Public Health Laboratories Research: Success and Strategies

From the Perspective of the City of Milwaukee Health Department Laboratory

Sanjib Bhattacharyya, PhD
Deputy Laboratory Director

Adjunct Faculty- UW- Milwaukee
ZSPH & Biomedical Science

APHL Annual Meeting- 2014

Outlines

* MHD Laboratory- *at-a-glance*
* Research Areas and Institutional Positions
* Community Engagements and Systems Partnerships
* Success and Challenges in PHL Research
* Future Directions

City of Milwaukee Health Department

City - 7,200 :: ~$1B

MHD – 275 :: ~$15M (1/2 grant)

MHD Lab :: ~$3M (1/4 grant)

MHD Divisions

- Disease Control & Environmental Health
- Family & Community Health
- **Laboratory**

3-Health Centers- **STD Clinic Laboratory**

Disease Control and Environmental Health

Home Environmental Health	• Lead, Asthma, Injury Prevention
Consumer Environmental Health	• Food Inspections (Food Safety & Security) • Weights and Measures • Tattoo and Body Piercing Inspections
Communicable Disease & Emergency Preparedness	• Communicable Disease Surveillance and Control • Milwaukee County CD Statistics (SurvNet) • Immunization Programs • Emergency Preparedness and Response
Community Environmental Health and Safety	• Air Quality • Animal Health • Hazardous/Toxic Materials (Ozone, pesticides, tobacco, etc.) • Water Quality (Potable/Recreational)
HIV, STD, and Tuberculosis Prevention	• Tuberculosis Surveillance, Clinic and Control • Refugee Health Screening • STD & HIV Clinic Services

MHDL History- Legacy of PH Research

Est. 1872

The Early Years ……

Milk Testing- 1910

1955- Polio Foundation supported research on Polio vaccine

Dr. Henry Wisniewski
Chief Virologist & Lab Director (1952- 1988)

1953- Diagnostic Virus lab

1955- NIH-supported Rickettsial Disease Research

1956- NIH-supported Q-Fever research

1958- Nobel Laureate Dr. Albert Sabin visited MHD- Polio vaccine work

City of Milwaukee Health Department
Today's Public Heath Laboratory

~ 100,000 tests/year

- 13,000 sq. ft.
- Totally rebuilt 1957 → 2000
- Dedicated one pass air: HEPA-in
- Dedicated exhaust: HEPA-out
- TB-designed BSL-3 Lab
  - Renovated 2003: added BSC/room
- STD Clinic Lab offsite (2 MLTs)

City of Milwaukee
Public Health Laboratory Strength

23 Staff

18 Scientists

- **Laboratory Director**
- **Deputy Laboratory Director**
- **Laboratory Operations Manager**

Clinical & Environmental Microbiology (BSL-3 facility)

- **5 Microbiologists**
- **2 MLTs (STD Clinic Lab)**

Virology, Chemistry & Molecular Science

- **5 Chemists**
- **3 Virologists**

1 LIS Coordinator

2 Office Staff

1 Lab Support Staff

1 Custodian

MHD Laboratory Programs

Sexually Transmitted Disease

- Resistance surveillance: NAAT, GS-AST-CDC

Foodborne Disease

Emergency Preparedness

Molecular diagnostics

- Real-time PCR (Bacterial and viral pathogens)
- Luminex (Respiratory virus surveillance; enteric pathogens)
- Molecular sequencing- Sanger & Pyroseq (ref. bacteria and fungus ID, TB, anti-viral resistance)

Communicable Disease

- Microbiology: Ref- Clinical, Env. & TB
- Virology: culture, NAAT & serology
- Surveillance programs: CDC, Wisc., WHO

Waterborne Pathogens

- Cryptosporidium/Giardia/ Culturable Viruses- EPA

Water Quality – Recreational and Potable- Colilert, qPCR

Chemistry- Analytical and Clinical

- Env. & Blood lead, Heavy metals, Asbestos, Household allergens- ELISA, MARIA
- AA's, GC/LC-MS- VOC/SVOC/Env. tox

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PH Research Projects

APHL Priority Areas

* Infectious Disease Detection and Technology Development
* Genetics & Newborn Screening
* Communicable Disease and Epidemiology
* Chronic Disease Detection and Prevention
* Health Research and Policy Development
* Primary care and Outcome-based Research
* Community Health
* Laboratory Information Systems and innovation in IT

MHD Supports to PH Research

MHD perspectives

- PH Surveillance and Preparedness Support
- Diagnostic Development- *Program support & Revenue generation*
- Academic Health Department- *Partnership Grants & Publications*
- Research committee- *IRB (human subject research) & IBC (general bio-safety and recombinant DNA work)*

Resources

- Leadership supports and promotes research- *basic, applied & translational*
- Funding availability- *operations budget, grants & collaborations*
- Qualified staff and state-of-the-art technology platforms

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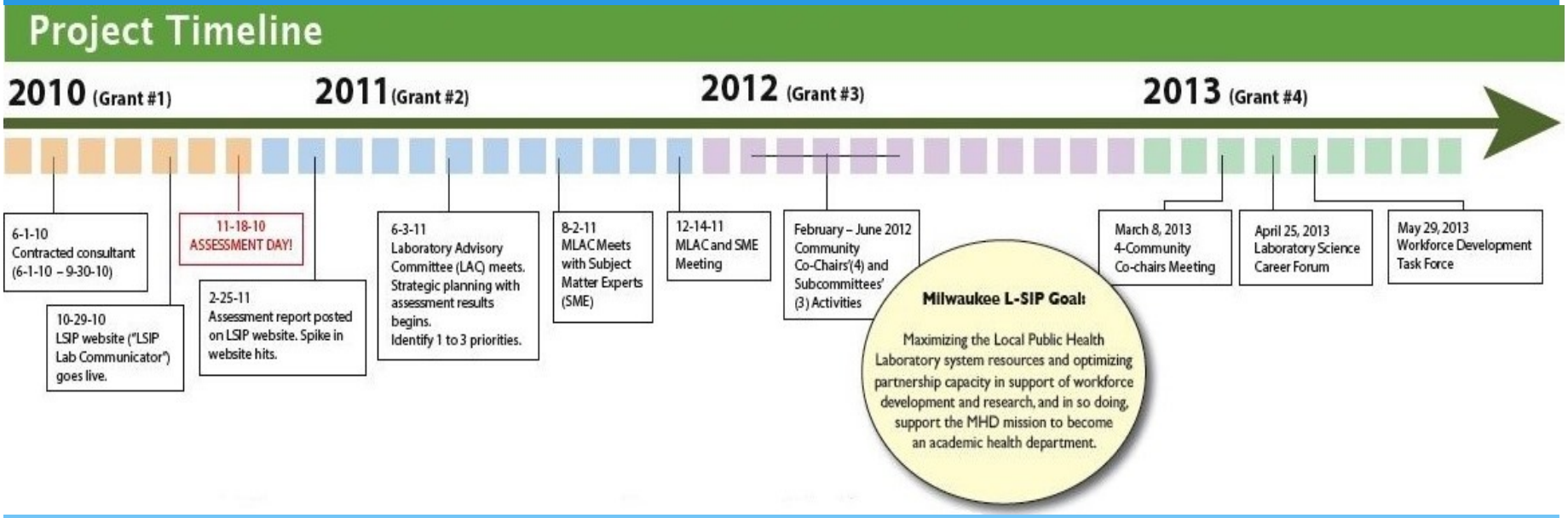
L-SIP- Impact on MHD Research



L-SIP actions have focused on responding to system gaps in **Workforce Development** and **Research**:

- Convened the Milwaukee Laboratory Advisory Committee (MLAC) to guide strategic planning efforts
- Selected Community Co-Chairs and Subject Matter Experts
  - ***Research sub-committee – Clinical & Environmental Health*** *(areas of basic, applied and translational)*

PHL Research Improvement Strategies

- An Innovative System's Approach

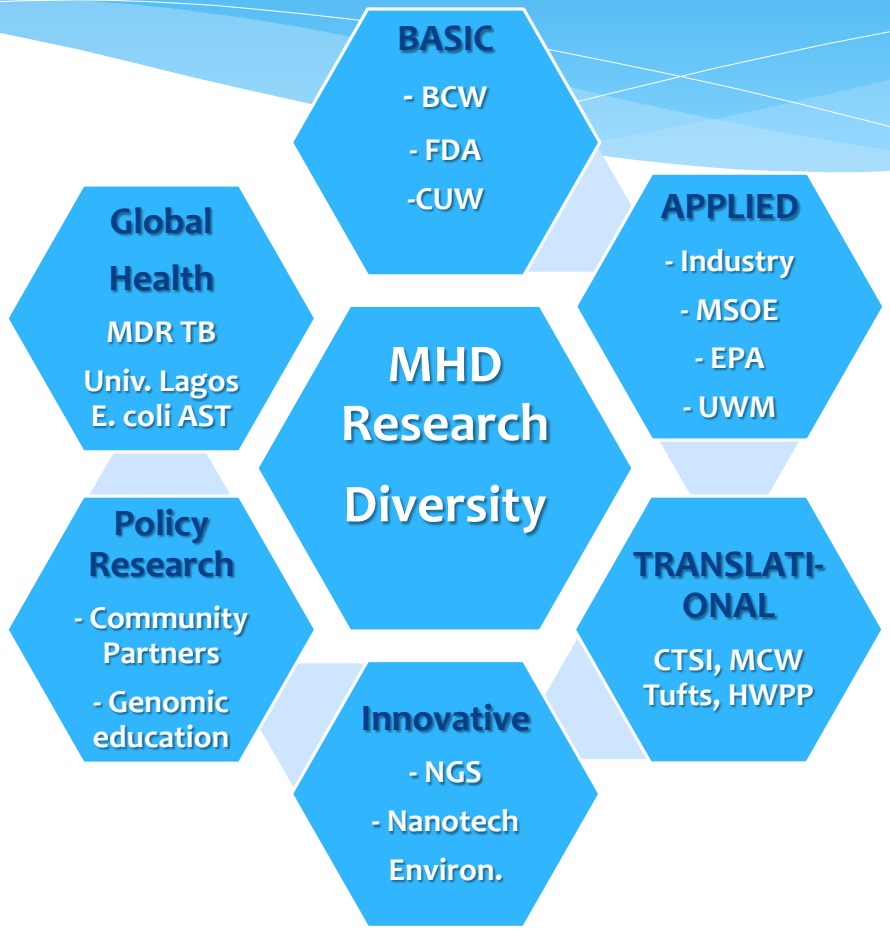
- **Diverse group of partners**
  - Diversity in research areas & innovations
  - Expanded research capabilities, themes, and collaborations
- **Identify laboratory systems research need & priorities**
- **Create an LPHL system research inventory**
  - Current research
    - Research methods (e.g., chemical, biological, microbial, engineering), biological systems, modeling, and surveillance
  - Research interests
    - Linking to other disciplines (outreach & interdisciplinary)- microbiology, genomic, molecular biology, environmental-toxicology and immunology
  - Resources
    - Models/centers of excellence, databases, specimen/sample repositories, instrumentation, students/interns and support staff

Research and Academic Partners

- CDC- Influenza, Picornavirus, STD and DPDx laboratories
- Environment Protection Agency (EPA)
  - Potable, Recreational water (*E. coli, Crypto/Giardia*) & waterborne viruses , env. toxins)
- Department of Natural Resources (DNR)
  - Beach monitoring (in partnership with EPA and UW-Milwaukee School of PH
- Milwaukee Medical Examiners- Infant death related
- Medical College WI (MCW)- CTSI, HWPP; Children's Hospital of WI (CHW)
- Milwaukee Water Works (MWW)
- WI State Lab and Racine Health Department
- Blood Center of WI, FDA and NIH-
  - Influenza Immunology (donor population) and herd immunity
- Tufts University, MA- InForMID- Influenza Seasonality
- UW-Milwaukee: School of PH; Biomedical Science; School of Freshwater Science- Milwaukee Water Council- Env. monitoring
- Biotech industry (Luminex, Cepheid, Life Tech; Hologic- GenProbe; Longhorn vaccine)- NGS
- Milwaukee School of Engineering- BioE, and Bio-Modeling (SMART program)
- Concordia University if WI (CUW)- TB MIC, adverse birth, nanotechnology (with UWM)
- University of Lagos, Nigeria (with UWM- Biomedical Science)- *E. coli* & AST

Diversity in Partnerships and Areas of Multidisciplinary Research

Research sub-committee meeting



Technology Advancement

- Evaluation of new platforms/technology

MHD lab uses and evaluates new instruments and provide input on the next generation of products

- Training on new technology

- Develop joint training courses (bio-safety)
- Provide opportunities for Corporate members to learn about PH preparedness and response capabilities at MHD/L

- Work together with partners for understanding the testing priorities (*e.g.* Clinicians, Epidemiologists, Law Enforcement, FBI-BioWatch, H1N1 and PH emergency)

Community Involvement in PHL Research Practice

1. **Community involvement** in PH research practice
2. **Communities feedback** in practice and priority of research topic
3. **Engage community partners** at different stages of research
4. **Celebrate community-PH research success**- visibility by community members and leaders

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Laboratory Diagnosis of Serious Diseases of Infancy

Impact of Picornaviruses on Infant Health

msn MSNBC News

Print | Email | Alerts

MSNBC

HEALTH

Children's Health

New virus suspected in two SIDS cases

Deaths of Wisconsin babies linked to new germ

The Associated Press
Updated: 5:37 p.m. ET Sept. 1, 2004

MILWAUKEE - A virus recently discovered in Japan is suspected in two "crib deaths" in Wisconsin, raising new questions about how many of these mysterious tragedies might be caused by germs.

The cases mark the first time the virus has been identified in the United States. Whether it killed the babies is not clear, but it was found before they died and had signs of disease in their lungs.

HUMAN PARECHOVIRUS TYPE 3
1st TIME IN USA

Sudden infant death syndrome — also called "crib death" for the devastating way it is usually discovered — is a catch-all term for unexplained deaths in children less than a year old. About 2,200 occur each year in the United States, mostly involving babies between 2 and 4 months old.

Brain or breathing abnormalities, genetic mutations and birth defects are possible causes. The risk rises if babies live with smokers, are put to sleep on their stomachs, or are bundled in too

Health
Health Library
Pets
Breaking Bioethics
Creature Comforts
Growing Up Healthy
Nutrition Notes
Sexploration
Smart Fitness
Special Reports

News
Business
Sports
Entertainment
Tech / Science
Health
Multimedia
Travel
Opinion
Weather
Local News
Newsweek
Today Show
Nightly News
Meet the Press
Dateline NBC
MSNBC TV
News Video
MSNBC Shopping
Classifieds
Newsbot

advertisement

Confused About Statin Medications?

Benjamin Ansell's Discussion

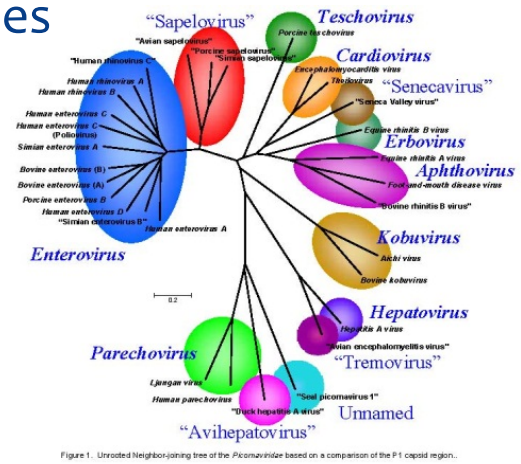
presented by AstraZeneca

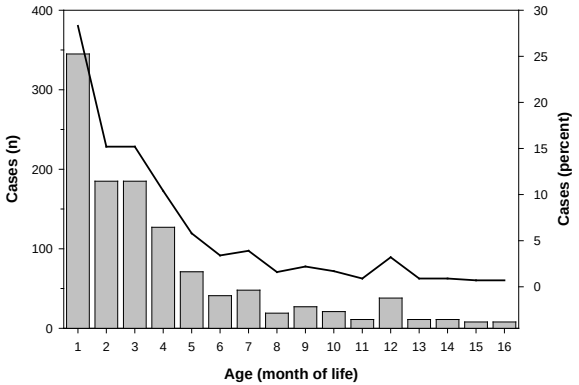
http://www.msnbc.msn.com/id/5888987/ 9/1/2004

Anti-picornaviral drugs not available, but early reporting of confirmed cases using molecular approach can help build the case for need

SIDS in Wisconsin: 1987-2004

- **Autopsies in 1263 unexplained deaths of children ≤ 2 y**
  - Median age, 2-3 mo
  - Virus isolates in 445 cases
    - 40% Enterovirus
    - 22% Adenovirus
    - 20% Rotavirus
    - 5% CMV
    - **5% Parechovirus**
    - 2% Rhinovirus
    - Others: HSV, RSV, flu, HPIV, reovirus
- **Specimens: NP swab, colon swab, lung tissue from all; some others**
  - **47% picornavirus (+)**





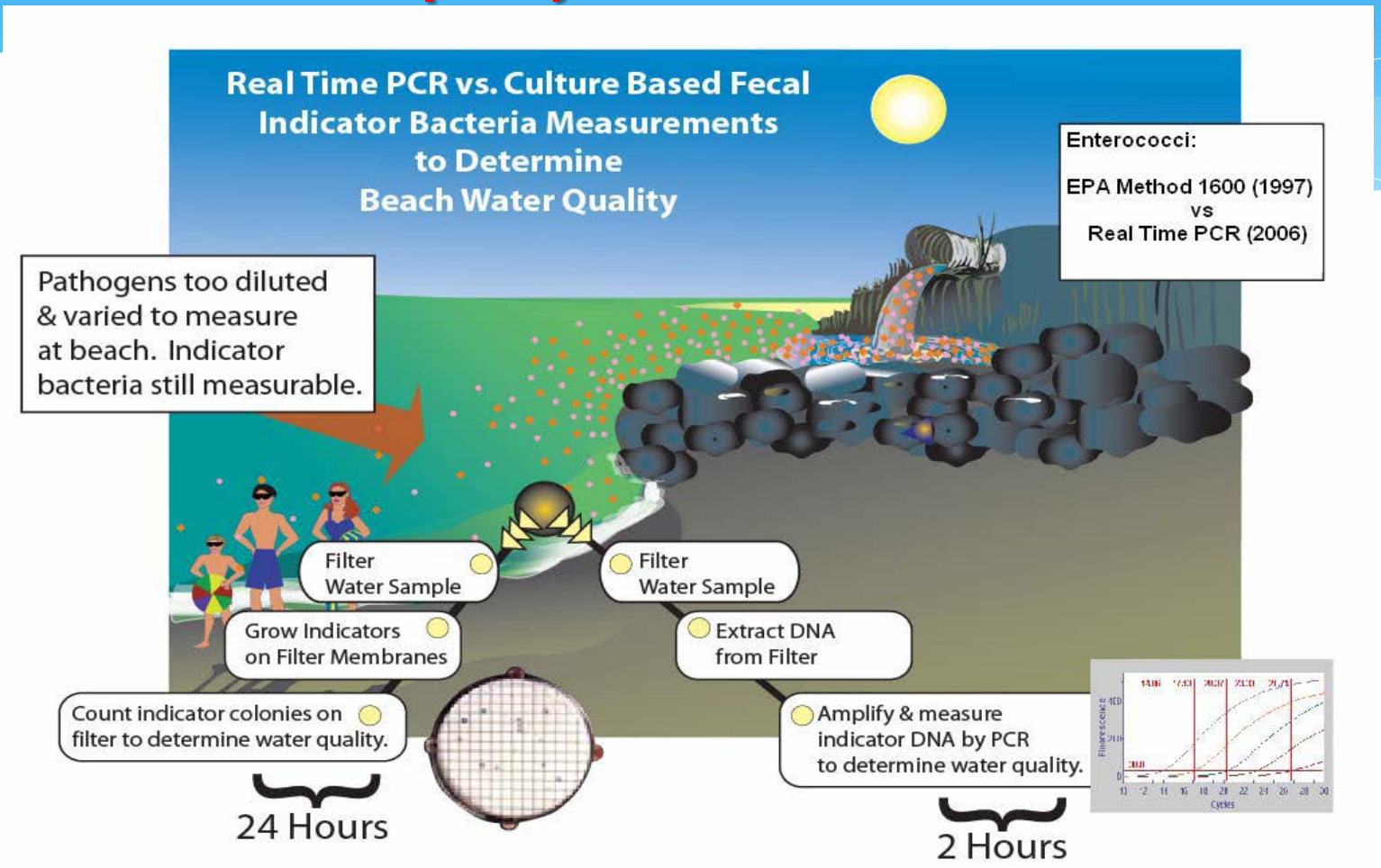
Sedmak *et al.*, 2010 CID

Sedmak G, Nix WA, Jentzen J, Haupt TE, Davis JP, Bhattacharyya S, Pallansch MA, and Oberste MS. 2010. Infant deaths associated with human parechovirus infection in Wisconsin. Clin. Infect. Dis. **50**(3):357-61.

Multi-lab, Multi-jurisdictions

EPA Validation Study of Rapid Method "qPCR"

Water Quality- Milwaukee Beaches



Spotlight on Member Research

Milwaukee Lab Investigates Beach Water:

Same-Day Direct Detection and Quantification of *Escherichia coli* from Recreational Water by Rapid Quantitative Polymerase Chain Reaction Assay at the City of Milwaukee Health Department Laboratory

By Sanjib Bhattacharyya, PhD, Chief Molecular Scientist; Manjeet Khubbar, MS, Microbiologist III; Valdis Kalve, MS, Microbiologist II; Steve Gradus, PhD, D(ABMM), Laboratory Director, City of Milwaukee Health Department Laboratory

MHDL staff Valdis Kalve filtering beach water samples.

polymerase chain reaction (qPCR) assays might allow faster public health actions. This article highlights the results.

As per the Beaches Environmental Assessment and Coastal Health Act of 2000 and Section 303(a) of the Clean Water Act, MHD adopted EPA water quality criteria and standards to issue public notifications on recreational water quality within 24 hours of water sampling using Colilert.[3] The Beach Protection Act of 2008 now allows EPA-approved labs to use a rapid-testing method.

Bridges
Issue 8: Summer 2011
Connecting the Nation's Environmental Laboratories

Research Partnership with EPA (since 2006)

Same-day Beach Closure Decisions Using Real-time Quantitative PCR Assay: Detection of *E. coli* in Milwaukee Area Beaches

Sanjib Bhattacharyya, Manjeet Khubbar, Valdis Kalve, Terri Linder, Anupa Gandhi, Steve Gradus
City of Milwaukee Health Department Laboratory, Disease Control and Environmental Health, Milwaukee, Wisconsin

Abstract

Objective: To compare the real-time quantitative PCR (qPCR) assay with conventional methods for same-day detection of microbial pollution indicators during beach monitoring in Milwaukee.

Study Design: Beach water samples are routinely examined at the City of Milwaukee Health Department Laboratory (MHDL) for the presence and quantitation of *E. coli* using the EPA-approved IDEXX method. We have validated and utilized the qPCR assay for detection of *E. coli* in beach water within 4-6 hours of sampling. During the summer of 2010-2011, the performance of E. coli qPCR assay was compared to culture (membrane filtration- EPA 1603) and defined-substrate (Colilert 18) methods by analyzing water samples collected from three frequently used Milwaukee area beaches.

Results: A significant correlation was found between the qPCR (cell equivalents) and conventional methods (CFU/MPN) during this study. Out of 88 samples tested in 2010 and 111 in 2011 (total 199), 75% were in agreement in 2010 and 87% in 2011. Only one warning was missed in 2011 (per EPA threshold standard, >1,000 MPN/100 mL results in beach closure decision) compared to three in 2010. (Figure 5, Table 3)

Conclusions: *E. coli* qPCR assay allows same-day beach closure decisions with follow-up confirmation in 24 hours by Colilert. Further optimization and evaluation of qPCR assay performance is needed to minimize the extent of disagreements between molecular and conventional techniques and to develop more defined water quality standards based on the qPCR assay performance and epidemiological data.

Introduction

Swimming associated illnesses mainly occur as a result of exposure to enteric bacteria, viruses, and protozoa. Fecal indicator bacteria such as *Enterococcus spp.* or *Escherichia coli* are ordinarily harmless microbes that are commonly found in sewage and other sources of fecal contamination. Multi-site epidemiological studies conducted by USEPA and other researchers have established a direct relationship between the density of these indicator bacteria in fresh water beaches and the occurrence of swimming-associated gastroenteritis[1,2].

Monitoring of recreational beaches for these fecal indicator bacteria is currently performed using culture-based methods Colilert and Enterolert and utilize defined-substrate technology. EPA's approved method 1600 for Enterococci and 1603 for *E. coli* utilize the membrane filtration technique and involve quantification by Most Probable Number (MPN) using serial dilution. These traditional culture-based methods are easier, inexpensive and allow detection in 18-24 hours. Because microbial water quality can change rapidly, guidelines based on indicator organisms that require 18 to 24 hours to develop are likely to result in both unnecessary beach closings and the exposure of swimmers to poor quality water. A recent study estimated that up to 40% of beach closures are in error[3,4].

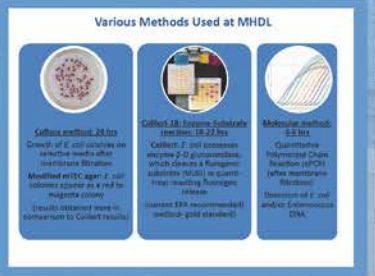
Here we present the data from the summer of 2010 and 2011 studies monitoring of area beach water quality using Colilert 18 and molecular methods (Table 1 and 2). The studies involved assessing equivalency with traditional water quality monitoring methods through simultaneous processing of water samples using both conventional and molecular methods (Figure 3).

Materials and Methods

Water Sampling
- Approximately 300 mL collected in two sterile containers are transported to the lab and refrigerated within 1 hour

Sample Processing
- Water collected from same site the same day was pooled and mixed before analysis

Calibration Standards and Quality Control
- *Escherichia coli* cell suspension: Prepared for solking calibrator samples and preparing DNA standard curve
- Sample processing controls (SPC) consisting of *Lactococcus lactis* cells: Prepared for solsing calibrator and test samples prior to extracting DNA
- *E. coli* and *L. lactis* stock cell suspensions containing approximately 10^7 cells per mL were made by diluting fresh cultures in PBS and storing them at -80°C

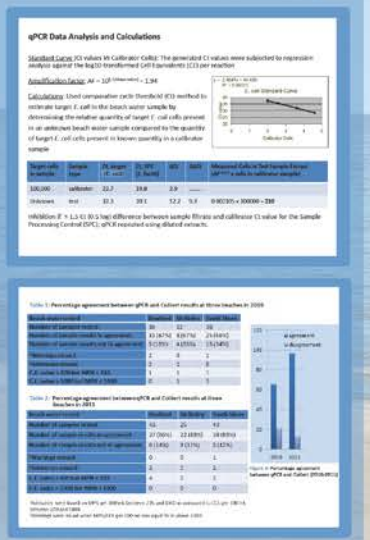


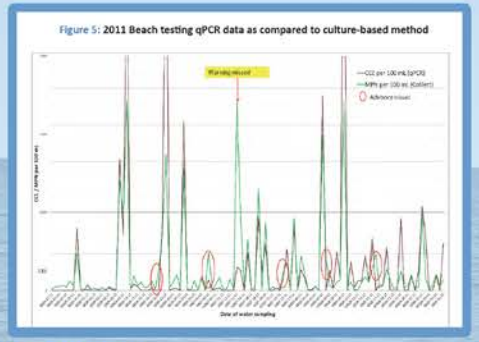
qPCR Assay Set up

DNA Extraction
- For *standard curve*: Crude DNA extracted by bead-beating was purified using commercially available Zymo DNA Clean and Concentrator kit (Irvine, CA).
- For *blank, calibrator and test samples*: DNA extractions were performed using commercially available Zymo Research Fecal DNA kit (Irvine, CA).

qPCR Setup
- Master mix: *E. coli* and *Lactococcus* SmartBeads (BioEx, Inc. Birmingham, AL)
- Templates: No template control (NTC) included all qPCR reagents plus nuclease-free water. Two calibrators consisted of DNA extracted from filters spotted with 10 µl of a suspension of 1 x 10^7 cells/ml.
- Real-time PCR Instrument: Cepheid Smart Cycler II (Sunnyvale, CA)
- Thermal cycling conditions: Stage 1: 95°C for 2 min; Stage 2: 45 two-temp cycles at 95°C for 15 s and 62°C for 43 s (optics ON). Cycle threshold (Ct) fluorescence value was manually adjusted to 8 units.

