الجمعية التونسية لطب الانعاش
ASSOCIATION TUNISIENNE DE REANIMATION

Organise

28ème CONGRÈS NATIONAL DE RÉANIMATION

&

18ème JOURNÉE DES PARAMÉDICAUX

PCR multiplex en réanimation : intérêts et implications thérapeutiques..

Zeineb HAMMOUDA/ Lamia BESBES

R2animation polyvalente

CHU F. Bourguiba Monastir

Pas de conflits d’intérêt

- La PCR multiplex est une technique de biologie moléculaire très répandue
- Principe : l'amplification de plusieurs cibles dans une seule expérience de PCR .

- **Les avantages:**

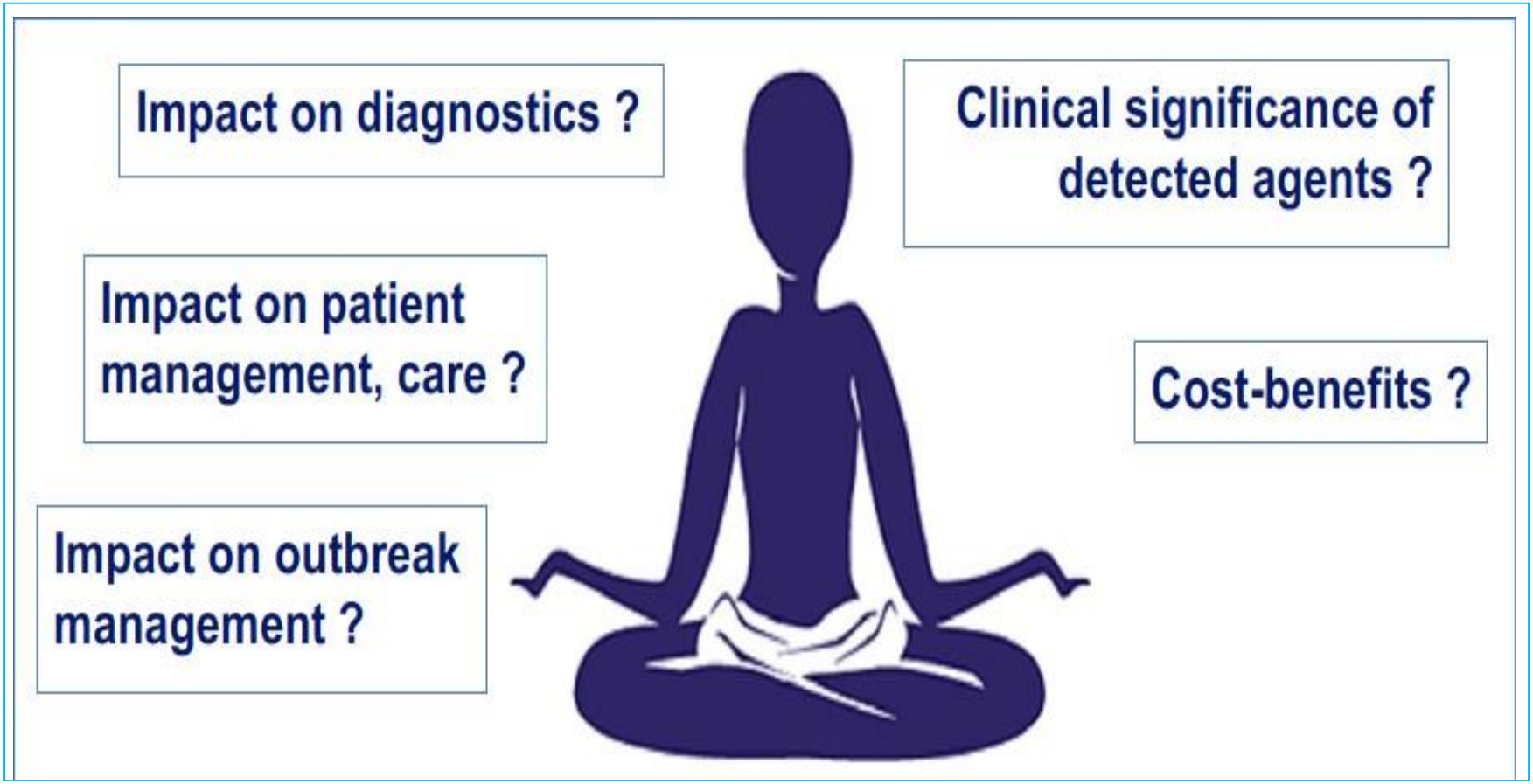
- Simplicité d’un prélèvement unique

- Identification simultanée des agents pathogènes et gènes de résistance

- Possibilité de détecter des bactéries (même si sous antibiothérapie)

