Emerging Technology in Clinical Microbiology

Vera Winn, MLS(ASCP)^{CM}
Technical Coordinator
IU Health Pathology Laboratory Microbiology
October 9, 2015

Disclosures

- No financial or research relationships to disclose

Backgrounds

- "Imported" from Taiwan in 1998
- An island in eastern Asia (Mandarin Chinese)
- Cases of Dengue Fever multiply in S. Taiwan







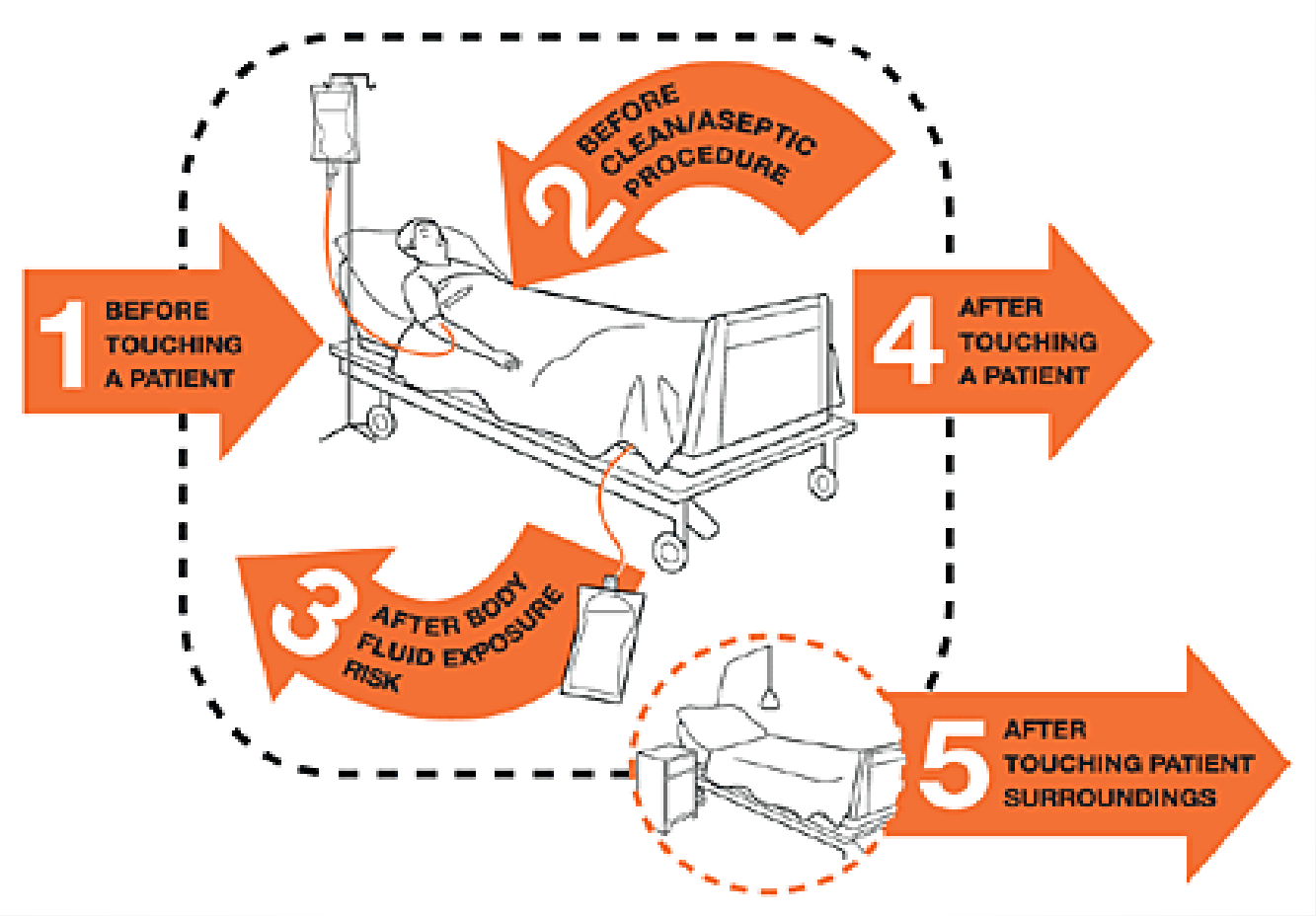
Backgrounds

- Indiana University Health Pathology Laboratory
  - Centralized testing facility
    - IU Health University Hospital
    - IU Health Methodist Hospital
    - Riley Hospital for Children at IU Health
    - Numerous other IU Health and non-IU Health hospitals in Indiana
    - Numerous IU Health and non-IU Health clinics and physician's offices
- 12 - 14 million laboratory tests per year

Objectives

- Identify and describe commonly concerned organisms that are related to healthcare-associated infections
- Describe the differences between traditional methods and molecular methods for clinical microbiology testing
- Describe the latest molecular technology in clinical microbiology

5 Moments of Hand Hygiene



World Health Organization

The "Big Players"

Abbrev.	Full Name
VRE	Vancomycin-resistant Enterococcus
MRSA	Methicillin-resistant Staph. Aureus
ESBL	Extended-spectrum β-Lactamases
CRE or CP-CRE	Carbapenem-resistant Enterobacteriacea or Carbapenemase-producing CRE
C diff	Clostridium difficile
TB	Mycobacterium tuberculosis
Influenza	Human influenza viruses A, B, and C