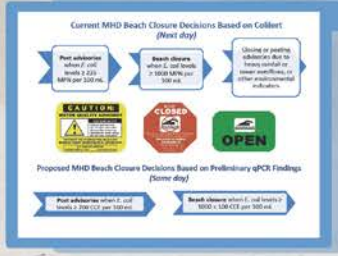
Results





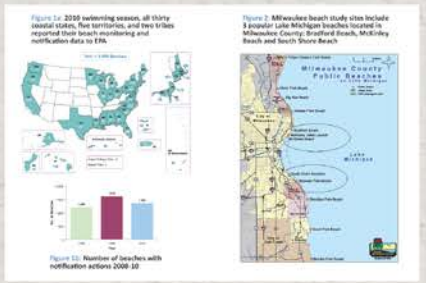
Limitations

- Ratio of viable and non-viable bacteria varies in different environments
- Environmental factors (e.g. wind direction, rainfall, and sewage overflow)
- Proper collection of water sample (representative sample preferred, by pooling water samples from different sites on same beach)
- Beach to beach variation (e.g. more inhibition seen in samples collected from South Shore Beach)
- Inhibition in PCR reaction (e.g. chemicals, excess nucleic acid from other species)
- Cost of reagents and labor for multiple methods
- No standardized cut-off values for cell equivalents (per 100 mL)



Conclusions

- Same-day beach closure decision
- qPCR results can be interpreted as prelim results (presumptive positives > 200 CE/100 mL; 1000 ± 100 CE)
- Follow up confirmation in 24 hours by Colilert for enumeration of *E. coli* in beach water
- Further optimization and evaluation of qPCR assay performance is needed to minimize the extent of disagreements between molecular and conventional techniques
- Need to develop more defined water quality standards based on the qPCR assay performance and epidemiological data



Manuscript under preparation-
Appl. Env. Micrbiol (ASM)

Best Poster Award (Local category- 2012 APHL AM)

Control of Influenza Virus by Immunity

NIH-funded study with BCW, Tufts Univ. & FDA

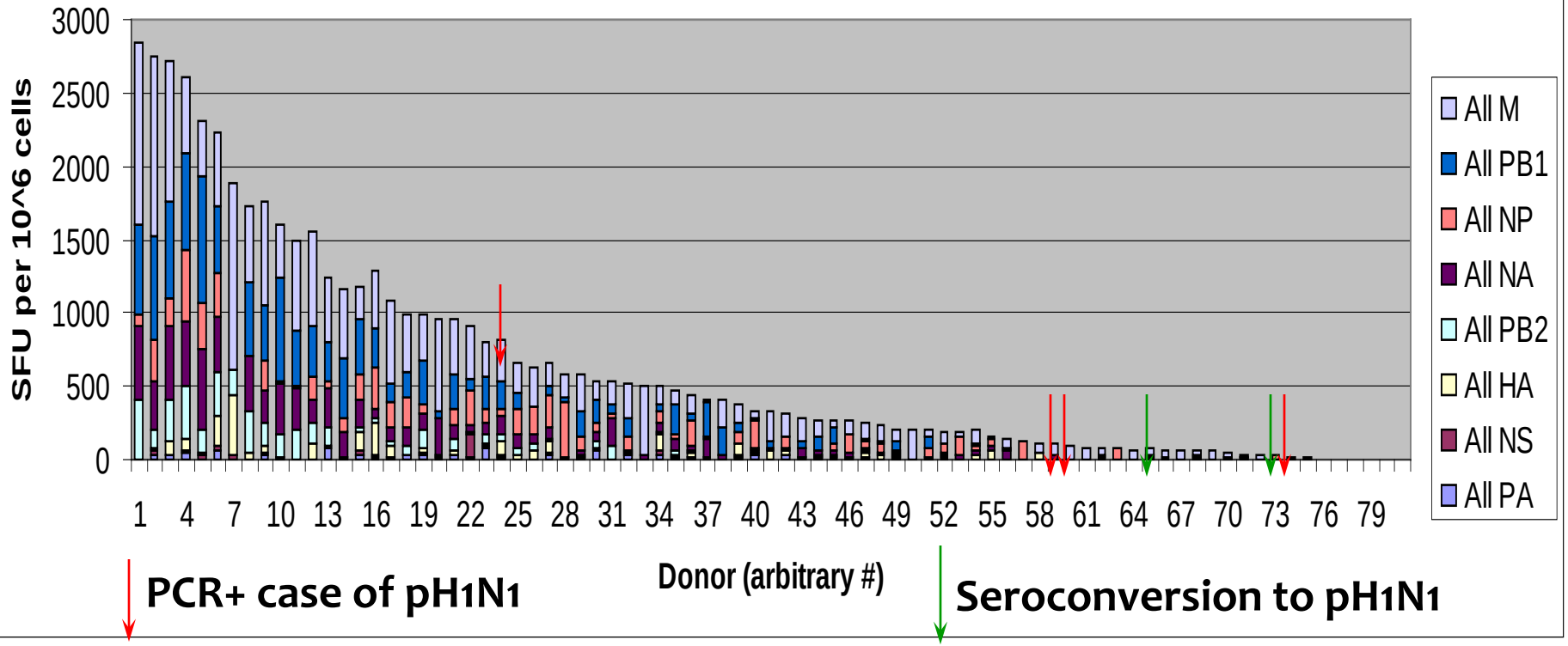
- **High rate of natural variation due to mutations, reassortment**
- **Current vaccine system: world-wide surveillance, strain predictions**
  - **Strain-matched vaccine lacking when predictions are wrong, or a pandemic emerges- Takes about 6 months to make available**

- **Can that public health gap be filled?**
- **Association with seasonality**
- **Universal influenza vaccines: based on cross-protection**
  - **For cross-protective vaccines, immune response (antibody, T cell) to conserved antigens, not HAI, might provide correlates**

M. Moorthy, D. Castronovo, A. Abraham, S. Bhattacharyya, S. Gradus, J. Gorski, Y. Naumov, N. Fefferman, E. Naumova. 2012. Deviation in Influenza Seasonality: Odd Coincidence or Obscure Consequence? Clin. Microbiol. Infect. (published online- DOI: 10.1111/j.1469-0691.2012.03959.x)

Individual Blood Donor Responses to Antigens

Reflections to the Herd Immunity?



Kumar P, Bartoszek AE, Moran TM, Gorski J, Bhattacharyya S, Navidad JF, Thakar MS, Malarkannan S. 2012. High-throughput Detection Method for Influenza Virus. J Vis Exp. 4;(60). pii: 3623. doi: 10.3791/3623.