- Sensibilité et spécificité accrues

Problématiques

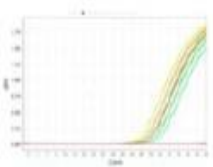


Évolution des techniques de PCR

PCR Conventionnelles

2004

PCR Spécifiques temps réel



2007

2010

PCR duplex/triplex rapides

2013

PCR multiplex

PCR multiplex syndromiques ultrarapides

JNI 20es Journées Nationales d'Infectiologie

Audrey Mérens

APPROCHE SYNDROMIQUE

Un syndrome

↓

Un seul prélèvement

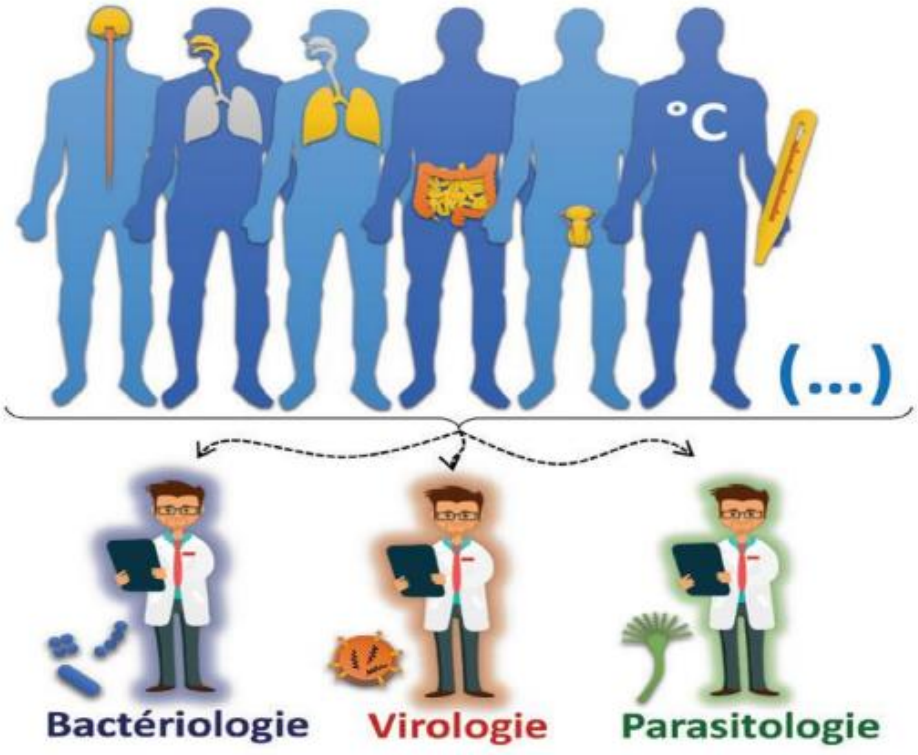
↓

Un seul test

↓

Un seul compte rendu

Plusieurs panels pour l'exploration de la quasi-totalité des infections +++



Système intégré de PCR multiplex: quels avantages?

Panels syndromiques unitaires		Techniques conventionnelles (PCR classique, cultures..)
+ Rapide Pour peu d'échantillons	VS	+ Efficacité Moins rapide mais traite un grand nombre d'échantillons
+ Facilité d'utilisation Manipulation simple		+ Quantification possible et plus fiable
+ Grands nombres d'agents pathogènes détectés simultanément		+ Sensibilité souvent meilleure
+ Détection de co-infections		+ Meilleure adaptabilité En cas d'émergence d'un nouvel agent

Ludovic Lemée et al, REVUE FRANCOPHONE DES LABORATOIRES • N° 541 • AVRIL 2022

MISE AU POINT / *UPDATE*

Approche syndromique multiplexe en réanimation

Multiplex PCR syndromic testing in intensive care units

B. Visseaux · L. Armand-Lefèvre

Reçu le 26 février 2019 ; accepté le 26 mai 2019

- Les nouveaux tests de diagnostic rapide par PCR multiplexe à visée syndromique, capables de détecter plusieurs dizaines de pathogènes en quelques heures, a entraîné un changement de paradigme en microbiologie et en pratique clinique.

- peuvent apporter une aide dans le diagnostic des infections chez les patients de réanimation.

- elles présentent cependant certains défis, comme l'évaluation de leurs performances réelles, leur coût très élevé, le choix des stratégies d'utilisation et l'interprétation clinico biologique des résultats.

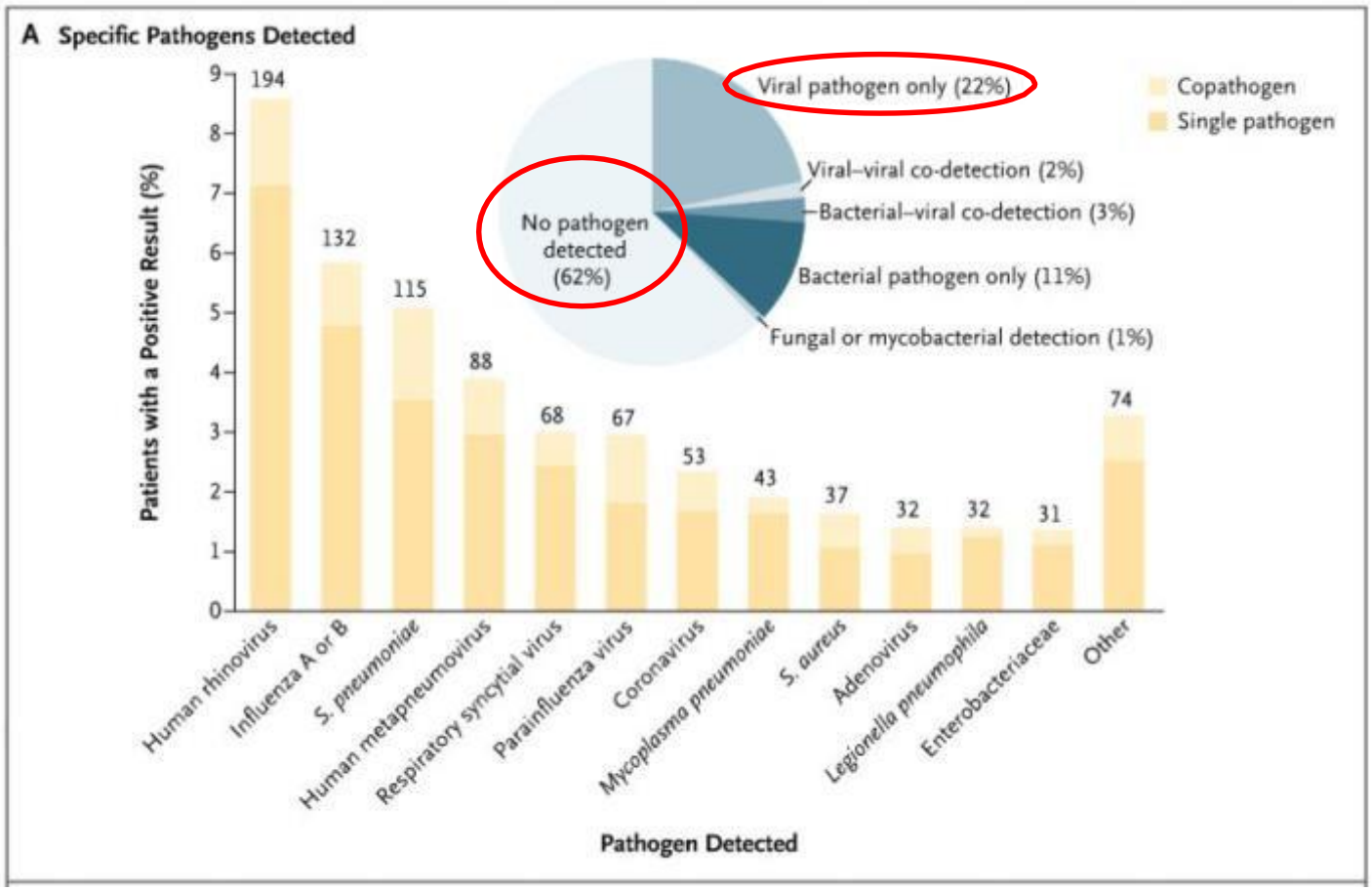
- Respiratoires +++
- Neuro méningés
- Bactériémies

