Network di Microbiologia e Virologia del Nord Est

INFEZIONI DELLE BASSE VIE RESPIRATORIE (LRTI):
ASPETTI CLINICI E DIAGNOSI MICROBIOLOGICA

14 dicembre 2012
Palazzo Montereale-Mantica
Pordenone

Dalla Diagnostica Tradizionale Ai Nuovi Metodi:

Appropriatezza del percorso analitico ed interpretativo

Manuela Avolio
Microbiologia Clinica e Virologia-Pordenone

- [x] WHY ETIOLOGIC DIAGNOSIS?
- [x] MICROBIOLOGICAL APPROPRIATENESS
- [x] CURRENT DIAGNOSTIC METHODS
- [x] ALTERNATIVE DIAGNOSTICS
- [x] PROSPECTIVES

WHY AN ETIOLOGIC DIAGNOSIS?

At present there is still a great deficit in the etiologic diagnosis of community-acquired lower respiratory tract infections (LRTI); in most studies more than 50% of cases remain without an etiologic diagnosis resulting in unnecessary or inappropriate antibiotic prescribing.

Ieven M, J Clin Virol 40 (2007) 259-276

JOURNAL OF CLINICAL MICROBIOLOGY, Jan. 2009, p. 21–31 Vol. 47, No. 1
0095-1137/09/$08.00+0 doi:10.1128/JCM.02037-08

MINIREVIEW

Optimal Sampling Sites and Methods for Detection of Pathogens Possibly Causing Community-Acquired Lower Respiratory Tract Infections▽†

K. Loens,* L. Van Heirstraeten, S. Malhotra-Kumar, H. Goossens, and M. Ieven

Acute respiratory tract infections (RTIs), both upper (URTIs) and lower respiratory tract infections (LRTIs), are the most common reason for consultation with a general practitioner. RTIs result in about 180 million antibiotic prescriptions per year in the EU-27 member states (ESAC website, 2008; www.esac.ua.ac.be), and 6.4 million antibiotic prescriptions were prescribed for acute bronchitis and cough in 2003 in adults between 16 and 64 years old in the United States (65).

The number of pathogens involved in LRTI, with various susceptibilities to antimicrobials, is large constituting an enormous challenge for diagnostic microbiology. In general, in only 50% of cases is an etiologic agent detected. Documented infection is uncommon in community-managed infection and is usually only defined in 25 to 50% of hospital-managed infections.

The number of pathogens involved in LRTI
Is LARGE constituting an enormous
challenge for diagnostic microbiolgy

WHY AN ETIOLOGIC DIAGNOSIS?

Microbiological diagnosis is crucial in the management of hospital-acquired pneumonia (HAP) and ventilator-associated pneumonia (VAP) [1,2]. Many studies argue that an early appropriate pathogen-directed antimicrobial therapy improves outcomes [3–5]. The episodes of bacterial cause in HAP/VAP due to the characteristics of prevailing bacteria and emerging drug resistance require a rapid and reliable diagnosis to help establish the most suitable treatment [6▪▪].