ESBL

- First discovered in Europe in the mid 80s
- GN bacteria, mostly Enterics such as *E. coli*, *K. pneumoniae,* & *K. oxytoca*
- Produce enzymes, **E**xtended **S**pectrum **B**eta-**L**actamases, breaks down and destroys:
  - Penicillins
  - Expanded-spectrum cephalosporins (Cefotaxime, Ceftriaxone, and Ceftazidime)
  - Oxyimino-Monobactam Aztreonam

→ Treatment failure

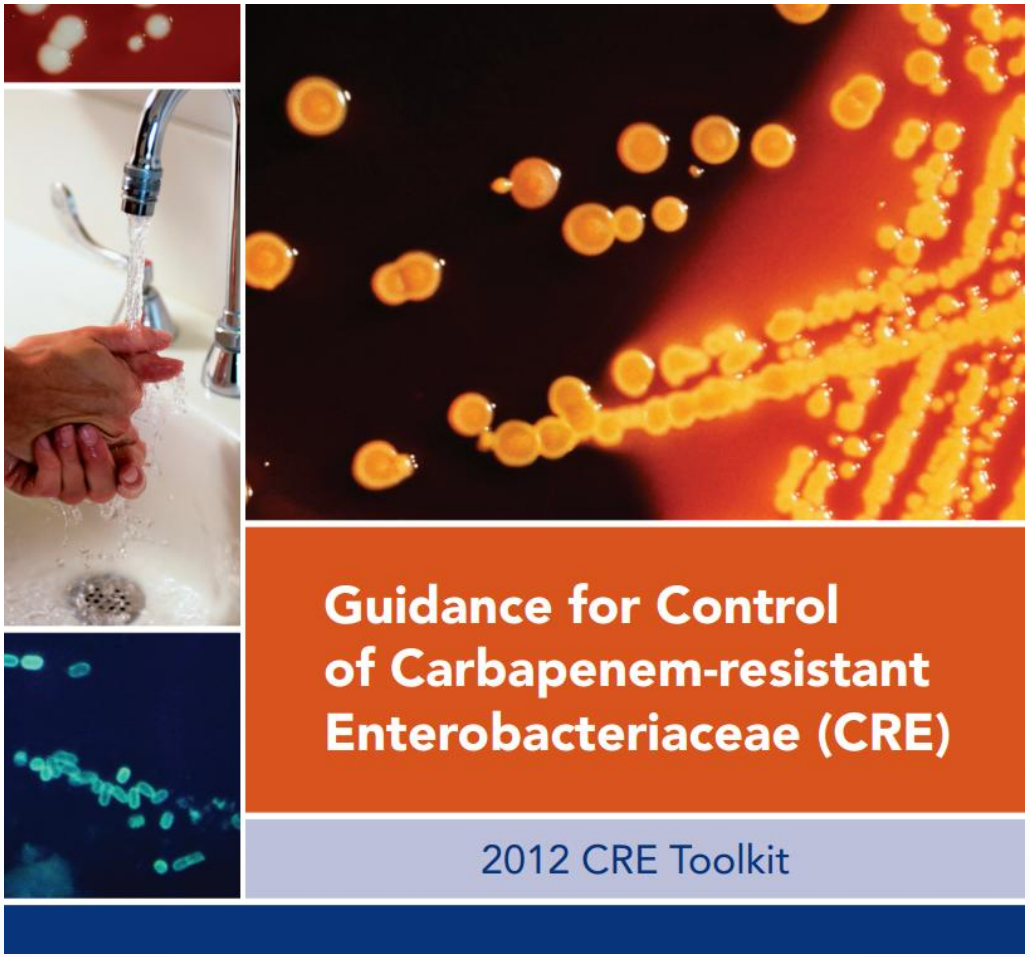
ESBL

- Derived from many plasmid encoded genes, such as TEM, SHV, CTX-M, OXA
  - Transferable—Plasmid transfer bacterial resistance
- Diagnose challenges:
  - Current best method still relies on traditional culture and susceptibility test
  - Many genes
  - Compete for attention
- Limited treatment options:
  - Nitrofurantoin
  - Fosfomycin (UTI only)
  - Chloramphenicol
  - For serious infections: Carbapenem (Ertapenem) or gentamicin injections

CRE or CP-CRE

- What are carbapenem antibiotics available in the US?
  - Primaxin (Imipenem, Merk, 1985)
  - Merrem (Meropenem, AstraZenica, 1996)
  - Invanz (Ertapenem, Merck, 2001)
  - Doribax (Doripenem, Johnson & Johnson, 2007)
  - All 4 drugs are injectable (IV) only
  - Broad-spectrum: active against GP, GN, anaerobes, non-fermenters
  - Drugs of choice for severe ESBL infections

CRE or CP-CRE



- http://www.cdc.gov/hai/pdfs/cre/CRE-guidance-508.pdf

CRE or CP-CRE

CDC CRE Toolkit 2012 Definition of CRE:

- Enterobacteriaceae that are:
  - Non-susceptible to one of the following antibiotics: Doripenem, Meropenem, or Imipenem**