Diagnostic microbiologique des infections respiratoires = véritable challenge

The NEW ENGLAND JOURNAL of MEDICINE

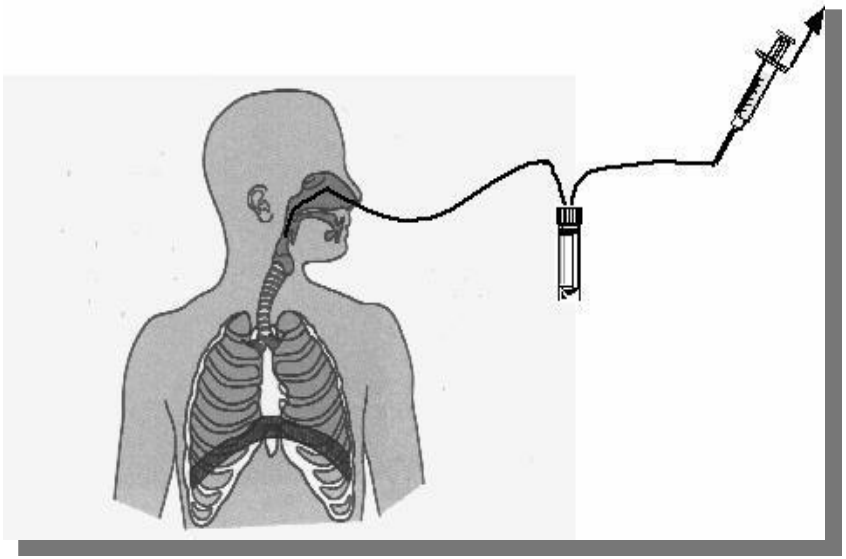
Community-Acquired Pneumonia Requiring Hospitalization among U.S. Adults

S. Jain, W.H. Self, R.G. Wunderink, S. Fakhran, R. Balk, A.M. Bramley, C. Reed,



Techniques de prélèvement

- ✓ Mise en œuvre facile et rapide
- ✓ Extraction des acides nucléiques + facile
- ✓ Ecouvillon spécifique ++++ → Nylon floqué
- ✓ milieu de transport virologique
- ✓ Emballage



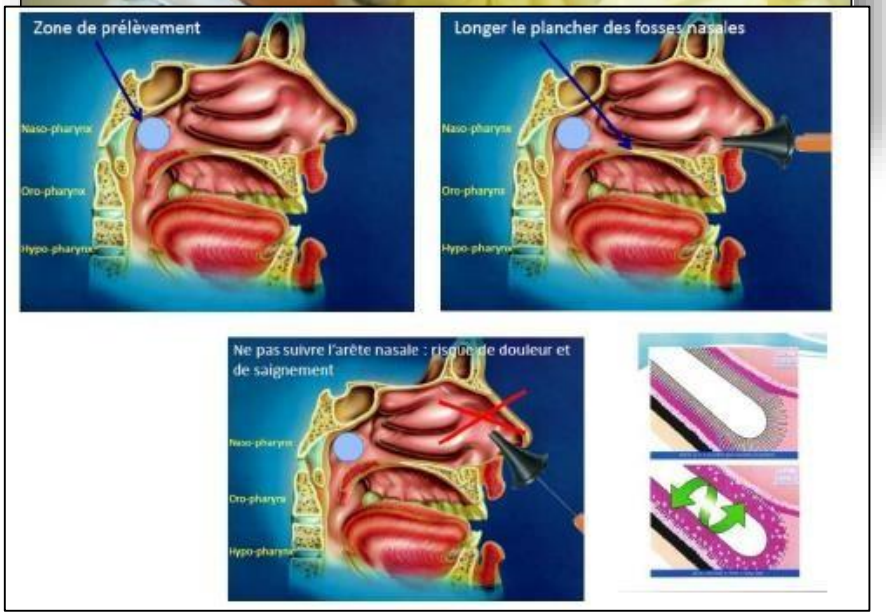


Table 1. Landscape of Food and Drug Administration–Cleared Diagnostic Tests for Acute Respiratory Tract Infection

Targets[a]	Approved Specimen Types	Time[b]	Cost[c]
CLIA-waived assays			
Influenza A/B only	NS direct, NPS direct, NP, NPS	15–30 minutes	$$–$$$
RSV only	NPS direct, NS, NPS	15 minutes	$$$
Flu A/B plus RSV	NS, NPS	20–30 minutes	$$–$$$
Multiple viruses plus atypical bacteria	NPS	60 minutes	$$$$
Moderate- to high-complexity assays			
Influenza A/B only	NS, NPS	0.5–2 hours	$$
PIV only	NPS	3.5 hours	$$
Flu A/B plus RSV	NS, NPS, NPA, NW	0.5–3.5 hours	$$–$$$$
RSV plus hMPV	NS, NPS	0.75 hours	$S
AdV, hMPV plus RV	NPS	3.5 hours	$$
Multiple viruses plus atypical bacteria	NPS	0.75–5 hours	$$$$
Multiple bacteria with resistance	ETA	4–5 hours	$$$$$
Multiple viruses and bacteria with resistance	S, ETA, BAL	60 hours	$$$$$

