past they have disagreed on how factors such as increases in impervious areas change the density of streams in the area. They have also debated whether the addition of storm drains affects flooding. A team of investigators from the University of Wyoming and University of Connecticut have gained new insights into how human and geological factors influence urban-area flooding. Using simulations, they discovered that the inability of surfaces to absorb water has a greater effect during heavy rainfall than during truly extreme events. In addition, small changes in drainage density--that is, the total length of streams and rivers in an area--significantly affect flood peaks in areas of already low drainage density. Subsurface storm drains can increase drainage density in areas lacking streams and rivers by providing more places for water to drain. However, the researchers found that depending on the existing stream network, watershed topography, and intensity and magnitude of a storm event, the addition of storm drains may have only a limited effect on the peak discharge.

PhysTEC Addresses Shortage of Physics Teachers

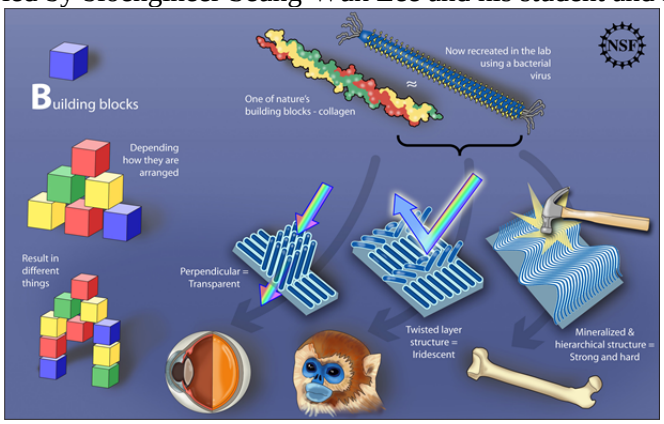
Credit: The Physics Teacher Education Coalition

School districts report a greater shortage of teachers in physics than in any other academic discipline. Only 35 percent of high-school physics teachers have a degree in physics or physics education. More than 250 colleges and universities have joined the Physics Teacher Education Coalition (PhysTEC), committing to educate greater numbers of highly qualified physics teachers. The PhysTEC project seeks to engage physics departments more deeply in teacher education so that every student will have the opportunity to learn physics from a qualified teacher. The PhysTEC members represent nearly one-third of all institutions offering physics degrees. Together these institutions graduate about 300 high-school physics teachers per year, addressing a significant fraction of the growing national need for 1400 new physics teachers per year. PhysTEC also organizes conferences and workshops, publishes articles and reports, and hosts listservs and websites (phystec.org and ptec.org) to more broadly connect with the physics community.

Highlights

Manufacturing Goes Viral

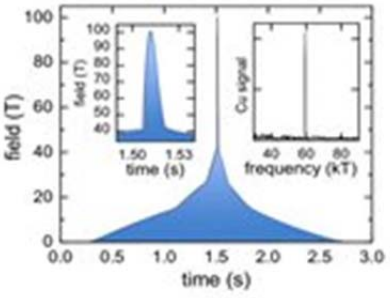
Using a simple, single-step process, engineers and scientists at the University of California at Berkeley, led by bioengineer Seung-Wuk Lee and his student and lead author Woo-Jae Chung, recently developed a technique to direct benign, filamentous viruses called M13 phages to serve as structural building blocks for materials with a wide range of properties. By controlling the physical environment alone, the researchers caused the viruses to self-assemble into hierarchically organized thin-film structures, with complexity that ranged from simple ridges, to wavy, chiral strands, to truly sophisticated patterns of overlapping strings of material. These results may also shed light on the self-assembly of biological tissues in nature. This novel, self-templating, biomaterials assembly process could be used in many other organic and inorganic materials to build hierarchical structures to tune optical, mechanical and even electrical properties from nano to macro scales. The reported approaches could be used to investigate mechanisms for diseases such as Alzheimer's, which is caused by amyloid aggregation in our brain tissues. More broadly, the breakthroughs could potentially yield scientific impacts in the area of tissue regeneration and repair.



Credit: Zina Deretsky, National Science Foundation

Super Magnet Breaks the Megagauss Barrier

Scientists and engineers at the National High Magnetic Field Laboratory (NHMFL) have successfully produced the highest nondestructive magnetic field ever – a field surpassing 100 tesla or 2 million times the Earth's magnetic field. Researchers will use this unprecedented tool to study a range of scientific activities--from unusual magnetic behaviors in materials to the quantum behavior of phase transitions in solids. The new magnet system--located at Los Alamos National Laboratory – achieved 100.75 tesla on March 22, 2012. The system is designed to pulse nondestructively in the intense 100-tesla realm on a regular basis. Magnets capable of higher field strengths have been created, but they explode after use because they cannot withstand the intense strength of the force created. The new magnet will also help researchers discover why superconductivity occurs in a newly discovered family of iron-based materials. This group of superconductors yields only to the highest magnetic fields. Superconducting magnets are used in everything from particle accelerators to magnetic resonance imaging machines. Nondestructive generation of 100 tesla magnetic fields has been a National Academy of Sciences Grand Challenge and a 15-year goal of the NHMFL. The project was jointly funded by the National Science Foundation and the Department of Energy.



Credit: Greg Boebinger, NHMFL, FSU

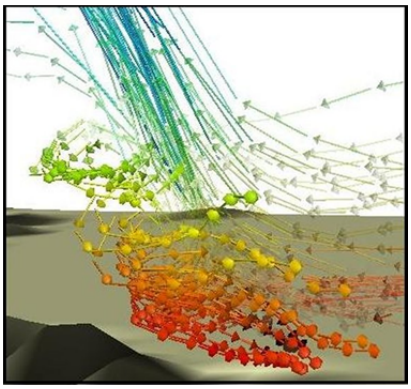
Highlights

State-of-the-Art Virtual Reality System is Key to Medical Discovery

Credit: Electronic Visualization Laboratory
University of Illinois at Chicago

A team of neurosurgeons from the College of Medicine at the University of Illinois at Chicago (UIC) recently stepped into CAVE2 – a next-generation, large-scale, virtual environment – to solve a vexing problem that presented itself in the arteries of the brain of a real patient. For years, the team had painstakingly used laptop and desktop computers to create three-dimensional, full-brain models that physiologically mirrored the brains of individual patients. These models were used for a patient whose cerebrovascular system they were trying to accurately model. But because of the limited image spatial-resolution of even today's best-quality computers, there was something the neurosurgeons couldn't see. That is, until they stepped into an automatic virtual environment, also known as a "CAVE" – a room in which images are seamlessly displayed so as to immerse an observer in a cyber-world of 3-D data. CAVE2 helped the team discover quickly that their model was “inconsistent with anatomy” – and with that revelation, their model could be corrected. The use of UIC's virtual reality system to make the discovery could help change the way surgeons are trained and greatly improve patient care – and the method could someday benefit hundreds of thousands of Americans who fall victim to brain aneurysms and strokes, the third leading cause of death in the United States. CAVE2 is funded through NSF's Major Research Instrumentation program and the Department of Energy.

