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

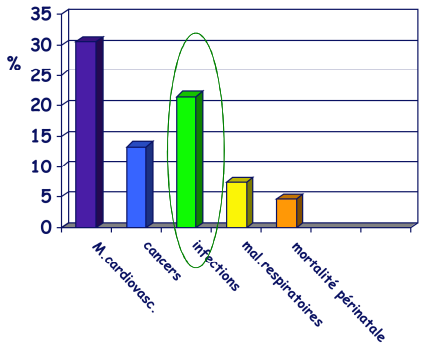
CONTENT

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

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

INTRODUCTION

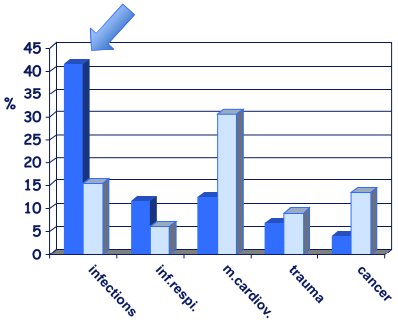
Causes of mortality *(WHO 2008)*



Global death rate related to infections = 20-25%

INFECTIONS = second cause

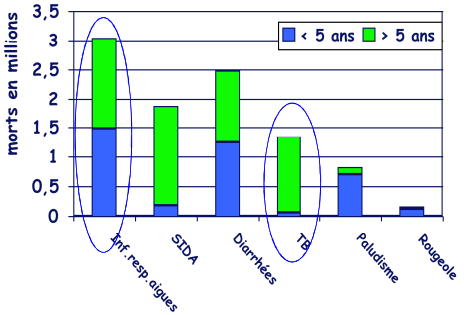
Causes of mortality *(WHO 2008)*



Low income countries (Africa, Asia, …) death rate related to infections = 40%

INFECTIONS = first cause

« Top » of deadly infectious diseases, *WHO 2008*



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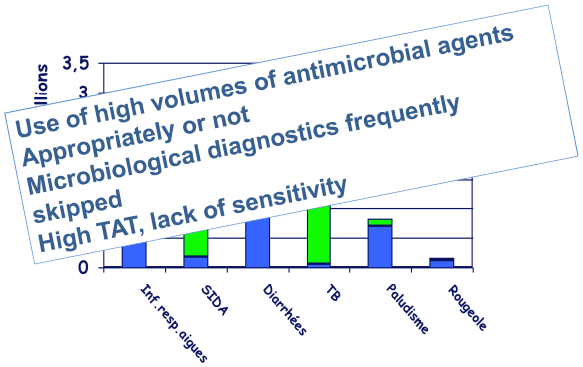
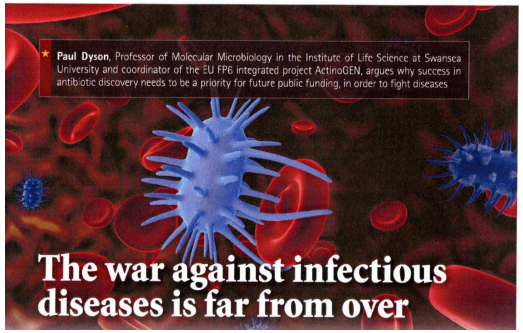


Worldwide major threat: Bacteria are doing resistance

Global increase of antimicrobial resistance
Emerging superbug



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 worlwide pandemic

- To facilitate stewardship for rational use of antimicrobial agents when needed
- ….

Clinical microbiology laboratory Primary missions

TO IMPROVE THE MANAGMENT 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

Theranostic approach

« Process of diagnostic therapy for individual patients »

€€€ Cost-effective

Turnaround time
collection of specimen

- Full automation
- With internal QC
- Easy to perform, to interprete
- Reduced training

Optimized management of patient and Infectious diseases

Specimen Analysis: Relevant pathogens

Identification
AST

- High Sensitivity
- High Specificity

Delayed r *for clinicians !*

Rapid identification of a pathogen

Prime importance for effective provision of care to patients with infections

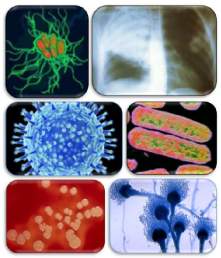
The faster you identify pathogens,
the quicker you can react to it, implementing

- **Treatment**
- **Preventive measures and control of infections**

Benefits are also for
The community, hospital and control measures

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 increased 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

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Huge challenges and synergies:
Biotechnologies, microtecnologies and clinical practice