En TUNISIE

BioFire® FilmArray®
Pneumonia Panel *plus*

BACTÉRIES
(Résultats semi-quantitatifs)
Acinetobacter calcoaceticus-baumannii complexe
Enterobacter cloacae complexe
Escherichia coli
Haemophilus influenzae
Klebsiella aerogenes
Klebsiella oxytoca
Groupe *Klebsiella pneumoniae*
Moraxella catarrhalis
Proteus spp.
Pseudomonas aeruginosa
Serratia marcescens
Staphylococcus aureus
Streptococcus agalactiae
Streptococcus pneumoniae
Streptococcus pyogenes

BACTÉRIES ATYPIQUES
(Résultats qualitatifs)
Chlamydia pneumoniae
Legionella pneumophila
Mycoplasma pneumoniae

VIRUS
Adénovirus
Coronavirus
Métapneumovirus humain
Entérovirus/rhinovirus humains
Virus de la grippe A
Virus de la grippe B
Coronavirus du syndrome respiratoire du Moyen-Orient (MERS CoV)
Virus parainfluenza
Virus respiratoire syncytial

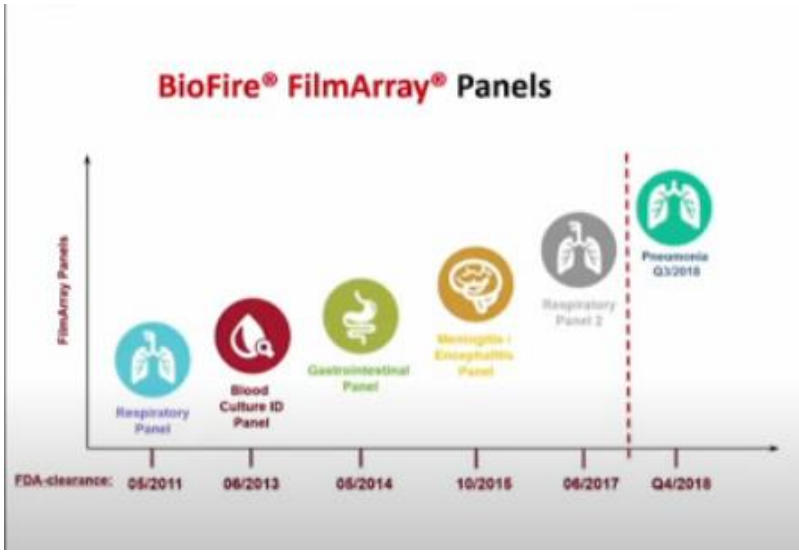
GÈNES DE RÉSISTANCE AUX ANTIBIOTIQUES

Résistance à la méticilline
mecA/C et MREJ

Carbapénémases
IMP
KPC
NDM
OXA-48-like
VIM

BLSE
CTX-M

34 cibles en 1 heure environ



Type d'échantillon :
Expectoration (AET compris) et LBA (mini-LBA compris)

Andrews et al. BMC Infectious Diseases (2017) 17:671
DOI 10.1186/s12879-017-2784-z

BMC Infectious Diseases

RESEARCH ARTICLE Open Access

Multiplex PCR point of care testing versus routine, laboratory-based testing in the treatment of adults with respiratory tract infections: a quasi-randomised study assessing impact on length of stay and antimicrobial use

Apport de l'approche syndromique
Durée d'hospitalisation
Bon usage des antibiotiques

- **Méthodologie:** Cohorte randomisée, ouverte n= 606 (contrôle 211, interventionnel 334). Technique PCR multiplex rapide VS techniques standards
- **Inclusion:** Adultes (consultants/hospitalisés) présentant syndrome grippal, IRA, +/- infection respiratoire basse.
- **Objectif principal:** Comparaison de la durée moyenne de séjour
- **Objectifs secondaires:** Impact sur l'utilisation des antibiotiques, les réadmissions, la mortalité, le délai admission résultat

Pas de différence sur la durée moyenne de séjour
Réduction du délai pour la mise en place des antiviraux
Meilleur traitement pour *Mycoplasma pneumoniae*

Bacterial and Viral Infection in Patients Hospitalized for Acute Exacerbation of Chronic Obstructive Pulmonary Disease: Implication for Antimicrobial Management and Clinical Outcome

Salma Messous[a,b], Aida Elargoubi[b], Sylvie Pillet[c], Alain Rajoharison[d], Jonathan Hoffmann[d], Imen Trabelsi[a], Mohamed Habib Grissa[a,e], Riadh Boukef[a,f], Kaouther Beltaief[a,e], Maha Mastouri[b], Gláucia Paranhos-Baccalà[c] Semir Nouira[a,e], and Bruno Pozzetto[c]

Table 2. Demographics together with clinical and biological characteristics at hospital admission of the 84 patients exhibiting an exacerbation of COPD included into the study.

Patients' characteristics	All patients
Age in years, mean (SD)	67.8 (10)
Male sex, no. (%)	78 (92.9)
Smoking history in packets/year, mean (SD)	52.3 (64)
Number of previous exacerbations in the last 12 months, mean (SD)	2.5 (1.7)
BMI in kg/m^2, mean (SD)	25.7 (4.9)
GOLD severity classification	
Stage 4, no. (%)	30 (35.7)
Stage 3, no. (%)	47 (56)
Stage 2, no. (%)	7 (8.3)
PaO_2 in kPa, mean (SD)[a]	14 (1.4)
$PaCO_2$, in kPa, mean (SD)[a]	5.9 (1.9)
pH, mean (SD)	7.3 (0.06)
WBC in cells/mm^3*10^3, mean (SD)	12.4 (6)
CRP in mg/l, mean (SD)	58 (56)

[a]These values were recorded while the patients were under oxygen supplementation.

Table 3. List of pathogens recovered from the sputum samples of the patients of the study.