Improving Tropical Cyclone Forecasts



Credit: Wallace Hogsett and Da-Lin Zhang
University of Maryland

Accurate tropical cyclone forecasts require prediction of tropical weather over vast tropical oceans; however, predicting cyclone formation is difficult due to the lack of direct observations in the formation regions and deficiencies in current models. The Weather Research and Forecasting (WRF) numerical model has captured the formation of a tropical cyclone within an area of disturbed weather associated with the Madden-Julian Oscillation (MJO) – a variable pattern of wind, rain, ocean temperature and cloudiness in the tropics. Results from the WRF research may lead the way to better modeling of tropical disturbances and improved forecasts to alert those in the tropics of potential cyclones. University of Maryland researchers have demonstrated that high-resolution models can describe slowly-evolving tropical weather patterns such as the MJO. Moreover, their results suggest that transient and small-scale weather phenomena such as tropical cyclones which develop within the disturbed weather associated with the MJO may be predictable.

NATIONAL SCIENCE FOUNDATION

FY 2014 Budget Request to Congress

April 10, 2013

SOCIAL, BEHAVIORAL AND ECONOMIC SCIENCES (SBE) $272,350,000
+$18,100,000 / 7.1%

SBE Funding
(Dollars in Millions)

	FY 2012 Actual	FY 2012 Enacted/ Annualized FY 2013 CR	FY 2014 Request	Change Over FY 2012 Enacted Amount	Change Over FY 2012 Enacted Percent
Social and Economic Sciences (SES)	$97.26	$97.18	$102.51	$5.33	5.5%
Behavioral and Cognitive Sciences (BCS)	92.47	92.69	97.43	4.74	5.1%
SBE Office of Multidisciplinary Activities (SMA)	28.22	28.23	30.65	2.42	8.6%
National Center for Science and Engineering Statistics (NCSES)	36.23	36.15	41.76	5.61	15.5%
Total, SBE	**$254.19**	**$254.25**	**$272.35**	**$18.10**	**7.1%**

Totals may not add due to rounding.

About SBE

SBE's mission is to promote the understanding of people and their lives by supporting research that reveals basic facets of human behavior; to encourage research that addresses important societal questions and problems; to work with other scientific disciplines to ensure that basic research and solutions to problems build upon the best multidisciplinary science; and to provide mission-critical statistical information about science and engineering (S&E) in the U.S. and the world through the National Center for Science and Engineering Statistics (NCSES). SBE supports long-term research across a diverse range of sciences that includes economics, psychology, sociology, geography, neuroscience, anthropology, archaeology, statistics, linguistics, and political science. SBE combines these sciences in a dynamic suite of interdisciplinary activities that link these fields to each other and to other science and engineering fields. Thus, SBE is a significant partner in cross-directorate programs that connect the social and behavioral sciences to priority investments across the agency.

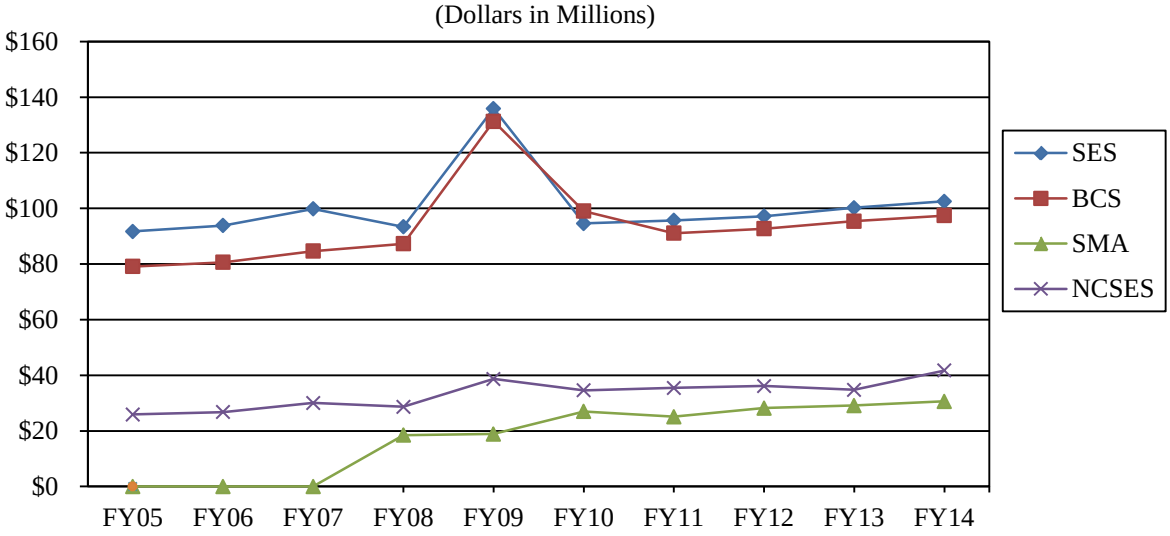
SBE's FY 2014 Request is informed by three key priorities: (1) participate in cross-directorate programs that integrate the social and behavioral sciences into priority NSF investments such as Science, Engineering, and Education for Sustainability (SEES); Comprehensive National Cybersecurity Initiative (CNCI) (via the Secure and Trustworthy Cyberspace (SaTC) investment); Cyberinfrastructure for 21st Century Science, Engineering, and Education (CIF21)/Big Data; and Innovation Corps (I-Corps); (2) sustain the directorate's ongoing strategic transformation through support for interdisciplinary research and training (via INSPIRE and SBE's own SBE 2020) and the emerging investment in cognitive science and neuroscience; and (3) protecting and enhancing core research programs and the commitment to the National Center for Science and Engineering Statistics (NCSES). These investments reflect both newly requested funds and a redeployment of resources previously committed to other areas.

SBE continues to strategically transform its scientific direction. These changes build on NSF's strategic plan, *Empowering the Nation Through Discovery and Innovation: NSF Strategic Plan for Fiscal Years 2011-2016;* and on the directorate's SBE 2020 visioning activity, which led to a report entitled *Rebuilding the Mosaic*, which was published by NSF in November 2011.

The SBE portfolio also includes major surveys that provide broad-based infrastructure for the research community while providing policy makers with needed information. NCSES is the designated federal statistical entity with responsibility for statistics about the S&E enterprise, and its data collections and analyses are important for evaluating overall U.S. competitiveness in science and engineering.

SBE provides 56 percent of the federal funding for basic research at academic institutions in the SBE sciences.

SBE Subactivity Funding
(Dollars in Millions)



FY 2009 funding reflects both the FY 2009 omnibus appropriation and funding provided through the American Recovery and Reinvestment Act of 2009 (P.L. 111-5).