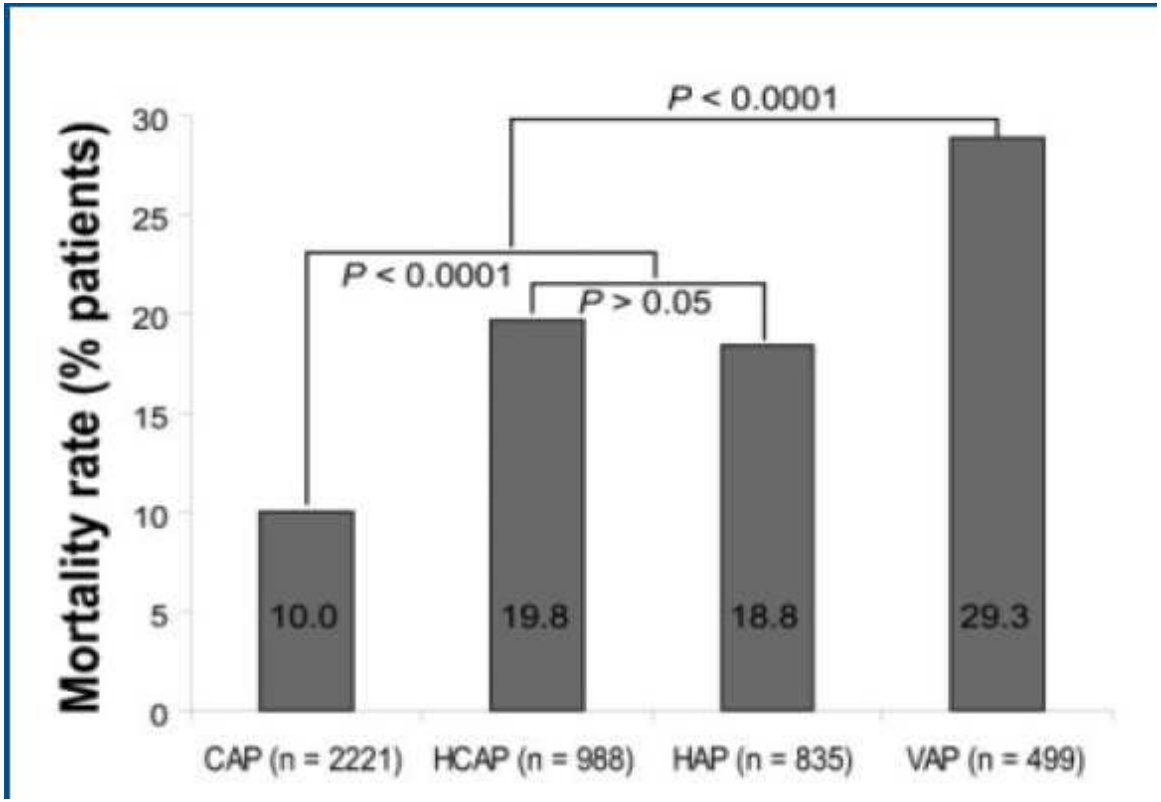
ACCURATE

RELIABLE

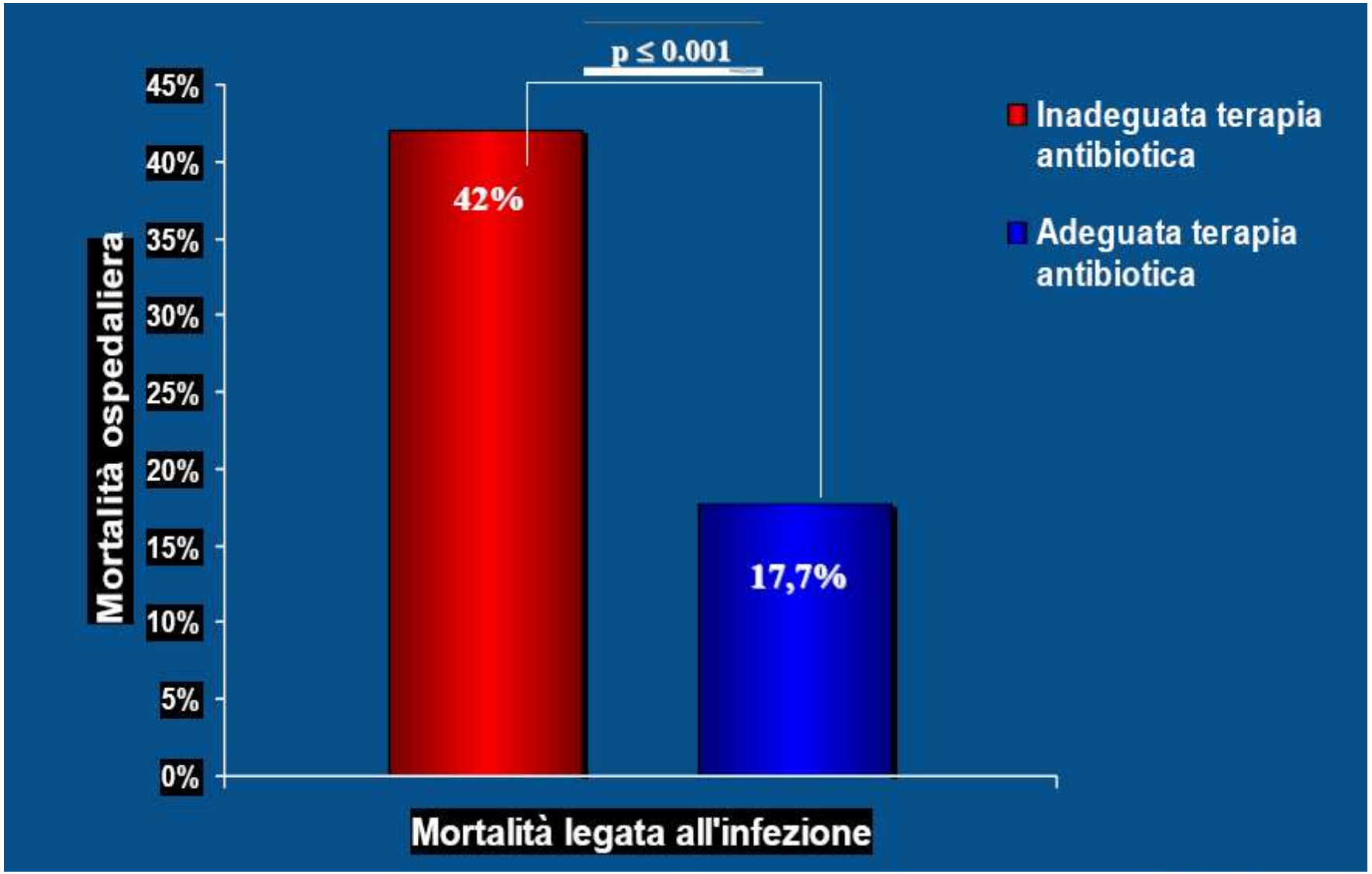
RAPID

Lung M, Codina G. CO Critical Care (2012), 18(5), 488-493

LRTI MORTALITY



Kollef MH et al., Chest 2005 Dec;128(6):3854-62.



Kollef MH et al., Chest 1999 Feb;115(2):462-74.

WHY AN ETIOLOGIC DIAGNOSIS?

THE DIFFICULTIES OF TREATING HOSPITAL-ACQUIRED PNEUMONIA

Acute bacterial pneumonia in hospitalized patients remains one of the most serious infections that physicians treat. Hospital-acquired pneumonia (HAP) is the second most common nosocomial infection and accounts for ~25% of all infections in the intensive care unit. According to the American Thoracic Society (ATS) and Infectious Diseases Society of America (IDSA), HAP occurs at a rate of 5–10 cases per 1,000 hospital admissions, with the incidence increasing by as much as 6–20-fold among mechanically ventilated patients [1]. Although the incidence of HAP varies depending on how each study defines this entity, ATS estimates that HAP accounts for >50% of the antibiotics prescribed [1–4]. Despite significant advances in antimicrobial chemotherapy (ie, the introduction of very potent antibiotics), patient support services, and radiological imaging, HAP still carries considerable morbidity and mortality (range, 25%–50%), and approximately one-half of all HAP-related deaths are directly attributable to pneumonia [2–4]. The microbiological identification of the pathogen lies at the center of this problem.

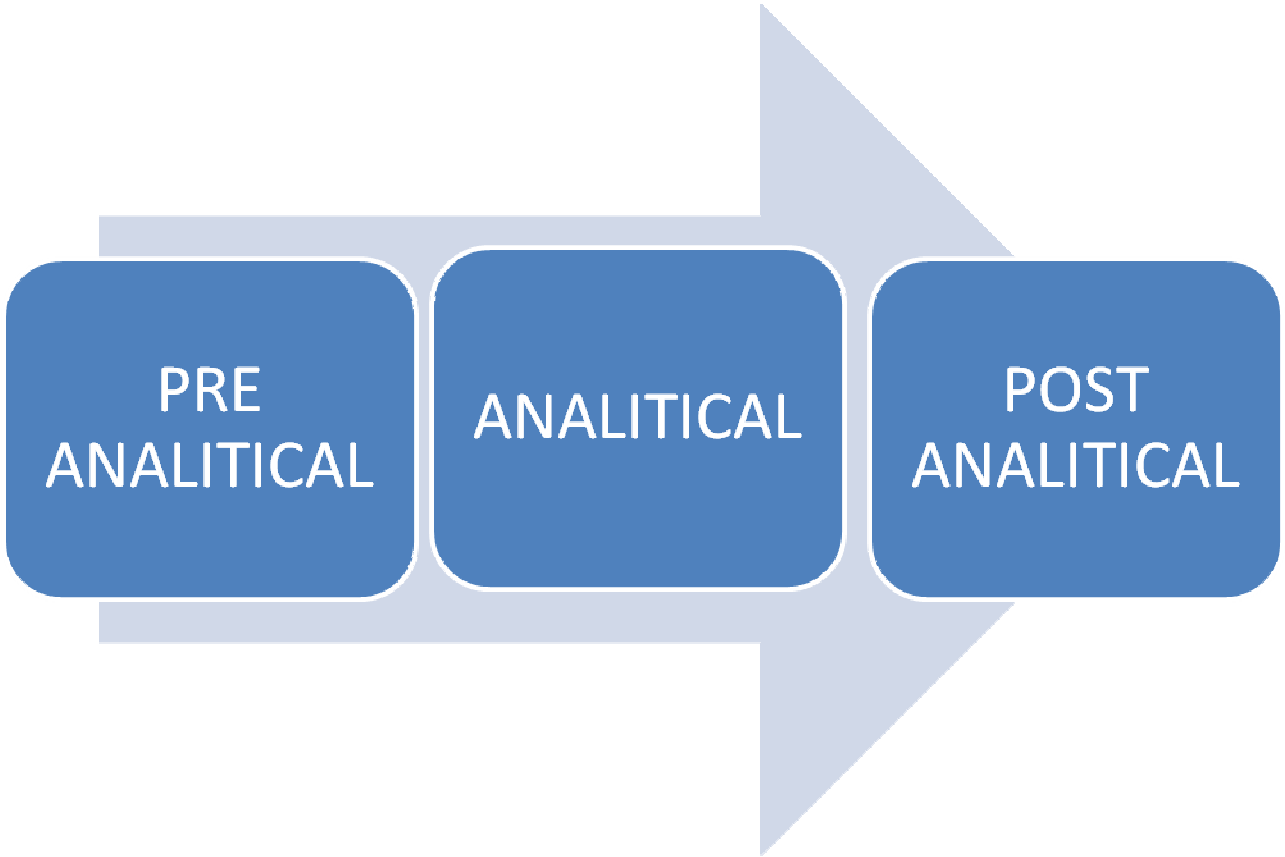
Endimiani et al. CID (2011) 25, 373-383

WHY A RAPID ETIOLOGIC DIAGNOSIS?

one key to reducing unnecessary or inappropriate antimicrobial use is the rapid identification of causative pathogens.

Rice L.B. CID 2011: 52 (4) 357-360

MICROBIOLOGICAL APPROPRIATENESS



UN REFERTO CLINICAMENTE EFFICACE

- **Identificare** l'agente eziologico e saggiare la sensibilità agli antibiotici per indirizzare il clinico verso la terapia ottimale.
- **Limitare** l'eccessivo uso di antibiotici qualora la diagnosi non venga confermata
- **Riconoscere** i germi resistenti per i quali vi sono pochi nuovi antibiotici efficaci
- **Monitorare** il trend eziologico e la sensibilità agli antibiotici

Clinical microbiology services involvement in AMS

The clinical microbiology service is an essential and integral part of a wide range of organisational initiatives that underpin antimicrobial stewardship efforts.

The Clinical Microbiology Services participate in a range of clinical AMS activities

- Establish guidelines to limit unnecessary susceptibility testing and to relate results to the site of infection;
- Update local antibiograms with pathogen-specific susceptibility data at least annually;
- Implement new technologies to enable rapid analysis of specimens to either rule out or rule in infection.
- Use selective reporting of antimicrobial susceptibilities;

Australian Commission on Safety and Quality in Healthcare, 2011.
http://www.safetyandquality.gov.au

SCELTA DELLE MOLECOLE, EQUIVALENZA E REFERTAZIONE

1. microrganismi, meccanismi di resistenza attesi, sede di infezione.
2. non è possibile testare tutti gli antibiotici su tutti i ceppi.
3. sarebbe auspicabile seguire una logica sequenziale (secondo i criteri CLSI/EUCAST) refertando solo gli antibiotici testati come “marker” di equivalenza.
4. Refertare molecole equivalenti e farmaci di ultima generazione solo quando strettamente necessario

Rapid Diagnostics and Appropriate Antibiotic Use

Louis B. Rice

Louis Stokes Cleveland Veterans Affairs Medical Center and Case Western Reserve University School of Medicine, Cleveland, Ohio

antibiotic choices

The first questionable assumption is that physicians and other antibiotic prescribers (the majority of whom lack fellowship training in infectious diseases) are interested in and moved by laboratory results that inform antibiotic choices. As a way of exploring this assump-

As noted above, the mere availability of a test will not change physician practices when it comes to antibiotic prescribing. Educational programs will be required to convince physicians of the reliability of the test and of the potential deleterious consequences (legal and otherwise) of continued antimicrobial therapy when data are available that suggest it is not necessary. As such, research

deleterious consequences

Rice L.B. CID 2011: 52 (4) 357-360

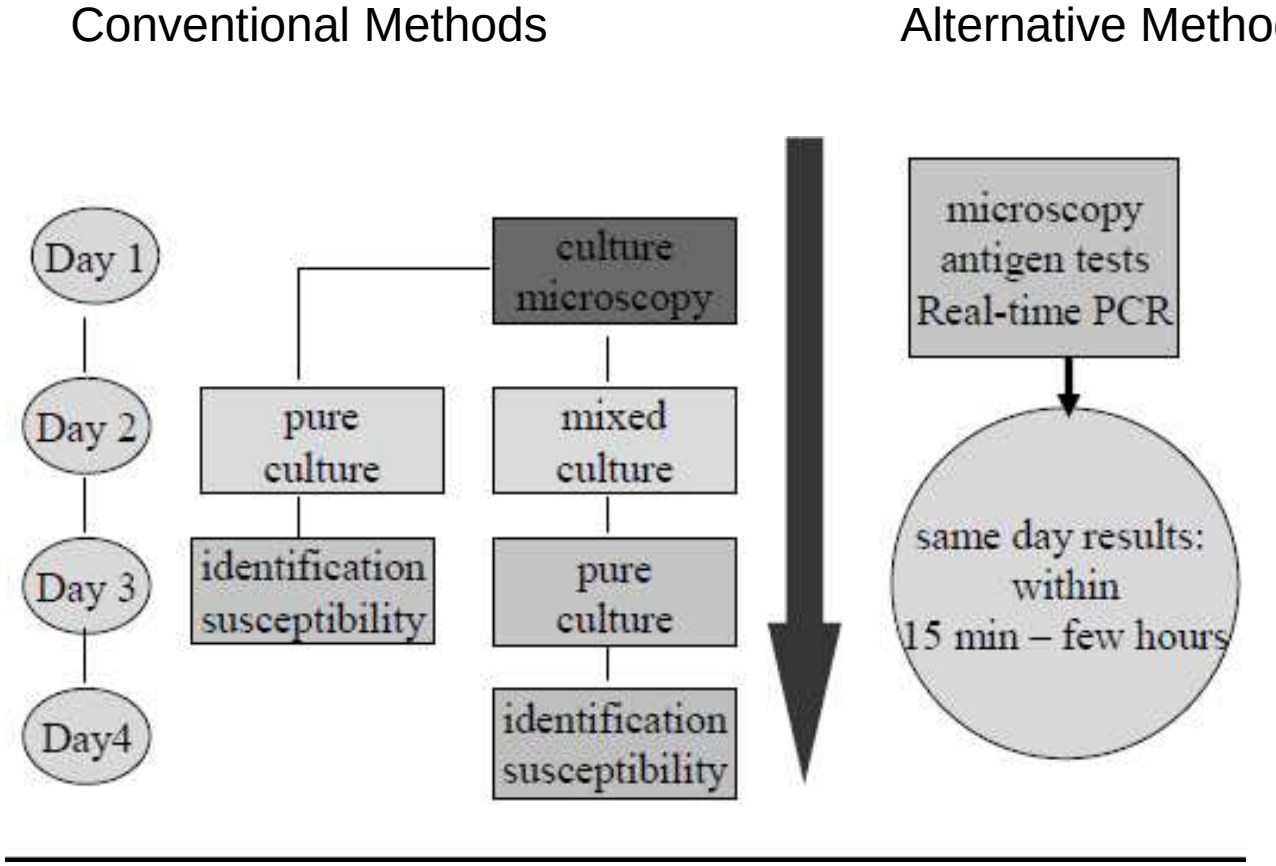
THE CHALLENGE IS A BETTER UNDERSTANDING OF

- **the relationship between use and resistance, to be able to adjust regimens in a way that will minimize resistance**
- **Availability of rapid and reliable tests**
- **How to use molecular and microbiological data to inform therapeutic choices.**

Rice L.B. CID 2011: 52 (4) 357-360


KNOWLEDGE AND SELECTION OF THE MOST APPROPRIATE METHODS OF INVESTIGATION AMONG STANDARD OR ALTERNATIVE

Time requirements for Microbiological Diagnostic Results

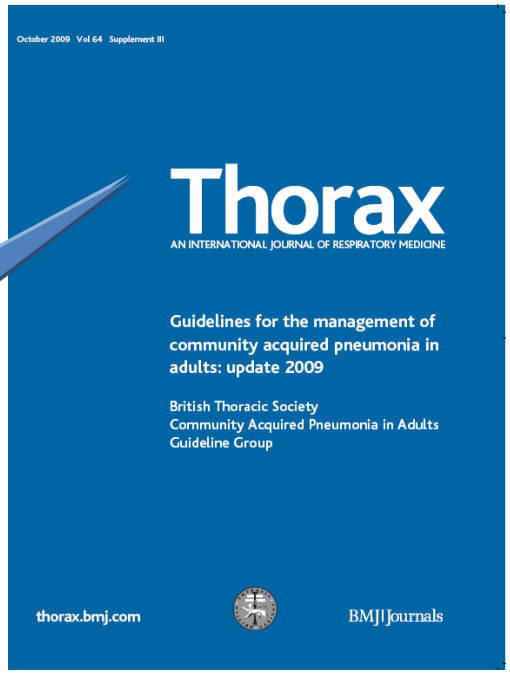


American Thoracic Society Documents

Guidelines for the Management of Adults with Hospital-acquired, Ventilator-associated, and Healthcare-associated Pneumonia

This official statement of the American Thoracic Society and the Infectious Diseases Society of America was approved by the ATS Board of Directors, December 2004 and the IDSA Guideline Committee, October 2004

2005



2009

Infectious Diseases Society of America/American Thoracic Society Consensus Guidelines on the Management of Community-Acquired Pneumonia in Adults

Lionel A. Mandell,[1,a] Richard G. Wunderink,[2,a] Antonio Anzueto,[3,4] John G. Bartlett,[7] G. Douglas Campbell,[8] Nathan C. Dean,[9,10] Scott F. Dowell,[11] Thomas M. File, Jr.[12,13] Daniel M. Musher,[5,6] Michael S. Niederman,[14,15] Antonio Torres[16] and Cynthia G. Whitney[11]

2007

2011 (update)

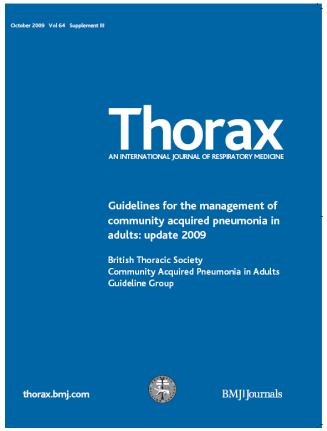
ORIGINAL ARTICLE 10.1111/j.1469-0691.2011.03602.x

Guidelines for the management of adult lower respiratory tract infections - Summary

M. Woodhead[1], F. Blasi[2], S. Ewig[3], J. Garau[4], G. Huchon[5], M. Ieven[6], A. Ortqvist[7], T. Schaberg[8], A. Torres[9], G. van der Heijden[10], R. Read[11] and T. J. M. Verheij[12] Joint Taskforce of the European Respiratory Society and European Society for Clinical Microbiology and Infectious Diseases

2011

COMMUNITY MANAGED CAP



What microbiological investigations should be performed in the community?

13. For patients managed in the community, microbiological investigations are not recommended routinely. [D]
14. Examination of sputum should be considered for patients who do not respond to empirical antibiotic therapy. [D]
15. Examination of sputum for *Mycobacterium tuberculosis* should be considered for patients with a persistent productive cough, especially if malaise, weight loss or night sweats, or risk factors for tuberculosis (eg, ethnic origin, social deprivation, elderly) are present. [D]
16. Urine antigen investigations, PCR of upper (eg, nose and throat swabs) or lower (eg, sputum) respiratory tract samples or serological investigations may be considered during outbreaks (eg, Legionnaires' disease) or epidemic mycoplasma years, or when there is a particular clinical or epidemiological reason. [D]

Eur Respir J 2005; 26: 1138–1180
DOI: 10.1183/09031936.05.00055705

ERS TASK FORCE IN COLLABORATION WITH ESCMID

Guidelines for the management of adult lower respiratory tract infections

M. Woodhead*, F. Blasi#, S. Ewig¶, G. Huchon+, M. Ieven§, A. Ortqvistƒ, T. Schaberg**, A. Torres##, G. van der Heijden¶¶ and T.J.M. Verheij¶¶

TABLE 9 Microbiological investigations in hospitalised patients with severe community-acquired pneumonia

Microbiological investigations
Blood culture
Sputum or lower respiratory tract sample for Gram stain and culture
Pleural fluid analysis
Urinary antigen test for *Legionella* spp. and *Streptococcus pneumoniae*
Respiratory samples for direct immunofluorescence for influenza and respiratory syncytial virus in winter months
Respiratory samples for culture or PCR for *Mycoplasma pneumoniae*, *Chlamydia pneumoniae* and *Legionella* spp. if well validated test available
Initial and follow-up serology for *Legionella* spp. and atypical pathogens if no PCR available; retrospective results

HOSPITAL MANAGED CAP

BLOOD CULTURE

Advantages

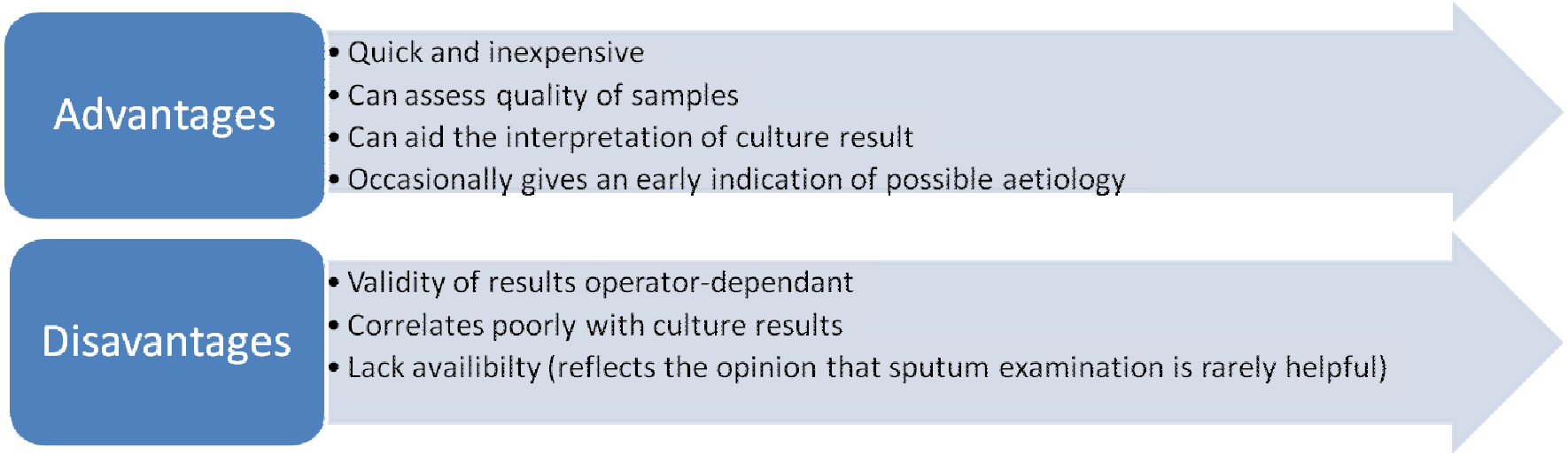
- Highly specific in determining the microbial aetiology
- Identify the causative agent including unexpected or antibiotic resistant pathogens
- Possibility to execute antimicrobial testing

Disavantages

- Prior to exposure to antibiotics
- Poor sensitivity (in pneumococcal pneumonia only 25%)

HOSPITAL MANAGED CAP

SPUTUM GRAM STAIN



HOSPITAL MANAGED CAP

SPUTUM CULTURES

Advantages

- Identify the causative agent including unexpected or antibiotic resistant pathogens
- Possibility to execute antimicrobial testing

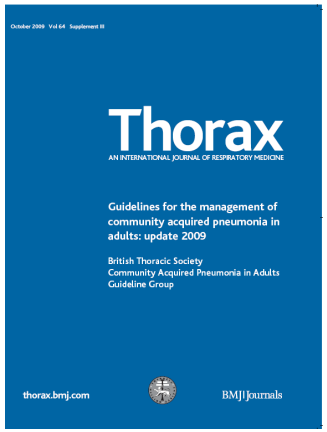
Disavantages

- Prior to exposure to antibiotics
- The inability of patient to produce good specimen
- Difficulty in interpretation dur to contamination by upper respiratory tract flora

Other tests for Streptococcus pneumoniae

25. Pneumococcal urine antigen tests should be performed for all patients with moderate or high severity CAP. [A−]
26. A rapid testing and reporting service for pneumococcal urine antigen should be available to all hospitals admitting patients with CAP. [B+]

97% specificità; 80% di sensibilità rispetto alla batteriemia da pneumococco, e 52 % rispetto alla coltura dell'espettorato



Tests for Legionnaires' disease

27. Investigations for legionella pneumonia are recommended for all patients with high severity CAP, for other patients with specific risk factors and for all patients with CAP during outbreaks. [D]
28. Legionella urine antigen tests should be performed for all patients with high severity CAP. [B+]
29. A rapid testing and reporting service for legionella urine antigen should be available to all hospitals admitting patients with CAP. [B+]
30. As the culture of legionella is very important for clinical reasons and source identification, specimens of respiratory secretions, including sputum, should be sent from patients with high severity CAP or where Legionnaires' disease is suspected on epidemiological or clinical grounds. [D] The clinician should specifically request legionella culture on laboratory request forms.
31. Legionella cultures should be routinely performed on invasive respiratory samples (eg, obtained by bronchoscopy) from patients with CAP. [D]
32. For all patients who are legionella urine antigen positive, clinicians should send respiratory specimens such as sputum and request legionella culture [D]. This is to aid outbreak and source investigation with the aim of preventing further cases.