Pathogen	Number
Conventional bacteriological cultures (n = 74)[a]	*15*
– *Acinetobacter baumannii*	2
– *Escherichia coli*	1
– *Klebsiella pneumoniae*	2
– *Haemophilus influenzae*	3
– *Haemophilus parainfluenzae*	1
– *Pseudomonas aeruginosa*	3
– *Staphylococcus aureus*	1
– *Streptococcus pneumoniae*	2
Molecular testing (n = 84)	
Bacteria	1
– *Chlamydia pneumoniae*	1
Viruses	63
– Influenza virus A/H1N1	7
– Influenza virus A/H3N2	5
– Influenza virus B	1
– Rhinovirus	33
– Respiratory syncytial virus B	1
– Parainfluenza virus type 1	2
– Parainfluenza virus type 3	1
– Human coronavirus 229E	2
– Human coronavirus OC43	2
– Human coronavirus NL63	2
– Human coronavirus HKU-1[b]	2
– Human bocavirus	4
– Adenovirus	1

Co Infections respiratoires
Pendant l’ère COVID

co-infections avec des virus respiratoires

RESEARCH LETTER

Rates of Co-infection Between SARS-CoV-2 and Other Respiratory Pathogens

JAMA Published online April 15, 2020

23/116 COVID+
292/1100 COVID-

Table 2. Proportions of Specimens Positive for Non–SARS-CoV-2 Respiratory Pathogens and Mean Patient Ages for Each Subgroup, by SARS-CoV-2 Result[a,b]

Pathogen	SARS-CoV-2 status			
	Negative (n = 1101)		Positive (n = 116)	
	Proportion positive for other respiratory pathogen, No. (%)[b]	Mean age of positive patients, y	Proportion positive for other respiratory pathogen, No. (%)[b]	Mean age of positive patients, y
Influenza				
A	29/1101 (2.6)	45.9	1/116 (0.9)	74.0
B	8/1101 (0.7)	21.6	0/116 (0)	
RSV	32/1101 (2.9)	26.0	6/116 (5.2)	52.3
Parainfluenza				
1	1/1101 (0.1)	71.0	1/116 (0.9)	43.0
2	0/1101 (0)		0/116 (0)	
3	2/1101 (0.2)	40.0	1/116 (0.9)	45.0
4	5/1101 (0.5)	26.6	1/116 (0.9)	36.0
Metapneumovirus	47/1101 (4.3)	41.1	2/116 (1.7)	67.0
Rhinovirus/enterovirus	133/1101 (12.1)	32.6	8/116 (6.9)	42.1
Adenovirus	10/1101 (0.9)	14.1	0/116 (0)	
Other Coronaviridae	39/1101 (3.5)	42.2	5/116 (4.3)	40.8
Chlamydia pneumoniae	0/1060 (0)		0/116 (0)	
Mycoplasma pneumoniae	6/1101 (0.5)	14.8	0/116 (0)	

Epidemiological and clinical characteristics of 99 cases of 2019 novel coronavirus pneumonia in Wuhan, China: a descriptive study

Nanshan Chen*, Min Zhou*, Xuan Dong*, Jieming Qu*, Fengyun Gong, Yang Han, Yang Qiu, Jingli Wang, Ying Liu, Yuan Wei, Jia'an Xia, Ting Yu, Xinxin Zhang, Li Zhang

www.thelancet.com Published online January 29, 2020

	Patients (n=99)
(Continued from previous column)	
Infection-related biomarkers	
Procalcitonin (ng/mL; normal range 0·0–5·0)	0·5 (1·1)
Increased	6 (6%)
Interleukin-6 (pg/mL; normal range 0·0–7·0)	7·9 (6·1–10·6)
Increased	51 (52%)
Erythrocyte sedimentation rate (mm/h; normal range 0·0–15·0)	49·9 (23·4)
Increased	84 (85%)
Serum ferritin (ng/mL; normal range 21·0–274·7)	808·7 (490·7)
Increased	62 (63%)
C-reactive protein (mg/L; normal range 0·0–5·0)*	51·4 (41·8)
Increased	63/73 (86%)
Co-infection	
Other viruses	0
Bacteria	1 (1%)
Fungus	4 (4%)

Data are n (%), n/N (%), mean (SD), and median (IQR). Increased means over the upper limit of the normal range and decreased means below the lower limit of the normal range. 2019-nCoV=2019 novel coronavirus. *Data available for 73 patients.

Table 3: Laboratory results of patients with 2019-nCoV pneumonia

Contou et al. Ann. Intensive Care (2020) 10:119
https://doi.org/10.1186/s13613-020-00736-x

Annals of Intensive Care

RESEARCH Open Access

Bacterial and viral co-infections in patients with severe SARS-CoV-2 pneumonia admitted to a French ICU

Damien Contou[1*], Aurore Claudinon[2], Olivier Pajot[1], Maïté Micaëlo[2], Pascale Longuet Flandre[3], Marie Dubert[4], Radj Cally[1], Elsa Logre[1], Megan Fraissé[1], Hervé Mentec[1] and Gaëtan Plantefève[1]

