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Title: Algorithm for rational use of FilmArray Pneumonia Panel in bacterial coinfections of critically ill ventilated COVID-19 patients

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Abstract

The FilmArray® Pneumonia Panel has proven to be an effective tool for rapid detection of main respiratory pathogens. However, its rational use needs appropriate knowledge and formation regarding its indication and interpretation. Herein, we provide some advices to help with success of its daily routine use, particularly in critically ill ventilated COVID-19 patients.

Clinical Trial registration number: NCT04453540.

Keywords: Covid-19 / bacterial coinfection / antimicrobial stewardship / pneumonia / critically ill / molecular diagnosis

Since the start of the pandemic Covid-19 outbreak, molecular respiratory panel such as FilmArray® Pneumonia Panel (FAPP; bioMérieux, France) has been widely used in critically ill patients for bacterial coinfections management. Regarding its performance for pathogens and antimicrobial resistance detection (Suppl. **Table 1**), all authors highlighted FAPP interest for antimicrobial stewardship, especially antibiotic sparing [1-6]. However, FAPP interpretation could be challenging [4,5]. Indeed, as evoked by Maataoui *et al.*, one of the reasons of a non-optimal use of FAPP was the “lack of knowledge and confidence in the test” [4]. The present study reports the lessons from the implementation of FAPP during the first COVID-19 outbreak, when a training on “how to use FAPP” could not be performed due to the work overload.

This is a multicenter retrospective analysis (clinicalTrial.gov NCT04453540) of all critically ill patients who were admitted to the Nancy and Reims University Hospitals (six ICUs) from March to May 2020, with COVID-19 and respiratory failure requiring invasive mechanical ventilation (IMV). The local institutional ethics committee approved this study (Comité d'éthique du CHRU de Nancy, N°CO-20). Informed consent was obtained from all participants and/or their legal guardians. Presence of SARS-CoV-2 was diagnosed using RT-PCR. All patients with suspicion of bacterial pneumonia were eligible. The decision to prescribe FAPP was at the discretion of the clinician. Only patients with concomitant FAPP, conventional culture (CC) and Gram stain were included. Samples were endotracheal aspirates (ETA) and bronchoalveolar lavages (BAL). Results of the FAPP and Gram stain were available for the intensivists within four hours. A first result of the CC was available after one day with a definitive result within five days. For quantitative culture, only the bacteria above the following threshold were considered: 10^4 CFU/mL for BAL and 10^5 CFU/mL for ETA. Phenotypic drug susceptibility testing was performed according to the

recommendations of the antibiogram committee of the French Society for Microbiology (CA-SFM)/European Committee for Antibiotic Susceptibility Testing (EU-CAST). A multidisciplinary expert committee (MEC) composed of intensivists, infectious disease specialists and microbiologists from both centers analyzed retrospectively the contribution of FAPP compared to CC in the treatment decision of pneumonia according to criteria from Weiss *et al.* [7]. Antibiotics used to treat any concomitant infection were not considered by the MEC. Early bacterial coinfections, represented by community-acquired pneumonia (CAP), were defined as infections occurring during the first 48h of ICU admission. The ventilator-associated pneumonia (VAP) were defined as infections occurring after 48h of IMV. Multiple tests from the same patient were considered independent when performed during distinct infectious episodes. Categorical data were analyzed using chi-square test or Fisher's exact test. Statistical analyses were performed by an independent statistician using SAS 9.4 software (SAS Institute, Inc, Cary, N.C.).

Overall, 344 patients with a positive SARS-CoV-2 RT-PCR were admitted in the participating ICUs of whom 90 fulfilled eligibility criteria. