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BRIEF REPORT

Assessment of Respiratory Bacterial Coinfections Among Severe Acute Respiratory Syndrome Coronavirus 2-Positive Patients Hospitalized in Intensive Care Units Using Conventional Culture and BioFire, FilmArray Pneumonia Panel Plus Assay

Camille Kolenda,[1] Anne-Gaëlle Ranc,[1] Sandrine Boisset,[2] Yvan Caspar,[2] Anne Carricajo,[3] Aubin Souche,[1] Olivier Dauwalder,[1] Paul O. Verhoeven,[3] François Vandenesch,[1] and Frédéric Laurent[1]; on behalf of the COVID-COINF study group

[1]Département de Bactériologie, Institut des Agents Infectieux, Hospices Civils de Lyon, Lyon, France, [2]Laboratoire de Bactériologie, Institut de Biologie et de Pathologie, Centre Hospitalo-Universitaire de Grenoble, Grenoble, France, [3]Laboratoire des Agents Infectieux et d'Hygiène, Centre Hospitalo-Universitaire de Saint-Etienne, Saint-Etienne, France

Background. Approximately 15% of patients infected by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) present with severe forms of the disease and require hospitalization in intensive care units, which has been associated with high mortality rates. The prevalence of bacterial infections in these patients is not well established, and more data are needed to guide empiric antibiotic therapy and improve patient outcomes.

Methods. In this prospective multicenter study, we assessed bacterial coinfections identified in culture from 99 French patients infected by SARS-Cov-2 and hospitalized in intensive care units. We concomitantly evaluated an innovative molecular diagnostic technology technique, the BioFire, FilmArray Pneumonia Panel plus (FA-pneumo) assay, to identify these coinfections at an early stage, and its concordance with conventional culture.

Results. We showed that a bacterial coinfection was detected in 15% of patients based on conventional culture. *Staphylococcus aureus* and *Haemophilus influenzae* were the most prevalent pathogens. The sensitivity of FA-pneumo compared with culture was 100%. In contrast, the specificity varied between 88.4% and 100% according to the pathogen, and our results highlighted that 60.5% of bacterial targets reported using this assay were not recovered by culture; 76.9% of discordant results corresponded to bacteria belonging to commensal oral flora and/or reported with $\leq 10^5$ copies/mL bacterial nucleic acids.

Conclusions. Based on its excellent sensitivity, the FA-pneumo assay is useful to rule out bacterial coinfections in the context of severe SARS-CoV-2 infection and avoid the inappropriate prescription of antibiotics. However, positive tests should be interpreted carefully, taking into consideration deoxyribonucleic acid bacterial load and all clinical and biological signs.

Keywords. bacterial coinfection; BioFire; COVID-19; FilmArray; intensive care units.

The severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) pandemic was declared by the World Health Organization on March 12, 2020 [1]. Based on the literature, approximately 15% of patients present with severe forms of the disease and require hospitalization in intensive care units (ICUs), which has been associated with high mortality rates [2]. In view of the poor prognosis of these severe forms, bacterial coinfections may be of importance. Their prevalence, nature, and impact on mortality in the context of other severe respiratory viral infections, such as influenza, have been well established [3], but these data are lacking for patients with coronavirus disease 2019 (COVID-19) [4]. Identification of coinfections in patients developing severe pulmonary manifestations in a very short time could be very helpful to initiate an early and appropriate antimicrobial treatment and thus improve their prognosis. In contrast, in the absence of clinical or radiological evidence of bacterial coinfection, absence of microorganisms in respiratory samples of those patients could preclude unnecessary antibiotic prescription. In this prospective multicenter study, we assessed bacterial coinfections identified in culture in the first low respiratory sample taken in patients infected by SARS-Cov-2 and hospitalized in an ICU. We also evaluated the use of an innovative molecular diagnostic technology to identify such coinfections, namely, the BioFire, FilmArray Pneumonia Panel plus ([FA-Pneumo] (bioMérieux) assay, and its concordance with conventional culture. This fully automated and multiplex polymerase chain reaction (PCR) assay allows rapid detection (approximately 1 hour) of a wide range of clinically relevant pathogens and a limited number of resistance markers (Table 1).

METHODS

In this study, 99 low respiratory track samples were prospectively collected, including 38 endotracheal aspirates, 12 bronchial aspirates, 13 bronchoalveolar lavage (BAL), and 36 mini-BAL

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Correspondence: Camille Kolenda, PharmD, Institut des Agents Infectieux – Département de Bactériologie, Hospices Civils de Lyon, 103 Grande rue de la Croix Rousse, 69004 Lyon, France (camille.kolenda@chu-lyon.fr).

