The Role of Rapid Diagnostics in Preventing Healthcare Infection

Hilary Humphreys

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Hosted by Prof. Jean-Yves Maillard
Cardiff University, Wales

Declaration

The views expressed are of a professional but personal nature and not necessarily those of the RCSI & Beaumont Hospital, Dublin.

I have recently received research funding from Pfizer & Astellas. I have also provided professional advice or education to Cepheid & Pfizer.
Objectives

• Provide a brief overview of healthcare-acquired infections (HCAI) & the specific current challenges
• Discuss current limitations in the techniques used to detect HCAI
• Explore the role of whole genome sequencing & other emerging technologies in detecting & defining spread
• Outline when & where such technologies will be used
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HCAI in USA, 2002

- Multiple datasets: 1.7m with HAI, 155,668 deaths & 98,987 due to HCAI; Mortality highest for pneumonia & BSI

HCAI in Europe, 2011-2012

- 1,000 hospitals in 30 countries
- 5.7% overall; 19.5% in ICU
- RTI 23.5% > SSI 19.6% > UTI 19% > BSI 10.7%
- 32.7% on antibiotics

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Current Limitations & Needs into the Future

Unlike with other laboratory diagnostic approaches, e.g. haematology, a result is not available in many cases for 1-2 days.

Therefore appropriate therapy may be delayed. Over-treatment may be given & transmissible multi-drug resistant infections may have resulted.
Bacterial Diagnosis - Traditional

Specimen Receipt
~ 1 h

Microscopy
18-24 h

Culture
18-24 h

Susceptibilities
Identification

Viral Diagnostics

Previous
Specimen Receipt
EM Immunofluorescence
Culture (~ 1 week)

Current
Specimen Receipt
PCR Film Array WGS

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Priorities

I. Acute, life-threatening infections such as bloodstream infections (BSI), meningitis

II. Multi-antibiotic resistant bacteria

III. Emerging, opportunistic & potential causes of HCAI, e.g. Zika virus, Ebola virus, astrovirus

IV. Fast tracking of HCAI spread within & between hospitals

What the future may bring
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## Current & Emerging Technologies

**Current**
- PCR
- Mass Spectrometry
- Whole genome sequencing (WGS)

**Emerging/Evolving**
- Electronic nose devices (volatile organic compounds)
- Infra-red spectroscopy
- Microfluids

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**Diagnostic Methods & Time Required**

<table>
<thead>
<tr>
<th>Diagnostic Method</th>
<th>Time for Pathogen Identification</th>
</tr>
</thead>
<tbody>
<tr>
<td>Microscopy</td>
<td>Morphology in minutes</td>
</tr>
<tr>
<td>Gram stain</td>
<td>General category in minutes</td>
</tr>
<tr>
<td>Culture and phenotypic biochemistry</td>
<td>Days to weeks</td>
</tr>
<tr>
<td>(or/with artificial media (bacterial, mycobacterial, fungal))</td>
<td></td>
</tr>
<tr>
<td>In vitro antimicrobial susceptibility</td>
<td>Days to weeks</td>
</tr>
<tr>
<td>Acute and convalescent antibody</td>
<td>Weeks</td>
</tr>
<tr>
<td>Monoclonal antibodies</td>
<td>Hours</td>
</tr>
<tr>
<td>Antigen detection</td>
<td>Minutes to hours</td>
</tr>
<tr>
<td>Real-time polymerase chain reaction for microorganisms and drug resistance genes</td>
<td>One to several hours</td>
</tr>
<tr>
<td>Mass spectrometry</td>
<td>Seconds to minutes, after growth on/in media</td>
</tr>
</tbody>
</table>

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Overview of Clinical Bacteriology

1. Molecular panels for BSI – emerging
   - May not detect all microbes if mixed infections
   - mecA may be from S. aureus or coagulase negative staphylococci
   - 24h staffing

2. Rapid identification & susceptibility testing
   - down from a day to hours or even minutes

3. Metagenomics (WGS)
   - human DNA “subtracted” & leftover DNA analysed


Overview of Clinical Bacteriology

What is the impact of this & other new emerging technologies on mortality, length of hospital stay & BSI duration?

Infectious Diseases Society of America (IDSA) Policy Paper

Federal priorities & incentives for tests
a) Directly on accessible specimens such as blood
b) Exclude infection, e.g. ≥ 98% negative predictive value
c) Incorporate biomarkers that indicate host response
d) Panels for clinical syndromes, e.g. CNS infections
e) Drug resistance
f) Point-of-care
g) Improved outbreak detection

Clin Infect Dis 2013; 57: S139-70

IDSA Policy Paper

Where time makes a difference
• HIV resistance & anti-viral choices
• Unrecognised/unculturatable organisms, e.g. HCV, Tropheryma whippelii
• Methicillin-susceptible or resistant S. aureus
• Middle East Respiratory Syndrome coronavirus (MERS-CoV)
• Genotyping, e.g. HPV 16 & 18 associated with neoplasia & in-outbreak investigation

Clin Infect Dis 2013; 57: S139-70
Point-of-Care Testing (POCT)

“Testing of specimens, whether in a laboratory or not, close to the patient, e.g. doctor’s office, 24h a day with a result within 2-4h”

- Simple, safe & quick tests
- Minimal equipment required
- Cheap or cost effective
- Less specialised equipment
- Bedside or satellite laboratory

POCT Set Up

Clin Microb Rev 2016; 29: 429-447

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POCT Options

Antigen assays for syndromes
- e.g. diarrhoea-rotavirus, adenovirus, *Clostridium difficile*, *Campylobacter* spp.
- Cheap, easy to use but low sensitivity such as 60%

Real-time PCR (RT-PCR)
- e.g. meningitis – *Neisseria meningitidis*, *Streptococcus pneumoniae*, enterovirus, herpes simplex virus, varicella-zoster virus

Variations accordingly to geography, patient categories (e.g. *Cryptococcus*) & possibly cheaper in resource-poor countries, e.g. TB

**Clin Microb Rev 2016; 29: 429-447**

Rapid BSI Detection

Challenges from +ve blood culture
- PCR inhibitors
- High quantity of non-microbial nucleic acids
- Contaminated DNA
- DNA from dead microbes
- Not a “catch all” approach

Other Issues
- Choice of probe dependent on Gram stain
- Bacterial load is usually $10^6 - 10^8$
- Turnaround time (TAT) of 1.5 – 4 h

**Clin Microb Infect 2015; 21: 313-322**
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Available Rapid BSI Systems (adapted)

<table>
<thead>
<tr>
<th>System</th>
<th>TAT</th>
<th>Organism detected</th>
<th>Sensitivity &amp; Specificity</th>
</tr>
</thead>
<tbody>
<tr>
<td>FISH (UC)</td>
<td>1-3h</td>
<td>Up to 4 Gr - &amp; 5 fungi</td>
<td>81-100% 90-100%</td>
</tr>
<tr>
<td>Microarray</td>
<td>2.5 -3.5h</td>
<td>Up to 60 bacteria &amp; 13 fungi</td>
<td>81-100% 95%</td>
</tr>
<tr>
<td>Multiplex – PCR</td>
<td>1-2 h</td>
<td>8 Gr +ve, 11 GR-ve, 5 fungi</td>
<td>91-100% 95-98%</td>
</tr>
<tr>
<td>Mass-spectrometry</td>
<td>&lt; 1h</td>
<td>&lt;1,000</td>
<td>76-99%</td>
</tr>
</tbody>
</table>

Clin Microb Infect 2015; 21: 313-322

SepTec™ Technology for BSI in ICU

- Microfluidic device that uses 2 ml
- Detects <10 colony forming units in < 25 minutes
- Categories as Gram positive, Gram negative, Fungal

