Better tests better care: Syndrome-based diagnostics for respiratory tract infections

Prof. Pierrette Melin
National Reference Centre for Streptococcus agalactiae
Clinical Microbiology, University Hospital of Liege, University of Liege

Infectious diseases in the XXIst century: Burden, threats and challenges

INTRODUCTION

CONTENT

- Introduction
- Desirable improvements
  - Theranostic approach
  - Syndrome-based diagnostic approach
- Respiratory tract infections
- Take home messages

Causes of mortality (WHO 2008)

Global death rate related to infections = 20-25%
INFECTIONS = second cause
Causes of mortality (WHO 2008)

- Low income countries (Africa, Asia, ...) death rate related to infections = 40%
- INFECTIONS = first cause

« Top » of deadly infectious diseases, WHO 2008

Worldwide major threat: Bacteria are doing resistance
Global increase of antimicrobial resistance
Emerging superbug

Use of high volumes of antimicrobial agents
- Appropriately or not
- Microbiological diagnostics frequently skipped
- High TAT, lack of sensitivity

The war against infectious diseases is far from over
Introduction

Fundamental to quality care

- Rapid and accurate establishment of a microbial cause
  - Whether caring for individual patients with infectious disease
  - Or responding to a worldwide pandemic
- To facilitate stewardship for rational use of antimicrobial agents when needed
  - ...

Clinical microbiology laboratory

Primary missions

TO IMPROVE THE MANAGEMENT OF INFECTIOUS DISEASE

CONTRIBUTION TO DIAGNOSTIC

- Presence/absence of pathogens
- Identification +/- quantification
  - Bacteria, fungi, virus, parasites

CONTRIBUTION TO CHOICE OF ANTIBIOTHERAPY

- Probabilistic, targeted
- Antimicrobial susceptibility testing, identification of resistance mechanisms and resistance genes

SUPPORT TO INFECTION CONTROL

To provide useful, accurate and relevant results

« Useful » results

POSITIVE IMPACT ON

- Therapeutic decision?
- Optimized management of patients?
- Morbidity, mortality?
- Length of hospitalization?

Reduction of Turn-Around-Time for result and its notification to clinician

- Control of nosocomial infections?
- Antibiotic consumption?
- Control of antimicrobial resistance?

XXIst century

Medical evolutionary background

Factors impacting on development and daily practice of microbiology

- Economic environment
  - Cost-effective use of available resources
  - Reimbursement system, regulation
- Trained human resources
  - Population pyramid and labour shortage
- Medical environment
  - Increasing emphasis on evidence-based medicine and adherence to guidelines
- Technological background
  - Exponential progress: molecular biology and robots
- Quality assurance, traceability
- Global increase of antimicrobial resistance
Reduction of time for microbial detection and identification

“NEED FOR SPEED”
Desirable improvement

Rapid identification of a pathogen

Prime importance for effective provision of care to patients with infections

The faster you identify pathogens, the quicker you can react to it, implementing
• Treatment
• Preventive measures and control of infections

Benefits are also for:
• The community
• Hospital
• Control measures

Theranostic approach

“Process of diagnostic therapy for individual patients”

Cost-effective

Turnaround time

Optimized management of patient and infectious diseases

Identification

AST

Delayed:
• High Sensitivity
• High Specificity for clinicians!

Cost-effective

Delayed:
• Full automation with internal QC
• Easy to perform, to interpret
• Reduced training

Specimen collection of specimen

Identification of relevant pathogens

Microbiological diagnostics of syndromic diseases

Syndromic diseases
• Characterized by the abnormal presence, simultaneously, of a group of signs and symptoms

- Respiratory tract infections
  - Pneumonia, bronchiolitis, sore throat, etc.
- Gastro-enteritis
- Sexually transmitted diseases
- Etc.