FY 2014 Summary by Division

- SES's FY 2014 request reflects its strong contribution to the unifying themes in the FY 2014 NSF Budget Request. This includes SEES, through investments in the Hazards, SEES Fellows, Water Sustainability and Climate, and Sustainability Research Networks activities; Secure and Trustworthy Cyberspace (SaTC) through the Cyber Economic Incentives theme within CNCI; and CIF21 through community research networks and research on virtual organizations and with an initial investment in CIF21's Big Data emphasis area. SES will continue efforts to build the scientific foundation and research evidence base needed for future programmatic efforts in broadening the participation in science and engineering (S&E) of women, underrepresented minorities, and people with disabilities (via SBE's Science of Broadening Participation (SBP)). SES will also maintain its commitment to existing programs and continue support for surveys that provide unique insights into U.S. social, economic, and political life, while providing funding for new research that has the potential to transform the social and economic sciences and contribute to effective policy development. SES will also enhance funding for the CAREER program. To further transform SBE by increasing interdisciplinary research, training, and integration with other parts of NSF, SES will sustain its investment in SBE 2020, funding more SBE Fellows. To enhance interdisciplinary research and training, SES will participate in the Interdisciplinary Behavioral and Social Science Research (IBSS) program and continue its role in international activities with increased investments in the European Open Research Area (ORA) and Science Across Virtual Institutes (SAVI) programs. SES will maintain investment in the National Nanotechnology Infrastructure Network (NNIN).

- In FY 2014, BCS will be a major partner in NSF-wide interdisciplinary activities such as SEES, CIF21, cognitive and neuroscience research, and CNCI. BCS will expand support for behavioral and cognitive research that informs our understanding of critical issues facing the Nation such as terrorism, pandemics, and sustainability. Increased SEES funding will focus on research with SBE-specific emphases, such as investments in understanding human behavior and decision making about energy use, interactions among natural and human systems, vulnerability and resilience, and participation in Hazards and Sustainability Research Networks. In its ongoing programs, BCS will operate in an interdisciplinary context; providing additional support for research on the complex ways people interact with climate and other natural systems; and research and methodological development on learning and adaptive systems. BCS support for CNCI will enable research on cognitive and behavioral aspects of threats to cybersecurity. BCS will contribute to broadening participation in S&E of women, underrepresented minorities, and people with disabilities in STEM (via SBP). Increased funding for the SBE 2020 activity will enable BCS to partner with other NSF directorates, increasing interdisciplinary research and training for behavioral and cognitive scientists. BCS will uphold its role in international activities by participating in SAVI, ORA, and other international partnerships. It will also support the Science of Learning Centers (SLC) program and continue investments in integrative interdisciplinary approaches to the understanding of human cultural and biological evolution over long time scales. BCS will also continue to fund basic research that advances understanding of the brain, cognition, and behavior through various research mechanisms.

- SMA provides a focal point for programmatic activities that cut across NSF and SBE boundaries. In addition, SMA assists with seeding interdisciplinary activities for the future. In FY 2014, SMA will continue to play an important role in the expansion of interdisciplinary training as part of SBE 2020, by expanding the SBE Postdoctoral Research Fellowship (SPRF) program to include interdisciplinary postdoctoral fellowships; SMA will provide overall management for the program. Support for enhancing the research experience for students will continue via investments in the Research Experiences for Undergraduates (REU) Sites and Supplements programs. SMA will fund interdisciplinary activities associated with CIF21; the Science of Science and Innovation Policy program (SciSIP); the interagency STAR METRICS pilot project; cognitive science and neuroscience; and SEES, including Hazards, Sustainability Research Networks, and SEES Fellows. SMA will participate in I-Corps, INSPIRE, and SaTC (through the Cyber Economic Incentives theme within CNCI, a multi-agency priority). SMA will continue to manage the agency-wide Science of Learning Centers program.

- For FY 2014, NCSES will close a growing gap in its national estimates for research and development funding and performance by developing and implementing a survey of nonprofit organizations. It will also expand the scope of administrative records sources being considered to augment the full suite of its existing surveys and proceed with a pilot project establishing collaboration between several federal agencies to assess the feasibility of using agencies' administrative records to measure research and development activity. NCSES will test new measures in the Survey of Doctorate Recipients (SDR) that address data gaps related to understanding the relationship between federal support for graduate education and outcomes, such as employment; increase the frequency of the Survey of State Government Research and Development; and develop and test effective data collection strategies for the nascent Microbusiness Innovation Science and Technology Survey (MIST).

Major Investments

SBE Major Investments
(Dollars in Millions)

Area of Investment	FY 2012 Actual	FY 2012 Enacted/ Annualized FY 2013 CR	FY 2014 Request	Change Over FY 2012 Enacted Amount	Change Over FY 2012 Enacted Percent
CAREER	8.58	5.54	6.01	0.47	8.5%
Cognitive Science and Neuroscience	1.00	1.00	3.00	2.00	200.0%
CIF21	5.50	5.50	7.50	2.00	36.4%
CTE	1.00	1.00	1.00	-	-
CNCI	7.58	6.00	6.00	-	-
I-Corps	0.20	0.50	0.50	-	-
INSPIRE	3.32	0.50	1.00	0.50	100.0%
Research Experiences for Undergraduate (REU) Sites and Supps	3.99	3.32	3.89	0.57	17.2%
SaTC	4.00	4.00	4.00	-	-
SEES	7.75	7.75	9.25	1.50	19.4%
SciSIP	12.80	13.50	11.05	-2.45	-18.1%

Major investments may have funding overlap and thus should not be summed.

- CAREER: SBE supports CAREER (an increase of $470,000 over FY 2012 Enacted, to a total of $6.01 million) with awards to young investigators in social and behavioral sciences who exemplify the role of teacher-scholar through the integration of education and research.

- Cognitive Science and Neuroscience: Support for this cross-foundation activity ($3.0 million total, $2.0 million above the FY 2012 Enacted level) will contribute to NSF's participation in the Office of the Science and Technology Policy's effort to coordinate federal research in this area. SBE and other NSF directorates work together informally through co-review of interdisciplinary proposals and formally through special solicitations, such as Collaborative Research in Computational Neuroscience. A Dear Colleague Letter (DCL) was issued in FY 2013 supporting research on neuroscience and cognitive science. In FY 2014, SBE, in conjunction with the Directorates for Computer and Information Science and Engineering (CISE); Engineering (ENG); Biological Sciences (BIO); and Mathematical and Physical Sciences (MPS), will continue to leverage existing investments in neuroscience, informed by the results of the DCL activity, and come together to call for a broad-based focus on understanding the brain and learning how to deploy that understanding.

- CIF21: Support for this NSF-wide investment ($7.50 million total, $2.0 million above FY 2012 Enacted) will support awards for data and cyberinfrastructure investments that create new opportunities for SBE researchers to understand human behavior and cognition and the effectiveness of virtual organizations in the context of the 21st century networked society. Also, SBE will make an initial investment in CIF21's Big Data emphasis area for research that advances the core scientific and technological means of managing, analyzing, visualizing, and extracting information from large, diverse, data sets, especially related to neuroscience, economics, and the integration of the human, social, and natural worlds.

- CTE: SBE's participation in Cyberlearning Transforming Education (CTE) remains at $1.0 million in FY 2014 for research on the development of technologies for cyberlearning, and for studying the impact of technologies on learning.

- Comprehensive National Cybersecurity Initiative (CNCI): In partnership with CISE, SBE will support multidisciplinary research in the science of cybersecurity, moving target defense, tailored trustworthy spaces, and cyber economic incentives. SBE's investment in this national priority is maintained at $6.0 million in FY 2014. SBE will devote resources to Secure and Trustworthy Computing (SaTC) through support for the Cyber Economic Incentives theme within CNCI. In addition, SBE's broad scientific base in the behavioral, social, and decision making sciences provides a wealth of opportunities to contribute to this national priority.