E. T. Lofgren, J. B. Wenger, N. H. Fefferman, D. Bina, S. Gradus, S. Bhattacharyya, Y. N. Naumov, J. Gorski, and E. N. Naumova. 2010. Disproportional effects in populations of concern for pandemic influenza: insights from seasonal pandemics in Wisconsin, 1967–2004. Influenza Other Respi Viruses. 4(4):205-12.

Milwaukee Healthy Homes Program

Developed Indoor Allergen Testing Capability
- Partner with Indoor Biotech- ELISA, MARIA

Started: Oct 2003:

Funded by HUD

April 2009 – 2012

PUPROSE:

reduce indoor asthma triggers

improve asthma control

PARTNERS:
Dominican Center For Women, Children's Hospital of Wisconsin, Fight Asthma Milwaukee

Outcomes:

Routine monitoring of allergen concentration along with nurse case management and home environmental intervention

- decreases the dust load,
- reduces children's exposure to allergens

Manuscript under prep. J. Asthma

Technology Development:
Multiplexing Approaches for Microbial Identification & Real-time Disease Surveillance

1. **Respiratory Virus Surveillance**
2. **Gastric pathogens (bacteria, virus and parasites)**
3. **Salmonella serotyping**
4. **Fungal identification (*in progress*)**

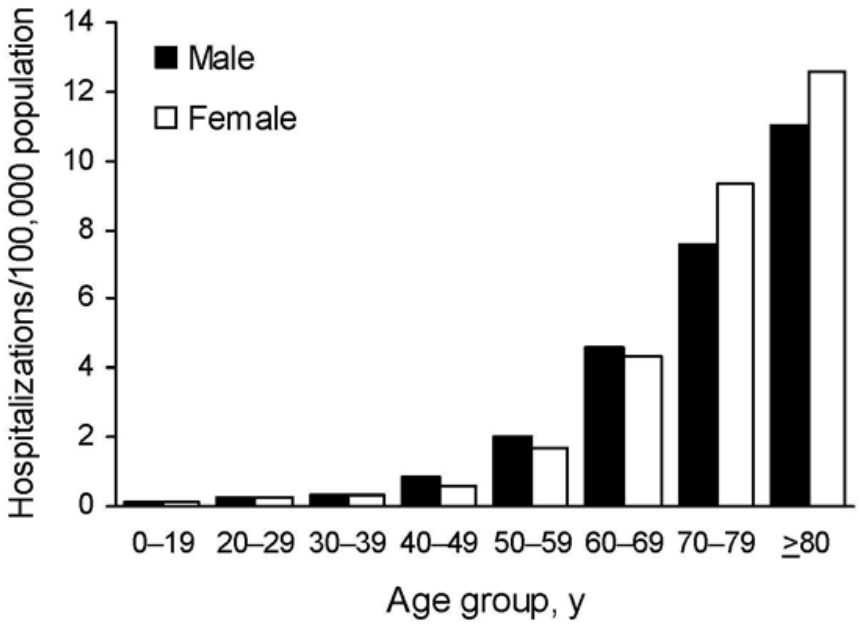
Partners:

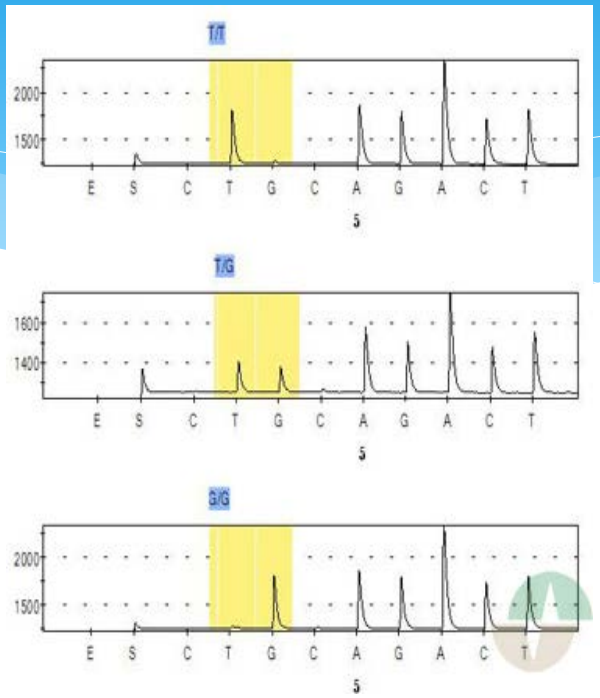
- Luminex Corporation, Canada; and Austin, TX
- Eragen Bioscience, Madison, WI
- Le Boehner Hospital, TN

J. Navidad, D. Griswold, S. Gradus, S. Bhattacharyya. 2013. Evaluation of Luminex xTAG Gastrointestinal Pathogen Panel Analyte Specific Reagents for high-throughput, simultaneous detection of multiple bacteria, viruses, and parasites of clinical and public health importance- J. Clin. Microbiol. 51 (9): 3018-3024

A. Patel, J. Navidad, S. Bhattacharyya. 2014. Site-specific Clinical Evaluation of the Luminex® xTAG® Gastrointestinal Pathogen Panel for the Detection of Infectious Gastroenteritis in Fecal Specimens. JCM Accepts (ePrint before publication)

Clinical Relevance of MOTT and MDR TB





Average annual prevalence of non-AIDS pulmonary non-tuberculous mycobacteria–associated hospitalizations by age group, sex and corresponding MDR TB ID

L. Daum, G. Fischer, J. Sromek, M. Khubbar, P. Hunter, M. S. Gradus, S. Bhattacharyya. 2013. Ion Torrent full-gene sequencing and phenotypic drug susceptibility testing (DST) confirm multi-drug resistance (MDR) *Mycobacterium tuberculosis* in the United States- Epidemiol. Infect.:1-6

Meeting Community Needs

Project Title: **Growing Healthy Soil for Healthy Communities**
HWPP Project #: **2013I-06**
Award Amount: **$749,999**
Start Date: **1/1/2014**
End Date**: 12/31/2018**

Project title: **Science Awareness Genomics & Ethics- Building SAGE Communities**
NSF Program- **Science, Technology & Society-** Div. of Social & Economic Science (*submitted 2014*)
Partners: **MCW-HWPP, MSOE, Community Partners**

Addressing Global Health

Expanding the PHL Research

* **Characterization, Antimicrobial Resistance and Molecular Profiling of Clinical and Environmental *E. coli* Isolated from Lagos, Nigeria and Milwaukee, WI** (PhD student from Univ. of Lagos- with UW-Milwaukee)

Igbokwe H, Bhattacharyya S, Gradus S, Khubbar M, Griswold D, Navidad J, Igwilo C, Masson-Meyers D, Azenabor AA. 2014. Preponderance of toxigenic Escherichia coli in stool pathogens correlates with toxin detection in accessible drinking-water sources. Epidemiol Infect.:1-11.