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Rapid RTI Diagnosis

Pneumonia (available) but MERS-CoV & TB (POCT) needed

<table>
<thead>
<tr>
<th>Time to result</th>
<th>Type of technology</th>
<th>Targets</th>
<th>Sensitivity</th>
<th>Specificity</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 h</td>
<td>Automated sample preparation of respiratory specimen, real-time PCR and detection using molecular beacon technology</td>
<td>MSSA and MRSA</td>
<td>99.9% compared with quantitative culture of endotracheal aspirates</td>
<td>72-2% compared with quantitative culture of endotracheal aspirates</td>
</tr>
<tr>
<td>4 h</td>
<td>Multiplex endpoint PCR and amplification detection by hybridisation to oligo probes spotted on membrane arrays direct from respiratory samples</td>
<td>Detection of 17 bacterial and fungal pathogens in addition to 22 antibiotic resistance genes</td>
<td>80-9% overall, target specific values 50-100%</td>
<td>99-0% overall, target specific values 72-3-100%</td>
</tr>
<tr>
<td>1 h</td>
<td>Pouch format comprising nucleic acid extraction, and nested PCR from nasopharyngeal swabs</td>
<td>20 targets including respiratory viruses, Bordetella pertussis, Mycoplasma pneumoniae and Chlamydophila pneumoniae</td>
<td>84-100%</td>
<td>98-100%</td>
</tr>
</tbody>
</table>

MSSA = methicillin-sensitive Staphylococcus aureus, MRSA = methicillin-resistant Staphylococcus aureus, SSTI = skin and soft tissue infection.

Lancet Infect Dis; 2014: 14: 1123-35

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Rapid Testing & Fungal Infections

- Mainly still microscopy, culture & histology
- Galactomannan & β-D-glucan screening of serum
- PCR in combination for aspergillosis but often systems are not validated
- MALDI-TOF assists in identification

Carbapenamase-Producing Enterobacteriaceae (CPE)

- A global problem, a national problem in many countries & a local one
- Hospital & community
- ≥ 1 mechanism, multiple genus & species
- Traditional methods cumbersome & slow
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ESBL/CPE – Rapid Biochemical Tests

<table>
<thead>
<tr>
<th>Test</th>
<th>Mechanism</th>
<th>Turnaround time</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>NDP</td>
<td>Cefotaxime hydrolysis</td>
<td>&lt;1 hour</td>
<td>&gt;98% S&amp;S</td>
</tr>
<tr>
<td>Carba NP</td>
<td>Imipenem hydrolysis &amp; change in pH</td>
<td>2 h</td>
<td>Low sensitivity to OXA-48</td>
</tr>
<tr>
<td>Blue-carba</td>
<td>Bromothmol blue indicator</td>
<td>Faster as no extract process</td>
<td>Better for OXA-48</td>
</tr>
</tbody>
</table>

*Infect Dis Clin N Am 2016, 323-45*

CPE – Molecular & Other Tests

- Ideally want to target
  - CTX-M, TEM, SHV, KPC, IMP, VIM, NDM, OXA
  - e.g. hyplex SuperBug, Curetis AG, GeneExpert
- In the future, the options will be
  - WGS
  - Micro-arrays – detect multiple genes & can be updated, e.g. Alere
  - Modifications to MALTI-TOF MS
  - Microfluidics & nanotechnology, lab-on chip

*Infect Dis Clin N Am, 2016; 323-345*
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Rapid Screening for MRSA

- Beaumont Hospital study of 462 (ward, emergency department & ICU) patients during 3 periods
  - 22-33% MRSA +ve
  - 27% not screened if culture used & 11% if PCR used (p<0.01)
  - 24% of patients pre-emptively isolated without PCR compared to 7% with PCR (p>0.001)

_Infect Control Hospital Epidem_ 2010; 31: 374-381

Does rapid detection of BSI & or identification of cause +/- resistance markers make a difference?
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**SeptiFast Blood Culture Detection**

<table>
<thead>
<tr>
<th>TABLE 1 Pathogens detectable using LightCycler SeptiFast test</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Gram-negative bacteria</strong></td>
</tr>
<tr>
<td>Escherichia coli</td>
</tr>
<tr>
<td>Klebsiella (pneumoniae/oxytoca)</td>
</tr>
<tr>
<td>Serratia marcescens</td>
</tr>
<tr>
<td>Enterobacter (cloacae/aerogenes)</td>
</tr>
<tr>
<td>Proteus mirabilis</td>
</tr>
<tr>
<td>Acinetobacter baumannii</td>
</tr>
<tr>
<td>Pseudomonas aeruginosa</td>
</tr>
<tr>
<td>Stenotrophomonas maltophilia</td>
</tr>
</tbody>
</table>

* Single probe detects a group of staphylococcal pathogens including *Staphylococcus epidermidis* and *Staphylococcus haemolyticus*.