AND…

  - Resistant to all of the extended-spectrum (3rd generation) cephalosporins tested (Cefotaxime, Ceftriaxone, Ceftazidime)

**Exceptions: *Proteus/Providencia/Morganella* are intrinsically resistant to imipenem

CRE or CP-CRE

Two general mechanisms cause CRE:

1) **Other non-carbapenemases in conjunction with other structural change of bacteria**
   - Such as ESBL or AmpC + porin loss
   - Low-level carbapenemase activity
   - Seen in most of the CRE before 2000

2) **Carbapenemase** production: β-lactamases that can destroy carbapenems
   - Carbapenemases = enzymes derived from plasmid encoded genes
   - Transferrable!
   - After 2000, CRE incidence increased rapidly
   - Much of this increase appears to be caused by the spread of Carbapenemase-Producing CRE

CRE or CP-CRE

- GNR Carbapenemase genes are:
  - **KPC**
    - First discovered in *K. pneumoniae*
    - Also commonly seen in other Enterics
  - **SME**
    - First discovered in *S. marcescens*
  - Metallo-β-Lactamases (MBL)
    - **NDM, VIM, IMP, GIM, SPM**
    - Enterics, *P. aeruginosa, Acinetobacter, S. maltophilia*
  - Carbapenemase genes are often linked to other resistance genes → Organisms often become **multi-drug resistant**
    - Most commonly quinolones, SXT, and at least one aminoglycoside

CRE or CP-CRE

- Diagnostic challenge:
  - CRE definition
  - Traditional Microbiology method vs. Molecular diagnostic method

- Treatment: Combo therapy
    - 2 drugs are better than 1

CRE or CP-CRE

- Positive patients may carry CRE for a **LONG** time even after discharge (as long as 387 days!)
- Patients carry CRE may also carry carbapenem resistant *Enterobacteriacea, Pseudomonas* and *Acinetobacter*
- LTACH and ECF are often the reservoir for local hospital's CRE patients.

C diff

- GP anaerobes, ubiquitous in the environment
- Produce spores to survive in harsh environment
- May become part of the flora in human colon (2-5% of adult population)
- **GDH** (glutamate dehydrogenase) enzyme produced by ALL strains of *C. difficile*
- **Toxins** produced by pathogenic (toxigenic) *C. difficile* strains cause CDI
  - Presence of toxin is necessary for CDI
  - Colonization of toxin-producing C. diff does not always causes CDI
  - Lead to confusing test results (more later…)

C. diff

- Diagnostic challenge:
  - Method selection
  - result interpretation

- Treatment:
  - Without symptoms is not recommended
  - Medications (Metronidazole, Vancomycin)
  - Probiotics
  - Fecal transplant
  - Colectomy

TB

- *Mycobacterium tuberculosis*
- "GP" or may not retain Gram stain
- Traditionally stained by acid fast stain (hence, acid fast bacilli, acid fast culture)
- MDR-TB = resist to Isoniazid (INH)and Rifampin (RIF)
- MDR plus TB = resist to INH, RIF, and Fluoroquinolone (FQ)
- XDR-TB (extensively drug-resistant TB) = resist to INH, RIF, FQ, and injectable aminoglycosides (Amikacin, Gentamycin, and Tobramycin)

TB

- Still with high prevalence in the world (1/3 of the world population)
  - No effective vaccination
  - Diagnostic challenge: slow grower
  - Treatment challenge: long term treatment and lack of new drug since 1968

    - Only 50% successful rate to treat MDR TB
    - On 12/28/12, FDA accelerated to approve Bedaquiline for MDR TB or XDR TB (Controversial!)

TB

- Out of all the positive acid fast culture, only 50% of their corresponding AFSM are negative.

- Rapid ID for TB is important:
  - Rapid ID of TB will improve diagnosis/treatment , prevent TB transmission, and help clinical trial of new TB drug development

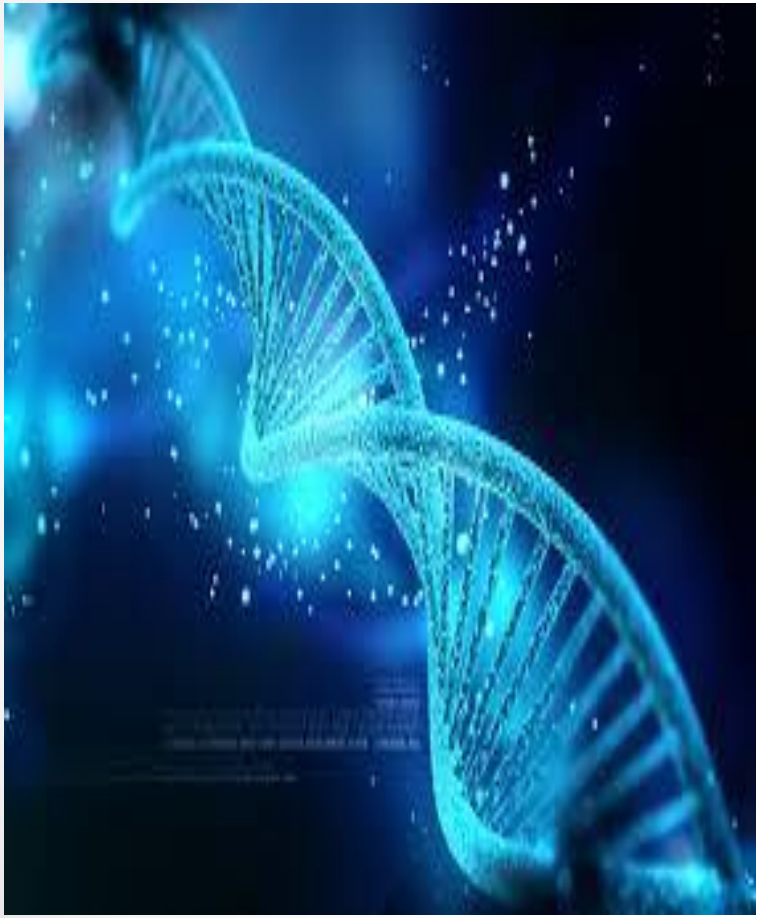
MDx v.s. Traditional Methods

- Traditional microbiology diagnostic methods
  - Inoculate patient samples to agar plates
  - Incubate for certain amount of time
  - Analyze growth on agars to determine additional testing
  - Additional testing = biochemicals
  - If applicable, perform susceptibility
  - TAT:
    - Typical bacteria 3-7 days if positive
    - Two to six weeks for fungus and AFB
  - Subjective
  - Cheaper
  - Allow to detect unexpected infection





MDx v.s. Traditional Methods



- Molecular diagnostic (MDx) methods
  - Different techniques to analyze or detect targets in genome or proteome
  - Some allow direct pathogen detection from a clinical specimen (eliminating culture/incubation process)
  - Specific, Sensitive
  - Some are high-throughput
  - Faster TAT
  - Objective
  - More expensive
  - Target specific

MALDI-TOF Mass Spectrometry

- **M**atrix-**a**ssisted **l**aser **d**esorption/**i**onization **t**ime-**o**f-**f**light (mass spectrometry)
- Identification based on protein contents of the organism
- Culture → Colony → MALDI → 10-15 minutes later → high-level of confidence identification





- Bruker Biotyper

MALDI-TOF Mass Spectrometry

Bruker Biotyper



bioMerieux Vitek MS



MALDI-TOF Mass Spectrometry

FDA approved for clinical use on:

- GN
- Yeasts (Vitek MS only)
- GP (Vitek MS only)
- Anaerobes (Vitek MS only)

Soon in the future:

- Mold identification
- Acid fast bacilli identification
- Bacteremia identification
- Identify presence of resistant enzymes, such as ESBL or Carbapenemases

Bacteremia/Sepsis Identification

- Bacteremia and fungemia are very serious infections
  - Mortality: 14% (community) – 34% (nosocomial)
  - >50% of bloodstream infections are nosocomial
- 2001 – 2007 condition with the highest increase in incidence2
  - 675,000 cases / year (increase of 97%)
- Financial burden

  $28,000 (community) - $105,000 (nosocomial)

  Average of $18,500 / patient

- Diekema et al. J Clin Microbiol. 2003 Aug;41(8):3655-60.
- Cost of Hospital Treatment for Blood Infection Surges, Especially for Uninsured Patients. AHRQ News and Numbers, June 9, 2010. Agency for Healthcare Research and Quality, Rockville, MD. http://www.ahrq.gov/news/nn/nn060910.htm

Bacteremia/Sepsis Identification

- Traditional culture methods rely on cultivation of pathogens:
  - 1-3 days: preliminary results
  - 3-5 days: definitive result, especially for MDRO such as CRE or VRE

- Not effective for modification/de-escalation of antimicrobial therapy

- Not effective for transmission prevention of MDRO

→ **Increased mortality, emergence of MDRO, chance to spread of MDRO**

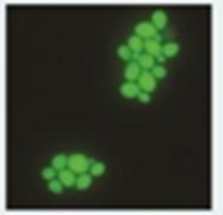
Bacteremia/Sepsis Identification

- Microbial identification based on detection of organism-specific nucleic acids
  - Microscopic detection - PNA-FISH®
  - NAAT - BioFire™ Diagnostics, Cepheid® Xpert® MRSA/SA BC
  - Nanoparticle-based detection – Nanosphere, Inc. Verigene® System
- Microbial identification using proteomic information
  - Mass spectrometry

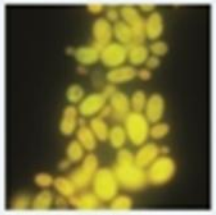
Note: A Gram stain is still required to guide decision of these rapid identification assay.