- I-Corps: With a sustained investment of $500,000, SBE will continue a multi-year effort to strengthen collaboration between social scientists and practitioners and improve social science students' understanding of innovation.

- INSPIRE: SBE support for this NSF priority is aligned with SBE Transformed Portfolio, SBE 2020. This support increases in FY 2014 (+$500,000 over FY 2012 Enacted, to a total of $1.0 million) to support interdisciplinary research and training.

- Funding for the Research Experiences for Undergraduates (REU) Sites and Supplements program is increased $570,000 over the FY 2012 Enacted. This additional funding will support enhanced research experiences for students in their first two years of college, as recommended by the President's Council of Advisors on Science and Technology (PCAST) in their report, *Engage to Excel: Producing One Million Additional College Graduates with Degrees in Science, Technology, Engineering, and Mathematics.*

- SEES: In FY 2014 SBE will continue its commitment to sustainability research by making significant investments across a variety of SEES activities, such as Water Sustainability and Climate (WSC), Coupled-Natural and Human Systems (CNH), SEES Fellows, Sustainability Research Networks (SRN), and Hazards. These investments further integrate the SBE sciences into research on energy and sustainability, while strengthening SBE's existing investments, and making new investments in decision making, coastal communities, and vulnerability and resilience. Funding increases by $1.50 million over FY 2012 Enacted (to a total of $9.25 million).

- Science of Science and Innovation Policy (SciSIP): decreases in FY 2014 (-$2.45 million below FY 2012 Enacted to a total of $11.05 million). SciSIP will continue to support research and data collections related to innovation and R&D spending.

SBE Funding for Centers Programs and Facilities

SBE Funding for Centers Programs
(Dollars in Millions)

	FY 2012 Actual	FY 2012 Enacted/ Annualized FY 2013 CR	FY 2014 Request	Change Over FY 2012 Enacted Amount	Change Over FY 2012 Enacted Percent
Centers Programs Total	**$17.32**	**$14.27**	**$14.20**	**-$0.07**	**-0.5%**
Nanoscale Science & Engineering Centers (SES & BCS)	1.16	0.60	0.60	-	-
Science of Learning Centers (SMA & BCS)	16.16	13.67	13.60	-0.07	-0.5%

For detailed information on individual centers, please see the NSF-Wide Investments chapter.

- Funding for the Nanoscale Science & Engineering Centers will continue at $600,000 in FY 2014.

- The Science of Learning Centers (SLC) program funding decreases (-$70,000) below the FY 2012 Enacted to a total of $13.60 million. Support includes annual increments to all six centers: the Center of Excellence for Learning in Education, Science, and Technology (CELEST); the Center for Learning in Informal and Formal Environments (LIFE); the Pittsburgh Science of Learning Center for Robust Learning (PSLC); the Spatial Intelligence and Learning Center (SILC); the Temporal Dynamics of Learning Center (TDLC); and the Visual Language and Visual Learning Center (VL2). Support is also included for SLC evaluation activities in FY 2014. Funding for Cohort 1 centers will end in FY 2014, and funding for Cohort 2 centers, approved for an additional five-year renewal by the National Science Board in February 2011, will end in FY 2015.

SBE Funding for Facilities
(Dollars in Millions)

	FY 2012 Actual	FY 2012 Enacted/ Annualized FY 2013 CR	FY 2014 Request	Change Over FY 2012 Enacted Amount	Change Over FY 2012 Enacted Percent
Facilities Total	**$0.40**	**$0.40**	**$0.40**	**-**	**-**
National Nanotechnology Infrastructure Network (NNIN)	0.40	0.40	0.40	-	-

For detailed information on individual facilities, please see the Facilities chapter.

The current NNIN ten-year cooperative agreement will close at the end of FY 2013. SBE's support for this activity will continue beyond this end point. The Directorate for Engineering (ENG) is currently evaluating the scope for future nanotechnology user facility support and working to identify a replacement program. NNIN is funded through the SES division and the funding level ($400,000) is sustained.

Summary and Funding Profile

SBE supports investment in core research and education as well as research infrastructure. In FY 2014, the number of research grant proposals is expected to be held constant with the FY 2013 Estimate level. SBE expects to award approximately 590 research grants in FY 2014. Average annualized award size will increase over the FY 2012 Enacted and duration will be held constant at the FY 2012 Enacted estimate level.

In FY 2014, funding for the centers accounts for 5 percent of SBE's Request. Center funding decreases $70,000 from the FY 2012 Enacted level, and includes the SLC program supporting six centers and support to the Centers for Nanotechnology in Society.

SBE Funding Profile

	FY 2012 Actual Estimate	FY 2012 Enacted/ Annualized FY 2013 CR Estimate[1]	FY 2014 Estimate
Statistics for Competitive Awards:			
Number of Proposals	4,775	5,500	5,500
Number of New Awards	1,018	1,190	1,220
Funding Rate	21%	22%	22%
Statistics for Research Grants:			
Number of Research Grant Proposals	3,209	3,700	3,700
Number of Research Grants	576	570	590
Funding Rate	18%	15%	16%
Median Annualized Award Size	$98,247	$100,350	$100,350
Average Annualized Award Size	$120,052	$115,450	$116,429
Average Award Duration, in years	2.5	2.6	2.6

[1]Award Estimates for FY 2013, such as numbers of awards and size/duration, are based upon the FY 2012 Enacted level.

Program Monitoring and Evaluation

Committees of Visitors (COV):

- No SBE COVs will convene in FY 2014.
- A COV to review the SES division will convene on June 3-5, 2013.
- In FY 2013, one COV convened on October 10-12, 2012 and reviewed programs under the Behavioral and Cognitive Sciences (BCS) division: Archaeology/Archaeometry; Biological Anthropology; Cultural Anthropology; Geography and Spatial Sciences; Linguistics; Documenting Endangered Languages; Perception, Action and Cognition; Cognitive Neuroscience; Developmental and Learning Sciences; and Social Psychology. The BCS COV recommended that: BCS experiment with new review cycles (inclusive of a mechanism for evaluating the effectiveness of the new cycle compared with the old); further development of transdisciplinary research across BCS disciplines; increase publicity of BCS-funded research (across NSF, and to the public and scientific community); further exploration of virtual conferencing; clearer definition of reviewer role in determining "broader impacts" and "intellectual merit" criteria for funding decisions; encourage BCS proposers to make better use of "broader impacts" to frame description of their research questions; and provide clearer

guidance on NSF's use of data management plans. The Chair of the BCS COV will present the report and response to the SBE Advisory Committee on May 20-21, 2013.

- In FY 2012, one COV convened on December 15-16, 2011 and reviewed programs under the Office of Multidisciplinary Activities (SMA): Research Experiences for Undergraduates (REU) Sites, SBE Minority Postdoctoral Research Fellowships (MPRF), and the Science of Science and Innovation Policy (SciSIP). The SMA COV recommended SBE management review the current placement of the multidisciplinary programs in the directorate, as well as the question of how many submissions a year are appropriate. The COV also recommended taking actions to broaden participation and increase capacity for research related to the Science of Science and Innovation Policy (SciSIP) program. The SMA COV report and response to the report were presented and approved before the SBE Advisory Committee (AC) on May 17-18, 2012.
- All SBE divisions are responding to and implementing recommendations from recent COVs.