**Rapid Detection of HCAI BSI in Critical Care with Multi-Pathogen PCR**

- SeptiFast real time PCR compared to blood cultures
- 25 pathogens detected in single blood samples
- 2129 citations →37 studies
- Study quality variable with bias a possibility
- 59% sensitivity & 89% specificity: better rule-in than rule-out potential
- SeptiFast unlikely to result in sufficient diagnostic utility of suspected sepsis-related HCAI

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Effectiveness of Improved Timelines of BSI Results

Compared rapid phenotypic tests & additional communication, rapid molecular tests & additional communications & rapid molecular tests only

- Identification: 1,800 non-duplicate records identified through electronic database search (PubMed, Embase, CINAHL)
- 27 additional records identified through other sources
  - Hand search, review of references, referrals: 20
  - Unpublished submissions: 7

- Screening: 1,827 records screened for topic relevance
  - 1,685 Excluded — Off topic
  - 142 records screened for practice effectiveness studies
  - 79 Excluded — Inclusion criteria not met (i.e., not a study, or no practice or outcome measure of interest)

- Eligibility: 63 full-text studies meeting inclusion criteria
  - 47 Excluded — study quality criteria not met

- Included: 16 studies included
  - Rapid molecular technique w/out additional direct comm: 7
  - Rapid molecular technique w/additional direct comm: 5
  - Rapid phenotypic technique w/additional direct comm: 4

Kerremans, 2008
Frye, UnPub
Bauer, 2010
Doern, 1994

R² = 0.4572

Effectiveness of Improved Timeline of BSI results

Rapid tests & additional communication leads to more timely treatment

Std diff in means and 95% CI

Favor Rapid Test
Favor Comparator

Clin Microb Rev 2016; 29: 59-103

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Application of Whole Genome Sequencing

Next Generation Sequencing (NGS)

1. Identify bacteria via sequence analyses of 16S rDNA & fungi via 18S rDNA
2. Single protocol, as primers needed & different platforms use different sequence technologies
3. Not clear how many alleles 2 genomes may vary to call them close to being or actually identical
4. NGS used for outbreak management, molecular case finding surveillance of pathogens, rapid identification & taxonomy
5. A metagenomic approach can be used to study the resistance

J Biotechnol 2017; 243; 16-24

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Next Generation Sequencing

Lots of data to analyse, e.g. faeces

J Biotechnol 2017; 243: 16-24

16S rDNA & Antimicrobial Stewardship

<table>
<thead>
<tr>
<th></th>
<th>Neurosurgical patients (27)</th>
<th>Other patients (33)</th>
</tr>
</thead>
<tbody>
<tr>
<td>16S rDNA detected</td>
<td>18 (53%)</td>
<td>15 (34%)</td>
</tr>
<tr>
<td>Antimicrobial details available</td>
<td>18 (87%)</td>
<td>11 (85%)</td>
</tr>
<tr>
<td>De-escalation</td>
<td>3 (23%)</td>
<td>3 (18%)</td>
</tr>
</tbody>
</table>

J Hosp Infect (in press)

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MRSA in Ireland – Hospital & Community

- 89 isolates, June 2013-2016; 78 HA & CA
- Mupirocin-resistant MRSA

Conclusions -1

1. Major changes in microbiology diagnostics
2. Greater accuracy & quicker results
3. Benefits in “downstream” value vs “upstream costs”
4. Costs will come down
5. Rationalisation of antibiotic use & earlier information to control outbreaks
Conclusions-2

6. Technology will drive centralisation & consolidation of laboratories

7. POCT will increase in importance due to consolidation & patient demands

8. The era of culture is not over yet, e.g. urines (cheap & fast enough)

9. Challenges are analysis & interpreting huge amounts of data

10. Clinical need & not availability of technology must drive developments

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