Bacteremia/Sepsis Identification

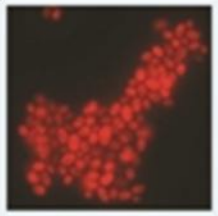
- PNA-FISH ® AdvanDx
  - Peptide Nucleic Acid Fluorescence In Situ Hybridization
  - Examine using fluorescence microscopy
  - Still somewhat subjective
  - Unable to determine resistant mechanism



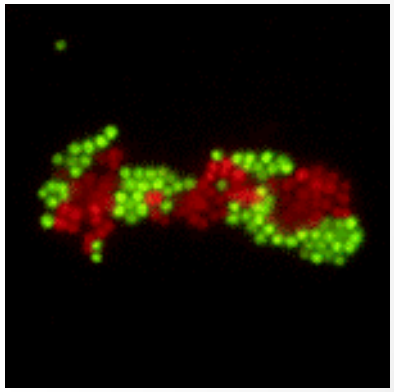
C. albicans and/or *C. parapsilosis*



C. tropicalis



C. glabrata and/or *C. krusei*



S. aureus (g) and CoNS (r)

Bacteremia/Sepsis Identification

- BioFire™ Diagnostics FilmArray BCID
  - Multiplex nucleic acid amplification-based platform
    - Blood Culture IDentification
    - Identification of 24 microbial pathogens, bacteria and yeast, as well as detection of 3 genes encoding antimicrobial resistance mechanisms
    - Uses a 100-µl aliquot of positive blood culture broth
    - Hands-on time, 2 minutes; assay time ~ 1 h





Bacteremia/Sepsis Identification

BioFire™ Diagnostics FilmArray BCID



Gram + Bacteria

Enterococcus
Listeria monocytogenes

Staphylococcus
Staphylococcus aureus

Streptococcus
Streptococcus agalactiae
Streptococcus pyogenes
Streptococcus pneumoniae



Gram – Bacteria

Acinetobacter baumannii
Haemophilus influenzae
Neisseria meningitidis
Pseudomonas aeruginosa

Enterobacteriaceae
Enterobacter cloacae complex
Escherichia coli
Klebsiella oxytoca
Klebsiella pneumoniae
Proteus
Serratia marcescens



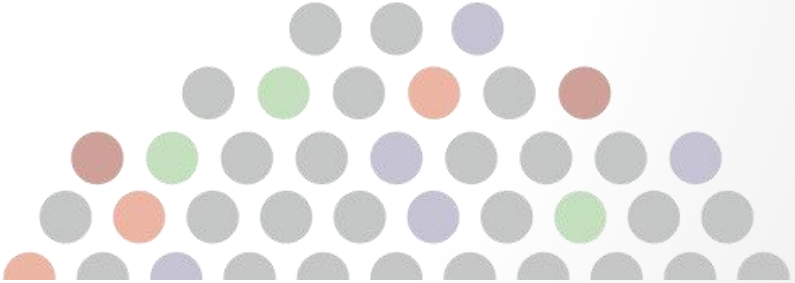
Yeast

Candida albicans
Candida glabrata
Candida krusei
Candida parapsilosis
Candida tropicalis



Antibiotic Resistance

mecA - methicillin resistant
vanA/B - vancomycin resistant
KPC - carbapenem resistant



Bacteremia/Sepsis Identification

- Cepheid® Xpert® MRSA/SA BC
  - Qualitative, real-time PCR for detection of methicillin-resistant *Staphylococcus aureus* and *S. aureus* (MSSA) in blood culture
  - Cartridge-based platform that offers
    - Automated/integrated sample extraction (cartridge manipulated, thermal cycled, etc. using the Cepheid® GeneXpert)
    - Uses a small sample volume
      - 50-µl aliquot of positive blood broth
    - 62-minute TAT
    - Targets include

      spa, mecA, and SCCmec



Bacteremia/Sepsis Identification

- Cepheid® Xpert® MRSA/SA BC



Note: Xpert® MRSA (nasal) & Xpert® MRSA/SA SSTI

Bacteremia/Sepsis Identification

- Nanosphere, Inc. Verigene® System
  - Array-based gold nanoparticle technology
  - Nanoparticles (gold, 13 – 20 nm) are coated with either oligonucleotides complementary to organism-specific DNA or RNA sequences **OR** they are coated with antibodies specific for protein targets
  - Uses a larger volume of blood culture broth for analysis
  - BC-GN kit: 700 µl
  - BC-GP kit: 350 µl
  - TAT is approximately 2 h



Bacteremia/Sepsis Identification

- Nanosphere, Inc. Verigene® System

Bacterial genera and species	Antimicrobial resistance genes
Acinetobacter spp.	bla_{CTX-M}
Citrobacter spp.	bla_{KPC}
Enterobacter spp.	bla_{NDM-1}
Proteus spp.	bla_{VIM}
Escherichia coli	bla_{IMP}
Klebsiella pneumoniae	bla_{OXA}
Klebsiella oxytoca	
Pseudomonas aeruginosa	
Serratia marcescens (not FDA-cleared in US)	

Antimicrobial resistance genes
mecA
vanA
vanB

Bacterial genera and species	Bacterial genera and species
Staphylococcus spp.	*Streptococcus pyogenes*
Staphylococcus aureus	*Streptococcus agalactiae*
Staphylococcus epidermidis	*Streptococcus anginosus* group
Staphylococcus lugdunensis	*Enterococcus faecalis*
Streptococcus spp.	*Enterococcus faecium*
Streptococcus pneumoniae	*Listeria monocytogenes*

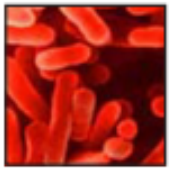
Bacteremia/Sepsis Identification

- All of these methods still rely on a sample from a positive blood culture.
- A positive blood culture may take 3 hours to days to be positive and detectable on a blood culture instrument.
- New MDx test—Direct detection from blood sample without the step of blood culture
  - T2Biosystem® T2 Candida Panel (FDA cleared)
  - T2Biosystem® T2Bactera Panel
  - Eliminate blood culture incubation