For all patient with high severity of CAP

Eur Respir J 2005; 26: 1138–1180
DOI: 10.1183/09031936.05.00055705

ERS TASK FORCE IN COLLABORATION WITH ESCMID

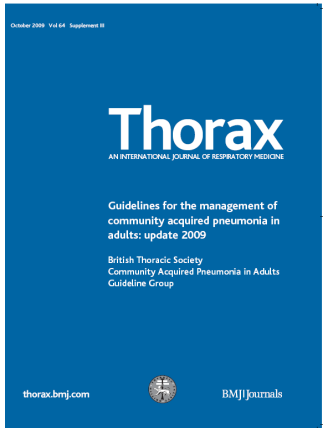
Guidelines for the management of adult lower respiratory tract infections

M. Woodhead*, F. Blasi#, S. Ewig¶, G. Huchon+, M. Ieven§, A. Ortqvistƒ, T. Schaberg, A. Torres##, G. van der Heijden¶¶ and T.J.M. Verheij¶¶**

TABLE 9 Microbiological investigations in hospitalised patients with severe community-acquired pneumonia

Microbiological investigations

- Blood culture
- Sputum or lower respiratory tract sample for Gram stain and culture
- Pleural fluid analysis
- Urinary antigen test for *Legionella* spp. and *Streptococcus pneumoniae*
- Respiratory samples for direct immunofluorescence for influenza and respiratory syncytial virus in winter months
- Respiratory samples for culture or PCR for *Mycoplasma pneumoniae*, *Chlamydia pneumoniae* and *Legionella* spp. if well validated test available
- Initial and follow-up serology for *Legionella* spp. and atypical pathogens if no PCR available; retrospective results



PCR and serological tests for other respiratory pathogens

38. Where PCR for respiratory viruses and atypical pathogens is readily available or obtainable locally, this is preferred to serological investigations. [D]

39. Where available, paired serology tests can be considered for patients with high severity CAP where no particular microbiological diagnosis has been made by other means (eg, culture, urine antigen, PCR) and who fail to improve, and/or where there are particular epidemiological risk factors. [D] The date of onset of symptoms should be clearly indicated on all serological request forms. [D]

40. Serological tests may be extended to all patients admitted to hospital with CAP during outbreaks and when needed for the purposes of surveillance. The criteria for performing serology tests in these circumstances should be agreed locally between clinicians, laboratories and public health. [D]

HCAP/HAP/VAP

American Thoracic Society Documents

Guidelines for the Management of Adults with Hospital-acquired, Ventilator-associated, and Healthcare-associated Pneumonia

THIS OFFICIAL STATEMENT OF THE AMERICAN THORACIC SOCIETY AND THE INFECTIOUS DISEASES SOCIETY OF AMERICA WAS APPROVED BY THE ATS BOARD OF DIRECTORS, DECEMBER 2004 AND THE IDSA GUIDELINE COMMITTEE, OCTOBER 2004

2005

Major Points and Recommendations for Diagnosis

3. Purulent tracheobronchitis may mimic many of the clinical signs of HAP and VAP, and may require antibiotic therapy, but prospective, randomized trials are needed (**Level III**) (180). Tracheal colonization is common in intubated patients, but in the absence of clinical findings is not a sign of infection, and does not require therapy or diagnostic evaluation (**Level II**) (40, 107).

Problema della colonizzazione della trachea nei pazienti intubati

5. All patients with suspected VAP should have blood cultures collected, recognizing that a positive result can indi-

Sempre emocolture

7. Samples of lower respiratory tract secretions should be obtained from all patients with suspected HAP, and should be collected before antibiotic changes. Samples can include an endotracheal aspirate, bronchoalveolar lavage sample, or protected specimen brush sample

Campioni respiratori: (TRASP, BAL,PSB)

ELSEVIER

Journal of Clinical Virology 40 (2007) 259–276

JOURNAL OF CLINICAL VIROLOGY

www.elsevier.com/locate/jcv

Review

Currently used nucleic acid amplification tests for the detection of viruses and atypicals in acute respiratory infections

Margareta Ieven*

Laboratory for Microbiology, Vaccine & Infectious Disease Institute (VIDI), University Hospital Antwerp, University of Antwerp, Wilrijkstraat 10, B-2650 Edegem, Belgium

Received 10 August 2007; accepted 20 August 2007

The availability and use of these new diagnostic tools in virology has contributed to a better understanding of the role of respiratory viruses in LRTI. The increasing importance of the viral agents, *Mycoplasma pneumoniae* and *Chlamydophila pneumoniae* in ARI is illustrated. A great proportion of ARI are caused by viruses, but their relative importance depends on the spectrum of agents covered by the diagnostic techniques and on the populations studied, the geographical location and the season. The discovery of new viruses is ongoing; examples are the hMPV and the increasing number of coronaviruses. Indications for the use of these rapid techniques in different clinical situations are discussed.

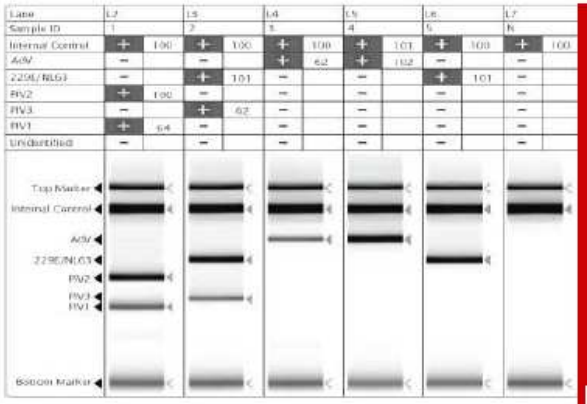
BETTER UNDERSTANDING OF THE ROLE OF RESPIRATORY VIRUSES IN LRTI

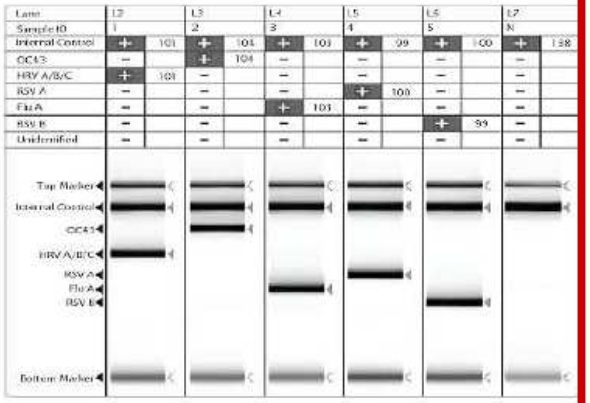
Seeplex®
RV 15 ONE STEP

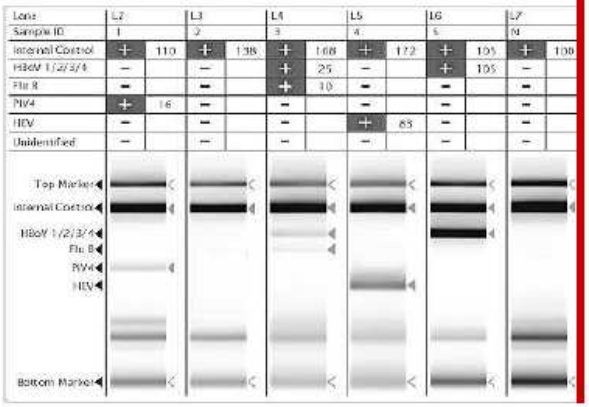
Parainfluenza virus 1
Parainfluenza virus 2
Parainfluenza virus 3
Adenovirus A/B/C/D/E
Coronavirus 229E/NL63

Coronavirus OC43
Rhinovirus A/B/C
Influenza A virus
RSV A
RSV B

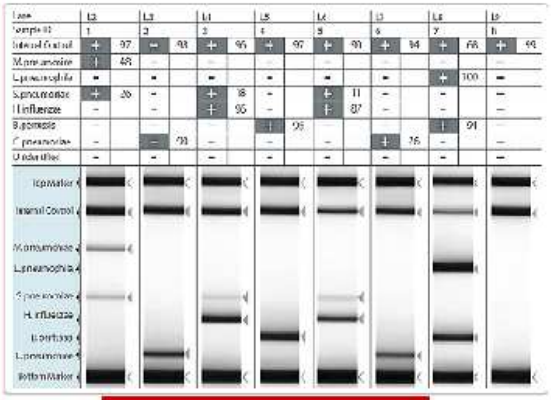
Bocavirus 1/2/3/4
Influenza B virus
Parainfluenza virus 4
Enterovirus







Dual Priming Oligonucleotide technology
(Seegene)



M.pneumoniae
C.pneumoniae
L.pneumophila
~~*S.pneumoniae*~~
~~*H.influenzae*~~
B.pertussis

Molecular diagnosis in HAP/VAP

Mayli Lung and Gema Codina

KEY POINTS

- The molecular diagnosis in hospital-acquired pneumonia (HAP)/ventilator-associated pneumonia (VAP) must provide accuracy and rapidity in the detection of pathogens in order to be considered as a valid tool to lead the antibiotic therapy.
- Significant advances have been made in the past years in the design of molecular diagnosis platforms based on respiratory infections. These platforms have become an additional option of diagnosis and sometimes have surpassed conventional methods.
- Potential applications have multiplex molecular methods in the detection of *Staphylococcus aureus*, nonfermenter Gram-negative bacilli, *Enterobacteriaceae* and antimicrobial resistance genes and/or virulence factors.
- It is still to be determined, on the basis of their performance characteristics, the most appropriate molecular methods for the microbiological diagnosis regarding the identification of potential pathogens in HAP/VAP.

CO Critical Care (2012), 18(5), 488-493

DIRECT DETECTION OF MULTIPLE ORGANISMS IN RESPIRATORY TRACT SAMPLES BY SYNDROME

Table 1. Potential Targets for Multiplex or Individual Molecular Amplification Assays by Syndrome [2, 4, 6, 8, 41]

CAP/exacerbations of COPD	HAP/VAP	Individual organisms
Streptococcus pneumoniae	*Staphylococcus aureus*	*Mycobacterium tuberculosis*
Haemophilus influenzae	*mecA* gene[a]	*Bordetella pertussis*
bla_{TEM} gene[b]	*Pseudomonas aeruginosa*	…
Moraxella catarrhalis	bla_{VIM}, bla_{IMP} genes[c]	…
Staphylococcus aureus	*Acinetobacter* spp	…
mecA gene[a]	bla_{OXA} genes[d]	…
Mycoplasma pneumoniae	Enterobacteriaceae	…
Chlamydophila pneumoniae	bla_{KPC} gene[c]	…
Chlamydophila psittaci	*Stenotrophomonas maltophilia*	…
Legionella pneumophila	…	…

NOTE. CAP, community-acquired pneumonia; COPD, chronic obstructive pulmonary disease; HAP, hospital-acquired pneumonia; VAP, ventilator-associated pneumonia.

[a] Mediates resistance to all β-lactam agents with the exception of the novel anti–methicillin-resistant *S. aureus* cephalosporins.

[b] Mediates resistance to penicillins and first-generation cephalosporins.

[c] Mediates resistance to cephalosporins and carbapenems; metallo-β-lactamases, such as VIM and IMP, typically do not mediate resistance to monobactams.

[d] Some OXA β-lactamases can mediate resistance to carbapenems.

Table 2. Summary of Selected Molecular Diagnostic Tests Discussed Here and Their Applications

Commercial kit/molecular assay (manufacturer)	Advantages	Application to bacterial pneumonia and/or point-of-care testing
GeneXpert System (Cepheid)	Detects MRSA in 1 h in blood cultures and wound swabs	Undetermined
AccuProbe (Gen-Probe)	Detects *Staphylococcus aureus, Streptococcus pneumoniae, Mycoplasma pneumoniae,* and *Legionella pneumophila*	Mostly for point-of-care *L. pneumophila* testing
GeneOhm (Becton-Dickinson)	Detects MRSA, MSSA, and CoNS	Undetermined
ResPlex and StaphPlex (Qiagen)	Detects *S. pneumoniae, Neisseria meningitidis, Haemophilus influenzae, L. pneumophila, M. pneumoniae, Chlamydophila pneumonia,* and *S.aureus*	Yes, but large clinical trials are needed for point-of-care *S. aureus* testing
Light Cycler (Roche)	Detects MRSA	Undetermined
MALDI-TOF MS/Autoflex II (Bruker Daltonic)	Protein-based assays with broad microbiological applicability	Undetermined
FilmArray systems (Idaho Technologies)	Detects *Bortedella pertussis, L. pneumophila, C. pneumoniae,* and *M. pneumoniae*	Undetermined
Check KPC/ESBL microarray (Check-Points)	Detects β-lactamase resistance genes conferring resistance to cephalosporins and carbapenems in 7–8 h	Undetermined
T5000 and PLEX-ID PCR/ESI-MS Biosensors (Abbott Molecular, Inc.)	Multiple species detected and typed and resistance genes mapped (*gyrA, parC, mecA,* and bla_{KPC})	Undetermined

Endimiani et al. CID (2011) 25, 373-383

LRTI: NEW PLATFORM FOR MOLECULAR BACTERIAL IDENTIFICATION

- GeneXpert (Cepheid), BD GeneOhm (Becton Dickinson): MRSA directly from nasal swab, blood, wound infections (1-2h).

- LightCycler (Roche): MRSA directly from blood (1-2h); 25 bacterial and fungal pathogens in blood (6h)

- Multiplex PCR FilmArray (Idaho Technologies): 17 respiratory viruses and Bordetella pertussis, Chlamydia pneumoniae, Mycoplasma pneumoniae: directly from unprocessed clinical samples (1h)

- MALDI-TOF MS Autoflex II (Bruker Daltonic): results in 1-2 min from nude bacteria or directly from some types of clinical samples.

Endimiani et al. CID (2011) 25, 373-383

WHAT DOES THIS ALL MEAN FOR US?

Right now, clinical trials are desperately needed to provide evidence to help us decide which methods are the best and how to apply this knowledge. Notwithstanding, we must also accept that

Endimiani et al. CID (2011) 25, 373-383

Prospects

Where available, PCR tests are an extremely useful addition to the diagnostic armamentarium and have the advantage of being rapid (relevant on occasions for both clinical and infection control purposes) and sensitive, and so are to be preferred over serological tests.

Pneumonia Guidelines Committee of the British Thoracic Society Standards of Care Committee.
British Thoracic Society guidelines for the management of community acquired pneumonia in adults: update 2009.
Thorax 2009; 64 (Suppl.III): iii1–iii55.

Optimization of laboratory strategy

Strategies will have to be developed adapting the evolution of the technology of the NAATs, the population of patients served (children, elderly, and immunocompromised patients) the resources available (infrastructure, staff, full-time service or service limited during some hours of the day, or some days of the week), the number and nature of the agents that can be covered.

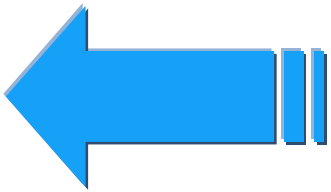
Ieven, M. J Clin Virol. 2007

In vivo 26: 469-472 (2012)

An Antibiotic Care Bundle Approach Based on Results of Rapid Molecular Screening for Nasal Carriage of Methicillin-resistant *Staphylococcus aureus* in the Intensive Care Unit

PAOLA STANO[1], MANUELA AVOLIO[1], RITA DE ROSA[1], MARIA LUISA MODOLO[1], STEFANO M.M. BASSO[1,2], FRANCO LUMACHI[4] and ALESSANDRO CAMPORESE[1]

concomitant bacterial infection (16). We found a significantly increased risk (RR=107.7, $p<0.0001$) of MRSA infection in patients with MRSA colonization, despite the relatively small population screened. Taken together, our results could underscore the importance of using the rapid molecular nasal screening for improving management and outcome of ICU patients. In fact, the real-time PCR assay is highly sensitive and quickly provides results to clinicians, leading to the better treatment of underlying infections (14).