Workshops and Reports:

- A recent report by the SBE directorate, *Rebuilding the Mosaic; Fostering Research in Social, Behavioral, and Economic Sciences at the National Science Foundation in the Next Decade* (issued November, 2011), sets forth a next generation model of research that is collaborative, data-intensive, and multi- or interdisciplinary. Based on 252 white papers from more than 500 individuals, together with consultation with professional associations, societies, and campus visits, the report explores the programmatic implications of this model of research for the directorate's programs and has been influential in setting priorities and framing discussions within the directorate, across the Foundation, and with other public and private agencies and organizations. Key areas of interest are interdisciplinary training and support for graduate students and young faculty; programs to foster interdisciplinary investigations; and efforts, within the directorate and in cooperation with CISE and other entities, to catalyze research communities around new data and computational infrastructures.
- Two recent workshops were convened by the SBE Advisory Committee's Subcommittee on the Science of Learning that have implications for programmatic portfolio development. The first was held October 4-5, 2012 and focused on the scientific achievements in the science of learning over the past decade; the results were reported at the SBE AC meeting on November 15, 2012. The second workshop was held February 28 and March 1, 2013 and focused on strategies and objectives to advance the science of learning into the future. The results will be discussed at the May 2013 SBE AC meeting with emphasis on future strategic and budget planning in SBE's investment in the Science of Learning.

The Performance chapter provides details regarding the periodic reviews of programs and portfolios of programs by external Committees of Visitors and directorate Advisory Committees. Please see this chapter for additional information.

Number of People Involved in SBE Activities

	FY 2012 Actual Estimate	FY 2013 Estimate	FY 2014 Estimate
Senior Researchers	3,646	3,100	3,200
Other Professionals	519	700	700
Postdoctorates	330	300	400
Graduate Students	2,097	2,300	2,300
Undergraduate Students	789	800	800
Total Number of People	**7,381**	**7,200**	**7,400**

DIVISION OF SOCIAL AND ECONOMIC SCIENCES (SES) $102,510,000
+$5,330,000 / 5.5%

SES Funding
(Dollars in Millions)

	FY 2012 Actual	FY 2012 Enacted/ Annualized FY 2013 CR	FY 2014 Request	Change Over FY 2012 Enacted Amount	Change Over FY 2012 Enacted Percent
Total, SES	**$97.26**	**$97.18**	**$102.51**	**$5.33**	**5.5%**
Research	**85.23**	**87.83**	**91.94**	**4.11**	**4.7%**
CAREER	2.38	2.82	3.11	0.29	10.3%
Centers Funding (total)	0.98	0.42	0.42	-	-
Nanoscale Science & Engineering Centers	0.98	0.42	0.42	-	-
Education	**3.73**	**3.79**	**3.17**	**-0.62**	**-16.4%**
Infrastructure	**8.30**	**5.56**	**7.40**	**1.84**	**33.1%**
Nat'l Nanotechnology Infrastructure Network (NNIN)	0.40	0.40	0.40	-	-
Research Resources	7.90	5.16	7.00	1.84	35.7%

Totals may not add due to rounding.

SES supports research and related activities, conducted within the U.S. and globally, that improve our understanding of economic, political, and social institutions and how individuals and organizations behave within them. SES also funds activities investigating risk assessment and decision-making by individuals and groups; the nature and development of science and technology and their impact on society; methods and statistics applicable across the social, economic, and behavioral sciences; scholarly career development; and broadening participation in the social, behavioral, and economic sciences. Its discipline-based programs include sociology, economics, and political science, while interdisciplinary programs support fields such as decision-making and risk; methods, measurement, and statistics; science of organizations; law and social science; and science and technology studies. In many of its programs, SES is the major, if not only, source of federal funding for fundamental research, making important investments in the data resources and methodological advances that produce transformative research.

SES also coordinates the Ethics Education in Science and Engineering program, supporting (with other NSF directorates) the Online Ethics Center for Engineering and Science, and manages the Centers for Nanotechnology in Society. SES is a participant in a number of Nanoscale Science and Engineering Centers. In addition, SES plays a major role in managing the Decision Making Under Uncertainty collaborative projects.

In general, 60 percent of the total SES portfolio is available for new research grants. The remaining 40 percent funds continuing grants made in previous years.

FY 2014 Summary

All funding decreases/increases represent change over the FY 2012 Enacted level.

Research

Overall, support for SES disciplinary and interdisciplinary research increases (+$4.11 million to a total of $91.94 million).

- Continued support ($7.10 million total) for interdisciplinary research, training, and integration opportunities through SBE's own SBE 2020 (via SBE's Interdisciplinary Behavioral and Social Science IBSS) program. Funding in this investment will require a reduction in core disciplinary research programs.
- CAREER funding in FY 2014 increases by $290,000, to a total of $3.11 million. This investment is consistent with SES's emphasis on supporting early career researchers.
- An increase of $1.97 million will expand SBE's international leadership role through participation in SAVI, the European Open Research Area program, and other international partnerships.
- SES continues its investments of $400,000 to the Ethics Education in Science and Engineering (EESE) cross-directorate program.
- CIF21: funding of $4.40 million (an increase of $1.75 million) will provide support for a Big Data ($1.50 million) emphasis area with research that aims to advance the core scientific and technological means of managing, analyzing, and visualizing, and extracting information from large, diverse, data sets. An additional $250,000 will further SES' investment in planning awards for the future data cyberinfrastructure investments that create new opportunities for SBE researchers in the context of the 21st century networked society.
- Increased funding (+$350,000, to a total of $4.0 million) for SEES will support research in expanded SEES activities through SBE-specific emphases, such as investments in understanding energy use and in decision making, coastal communities, and vulnerability and resilience, through the enhancement of existing programs and new solicitations; funding will also support Hazards, Sustainability Research Networks, SEES Fellows, and Water Sustainability and Climate.
- Continued support of $2.0 million for SaTC is provided through support for the Cyber Economic Incentives and other themes within CNCI.
- Funding for SES' Science of Broadening Participation investment increases by $250,000 to a total of $750,000. SES' SBP investment supports efforts to build the scientific foundation and research evidence base needed for future broadening participation efforts. Investing in research that informs the science of broadening participation spans education and the SBE sciences, and engages all of NSF.
- Support for the Coupled Natural and Human Systems program decreases by $250,000, to $2.25 million total.
- Permanent termination of SES' investment in the Enhancing Access to the Radio Spectrum (EARS) program, a partnership with ENG, MPS, and CISE (-$500,000). Funding is redeployed to establish or increase funding for NSF and SBE priorities.