* **Responses to Global Multi-drug Resistance TB- Use of Next-Generation Sequencing for Identifying Pyrazinamide Resistance in *M. tuberculosis*** (with Longhorn Vaccines, TX; Univ. of Pretoria, & South African Medical Research Council, South Africa)

L. Daum, P. B. Fourie, S. Bhattacharyya, N. A. Ismail, S. Gradus, N. E. Maningi, S. V. Omar, and G. W. Fischer. 2014. Next-Generation Sequencing for Identifying Pyrazinamide Resistance in *Mycobacterium tuberculosis.* Clin. Infect Dis. 58 (6): 903-904

Challenges in PHL Research

Potential Road blocks

1. Leadership buying
   - Perception
   - Legal issues- sharing clinical materials, safety and patient confidentiality
2. Admin support
   - Justifying the need
   - Research areas
3. Sustained funding
   - Limited operations cost
   - Challenges in obtaining grant
4. Personnel
   - Staff Vs. Researcher
   - Motivation & expertise
5. PH routine response & emergencies
   - How do you manage and sustain the demands of day-to-day service and surges while also continuing research projects?

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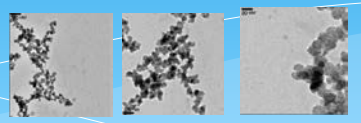
* Community Engagements and Systems Partnerships
* Success and Challenges in PHL Research
* **Future Directions**

Potential Research Areas

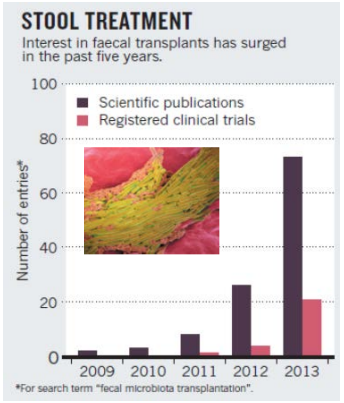
……but not limited too…

* **Nanotechnology- Impact of Human and Environmental Health-**
  * TEM study to analyze the nanoparticle in water (with UW-Milwaukee)
  * Public health impact and understanding



TEM- 5-50 nm

* **Microbiome approach- Complex matrix analysis for potential microbial impact on chronic diseases- Use of NGS for WGS**
  * Fecal transplant and biome analysis- pathogen ID & interactions
  * Impact of pathogen load in gut microbiota & obesity
  * WGS for MDR- TB, MRSA (mechanism of resistance during photo-therpy)
* **Environmental Health Genomics-** New Paradigm to address children's environmental health- NIEHS priority areas
* **Immune Protection in HIV Disease-** Partner with UW-Milwaukee and University of South Africa
* **Genomic and Society-** Community understanding of genomic application- Scientific citizen and Citizen Scientists- Partner with MCW, CTSI and HWPP



Nature, 2014

Quality Control and Critical Workforce for PHL Research

1. **Adhere to the LEAN and Quality Control Practices**
2. **Workforce development- students, interns and faculty development**
3. **Partnership with industry, academic- explore non-traditional partners**
4. **Sustained funding**
5. **Publication, seminars**

ASQ – LEAN Tools

Students & Workforce Development

Collaborations for Applied Research: Developing Public Health Tools

Characterization of Multi-drug Resistant *Mycobacterium tuberculosis* from Immigrants Residing in the United States using Next Generation Sequencing

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S. Bhattacharyya, M. Khubbar, J. Sromek, P. Hunter, S. Gradus: City of Milwaukee Health Department Laboratory, Milwaukee, WI

Abstract

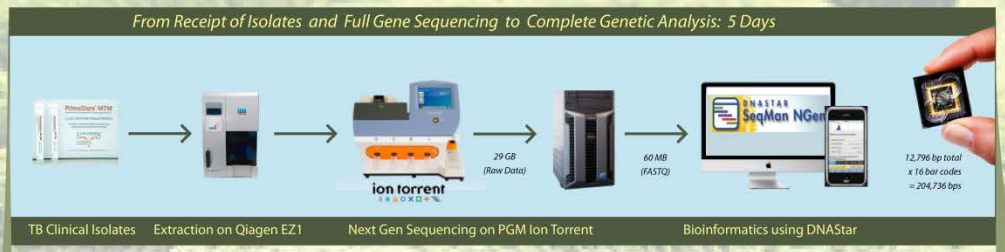
Objective: Drug-resistant *Mycobacterium tuberculosis* (MTB) is spreading worldwide. While this is a global threat, there is a growing concern in this country as the majority of MTB cases are from foreign-born persons currently residing in the United States. The use of Next Generation Sequencing (NGS) can be a practical tool to enhance early intervention and potentially provide a more meaningful characterization of multi-drug resistant (MDR) TB in the local community.

Study Design: The City of Milwaukee Health Department Laboratory isolated three drug-resistant strains from Burmese, Hmong, and Indian immigrants residing in Milwaukee, WI. Ion Torrent full-gene sequencing and complete genetic analysis was performed within five days of the receipt of isolates. Genotypic results obtained by NGS were compared to phenotypic results obtained by classical drug sensitivity testing (DST).

Results: Genetic characterization of seven, full-length resistance-associated genes revealed two MDRs and one highly resistant strain with important drug-resistant mutations that were subsequently confirmed by traditional DST. NGS revealed a novel Lys96Arg (K96R) *pncA* gene substitution mutation in one isolate that had not been formerly reported.

Conclusions: The rapid turnaround time from sample-to-sequence underscores the public health value of using an NGS approach for full-gene analysis of MDR cases from epidemiologically significant clinical isolates. This novel, non-culture based approach could be used for routine MTB drug resistance characterization as well as for potential discovery of new resistance-associated mutations, and would allow for improved understanding and characterization of drug-resistant mycobacteria.

Nature, Jan. 2013 Vol. 493, 7430 Source: WHO



Introduction

Increasing cases of multidrug-resistant (MDR) and extensively drug-resistant (XDR) strains of *Mycobacterium tuberculosis* (MTB) continue to emerge, particularly from Asia and Africa[1]. In the United States, the majority of reported MTB cases have occurred in foreign-born persons[2]. MDR strains are resistant to rifampin and isoniazid, while XDR strains are resistant to rifampin and isoniazid plus one fluoroquinolone and one of the injectable drugs – amikacin, kanamycin, or capreomycin. However, some MDR strains have additional drug resistance, *i.e.* pyrazinamide and/or streptomycin that might decrease treatment efficiency. Recently, an Ion Torrent (Life Technologies, Carlsbad, CA) full-gene sequencing approach was developed for rapid characterization of MTB resistance mutations[3] that can be performed for clinical isolates within days.

Specific Aim

Between April 2009 and November 2011, sputum specimens from three patients (Burmese, Hmong, and Indian immigrants residing in Milwaukee, WI) yielded antibiotic resistant strains of MTB. Antibiotic resistance profiles were deduced by full-length gene sequencing of three clinical isolates (two MDRs and one unique drug-resistant strain) on the Ion Torrent Next Generation Sequencing (NGS) platform. Sequencing data was compared to phenotypic data obtained by conventional drug sensitivity testing (DST).