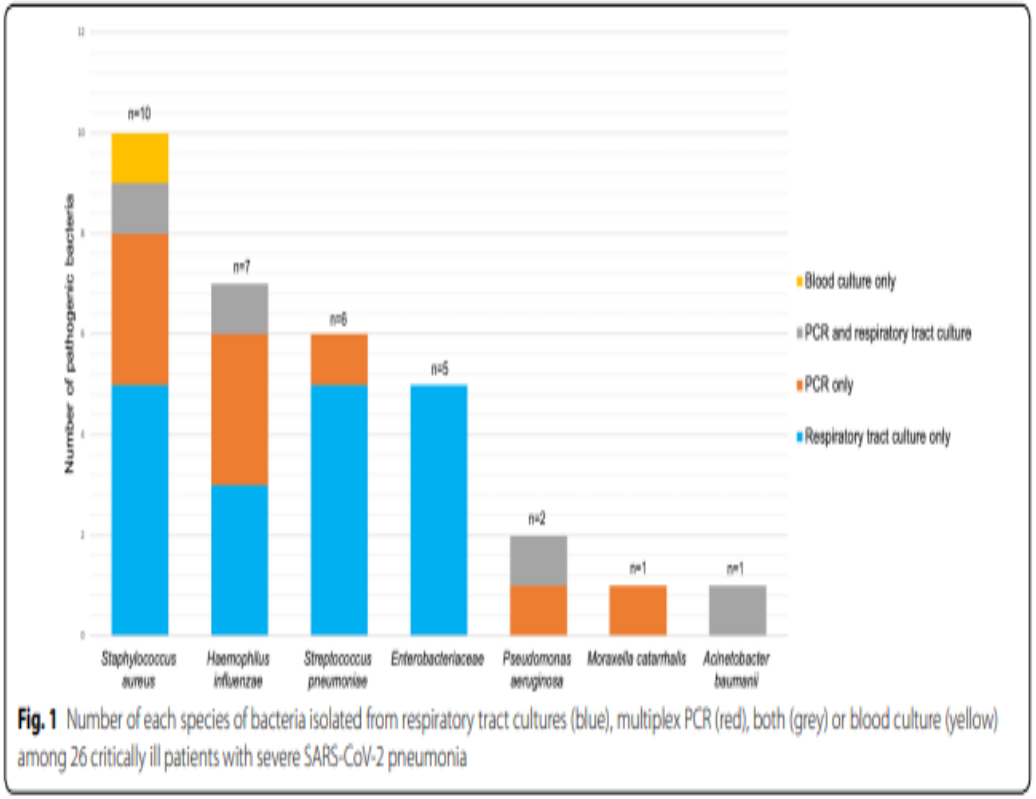


Fig. 1 Number of each species of bacteria isolated from respiratory tract cultures (blue), multiplex PCR (red), both (grey) or blood culture (yellow) among 26 critically ill patients with severe SARS-CoV-2 pneumonia

We report on a 28% rate of bacterial co-infection at ICU admission of patients with severe SARSCoV-2 pneumonia

our results encourage the systematic administration of an empiric antibiotic monotherapy with a 3rd generation cephalosporin, with a prompt de-escalation as soon as possible.

Further larger studies are needed to assess the real prevalence and the predictors of co-infection together with its prognostic impact on critically ill patients with severe SARS-CoV-2 pneumonia.

antibiotics

MDPI

Systematic Review

The Assessment of Multiplex PCR in Identifying Bacterial Infections in Patients Hospitalized with SARS-CoV-2 Infection: A Systematic Review

Iulia Bogdan [1,2,3,†], Tejaswi Gadela [4], Felix Bratosin [2,3], Catalin Dumitru [5,*], Alin Popescu [5], Florin George Horhat [6], Rodica Anamaria Negrean [7,†], Razvan Mihai Horhat [8], Ion Cristian Mot [9], Adrian Vasile Bota [3], Carmen Nicoleta Stoica [10], Bogdan Feciche [11,*], Andrei Nicolae Csep [12], Roxana Manuela Fericean [1], Gratiana Nicoleta Chicin [13,14,*] and Iosif Marincu [3]

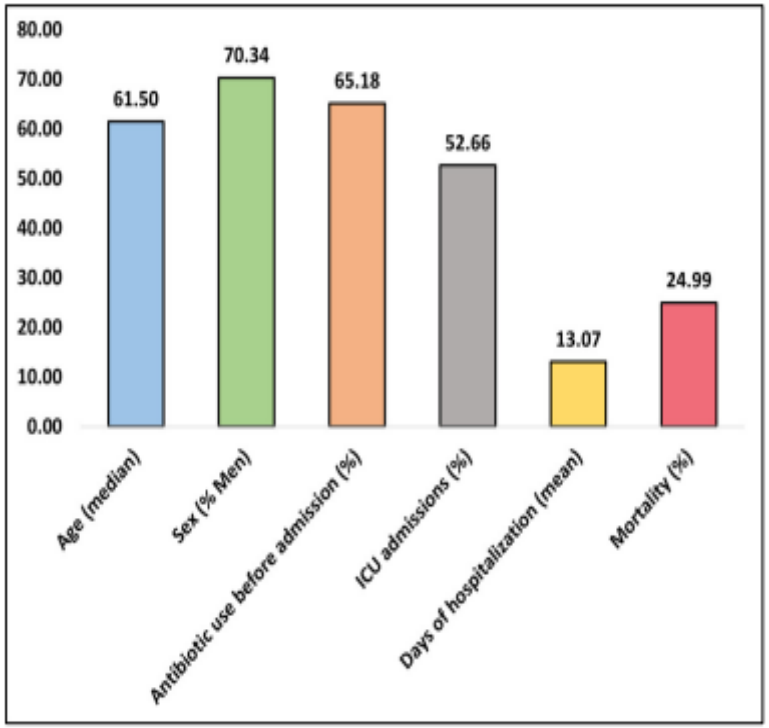


Figure 2. Summary of findings.

- Bacterial infections that are identified in COVID-19 patients can lead to more negative outcomes, particularly with a severe infection who are hospitalized with COVID-19.
- effective methods of timely diagnosis and treatment need to be implemented in order to decrease the mortality rate and prevent the misdiagnosis of infections.
- This will allow for a reduction in the number of infections that are incorrectly diagnosed.

Infections respiratoires
Après l'ère COVID

Journal of Infection 86 (2023) 462–475

Contents lists available at ScienceDirect

Journal of Infection

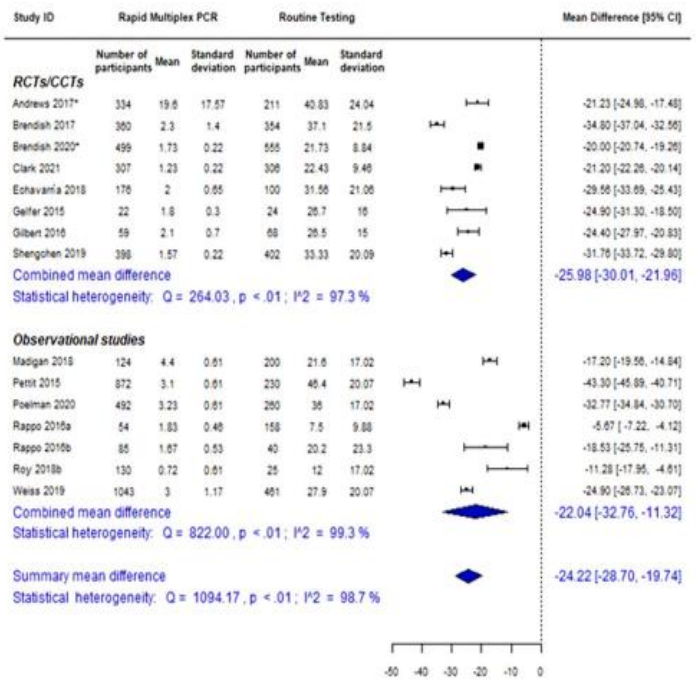
journal homepage: www.elsevier.com/locate/jinf