MDx Fecal Tests

- BioFire™ Diagnostics FilmArray GI Panel



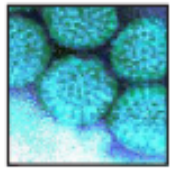
Bacteria

Campylobacter (jejuni, coli and upsaliensis)
Clostridium difficile (toxin A/B)
Plesiomonas shigelloides
Salmonella
Yersinia enterocolitica
Vibrio (parahaemolyticus, vulnificus and cholerae)
Vibrio cholerae
Diarrheagenic E. coli/Shigella
Enteroaggregative *E. coli* (EAEC)
Enteropathogenic *E. coli* (EPEC)
Enterotoxigenic *E. coli* (ETEC) *lt/st*
Shiga-like toxin-producing *E. coli* (STEC) *stx1/stx2*
E. coli O157
Shigella/Enteroinvasive *E. coli* (EIEC)



Parasites

Cryptosporidium
Cyclospora cayetanensis
Entamoeba histolytica
Giardia lamblia



Viruses

Adenovirus F 40/41
Astrovirus
Norovirus GI/GII
Rotavirus A
Sapovirus (I, II, IV and V)

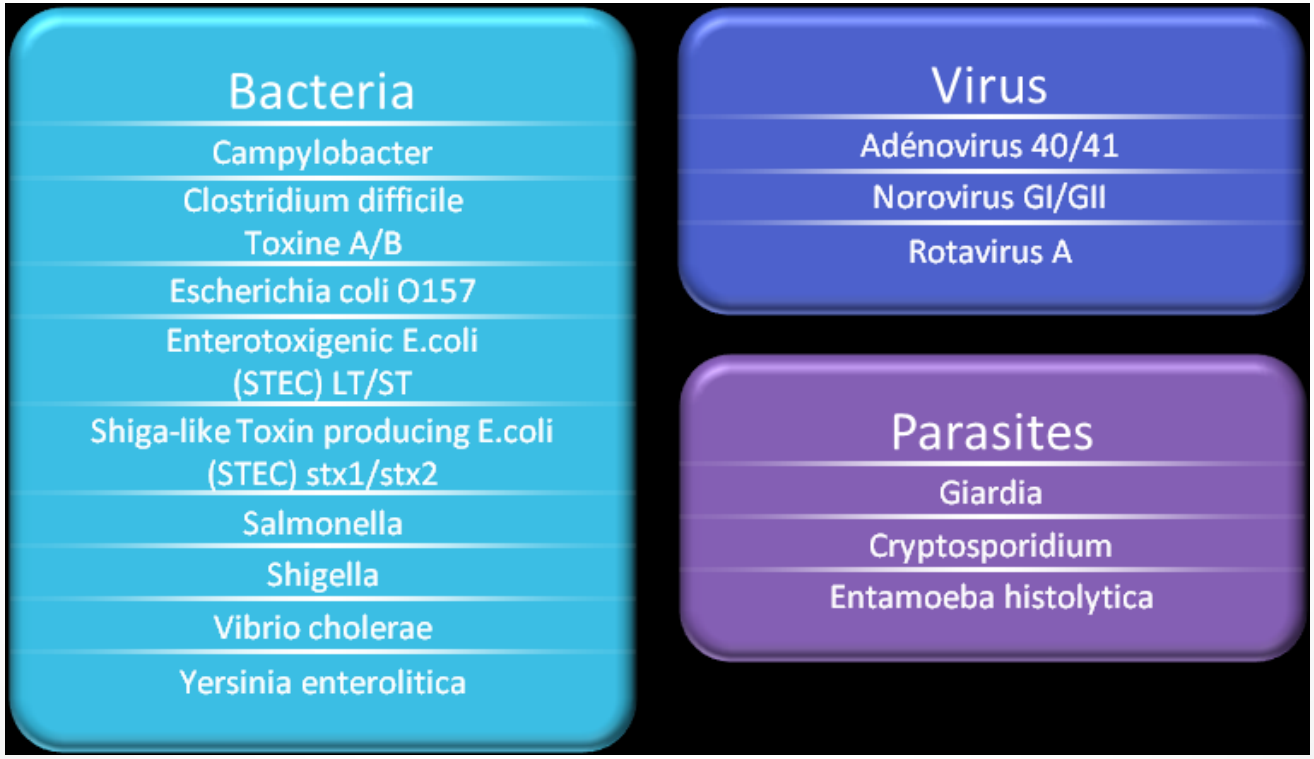
MDx Fecal Tests

- Nanosphere, Inc. Verigene® System Enteric Panel

TARGETS	US/FDA Cleared
Bacteria	
Campylobacter Group	●
Salmonella spp.	●
Shigella spp.	●
Vibrio Group	●
Yersinia enterocolitica	●
Toxins	
Shiga Toxin 1 (*stx1*)	●
Shiga Toxin 2 (*stx2*)	●
Viruses	
Norovirus	●
Rotavirus	●

MDx Fecal Tests

- Luminex xTAG® Gastrointestinal Pathogen Panel (GPP)