Education

- FY 2014 support for ADVANCE ($790,000) and REU supplements ($500,000) remain constant with the FY 2012 Enacted level.
- In an effort to establish a better balance between the responsibilities and demands of work lives and family lives for social and behavioral scientists, an investment of $130,000 provides support to the Career-Life Balance (CLB) Initiative.
- SBE will invest $1.75 million in graduate traineeships as IGERT evolves into a new program, NSF Research Traineeships (NRT), which will encourage strong, well-documented efforts at innovation and design of graduate programs to support growth within specific disciplines and solid preparation of the trainees.

Infrastructure

- The existing National Nanotechnology Infrastructure Network (NNIN) comes to a close at the end of FY 2013 at the completion of its ten-year cooperative agreement. ENG is currently evaluating the scope for future nanotechnology user facility support and working to identify a replacement program, and SBE expects to sustain its investment of $400,000 in NNIN beyond the end point of the current cooperative agreement.
- Funding for other Research Resources activities increases (+$1.84 million, to a total of $5.16 million). Funding supports multi-million dollar survey awards such as the American National Election Studies (ANES), the Panel Study of Income Dynamics (PSID), and the General Social Survey (GSS). These surveys are national resources for research, teaching, and decision-making and have become models for similar undertakings in other fields. Funding supports SES' CIF21 investment inclusive of support for the Building Community and Capacity for Data-Intensive Research in the Social, Behavioral, and Economic Sciences and in Education and Human Resources (BCC-SBE/EHR) initiative (in partnership with EHR and CISE). This investment seeks to enable research communities to develop visions, teams, and capabilities dedicated to creating new large-scale, next-generation data resources and relevant analytic techniques to advance fundamental research for SBE and EHR sciences.

DIVISION OF BEHAVIORAL AND COGNITIVE SCIENCE (BCS) $97,430,000
+$4,740,000 / 5.1%

BCS Funding
(Dollars in Millions)

	FY 2012 Actual	FY 2012 Enacted/ Annualized FY 2013 CR	FY 2014 Request	Change Over FY 2012 Enacted Amount	Change Over FY 2012 Enacted Percent
Total, BCS	**$92.47**	**$92.69**	**$97.43**	**$4.74**	**5.1%**
Research	**87.78**	**89.73**	**93.77**	**4.04**	**4.5%**
CAREER	5.54	2.82	3.11	0.29	10.3%
Centers Funding (total)	5.05	5.78	5.78	-	-
Nanoscale Science & Engineering Centers	0.18	0.18	0.18	-	-
Science of Learning Centers	4.87	5.60	5.60	-	-
Education	**3.56**	**2.92**	**2.52**	**-0.40**	**-13.7%**
Infrastructure	**1.14**	**0.04**	**1.14**	**1.10**	**2750.0%**
Research Resources	1.14	0.04	1.14	1.10	2750.0%

Totals may not add due to rounding.

BCS supports research and related activities that advance fundamental understanding in the behavioral, cognitive, anthropological, and geographic sciences. Strong core programs are complemented by active involvement in competitions that support collaborative and cross-disciplinary projects. The division seeks to advance scientific knowledge and methods focusing on human cognition and behavior, including perception, thought processes, language, learning, and social behavior across neural, individual, family, and group levels. BCS also supports activities focusing on human variation at the scales of society, culture, and biology, and how these variations and related patterns develop and change across time and space. The division aims to increase basic understanding of geographic distributions and relationships as well as the capabilities to explore them, with an emphasis on interactions among human and natural systems on the Earth's surface. BCS research is helping us prepare for and mitigate the effects of natural and human-initiated disasters, predict and address how people respond to stressors, improve methods for effective learning, enhance the quality of social interaction, and respond to issues such as globalization, terrorism, and climate change. BCS investments in SEES advance our understanding of sustainability and contribute to energy research.

In general, 59 percent of the BCS portfolio is available for new research grants. The remaining 41 percent funds continuing grants made in previous years.

FY 2014 Summary

All funding decreases/increases represent change over the FY 2012 Enacted. In the FY 2014 Request there is a general reduction for core programs to provide resources for enhancement and implementation of other programs related to directorate priorities.

Research
Overall, support for BCS disciplinary and interdisciplinary research increases (+$4.04 million to a total of $93.77 million).

- Support increases (+$2.28 million, to a total of $5.40 million) for SBE 2020 (via SBE's IBSS initiative and Dear Colleague Letter (DCL)) to support interdisciplinary research, training, and integration opportunities for behavioral and cognitive scientists.
- CAREER funding will increase by $180,000, to a total of $2.90 million. This investment is consistent with BCS' emphasis on supporting early-career researchers.
- Cognitive Science and Neuroscience: BCS support for this cross-foundation activity totals approximately $2.0 million in FY 2014 (+$1.0 million). BCS funding will support NSF's commitment to making targeted investments in collaborative science and innovative technologies for understanding the brain.
- An increase of $1.0 million will expand SBE's international leadership role through participation in SAVI, the European Open Research Area program, and other international partnerships.
- Increased funding (+$80,000, to a total of $3.33 million) for SEES to support research with SBE-specific emphases, such as investments in understanding human behavior and decision making about energy use, interactions among natural and human systems, vulnerability and resilience, and to participate in Hazards, Sustainability Research Networks, SEES Fellows, and Water Sustainability and Climate (WSC).
- Increased support (+$250,000, to a total of $2.10 million) for CIF21 will create new opportunities for BCS researchers to understand human behavior and cognition.
- $1.20 million will be used for SaTC to support the Cyber Economic Incentives theme within CNCI. An additional $1.0 million is provided for multidisciplinary research in other CNCI activities.
- Support for the Enhancing Access to the Radio Spectrum (EARS) program is permanently terminated (-$500,000) at the FY 2014 Request level as a result of SBE's redeployment of funding to establish or increase investments in NSF and SBE priorities.
- Centers Funding: As planned, support for the SLC program ($5.60 million total) and the Nanotechnology Centers ($180,000) remains constant with the FY 2012 Enacted level.
- Funding for BCS' Science of Broadening Participation (SBP) investment increases by $250,000 to a total of $750,000. BCS's SBP investment supports efforts to build the scientific foundation and research evidence base needed for future broadening participation efforts. Investing in research that informs the science of broadening participation spans education and the SBE sciences, and engages all of NSF.
- Continued investment in support of integrative and interdisciplinary approaches to the understanding of human cultural and biological evolution over long time scales.

Education

- BCS support for ADVANCE will remain at the FY 2012 Enacted level ($680,000).
- With an initial investment of $120,000, BCS will support NSF's Career-Life Balance activity.
- BCS will invest $1.28 million in graduate traineeships as IGERT evolves into a new program, NSF Research Traineeships (NRT), which will encourage strong, well-documented efforts at innovation and design of graduate programs to support growth within specific disciplines and solid preparation of the trainees.
- Support for Research Experiences for Undergraduates (REU) Supplements ($440,000) is sustained.

Infrastructure

- FY 2014 support for infrastructure activities increases by $1.10 million (to a total of $1.14 million). Funding supports BCS' CIF21 investment inclusive of support for the Building Community and Capacity for Data-Intensive Research in the Social, Behavioral, and Economic Sciences and in Education and Human Resources (BCC-SBE/EHR) initiative (in partnership with EHR and CISE) which seeks to enable research communities to develop visions, teams, and capabilities dedicated to creating new large-scale, next-generation data resources and relevant analytic techniques to advance fundamental research for SBE and EHR sciences.