Rapid multiplex PCR for respiratory viruses reduces time to result and improves clinical care: Results of a systematic review and meta-analysis

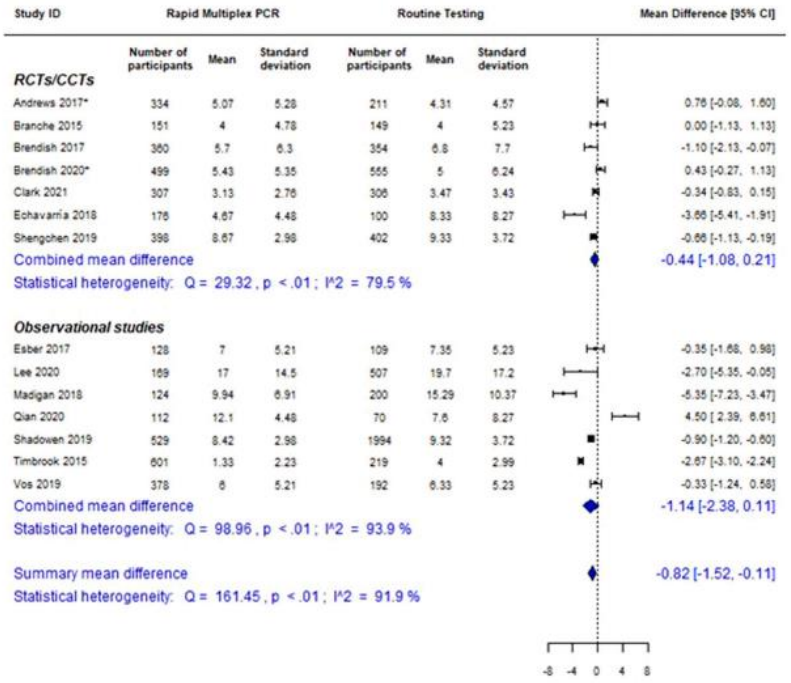
Apport de l'approche syndromique

Delai diagnostic

Durée du séjour



Délai de résultat du test



Durée du séjour

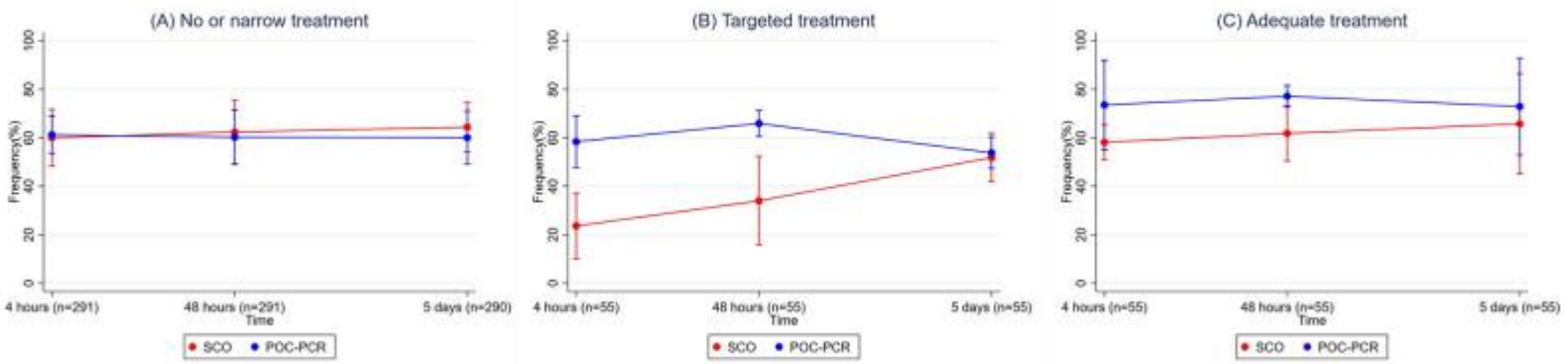
T.W. Clark, K. Lindsley, T.B. Wigmosta et al., Journal of Infection 86 (2023) 462–475

PLOS MEDICINE

RESEARCH ARTICLE

Evaluation of point-of-care multiplex polymerase chain reaction in guiding antibiotic treatment of patients acutely admitted with suspected community-acquired pneumonia in Denmark: A multicentre randomised controlled trial

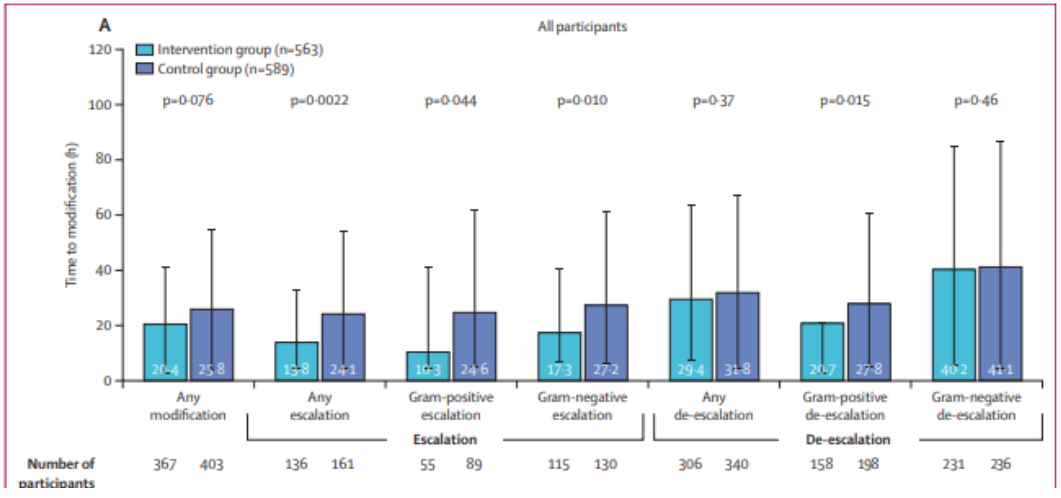
Mariana Bichuette Cartuliares[1,2]*, Flemming Schønning Rosenvinge[3,4], Christian Backer Mogensen[1,2], Thor Aage Skovsted[5], Steen Lomborg Andersen[6], Claus Østergaard[7], Andreas Kristian Pedersen[8], Helene Skjøt-arkil[1,2]