LightCycler ® SeptiFast

Med Microbiol Immunol
DOI 10.1007/s00430-007-0063-0

ORIGINAL INVESTIGATION

A multiplex real-time PCR assay for rapid detection and differentiation of 25 bacterial and fungal pathogens from whole blood samples

Lutz Eric Lehmann · Klaus-Peter Hunfeld · Thomas Emrich · Gerd Haberhausen · Heimo Wissing · Andreas Hoeft · Frank Stüber

Received: 29 June 2007

Septi*Fast* Test validation

LightCycler ® SeptiFast

Gram (-)	Gram (+)	Miceti
Escherichia coli	*Staphylococcus aureus*	*Candida* spp. *(albicans, tropicalis, parapsilosis, glabrata, krusei)*
Klebsiella pneumoniae /oxytoca	*CoNS* (stafilococchi coagulasi neg)	*Aspergillus fumigatus*
Serratia marcescens	*Streptococcus pneumoniae*	
Enterobacter cloacae/aerogenes	*Streptococcus spp*	
Proteus mirabilis	*Enterococcus faecium*	
Pseudomonas aeruginosa	*Enterococcus faecalis*	
Acinetobacter baumanii		
Stenotrophomonas maltophila		

6h

Fig. 1.
DNAemia in patients with strong clinical suspect of sepsis.

Microrganism-DNA detected	N°	(SeptiFast+/BC+)	(SeptiFast+/BC-)	(BC not requested)
Asper.fumigatus	2	1	1	0
Candida albicans	7	1	1	5
Candida tropicalis	3	0	0	3
Coagulase Negative Staphylococci	2	1	0	1
Escherichia coli	67	41	16	10
Klebsiella pneumoniae/oxytoca	11	4	4	3
Enterobacter cloacae/aerogenes	6	3	1	2
Enterococcus faecalis	4	0	4	0
Proteus mirabilis	2	1	1	0
Pseudomonas aeruginosa	7	4	0	3
Serretia marcescens	1	0	0	1
Staphylococcus aureus	20	9	5	6
Streptococcus spp.	11	5	1	5
Stenotrophomonas maltophilia	1	1	0	0
Streptococcus pneumoniae	15	7	5	3
Staph.aureus/Kleb.spp	1	0	0	1
Staph.aureus/Str.pneum.	1	0	0	1
Staph.aureus/Ps.aeruginosa	1	0	0	1
Kleb.spp/Enterobacter spp.	1	0	0	1
E.coli / Kleb.spp	3	1	1	1
E.coli/ Ent.faecium	1	1	0	0
E.coli/Ps.aeruginosa	1	1	0	0
E.coli/Serr. marcescens	1	1	0	0
E.coli/Str.spp	1	1	0	0
tot	170	83	40	47

