Diagnostic Microbiology Challenges in Infection Prevention Across the World

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LEAST-STRESSED CITIES IN AMERICA

1. Iowa City, IA
2. Madison, WI
3. Duluth, MN
4. Boulder, CO
5. Columbia, MO
6. Fargo, ND
7. Fort Collins, CO
8. Lawrence, KS
9. Ann Arbor, MI
10. Bloomington, IL

Outline

• Define essential elements of lab support for IP
• Key challenges to effective lab support
  – Limitations of existing technology
  – Obstacles to realizing potential of tech advances
• Future directions
  – Bringing technologic advances to the point-of-care
  – Strengthening regional/national lab networks
Diagnostic Microbiology: Essential partner in infection prevention

• **Surveillance**: *cornerstone of prevention*!
  – Accurate detection of organisms and resistance

• **Outbreak detection and management**
  – Case finding, molecular typing, additional culturing

• **Antimicrobial stewardship**
  – Antibiograms, timely ID and AST data to direct therapy
WHO Core Components for IPC

• “Good quality microbiology laboratory support is a very critical factor in an effective IPC programme”
• “Quality microbiology and laboratory capacity is essential to enable reliable HAI surveillance”
• “Microbiology and laboratory capacity and quality are critical for national and hospital-based HAI and AMR surveillance”
• “Good quality microbiology support provided by at least one national reference laboratory is a critical factor for an effective national IPC surveillance programme”

Urgent need for timely detection of pathogens and resistance patterns at all levels

WHO. Worldwide country situation analysis, 2015.
What is “good quality....support”? 

• **Timely organism detection and reporting**
  – Accurate ID and AST (if applicable)

• **Cumulative data availability**
  – Regularly updated, accessible to providers
  – Susceptibility + pathogen incidence

• **Access to molecular typing/advanced testing**
  – Regional and national referral laboratories
Limitations of existing technology
Our methods are so “last century”!

“In everyday life when we deal with infectious diseases, we still rely on culture based methods that were developed in the 19th century”

Stephan Harbarth, MD
Culture-based methods: Too slow!

Current turnaround times for culture-based diagnostics (2-4 days from sample collection to antimicrobial susceptibility testing), have limited clinical relevance.

Limitations of 20\textsuperscript{th} century testing

• Need to grow organisms (time, cultivability)
• Accuracy: e.g. species identification
• Discrimination: e.g. older typing methods
• Sensitivity: e.g. \textit{C. difficile} toxin EIA testing
• Infrastructure and personnel requirements
  – Equipment, maintenance, reagents, trained staff
Consequences for IP and stewardship

• Delays important interventions
  – Transmission based precautions
  – Outbreak recognition and response
  – Optimizing antibiotic therapy

• Failure to recognize and track epidemiologically-important pathogens

• Basic services unavailable if limited resources
The “culture of culturing”

• In many low-middle income settings, culture prior to antibiotic therapy is not standard
  – Costs, turnaround time, lab capacity/quality
  – Empiric therapy, culture only if treatment fails

https://www.asm.org/index.php/public-outreach/agar-art
The future: NGS and metagenomics

- **Next-generation sequencing**: DNA-sequencing methods that produce more data in a shorter time, with less manual intervention, than previous methods.

- **Metagenomics**: Analyzing all genetic material in a sample, without separating genomes or culturing.

- **Metagenomic whole genome sequencing**: Applying WGS to a metagenomic sample—DNA is extracted from a sample, producing a mixture of genomes, which are subjected to WGS en masse.
The promise of next generation sequencing

- Still aspirational
- Technical hurdles:
  - Standardization
  - Automation
  - Validation methods
  - Curated databases
- Clinical hurdles:
  - Reporting
  - Interpretation

Interpretation: So much information!

• High noise-to-signal ratio
  – Skin commensals, lab contaminants, environmental contaminants during collection

• Obtaining a sequence ≠ identifying a pathogen

• Resistance genotype may not = phenotype
  – Incomplete understanding of genotypic basis for phenotypic resistance

All culture based

NGS/metagenomics

Species identification

In silico antibiogram

Sequence type

“Well, this is just going from bad to worse.”
Emerging diagnostic technologies: Speed, accuracy, sensitivity

• Mass spectrometry (MALDI-TOF)
  – Matrix-assisted laser desorption/ionization-TOF

• Nucleic acid amplification/detection (NAAT)
  – PNA-FISH, PCR approaches (microarrays, multiplex)

• Magnetic resonance
  – Applied directly to sample after short amplification step

• Automated microscopy + FISH
  – “Rapid phenotypic testing”
Sample-to-answer technology

- MTB + rifampin R
- *C. difficile* toxin genes
- Bacterial resistance genes
  - mecA, vanA, carbapenemases
- Microarray/multiplex panels
  - Respiratory
  - CNS infection
  - Gastroenteritis
  - Bloodstream infection (culture)
<table>
<thead>
<tr>
<th>Gastroenteritis</th>
<th>Meningitis/Encephalitis</th>
<th>Respiratory tract</th>
<th>Bacteremia</th>
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<tbody>
<tr>
<td>Campylobacter spp.</td>
<td>Escherichia coli K1</td>
<td>Adenovirus</td>
<td>Enterococcus spp.</td>
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<tr>
<td>Clostridium difficile</td>
<td>Haemophilus influenzae</td>
<td>Coronavirus 229E</td>
<td>Listeria monocytogenes</td>
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<tr>
<td>Plesiomonas shigelloides</td>
<td>Listeria monocytogenes</td>
<td>Coronavirus HKU1</td>
<td>Staphylococcus (aureus)</td>
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<tr>
<td>Salmonella spp.</td>
<td>Neisseria meningitidis</td>
<td>Coronavirus OC43</td>
<td>Streptococcus (4 species)</td>
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<td>Streptococcus agalactiae</td>
<td>Coronavirus NL63</td>
<td>Acinetobacter spp.</td>
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<tr>
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<td>Streptococcus pneumoniae</td>
<td>Human metapneumovirus</td>
<td>Haemophilus influenzae</td>
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<td>Vibrio cholerae</td>
<td>Cytomegalovirus</td>
<td>Rhinovirus/Enterovirus</td>
<td>Neisseria meningitidis</td>
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<td>Enteroaggregative E. coli</td>
<td>Enterovirus</td>
<td>Influenza A</td>
<td>Pseudomonas aeruginosa</td>
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<td>Enteropathogenic E. coli</td>
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<td>Influenza A/H1</td>
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<td>Human herpes virus 6</td>
<td>Influenza A/H3</td>
<td>Escherichia coli</td>
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<td>Human parechovirus</td>
<td>Influenza B</td>
<td>Klebsiella (oxytoca/pneu)</td>
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<tr>
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<td>Varicella zoster virus</td>
<td>Parainfluenza 1</td>
<td>Proteus spp.</td>
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<td>Astrovirus</td>
<td>Cryptococcus neoformans</td>
<td>Parainfluenza 2</td>
<td>Serratia marscescens</td>
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<td>Rotavirus A</td>
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<td>Parainfluenza 4</td>
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<td>Respiratory syncytial virus</td>
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<td>Cryptosporidium, Giardia lamblia</td>
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<td>Candida parapsilosis</td>
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<td>Chlamyphila pneumoniae</td>
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<td>Entameoba histolytica</td>
<td>Mycoplasma pneumoniae</td>
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<td>mecA, vanA, KPC</td>
</tr>
</tbody>
</table>
Microarrays: changing a paradigm

1. Recognize syndrome
2. Formulate Diff Dx
3. Send tests for most likely etiologies
   + Make diagnosis
4. Send tests for less likely etiologies
5. SYNDROMIC TESTING
"I've written my diagnosis on this piece of paper. I'm going to slide it over to you, and I want you to tell me if you're interested."
Infection prevention implications

- Organisms require transmission-based precautions, but are not routinely or rapidly detected.
- Allows for more rapid detection of outbreaks, early intervention to prevent transmission.
- Provides valuable information to public health.
Stewardship implications

- Meta-analysis of rapid tests
  - NAAT, PNA-FISH, MALDI
- 31 studies, 5920 patients
- All cause 30 day mortality
- 20 of 31 supported by ASP
- Most quasi-studies
- OR: 0.66 (0.54, 0.80)
- # needed to treat = 20
- 5 hrs \(\downarrow\) time to effective tx

Timbrook, et al. CID 2017
Reality check: Recent diagnostic advances often not accessible in LMIC

- Instrument costs
- Supply chain
  - reagents, cartridges
- Infrastructure (electricity)
- Maintenance and technical support
- Pair with a core/regional lab for support
Lack of lab capacity globally!

Figure 1.4 – Percentages of Member States in which laboratory sensitivity was tested and which participated in external quality assessment, all regions.
Laboratory response network

How to bring quality diagnostic microbiology to the point of care in all acute-care settings?
Lab assessment for improvement

• Essential for monitoring and improving lab capacity in all settings, urgent need in LMIC
  – Site capacity, supply chain and resources
  – Dedicated personnel (training, communication)
  – Quality management systems in place
Vietnam lab assessments

- APHL and CDC recently conducted assessment of 19 labs in July 2016
- All candidates for MOH-led AMR surveillance system
- High variability of capacity
  - Some with advanced capacity, well-developed SOPs and QC/ EQAS programs
  - Some with scarce supplies, poor equipment
  - Access to microbiologists not uniform
  - Some had weak demand for culturing
Rapid Point of Care (POC) testing

- Expanding menu
  - malaria, filariasis, HIV, influenza, RSV, SPN, Legionella, GAS, STDs

- Expands test settings
  - Resource limited
  - Cruise ship/Air travel/Space

- Technology advancing

ASSURED guidelines for POCT

- Affordable
- Sensitive
- Specific
- User-friendly
- Rapid and Robust
- Equipment free
- Delivered (to end users)

POC lab connected to a core/regional lab for:
- Interpretation
- Validation
- Quality control
- Feedback of data
- Surveillance
- Outbreaks
- Antibiogram
Antibiotics prescribed to 69% with negative RDT (versus 40% with a positive RDT)

Infection prevention wish list for POC

• Bacterial vs. viral vs. parasitic/protozoal/fungal

• Rapid detection of key pathogen targets
  – Most common HAI pathogens and R genes
    • meca, vanA, carbapenemases, mcr

• Technologic advances to increase availability
  – Microfluidic PCR, smart phone applications

• Core/regional lab provides advanced support
  – Cultures, standard AST, molecular typing/WGS
Summary:
Diagnostic microbiology challenges for IP

• Provide the essential support:
  – Timely organism detection and susceptibility
  – Cumulative data availability/feedback
  – Access to molecular typing/advanced testing
• Bring this essential support close to the POC
• Strengthen the laboratory response network
"We’ve tried all the news channels—this guy is unbreakable."