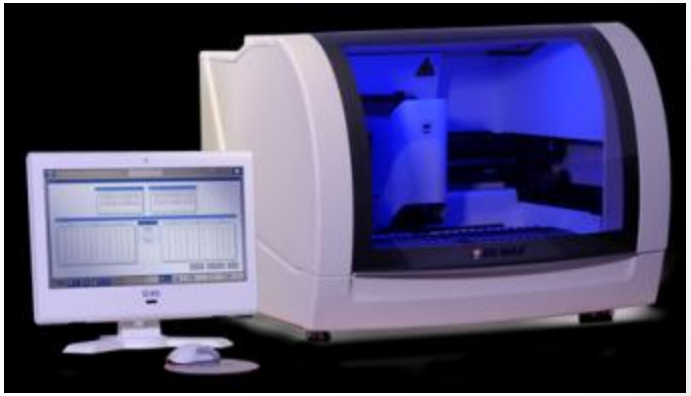
MDx Fecal Tests

- Cepheid® Xpert® C. difficile
  - Detect toxigenic *C. difficile*
- Cepheid® Xpert® C. difficile/Epi
  - Detect toxigenic *C. difficile*
  - If positive, detect 027/NAP1/BI strain



MDx Fecal Tests

- BD MAX™ System C. difficile
- BD MAX™ System Enteric Bacterial Panel
  - Salmonella spp.
  - Campylobacter spp. (jejuni / coli)
  - Shigellosis disease causing agents
    - Shigella spp. and Enteroinvasive E. coli (EIEC)
  - Shiga-toxin producing E. coli



Note: BD MAX also has panels for MRSA (nasal) & StaphSR (SSI)

CDI Diagnosis

Currently available *C. difficile* Laboratory Tests:

Test	Target Detected	TAT	Sensitivity (%)	Specificity (%)
Culture with Cytotoxin	Toxigenic *C. difficile*	3-5 days	>95	80-90
Cytotoxin	Toxin B	1-3 days	95	90-95
EIA Toxin A or A/B	Toxin A or Toxin A&B	Hours	75-80	97-98
GDH	*C. difficile*	Hours	95-100	70-80
GDH + EIA Toxin A/B	*C. difficile* + Toxin A&B	Hours	95-100	97-98
MDx (PCR)	Toxigenic *C. difficile*	Hours	>98	80-99

- Stamper P, et al. *J. Clin. Microbiol.* 2009;47:373
- Barlett J. *ICHE* 2010, 31:S35

CDI Diagnosis

- What do the results mean?
  - Positive GDH = *C. difficile* present (non-toxigenic and/or toxigenic strains)
  - Positive Toxin A/B = the toxin(s) that causes CDI present (true positive CDI)
  - Positive PCR = Toxigenic strain *C. difficile* present

- Presence of toxin(s) = CDI
- Presence of toxigenic strain *C. difficile* does not necessary = CDI

CDI Diagnosis

- MDx (PCR) C diff test will pick up asymptomatic carriers
  - Carrier of the toxigenic C. diff without active CDI
  - >50% of CDI patients after treatment continue to shed C. difficile organisms
- From infection prevention standpoint
  - An asymptomatic C. diff carrier may still present a risk of transmission
  - The current best way to screen for carriers is MDx method
  - Screen all patients for C. diff? Isolate asymptomatic carriers? Controversial!
- From treatment standpoint
  - MDx (PCR) test overdiagnoses CDI → Set clinical criteria
  - A patient with GDH +/Toxin – and/or PCR + C. diff result should not be treated

Riggs, et al. 2007 CID. 45: 992-998.

TB Diagnosis

Direct respiratory sample test:

- **Roche COBAS® *TaqMan*® MTB test & Amplicor® MTB**



- **Hologic (Gen-Probe) Amplified MTD® test**



- **Cepheid® Xpert® MTB/RIF**



TB Diagnosis

- CDC's "Report of an Expert consultation on the Uses of Nucleic Acid Amplification Tests for the Diagnosis of Truberculosis"

  http://www.cdc.gov/tb/publications/guidelines/amplification_tests/default.htm

- Advantages of MDx TB Diagnosis:
  - Faster diagnosis (hours versus weeks)
  - Initiation of earlier treatment
  - Faster reporting to TB programs
  - Reduce transmissions

Influenza & Respiratory Viruses

- Large panels include different strains of respiratory viruses and/or bacteria:

Nanosphere Verigene® RP Flex	BioFire™ FilmArray Respiratory Panel	GenMarkDx® eSensor® RVP
Adenovirus Human Metapneumovirus Influenza A • & Subtype H1, H3 Influenza B Parainfluenza 1,2,3,4 Rhinovirus RSV A & B Bordetella pertussis B. parapertussis/bronchiseptica B. holmesii	Adenovirus Human Metapneumovirus Influenza A • & Subtype H1, H3, H1-2009 Influenza B Parainfluenza 1,2,3,4 Rhinovirus/Enterovirus RSV Coronavirus • HKU1, NL63, 229E, OC43 Bordetella pertussis Chlamydophila pneumoniae Mycoplasma pneumoniae	Adenovirus B/E, C Human Metapneumovirus Influenza A • & Subtype H1, H3, H1-2009 Influenza B Parainfluenza 1,2,3 Human Rhinovirus RSV A & B

Influenza & Respiratory Viruses

Influenza based test:

- **Roche COBAS® Liat Influenza A/B**



- **Alere™ i Influenza A & B**



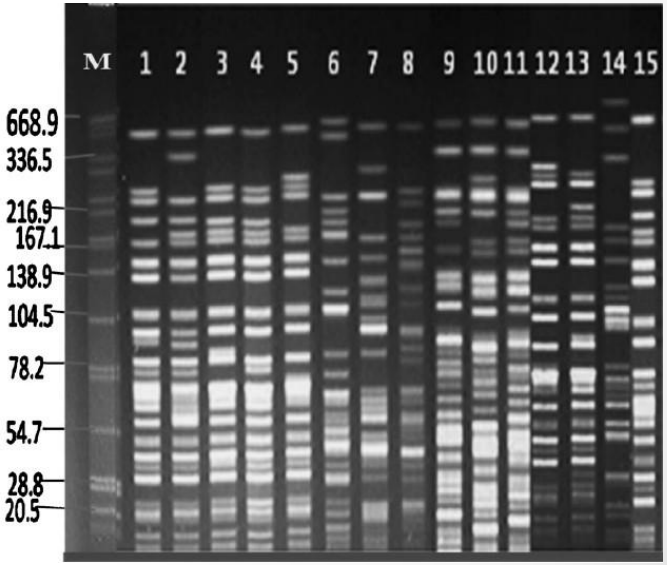
- **Cepheid® Xpert® Flu & Flu/RSV XC**



PFGE

- PFGE = **P**ulsed –**F**ield **G**el **E**lectrophoresis

- Relatively "older" technology
- Compare strains of bacteria at genomic level
- Applications:
  - Outbreak studies (foodborne)
  - Bacterial typing or characterization
  - Nosocomial person-to-person transmission



More in the future…

- Direct sample to detect sepsis (such as T2Biosystem®)
- Rectal sample to screen for CRE by non-culture method(such as Cepheid®)
- Direct sample to organism identification/susceptibility without cultivation (GeneWEAVE™ Smarticles™)

Applications for MDx

- Specific pathogen identification for
  - Surveillance and other epidemiologic purposes
  - Organisms associated with nosocomial transmission
  - Organisms associated with disease outbreak
  - Organisms ID when conventional methods fail to achieve high-level of confidence

- Challenge to implement MDx in your lab:
  - COST, COST, COST!
  - Validation difficulty (need assistance from ID docs, ICP practitioners, and hospital epidemiologists to acquire samples)
  - Consider complexity and throughput of newly developed technology→ may only be available to large commercial or university-based diagnostic laboratories
  - Middleware allow for interfacing of HIS/LIS from multiple institutions

Acknowledgements

- Dr. Ryan Relich, Ph.D, MLS(ASCP) IUHPL
- Deidre Jarrett, MT(ASCP) Cepheid
- Steve Smith, Nanosphere

Additional References

- World Health Organization Clean Care is Safer Care: My 5 Moments for Hand Hygiene. Retrieved from http://www.who.int/gpsc/5may/background/5moments/en/
- Centers for Disease Control and Prevention: Healthcare Infection Control Practices Advisory Committee (HICPAC). Retrieved from http://www.cdc.gov/hicpac/index.html
- VanDuin, D., et al. 2013 Carbapenem-resistant enterobacteriacease: a review of treatment of outcomes. Diag. Microbial. Infect. Dis. 75:115-120.
- Gupta, N. et al. 2011. Carbapenem-resistant enterobacteriacea: epidemiology and prevention. CID 53:60-67.
- Zimmerman, F.S. et al. 2013 Duration of carriage of carbapenem-resistant enterobacteriaceae following hospital discharge. Am. J. Infect. Control 41:190-194.
- Vasoo, S. et al. 2014. Cost-effectiveness of a modified two-step algorithm using a combined glutamate dehydrogenase/toxin enzyme immunoassay and real-time PCR for the diagnosis of clostridium difficile infection. J. Microbiol. Immunol. Infect. 47:75-78.
- Ryan, K., Ray C. (editors) Sherris Medical Microbiology 4th ed. 2004 pp322—324.
- Centers for Disease Control and Prevention (2013). MMWR weekly: Provisional CDC guidelines for the use and safety monitoring of bedaquiline fumrate (sirturo) for the treatment of multidrug-resistant tuberculosis. Retreived from http://www.cdc.gov/mmwr/preview/mmwrhtml/rr6209a1.htm?s_cid=rr6209a1_x

Additional References

- Khot, P.D. and M.A. Fisher. 2012. Mass Spectrometry in the Clinical Microbiology Laboratory, Part II: MALDI-TOF MS. Clin Micro News. 34:135-142

- APIC FAQ on testing asymptomatic patients for C. difficile, April 26, 2013. Retrieved from http://www.apic.org/Resource_/TinyMceFileManager/FAQ_on_Testing_Asymptomatic_Patients_for_Cdiff_04262013.pdf
- Riggs, M.M., et al. 2007. Asymptomatic carriers are a potential source for transmission of epidemic and nonepidemic clostridium difficile strains among long-term care facility residents. CID. 45: 992-998.

- Centers for Disease Control and Prevention: Report of an expert consultation on the uses of nucleic acid amplification tests for the diagnosis of tuberculosis. Retrieved from: http://www.cdc.gov/tb/publications/guidelines/amplification_tests/default.htm