Severe LRTI

1

6

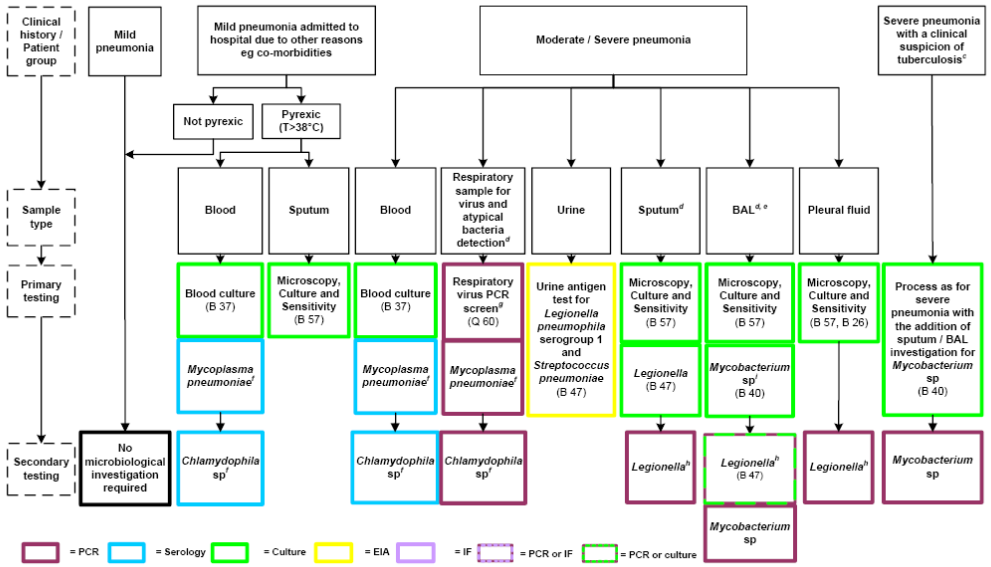
1

3

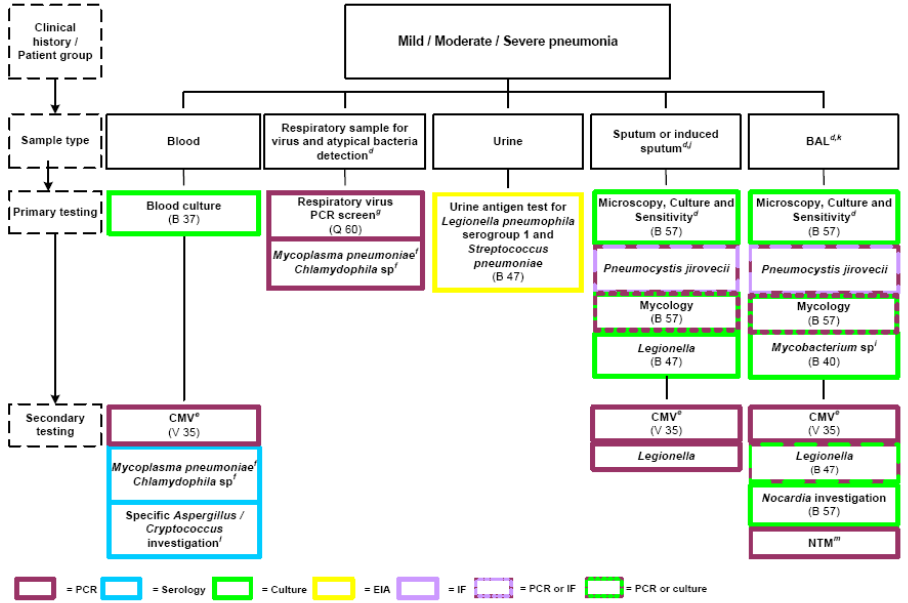
4

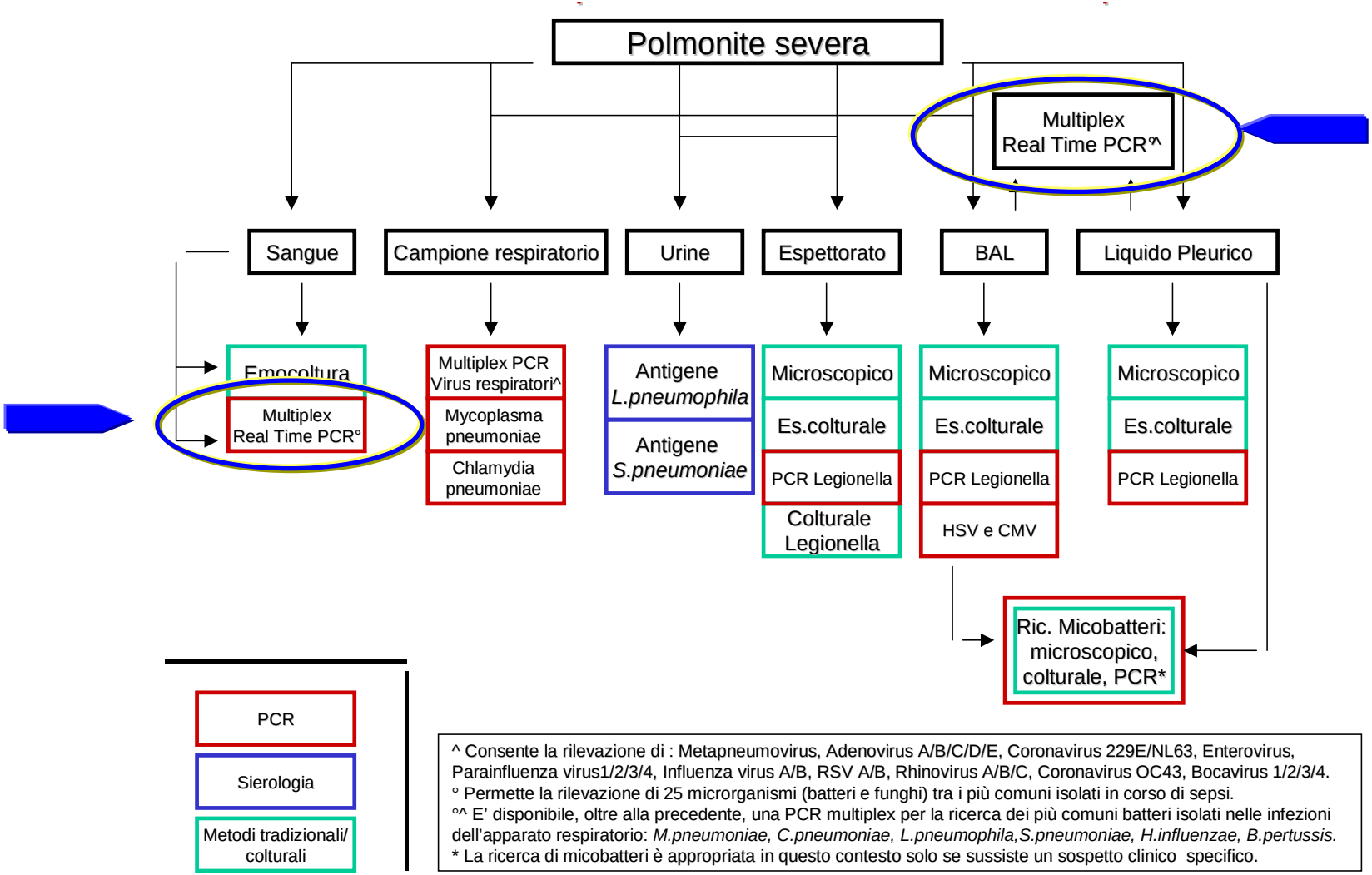
Avolio M. et al, 23°ECCMID, Berlin 2013, submitted

Pneumonia in immunocompetent adults[1-8, b]



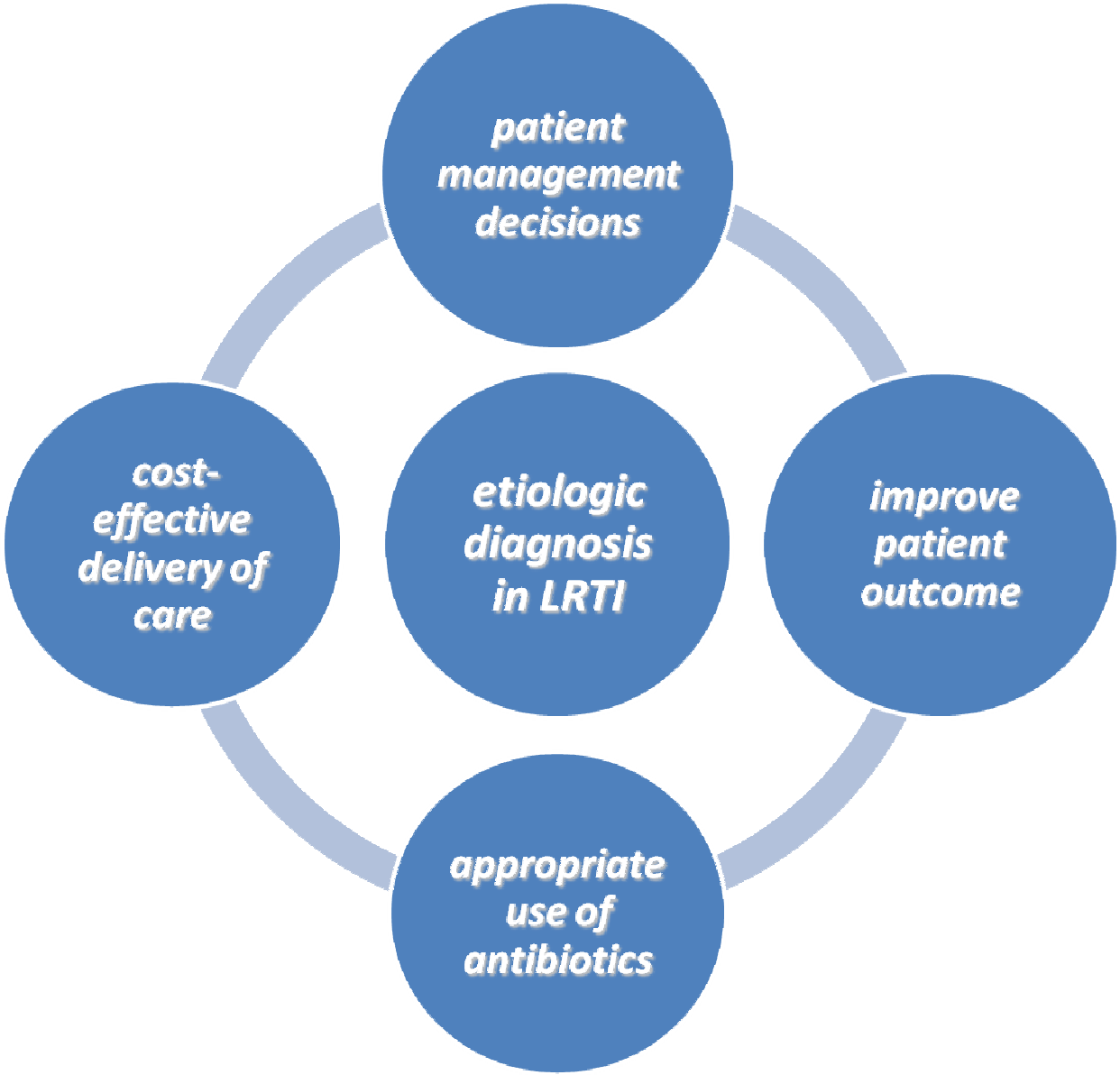
Pneumonia in immunocompromised adults[1-8, b]





^ Consente la rilevazione di : Metapneumovirus, Adenovirus A/B/C/D/E, Coronavirus 229E/NL63, Enterovirus, Parainfluenza virus1/2/3/4, Influenza virus A/B, RSV A/B, Rhinovirus A/B/C, Coronavirus OC43, Bocavirus 1/2/3/4.
° Permette la rilevazione di 25 microrganismi (batteri e funghi) tra i più comuni isolati in corso di sepsi.
°^ E' disponibile, oltre alla precedente, una PCR multiplex per la ricerca dei più comuni batteri isolati nelle infezioni dell'apparato respiratorio: *M.pneumoniae, C.pneumoniae, L.pneumophila,S.pneumoniae, H.influenzae, B.pertussis.*
* La ricerca di micobatteri è appropriata in questo contesto solo se sussiste un sospetto clinico specifico.

CONCLUSIONS



“Breathing New Life Into Pneumonia Diagnostics”

Murdoch D.R. et al J. Clin Microb, 2009

GRAZIE PER L'ATTENZIONE!

Manuela Avolio
Microbiologia Clinica e Virologia-Pordenone