Methods

1. Sputum samples were confirmed for *M. tuberculosis* at the MHD laboratory using smear microscopy, MTB specific real-time PCR[4], culture and DST for phenotypic resistance using the BACTEC™ MGIT™ system (BD Diagnostic, USA)[5] with isoniazid (0-2 and 1-0 µg/ml), rifampin (1-0 µg/ml), and pyrazinamide (100 µg/ml).
2. H37Rv strain was used as an internal sequencing control. The MTB colonies grown on solid Lowenstein–Jensen medium was inactivated and transported in PrimeStore Molecular Transport Medium (MTM; Longhorn Vaccines & Diagnostics, USA) at ambient temperature for sequencing.
3. Total DNA was purified from 200 µl aliquots of PrimeStore MTM. PCR amplification using full-gene primers for *rpoB, katG, pncA, gyrA,* and *rrs* was performed[3]. Additional primers for full-gene amplification of *rpsL* and *inhA* were used for streptomycin and isoniazid resistance. PCR amplicons from clinical isolates were pooled, sheared, size-selected, and subjected to adaptor/barcoding ligation[3].
4. Ion Torrent sequencing was performed using 314 V2 chips with 200-bp sequencing chemistry (Life Technologies, Carlsbad, CA). Sequence assembly, alignments, and protein translations were performed using SeqMan NGen (V4) and LaserGene (V10) Core Suite (DNAStar, USA).

Results

PCR was performed using seven novel primer pairs for full-length amplification of *rpoB, katG, inhA, gyrA, pncA, rpsL,* and *rrs* genes associated with MDR and XDR resistance in MTB *(Figure 2)*.

Full-length *rpoB, katG, inhA, pncA, rpsL, gyrA,* and *rrs* genes sequences showed 100% concordance with phenotypic DST through direct identification of amino-acid substitutions known to confer antibiotic resistance *(Table 1)*. All three cases were resistant to isoniazid at both low and high levels (0.1 µg/ml and 0.4 µg/ml) by phenotypic testing *(Table 1)*.

Cases 1 and 3 contained a characteristic Ser531Leu mutation associated with rifampin resistance[6], while case 2 contained the wild type residue. NGS revealed that cases 2 and 3 exhibited the common Ser315Thr katG mutation known to confer between 75% and 94% of isoniazid resistance worldwide[7]. Case 1 did not contain any *katG* gene mutations, but exhibited a much rarer Iso21Thr (I-21-T) substitution in the *inhA* gene that was previously correlated with isoniazid resistance, and a K96R pyrazinamide gene mutation *(Table 2)*.

Table 1. *Comparison of genotypic and phenotypic drug resistance[a] Ion Torrent sequencing[c] and drug sensitivity testing*

	Case 1		Case 2		Case 3	
	Ion Torrent	Bactec MGIT 960	Ion Torrent	Bactec MGIT 960	Ion Torrent	Bactec MGIT 960
Rifampin	Resistant	Resistant	Sensitive	Sensitive	Resistant	Resistant
Isoniazid	Resistant	Resistant	Resistant	Resistant	Resistant	Resistant
Pyrazinamide	Resistant	Resistant	Sensitive	Sensitive	Sensitive	Sensitive
Fluoroquinolone	Sensitive	Sensitive	Sensitive	Sensitive	Sensitive	Sensitive
Streptomycin	Resistant	Resistant	Resistant	Partial resistance[b]	Sensitive	Sensitive
Resistance type	*Multidrug resistant*		*Drug resistant*		*Multidrug resistant*	

[a] Critical concentrations for indicated antibiotics are: rifampin (2-0 µg/ml); isoniazid (0-1 µg/ml); pyrazinamide (100 µg/ml); fluoroquinolone (2 µg/ml); streptomycin (2-0 µg/ml and 6-0 µg/ml).
[b] Previously confirmed amino-acid substitutions giving rise to antibiotic resistance.
[c] Resistant at 2-6 µg/ml but sensitive at 6-0 µg/ml.

Table 2. *Amino-acid substitutions in drug resistance genes from* M. tuberculosis *samples isolated from Burmese, Hmong, and Indian immigrants residing in the USA using Ion Torrent sequencing[a]*

	Amino-acid substitution(s)[c] in full-length genes (antibiotic)[d]							
Isolate no.	*rpoB* (rifampin)	*katG* (isoniazid)	*inhA* (isoniazid)	*pncA* (pyrazinamide)	*gyrA* (fluoroquinolones)	*rpsL* (streptomycin)	*rrs* (16S) (aminoglycosides/ streptomycin)
Case 1	**S531L**	R463L	**I21 T**	**K96R**	S95 T, E21Q, G688D	**K43R**	WT
Case 2	WT	**S315T,** R463L	WT	WT	WT	**K43R**	WT
Case 3	**S531L**	**S315T,** R463L	WT	WT	WT	WT	WT

[a] WT refers to the "wild type/ H37Rv reference strain.
Amino-acid changes in bold indicate a known resistance mutation.
[c] Compared to previously confirmed amino-acid substitutions known to confer antibiotic resistance.
[d] Compared to H37Rv wild-type reference strain.
[e] Gene sequences: Case 1 (TB11-91), Case 2 (TB11-111), and Case 3 (TB11-153) were deposited in GenBank (accession numbers KC692347- KC692364).

Figure 2. Representative amplicons for 4 MTB genes (*rpoB, katG, gyrA,* and *rrs*). Seven genes were sequenced for each clinical isolate.

Current Practices

Phenotypic Drug Resistance Testing (DST): Bactec MGIT (320/960) (BD Diagnostics, MD)- BACTEC PANTA (Polymyxin B, Amphotericin B, Nalidixic acid, Trimethoprim and Azlocillin) for growth-Streptomycin (STR), Isoniazid (INH), Rifampin (RIF), Ethambutol (EMB) (collectively known as SIRE), and Pyrazinamide (PZA)

Molecular Detection of Drug Resistance:

1. GeneXpert TB/RIF assay for MTB and Rifampin (rpoB mutations)
2. Molecular Detection of Drug Resistance (MDDR) for rapid identification of MDR TB-DNA sequencing (PSQ) for detection of mutations (rifampin and isoniazid), followed by Sanger sequencing of comprehensive panel (resistance to INH, RMP, EMB, PZA, FQ, and second-line injectable drugs) at CDC

Challenges

1. Phenotypic drug susceptibility testing (DST)-growth characteristics of MTB make it difficult to establish resistance (e.g. PZA)
2. Limited availability of commercial NAAT assays for MTB drug resistance
3. Inability to detect novel or unusual mutations

Advantage of NGS

1. Improved TAT, workflow, consistency, and potential for library creation
2. Depth of coverage and characterization of mixed strain populations allows detection of hetero-resistance in populations (both resistant and susceptible subpopulations)
3. Potential for discovery of new mutations that may be associated with resistance
4. No laser detection or light-sensitive chemistries require fluorescent-labelled moieties
5. A small working footprint

Limitations

1. Initial DNA concentration AND no direct amplification/library prep from primary sputum
2. Read-length varies for platforms
3. Lack of phenotypic Minimal Inhibitory Concentration (MIC) study for new resistance
4. Understanding type of amino-acid substitution-important for phenotypic DST Vs. NGS
5. Bioinfomatic capability limited

Conclusions

- Global travel and mobility of pathogens will continue to increase the spread of TB and other communicable diseases.
- Drug-resistant TB continues to emerge globally; however, characterization of these strains remains incomplete.
- Improved and more rapid understanding of TB resistance-associated genes should decrease inappropriate treatment and transmission of MDR-TB globally.

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Abstract # 72

Thank You

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