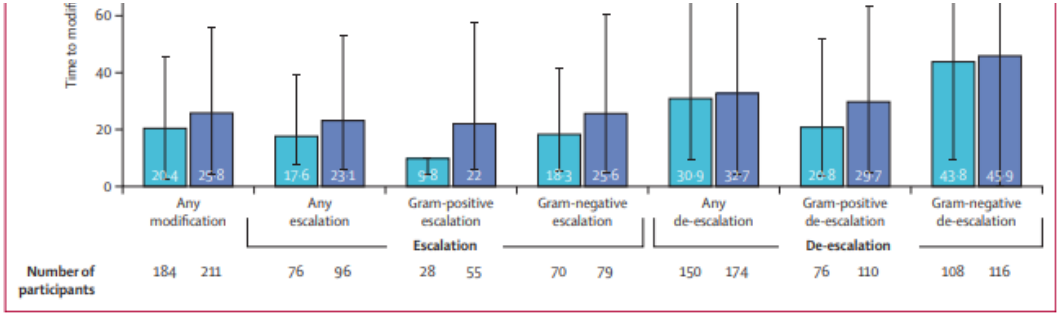
In this randomised study, adding sputum-POC-PCR to our diagnostic setup did not affect prescriptions of no or narrow-spectrum antibiotics during the first 2 days of admission, but less patients in the POC-PCR-group were treated with no or narrow-spectrum antibiotics after 5 days. Interestingly, patients in the POC-PCR-group were more likely to receive early targeted and adequate treatment. Number of readmissions, ICU admissions, and mortality were unchanged but we found a nonsignificant one-day reduction in LOS.

Rapid multiplex PCR panel for pneumonia in hospitalised patients with suspected pneumonia in the USA: a single-centre, open-label, pragmatic, randomised controlled trial

Abinash Virk, Angel P Strasburg, Kami D Kies, Alexander D Donadio, Jay Mandrekar, William S Harmsen, Ryan W Stevens, Lynn L Estes, Aaron J Tande, Douglas W Challener, Douglas R Osmon, Madiha Fida, Paschalis Vergidis, Gina A Suh, John W Wilson, Nipunie S Rajapakse, Bijan J Borah, Ruchita Dholakia, Katelyn A Reed, Lisa M Hines, Audrey N Schuetz, Robin Patel



Interpretation Clinical use of the BioFire FilmArray pneumonia panel might lead to faster antibiotic escalations, including for Gram-negative or Gram-positive bacteria, and faster antibiotic de-escalations directed at Gram-positive bacteria. Additional research is needed regarding antimicrobial de-escalation, especially when antibiotics with broad Gram-negative spectrum are being used, by use of rapid diagnostics in patients with lower respiratory tract infection.



Recommandations de l'IDSA


Clinical Infectious Diseases

VIEWPOINTS ARTICLE

IDSA Infectious Diseases Society of America hivma hiv medicine association

Molecular Testing for Acute Respiratory Tract Infections: Clinical and Diagnostic Recommendations From the IDSA's Diagnostics Committee

Kimberly E. Hanson,[1,2] Marwan M. Azar,[3] Ritu Banerjee,[4] Andrew Chou,[5] Robert C. Colgrove,[6] Christine C. Ginocchio,[7,8] Mary K. Hayden,[9,10] Mark Holodiny,[11,12] Seema Jain,[13] Sophia Koo,[14] Jaclyn Levy,[15] Tristan T. Timbrook,[16,17] and Angela M. Caliendo[18]; for the Diagnostics Committee of the Infectious Diseases Society of America (IDSA)

Clinical Infectious Diseases® 2020;XX(XX):1–8

PCRm virologiques en compléments des tests bactério de routine

L'approche syndromique moléculaire dans les infection respiratoire dépend de :

Sévérité intermédiaire

- **Test négatif**: arrêter le ttt empirique
- **Test positif**: ttt ciblé, réduire la toxicité des ttt évitables, réduit le recours aux tests inutiles

Sévérité élevée

ces tests peuvent se révéler dangereux en cas d'absence de ttt empirique contre un agent mortel

Sévérité faible

Tests non adaptée aux pays à faible ressources

D'autres études coût/bénéfice sont nécessaires

PERSPECTIVES

Table 2. Committee Recommendations for Future Respiratory Diagnostic Studies

Development of New and Innovative Diagnostics	Cost-effectiveness Studies of Available Tests	Definition of Optimal Testing Algorithms and AS Interventions
Novel biomarker discovery and host-response signatures that help separate viral, bacterial, fungal, and coinfections from colonization or no infection.	Prospective studies that capture both clinical outcomes and costs.	Studies combining host-response signatures or biomarkers with pathogen detection and active AS.
Continued refinement and analytical evaluation of unbiased next-generation sequencing platforms for use in clinical settings. Targeted tests for fungi, nontuberculous mycobacteria, and Nocardia.	Specific assessments of the impact of non–influenza virus detections, mixed infections, and bacterial pneumonia panels with antibiotic-resistance markers.	Prospective studies of AS interventions in conjunction with NAAT results and testing algorithms in the outpatient clinic, intensive care unit, and immunocompromised host settings.

Abbreviations: AS, antimicrobial stewardship; NAAT, nucleic acid amplification test.

Conclusion

- **PCR multiplex et approche syndromique moléculaire**

- une innovation technologique indiscutable : une révolution diagnostique et thérapeutique en réanimation.

- une démarche clinique indispensable et un dialogue étroit entre cliniciens et microbiologistes pour maximiser leur impact.

-problème: coût et la disponibilité.

-une opportunité d'innover et de redéfinir l'avenir de la réanimation, avec audace et pragmatisme