SBE OFFICE OF MULTIDISCIPLINARY ACTIVITIES (SMA) **$30,650,000 +$2,420,000 / 8.6%**

SMA Funding
(Dollars in Millions)

	FY 2012 Actual	FY 2012 Enacted/ Annualized FY 2013 CR	FY 2014 Request	Change Over FY 2012 Enacted Amount	Change Over FY 2012 Enacted Percent
Total, SMA	**$28.22**	**$28.23**	**$30.65**	**$2.42**	**8.6%**
Research	**22.47**	**22.56**	**22.80**	**0.24**	**1.1%**
CAREER	0.66	-	-	-	N/A
Centers Funding (total)	11.29	8.07	8.00	-0.07	-0.9%
Science of Learning Centers	11.29	8.07	8.00	-0.07	-0.9%
Education	**3.47**	**3.38**	**5.95**	**2.57**	**76.0%**
Infrastructure	**2.28**	**2.29**	**1.90**	-0.39	**-17.0%**
Research Resources	2.28	2.29	1.90	-0.39	-17.0%

Totals may not add due to rounding.

SMA provides a focal point for programmatic activities that cut across SBE disciplinary boundaries, including the agency-wide Science of Learning Centers (SLCs). SMA also funds the Science of Science and Innovation Policy (SciSIP) program, Research Experiences for Undergraduates (REU) Sites, and SBE Postdoctoral Research Fellowships (SPRF). SMA will play a critical role in several NSF areas of emphasis for FY 2014, such as clean energy and sustainability (via the SEES investment); cyberinfrastructure and computer science (via the CIF21 investment); national security (via the CNCI investment); international leadership and interaction (via support to the Open Research Area (ORA) activity); innovation (via the Innovation Corps (I-Corps) investment); interdisciplinary research and training (via the INSPIRE investment and full implementation of the SBE Transformed Portfolio, SBE 2020 through the Interdisciplinary Behavioral and Social Science Research (IBSS) solicitation); and the developing investment in cognitive science and neuroscience. These investments reflect both newly requested funds and a significant redeployment of resources previously committed to other social, behavioral and economics science disciplines within SBE. Co-funding with other divisions in SBE and with other directorates is typical for SMA, as is participation in interagency activities. While all SBE divisions pursue interdisciplinary work, SMA assists with seeding multidisciplinary activities for the future. All areas of SBE sciences are represented in the SMA portfolio.

In general, 39 percent of the SMA portfolio is available for new research grants. The remaining 61 percent funds continuing awards made in previous years, including funding for the SLCs.

FY 2014 Summary

All funding decreases/increases represent change over the FY 2012 Enacted. In the FY 2014 Request there is a general reduction for core programs to provide resources for enhancement and implementation of other programs related to directorate priorities.

Research
Overall, support increases for basic research activities (+$240,000 to a total of $22.80 million).

- $1.0 million (an increase of $500,000) supports INSPIRE, an NSF priority aligned with SBE 2020.

- $500,000 (to a total of $500,000) supports the I-Corps investment, strengthening collaboration between social scientists and academe and improving social science students' understanding of innovation.
- In FY 2014, SMA will continue to support six active Science of Learning Centers and funding decreases by $70,000 (to a total of $8.0 million). A gradual phase down of the program continues as centers reach their endpoints in FY 2014 and FY 2015.
- Funding for the SciSIP disciplinary research activities decreases by $2.45 million, to a total of $5.10 million. Funding is redeployed to establish or increase funding for NSF and SBE priorities.
- $1.0 million of resources from SMA SciSIP funding will be redirected to initiate an investment in cognitive science and neuroscience research. At this level SMA, in partnership with SBE's BCS division and other NSF directorates (ENG, BIO, MPS, and EHR), will further its efforts toward defining a broad-based focus on understanding the brain and learning how to deploy that understanding through community building activities such as; workshops, Dear Colleague Letters, and research coordination networks.
- SEES funding, $1.92 million total (+$1.07 million) supports research with SBE-specific emphases, such as investments in understanding human behavior and decision making about energy use, interactions among natural and human systems, and vulnerability and resilience. SMA will participate in Hazards, Sustainability Research Networks, and SEES Fellows.
- SMA provides continued support of $1.0 million for Cyberinfrastructure Framework for 21st Century Science and Engineering (CIF21). Of particular interest to SMA are new opportunities for SBE researchers to understand the 21st century networked society.
- With a continued investment of $800,000, SMA will partner with CISE in devoting resources to the Secure and Trustworthy Cyberspace (SaTC) initiative through support for the Cyber Economic Incentives theme within CNCI. This investment will support research at the interstices of the economic and computer sciences to achieve secure practices through the development of market forces that incentivize good behavior.

Education

Overall, support for Education activities in SMA increases by $2.57 million, to a total of $5.95 million.

- Support for Research Experiences for Undergraduates (REU) Sites increases by $570,000, to a total of $2.89 million. This additional funding will support enhanced research experiences for students in their first two years of college, as recommended by the President's Council of Advisors on Science and Technology (PCAST) in their report, *Engage to Excel: Producing One Million Additional College Graduates with Degrees in Science, Technology, Engineering, and Mathematics.*
- In FY 2012, NSF/SBE expanded an existing postdoctoral fellowship program to include interdisciplinary post-doctoral fellows. It is called the SBE Postdoctoral Research Fellowship (SPRF) program, and has two tracks – broadening participation (SPRF-BP), which replaces the former SBE Minority Postdoctoral Fellowships; and interdisciplinary research (SPRF-IBSS) which aligns with SBE 2020 activities. At the FY 2014 Request level, funding for SPRF-BP increases by $500,000, to a total of $1.50 million. An investment of $1.50 million will support the SPRF-IBSS activity.

Infrastructure

- Support for infrastructure activities decreases (-$390,000 to a total of $1.90 million). Funding is primarily for data and tool development. Data development includes such databases as: the National Bureau of Economic Research/Harvard patent database; the University of California, Davis database on initial public offerings; and two surveys, "Management and Organizational Practices Across the U.S.", and the "Division of Innovative Labor." Tool developments include such projects as Open Researcher and Contributor ID (ORCID) unique researcher identifiers and Publication Harvester: An Open-Source Software Tool for Science Policy Research.

NATIONAL CENTER FOR SCIENCE AND ENGINEERING STATISTICS (NCSES) $41,760,000 +$5,610,000 / +15.5%

NCSES Funding

(Dollars in Millions)

	FY 2012 Actual	FY 2012 Enacted/ Annualized FY 2013 CR	FY 2014 Request	Change Over FY 2012 Enacted Amount	Change Over FY 2012 Enacted Percent
Total, NCSES	**$36.23**	**$36.15**	**$41.76**	**$5.61**	**15.5%**
Research	**0.46**	**0.55**	**0.50**	**-0.05**	**-9.1%**
Infrastructure	**35.77**	**35.60**	**41.26**	**5.66**	**15.9%**

Totals may not add due to rounding.