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Table 1. Targets Identified by the FA-pneumo Assay

Category (Result Type)	Targets
Viruses (qualitative)	Adenovirus, coronavirus, human metapneumovirus, human rhinovirus/enterovirus, influenza A virus, influenza B virus, parainfluenza virus, respiratory syncytial virus
Bacteria (qualitative)	*Chlamydia pneumoniae*, *Legionella pneumophila*, *Mycoplasma pneumoniae*
Bacteria (semiquantitative[a])	*Acinetobacter calcoaceticus-Acinetobacter baumannii* complex, *Enterobacter cloacae* complex, *Escherichia coli*, *Haemophilus influenzae*, *Klebsiella aerogenes*, *Klebsiella oxytoca*, *Klebsiella pneumoniae*, *Moraxella catarrhalis*, *Proteus* spp, *Pseudomonas aeruginosa*, *Serratia marcescens*, *Staphylococcus aureus*, *Streptococcus agalactiae*, *Streptococcus pneumoniae*, *Streptococcus pyogenes*
Antimicrobial resistance markers (qualitative)	
Carbapenemases	KPC, NDM, IMP, VIM, OXA-48 like
Extended-spectrum beta-lactamases	CTX-M
Methicillin resistance genes	*mec*A/*mec*C and MREJ

Abbreviations: FA-pneumo, FilmArray pneumonia.

[a]Reported as 10^4, 10^5, 10^6, or $\geq 10^7$ copies/mL.

specimens, between March 1 and April 15, 2020 from patients with SARS-CoV-2 infection confirmed by reverse-transcription quantitative PCR and admitted to the medical ICU of 3 French university hospitals (Lyon, Grenoble, Saint-Etienne). These samples were taken in absence of mechanical ventilation or within 48 hours after this was initiated. This observational study was approved by the national data protection commission (Commission Nationale de l'Informatique et des Libertés, no. 20_133).

All specimens were subjected to Gram staining, and conventional cultures were performed by inoculating blood, chocolate, and McConkey or Bromocresol Purple or Drigalski agar plates according to the hospital, incubated at 35°C in an aerobic atmosphere, and enriched with 5% CO_2 for blood agar plates for 2 days. Microorganisms that grew in significant amounts according to the guidelines of standard laboratory procedures [5] were identified using matrix-assisted laser desorption ionization time-of-flight mass spectrometry (VITEK MS, bioMérieux, Marcy l'Etoile, France; or Biotyper-Microflex, Bruker Daltonics, Billerica, MA). Susceptibility testing was performed using VITEK 2 (bioMérieux), BD Phoenix M50 (Becton Dickinson, Franklin Lakes, NJ), or disk diffusion method as recommended by CASFM/EUCAST. In parallel, the BioFire, FA-Pneumo assay was performed according to the manufacturer's instructions from 200 µL sample. Results obtained from the 2 approaches were compared for detection of bacteria and antibiotic resistance. Sensitivity and specificity of FA-Pneumo for bacterial identification were assessed considering culture as the gold standard. A retrospective chart review was performed for each patient to determine the type and duration of antibiotic therapy administered before the sample was collected.

Patient Consent Statement

The design of this work has been approved by local ethical committees or conforms to standards currently applied in the country of origin, and it includes the name of the authorizing body that should be stated in the paper.

RESULTS

Cultures identified 17 bacteria in 15 of 99 samples (15.1%) including Staphylococcus aureus (n = 7), Haemophilus influenzae (n = 4), Streptococcus pneumoniae (n = 2), Enterobacteriaceae (n = 2), Moraxella catarrhalis (n = 1), and Legionella pneumophila (n = 1). Only few other studies described coinfections in patients infected by SARS-CoV-2, reporting lower percentages of bacterial coinfections, but these studies were not specifically dedicated to severe forms of SARS-CoV-2 infection [6, 7].

The sensitivity of FA-pneumo assay was 100% because all of the bacteria isolated in culture were also detected using FA-pneumo. The overall specificity was 98.7% with a percentage ranging between 88.4% and 100% according to the pathogen (Table 2). In total, 26 additional bacteria in 20 samples were detected using FA-pneumo but not in culture. Of note, coinfection with a picornavirus was also identified in 1 sample using FA-pneumo. Among 16 bacteria reported in culture, 15 (93.8%) showed $\geq 10^6$ copies/mL bacterial nucleic acids using FA-Pneumo, but the load of *L pneumophila* was not reported because this species is strictly pathogen (Table 3). In contrast, among the 26 bacteria detected using FA-Pneumo but not reported in culture, 20 (76.9%) had $\leq 10^5$ copies/mL bacterial nucleic acids using FA-Pneumo. Overall, the percentage of FA-pneumo-positive results concordant with culture increased in function of the bacterial nucleic acid load threshold reported using FA-Pneumo: 10^4 copies/mL - 38.1%, 10^5; 59.2%, 10^6; 71.4%, 10^7; 92.9%. None of the targeted resistance genes was detected using the FA-Pneumo assay, whereas all *S aureus* and *Enterobacteriaceae* (species possibly harboring the targeted resistance genes) found in culture were susceptible to methicillin