Bacteria, fungi, viruses, parasites
Microbiological diagnostic approaches

- Conventional (aetiological) approach
  - « Is a specific pathogen present in the specimen? »
  - Step by step, on demand (primarily directed to typical bacteria)
  - Varied individual methods
  - TAT: minutes to days or even weeks

- Syndrome-based approach
  - « Which pathogen is causing this syndrome? »
  - Broad panel diagnostic method (including atypical agents, viruses, fungi, parasites)
  - All inclusive testing system
  - TAT: hour(s)

Point of-care-test platforms for early diagnosis of infection

To provide an integrated, holistic solution addressing technological challenges

- For rapid detection of bacteria, mycobacteria, fungi, viruses, host markers and resistance to antimicrobial drugs
- To enhance clinical decision-making
- To improve quality of care and clinical outcomes
- To improve targeted therapy and reduce overuse
  - Specific probes
  - Novel methods of sample preparation
  - Ultra-high sensitive detection methods

Results availability in less than 2 hours/30 min for IN/OUT patients

Current diagnostic landscape, unmet needs and emerging technologies

LOWER RESPIRATORY TRACT INFECTIONS
### Aetiological agents of LRTI in the community (%)

<table>
<thead>
<tr>
<th>Reference</th>
<th>n</th>
<th>S. pn</th>
<th>H. infl</th>
<th>M. pc</th>
<th>C. pn</th>
<th>Virus</th>
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<tbody>
<tr>
<td>Boldt et al. 1990</td>
<td>42</td>
<td>3.0</td>
<td>3.0</td>
<td>8.0</td>
<td>0</td>
<td>21.0</td>
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<tr>
<td>Creer et al. 2006</td>
<td>80</td>
<td>18.8</td>
<td>6.3</td>
<td>1.2</td>
<td>61.3</td>
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<tr>
<td>Graffelman et al. 2004</td>
<td>145</td>
<td>6.2</td>
<td>9.0</td>
<td>9.0</td>
<td>1.3</td>
<td>39.0</td>
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<tr>
<td>Holm et al. 2007</td>
<td>364</td>
<td>48.4</td>
<td>9.4</td>
<td>1.2</td>
<td>&lt;1</td>
<td>24</td>
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<tr>
<td>Hopstaken et al. 2005</td>
<td>247</td>
<td>2.9</td>
<td>13.8</td>
<td></td>
<td></td>
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<tr>
<td>Macfarlane et al. 1993</td>
<td>206</td>
<td>30.0</td>
<td>8.0</td>
<td>0.5</td>
<td>8.0</td>
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<tr>
<td>Meier-Leibnitz et al. 2001</td>
<td>316</td>
<td>17.1</td>
<td>9.8</td>
<td>7.3</td>
<td>17.4</td>
<td>19.3</td>
</tr>
<tr>
<td>GRACE study, 2012</td>
<td>3059</td>
<td>9.1</td>
<td>14.8</td>
<td>2.9</td>
<td>2.2</td>
<td>51.1</td>
</tr>
</tbody>
</table>

Range: 3-30 to 3-15, 0.5-9 to 0.0-17, 8-61

* C. pn is in some studies reported in a large nr of cases: 0-20%
* Early data are largely based on serological analysis only


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### Conventional diagnostic methods

**Aetiological agents**

- **Classical bacteria**
  - *Streptococcus pneumoniae* *
  - *Haemophilus influenzae* *
  - *Moraxella catarrhalis* *
  - *Mycobacterium tuberculosis* *

- **Atypical bacteria**
  - *Mycoplasma pneumoniae*
  - *Chlamydophila pneumoniae*
  - *Legionella pneumophila*
  - *Chlamyd. trachomatis*
  - *Coxiella burnetii*

- **Opportunistic bacteria**
  - *Pseudomonas aeruginosa*
  - *Staphylococcus aureus* *
  - *Enterobacteriaceae*

- **Virus**
  - Influenza
  - Parainfluenza
  - RSV
  - Adenovirus
  - Human metapneumovirus
  - Rhino-enterovirus
  - Coronavirus
  - Bovivirus
  - Etc.

- **Fungi**
  - *Aspergillus spp*
  - *Candida spp*
  - *Pneumocystis jiroveci*
  - *Cryptococcus neoformans*
  - *Autres fungi*

* Frequent transient colonization of upper RT

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**Conventional culture-based methods**

(addressed mainly to Bacteria)

**Value of sputum cultures**

- Good Sensitivity ang NPV, if predominant morphotype
- Low specificity and PPV, due to contamination
- except for bacteria not part of normal flora (eg. *Legionella, M. tuberculosis*)
- Relevant for colonizing organisms: if correlated with predominant organisms identified on Gram stain

**Value of blood cultures in the diagnosis of CAP**

- Specificity: very high (100%)
- Sensitivity: low, positive in 4-29% of untreated cases
  - Most sensitive for *S. pneumoniae*, less for *H. influenzae* and other pathogens
  - But... easy to sample and often the only source of information!
Conventional diagnostic methods
Rapid Antigen testing for LRTI

- Urinary antigen tests
  - *Legionella pneumophila* serogroupe 1
  - *Streptococcus pneumoniae* (not in children)
- Antigen tests for respiratory specimens
  - Influenza A and B
  - RSV
  - Para-influenza1-4
  - Adenoviruses
  - Human-metapneumovirus

Sensitivity depends on specimen type, sampling method and microscopist's competence.

Diagnostic tests for *Legionella*

<table>
<thead>
<tr>
<th>Test</th>
<th>Time needed</th>
<th>Specimen</th>
<th>Sensitivity</th>
<th>Specificity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blood</td>
<td>3-7 days</td>
<td>LRT</td>
<td>&lt;10-80</td>
<td>100</td>
</tr>
<tr>
<td>Fluorescent antibody staining</td>
<td>&lt;4 hrs</td>
<td>LRT</td>
<td>25-70</td>
<td>&gt;95</td>
</tr>
<tr>
<td>Antigen detection</td>
<td>&lt;1 hr</td>
<td>Urine</td>
<td>70-90</td>
<td>&gt;99</td>
</tr>
<tr>
<td>PCR</td>
<td>&lt;4 hrs</td>
<td>Serum</td>
<td>80-100</td>
<td>&gt;90</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Urine</td>
<td>40-60</td>
<td>&gt;90</td>
</tr>
</tbody>
</table>

Ag *L. pneumophila* Positive influence on management, correct treatment immediately started but many drawbacks.

Molecular diagnostic methods
Choice of platforms and assays

- Commercial versus "in house tests"
  - Degree of validation and standardization
  - Variability between laboratories
  - Internal controls
  - Quantification standards
  - Requirements ISO 15189
- Mainly for viral, atypic bacterial and fungal targets
  - Qualitative test
From Pr. Greet Ieven

**Multiplex all inclusive tests and system**

**GenMark Diagnostics**
- Respiratory panel of 14 viral targets
  - Multiplex PCR
  - Electrochemical detection
  - 3.5 hours

**Curetis Unvero system**
- Pneumonia panel: 16 bacteria + 1 Fungus and 22 antibiotic resistance markers
  - Endpoint PCR
  - Array format
  - 4 hours

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**Biofire (bioMerieux)**

**JVDs: All Inclusive Systems for Multiplex syndromic approach**
- System integrates sample preparation, amplification, detection and analysis
- All reagents freeze dried in one pouch
- Closed system prevents cross-contamination
- Internal controls for each step
- Advanced software runs the system, automatically analyzes and reports results
- Multiplexed testing analyzes up to >20 targets per sample
- Rapid results in 1 hour from sample injection

**C4L platform Coris (EU funded project)**
- **C4L Prototype**
  - Influenza A, Influenza B, RSV A, RSV B, Human metapneumovirus, Rhinovirus
  - *Bordetella pertussis, Mycoplasma pneumoniae*

- **C4L Prototype compared with the RespiFinder 2SMART assay**
  - In CHU Liège
    - Bronchoalveolar lavages (adults) and nasopharyngeal aspirates (mixed population)
    - 82% agreement
  - In UZ Antwerpen
    - Mainly nasopharyngeal aspirates (children)
    - 84% agreement
**C4L platform Coris (EU funded project)**

- **C4L Prototype**
  - Influenza A, Influenza B, RSV A, RSV B, Human metapneumovirus, Rhinovirus
  - *Bordetella pertussis, Mycoplasma pneumoniae*

- **C4L Prototype compared with the RespiFinder 2SMART assay**
  - Lack of sensitivity
    - for Rhinovirus
    - in mixed infection
  - Improved rate of detection of clinically significant pathogens when compared to conventional approach
    - For viral and atypical targets

**TAKE-HOME MESSAGES**

*Multiplex syndromic approach*

- Reduction of TAT
- Increased rate of detection for a wide panel of aetiological agents
  - Improved management of patients with severe infections
  - Initiation more rapidly the appropriate rational use of antibiotics
  - Avoidance of unnecessary antibiotherapy
    - Cost avoidance
  - Implementation of control measures for contagious agents
- Complementary to conventional methods
- C4L prototype platform and chips
- Could be used in the lab as a POCT