The National Center for Science and Engineering Statistics (NCSES) was established within the National Science Foundation by Section 505 of the America COMPETES Reauthorization Act of 2010 (P.L. 111-358). The Act provides NCSES with the legislative mission to "…serve as the central federal clearinghouse for the collection, interpretation, analysis, and dissemination of objective data on science, engineering, technology, and research and development." NCSES is called on to support the collection of statistical data on research and development trends, the science and engineering workforce, U.S. competitiveness, and the condition and progress of the Nation's STEM education; to support research using the data it collects and on methodologies in areas related to the work of the Center; and to support the education and training of researchers in the use of its own and other large-scale, nationally representative data sets.

As one of the thirteen principal federal statistical agencies, NCSES has broad responsibility for statistics about the science and engineering enterprise. NCSES designs, supports, and directs a coordinated collection of periodic national surveys and performs a variety of other data collections and research, providing policymakers, researchers, and other decision makers with high quality data and analysis on R&D, innovation, the education of scientists and engineers, and the science and engineering workforce. The work of NCSES involves survey development, methodological and quality improvement efforts, data collection, analysis, information compilation, dissemination, web access, and customer service to meet the statistical and analytical needs of a diverse user community. It also prepares two congressionally mandated biennial reports — *Science and Engineering Indicators (SEI)* and *Women, Minorities, and Persons with Disabilities in Science and Engineering*. The data collected by NCSES also serve as an important resource for researchers in SBE's Science of Science and Innovation Policy (SciSIP) program.

The funding portfolio for NCSES includes ongoing, cyclical surveys; reports and other products; and projects accomplished primarily through contracts and grants.

FY 2014 Summary

All funding decreases/increases represent change over the FY 2012 Enacted.

Infrastructure

FY 2014 support for core NCSES infrastructure activities increases by $5.61 million to an overall total of $41.76 million. The additional funds support targeted improvements in NCSES' statistical programs and are as follows:

- $2.0 million to plan and conduct a survey of research and development funding and performance by nonprofit organizations. NCSES has not conducted such a survey in more than 15 years, though the level and importance of R&D activity in this sector is thought have grown significantly.
- $500,000 to increase the frequency of the Survey of State Government Research and Development
- $500,000 to develop and test effective data collection strategies for the Microbusiness Innovation Science and Technology Survey, to fill a void in our knowledge of the smallest employers, often the very businesses believed to fuel innovation in the U.S.
- $500,000 for the Survey of Doctorate Recipients to develop and test new measures that address data gaps related to understanding the relationship between Federal support for graduate education and outcomes, such as employment.
- $1.50 million to expand the scope of administrative records sources that NCSES is exploring to augment the full suite of its existing surveys. NCSES will proceed with a pilot project establishing collaboration between several federal agencies to test the feasibility of using agencies' administrative records to measure research and development activity. NCSES will explore approaches to improving other agencies' data sets, closely coordinating such activities with relevant offices in OMB. This work will also include outcome data for STEM graduates whose education is funded in whole or in part via federal research grants and improved innovation measures for the Business R&D and Innovation Survey (BRDIS) and NCSES' other R&D surveys.
- $610,000 to plan and design program modifications to respond to recommendations received from the National Academy's Committee on National Statistics Panel on Developing Science, Technology and Innovation Indicators for the Future.
- Funding for NCSES SciSIP activities will decrease to $4.95 million total ($1.0 million below the FY 2012 Enacted level). Current SciSIP funding is used to support the Business R&D and Innovation Survey, the federal statistical system's primary survey on business domestic and global R&D expenditures and workforce; and the National Survey of Recent College Graduates (NSRCG), the federal statistical system's primary survey of the nation's science and engineering workforce. This reduction will be offset primarily with savings from the termination of the NSRCG.

Dear NSF I'm Jiwon Kim
From: 김지원 <[redacted]
Date: Tue, April 23, 2013 10:15 am
To: pcast@ostp.gov

Dear NSF

I'm Jiwon Kim who is university student in Korea

With LG, one of the leading companies in South Korea, we are now doing a project named "Global Challenger". The project members consist of one of the most talented Korean university students. We want to learn advanced technologies and policies from other countries and try to apply to Korea in the best way.

Our team and LG are interested in the way of using public data by the government in the United States. We have the responsibility to introduce the advanced model from the US to Korean government.

That is why our team and LG are interested in integrating and managing big public data from each government.

Currently Korean government is paying attention to the 2012 US policy which was announced by US government-"Big Data Research and Development Initiative".

Since Korea is now in early stage of the big data R&D, we would like to ask PCAST for an advice,- an organization that introduces 'Big Data Initiative' policy to the White House.

We saw at your homepage that there will be a student volunteering program in OSTP this summer, and we would like to visit the program.

In particular, we would like to meet with Executive Director, PCAST co-chair, and ask for an advice for the role of the government.

Giving us an opportunity to visit volunteering program really will help Korean government in the future. We need your big help. We thank you for your kind attention and look forward to your positive reply.

DEPARTMENT OF
CHEMISTRY & BIOCHEMISTRY

[redacted]

UNIVERSITY OF NOTRE DAME
[redacted]

May 1, 2013

On March 15th, 2013 I had the privilege to attend the PCAST Meeting at the National Academy of Sciences. The agenda included a presentation of the American Chemical Society report on the future of graduate education in chemical sciences. This topic was of particular interest to me as Professor of Chemistry and Biochemistry and Associate Dean for Research at the University of Notre Dame. The presentation and report itself carefully and accurately presented the current state of graduate education in the chemical sciences and serious issues university programs are currently facing including but not limited to graduate student funding, laboratory safety, and employment prospects for trainees.

Throughout the written document as well as the presentation to PCAST on March 15th, graduate education is most strongly justified by benefits to the individual, to employers, and to society through the student's training and development as an independent scientist. The report includes recommendations that evolve graduate education and training to benefit all three of these constituencies in the education of future scientific investigators in industry and academic research laboratories.

I have no doubt in my mind that the members of the ACS Commission including the March 15th representatives, Professors Barton and Shakhashiri as well as the distinguished members of the President's Council of Advisors on Science and Technology, are well aware of this history of scientific research in our country and its influence on our current university research enterprise. Unfortunately, I believe the ACS commission, as well as the specific individuals who presented to the Council, missed an important opportunity to reaffirm the social contract that generated current model for graduate education. Vannevar Bush, in his report to President Roosevelt, stressed the importance of concurrent training and research studies. As such graduate education is not a system that only provides future benefits to the individual, to potential employers, and, in turn, society. In fact, graduate student's active participation in research is the vehicle that simultaneously generates fundamental discoveries that advance our understanding of specific fields, provides the foundation for future technological advances, solves important translational problems in biomedical research and engineering, and generates the intellectual property that is the basis for economic development.

However, I believe that much of the recent constriction of federal funding for university research is due to the public's and their governmental representative's misunderstanding of the value of graduate education and research and its contribution to society. As we evolve graduate education in the STEM disciplines in support of an atmosphere of continuous improvement, we should highlight aspects of the current system that work well and make it the foundation on which to build.

Sincerely,

Richard Taylor
Professor of Chemistry & Biochemistry
Associate Dean for Research; College of Science
University of Notre Dame
[redacted]