Table 2. Bacteria Identified in Culture and by the FA-pneumo Test

Bacterial Species	Number of Samples													Sensitivity	Specificity
	Culture + FA-pneumo +					Culture - FA-pneumo +					Culture + FA-pneumo -	Culture - FA-pneumo -			
		Bacterial Load[a]					Bacterial Load[a]								
	Total	$\geq 10^7$	10^6	10^5	10^4	Total	≥p7	p6	p5	p4					
Haemophilus influenzae	4	4				11	1	2	2	6	0	84		100%	88.4%
Staphylococcus aureus	7	5	2			6			2	4	0	86		100%	93.5%
Streptococcus pneumoniae	2	2				2		1	1		0	95		100%	97.9%
Moraxella catarrhalis	1	1				2				2	0	96		100%	97.9%
Streptococcus agalactiae	0					2		1		1	0	97		100%	98.0%
Streptococcus pyogenes	0					2		1		1	0	97		100%	98.0%
Enterobacteriaceae	2	1		1		0					0	97		100%	100%
Pseudomonas aeruginosa	0					1				1	0	98		100%	99.0%
Legionnella pneumophila	1 (not quantified)					0					0	98		100%	100%
Total	17	13	2	1		26	1	5	5	15	0	848		100%	98.7%

Abbreviations: FA-pneumo, FilmArray pneumonia.

[a]FA-pneumo copies/mL.

or cephalosporins/carbapenems, respectively. The retrospective medical chart review showed that 72 of 99 patients received antibiotics (mainly amoxicillin and clavulanic acid or third-generation cephalosporins associated to macrolides) before sampling. The FA-Pneumo positivity rate was 19.4% (14 of 72) and 51.9% (14 of 27) in patients with or without prior administration of antibiotics, respectively ($P = .001$). It is interesting to note that the percentage of FA-pneumo-positive results concordant with culture was not affected by antibiotic administration (9 of 20 in the group with prior administration of antibiotics vs 8 of 23 without).

DISCUSSION

To the best of our knowledge, this the first published study assessing the performance of the FA-pneumo assay in the context of the SARS-CoV-2 pandemic. The present study found that the sensitivity of the FA-pneumo assay was excellent and would allow the initiation or the escalation of antimicrobial therapy to be precluded in patients transferred to the ICU presenting a FA-Pneumo negative test. However, the results presented herein indicate that 60.5% of bacterial targets reported positive using this assay were not found in culture. It is interesting to note that an important proportion of positive FA-pneumo results not concordant with culture corresponded to oral commensal species and was reported with $\leq 10^5$ copies/mL bacterial nucleic acids loads. This suggests that such results should be interpreted with caution. Conversely, results with $\geq 10^6$ copies/mL can be used for early adaptation of antibiotic therapy. The performances described herein are in line with the findings of previous studies evaluating the FA-pneumo assay in a more general context of bacterial pneumonia and reporting high sensitivities but variable specificities depending on the pathogen [8–10]. Of note, in the study by Buchan et al [10], 69.9% of bacteria reported using FA-pneumo but not found in culture also showed $\leq 10^5$ copies/mL bacterial nucleic acids.

We acknowledge that the absence of discrimination between colonization and true infection for the bacteria detected by the FA-pneumo assay but not in a culture is a major limitation of the present study. However, this was not feasible in this context. Indeed, because of the ongoing infection by SARS-CoV-2, clinical (eg, fever, cough) and x-ray data were not useful to suspect bacterial infection. Biological markers of bacterial infections (leukocytes, neutrophils counts, procalcitonin obtained within 24 hours before or after the respiratory sample was taken) were not useful either because they were not different in patients with negative or positive FA-pneumo assays. We were surprised to find that elevated procalcitonin values (>1 µg/L) were more frequently observed in patients with negative (45%) than positive (33%) FA-pneumo assays, and the highest values were observed in patients without any evidence of bacterial infection. Another limit of the present study is the percentage of patients receiving antibiotics before the collection of respiratory

Table 3. Semiquantitative Analysis of Bacterial Nucleic Acid Loads Reported Using FA-pneumo and Bacterial Counts in Culture

		FA-pneumo (Copies/mL)[a]				
		$\geq 10^7$	10^6	10^5	10^4	Not Quantified
Culture (CFU/mL)	$\geq 10^7$	2				
	10^6	5				
	10^5	4	1	1		
	10^4	2	1			
	Not quantified					1 (*Legionnella pneumophila*)
	Not reported	1	5	5	15	

Abbreviations: CFU, colony-forming units; FA-pneumo, FilmArray pneumonia.

[a]A total of 10× copies/mL is reported by the system for deoxyribonucleic acid load ranging from $10^{x-0.5}$ to $10^{x+0.5}$ copies/mL.

samples. The significance of the FA-pneumo assay to detect bacterial coinfections should be evaluated at an earlier stage of SARS-CoV-2 infection to avoid massive empiric prescription of antibiotics, as in the present cohort of patients.

CONCLUSIONS

In conclusion, in the present study, we found that the FA-pneumo assay can be used to rule out bacterial coinfections in SARS-CoV-2-positive patients after their admission to the ICU to limit the prescription of antibiotics, but that positive tests with $\leq 10^5$ copies/mL bacterial nucleic acids should be interpreted carefully.

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Potential conflicts of interest. All authors: No reported conflicts of interest. All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest.

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