Current diagnostic landscape ,
unmet needs and emerging technologies

LOWER RESPIRATORY TRACT INFECTIONS

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***

* *Frequent transient colonisation of upper RT*

- **Virus**
  - Influenza
  - Parainfluenza
  - RSV
  - Adenovirus
  - Human metapneumovirus
  - Rhino/enterovirus
  - Coronavirus
  - Bocavirus
  - Etc.
- **Fungi**
  - *Aspergillus spp*
  - *Candida spp*
  - *Pneumocystis jirovecii*
  - *Cryptococcus neoformans*
  - Autres *fungi*

Aetiological agents of LRTI in the community (%)

Reference	n	S. pn	H. infl	M. pn	C.pn	Virus
Boldy et al. 1990	42	3.0	3.0	8.0	0	21.0
Creer et al. 2006	80	18.8	6.3	1.2		61.3
Graffelman et al. 2004	145	6.2	9.0	9.0	1.3	39.0
Holm et al. 2007	364	6	4	3	<1	24
Hopstaken et al. 2005	247	2.9	13.8			
Macfarlane et al. 1993	206	30.0	8.0	0.5		8.0
Macfarlane et al. 2001	316	17.1	9.8	7.3	17.4	19.3
GRACE study, 2012	3059	9.1	14.8	2.9	2.2	51.1
Range		**3-30**	**3-15**	**0.5-9**	**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**

Woodhead M et al. Clin. Microbiol.Infect. 2011; 17, E1-E59

Conventional diagnostic methods

- **Direct microscopy - Gram stain**
  - **For detection of *S.pneumoniae, H.influenzae***
    - **Low Sensitivity ang NPV**
    - **High specificity and PPV**
  - **Quality control of sputum sample**

Old, simple, cheap & rapid diagnostic test for pneumonia

Helps in guiding antibiotic treatment in +/- 25% of patients

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*

Test	Time needed	Specimen type	Sensitivity %	Specificity %
Culture	3-7 days	LRT Blood	<10-80 < 10	100 100
Fluoresc antibody staining	< 4 hrs	LRT	25-70	>95
Antigen detection	**< 1 hr**	**Urine**	**70-90**	**>99**
Serology	3-10 weeks	Serum	60-80	>95
PCR	**< 4 hrs**	**LRT** Serum Urine	**80-100** 30-50 46-86	**> 90** > 90 > 90

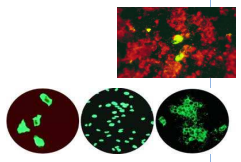
Limited to *L.pneumophila* serogroup1

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

Conventional diagnostic methods
Rapid Antigen testing for LRTI

- **Urinary antigen tests**
  - *Legionella pneumophila* serogroupe 1
  - *Streptococcus pneumoniae*
- **Antigen tests for respiratory specimens** (single or pooled)
  - Influenza A and B
  - RSV
  - Para-influenza1-4
  - Adenoviruses
  - Human-metapneumovirus

Sensitivity depends on specimen type, sampling method and microscopist's competence or other immuno-assay methods



Molecular diagnostic methods
Choice of platforms and assays

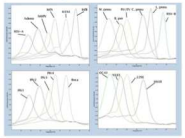
- Commercial **versus** « in house tests »
  - **Degree of validation and standardization**
  - **Variability between laboratories**
  - **Internal controls**
  - **Quantification standards**
  - **Requirements ISO 15189**
- **Mainly for viral, atypic bacterial and fungal targets**
  - **Qualitative test**

Some commercially Multiplex tests

Cepheid GeneXpert

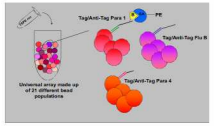
Single use cartridge based
Up to 6 targets
TAT 1.5 to 2/5 hr

RespiFinder Mx, Pathofinder



Mx amplification and detection by melting curve analysis
Up to 25 targets
TAT 6 hr

Luminex xTAG Universal Bead Array



Liquid microarray based
Up to 20 targets
RVP 10-12 hr to RVP Fast 4-5 hr

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IVDs: 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

Viral: Adeno, Boca, Corona (229E, HKU1, OC43,NL63), Flu A, Flu A H1, Flu A H1 2009, Flu A H3, Flu B, hMPV, Para (1, 2, 3, 4), RSV, Rhino
Bacterial: *B. pertussis, C. pneumoniae, M. pneumoniae*

Biofire (bioMerieux)

From Pr. Greet Ieven

Multiplex all inclusive tests and system

GenMark Diagnostics

Respiratory panel of 14 viral targets
- Multiplex PCR
- Electrochemical detection
- 3 1⁄2 hours

Curetis Univero system

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

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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**
    - **Broncho alveolar lavages (adults) and nasopharyngeal aspirates (mixed population)**
    - **82 % agreement**
  - **In UZ Antwerpen**
    - **Mainly nasopharyngeal aspirates (children)**
    - **84 % agreement**

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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**

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TAKE-HOME MESSAGES

Mutations & a new culture are necessary to enjoy over the future of microbiology

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**

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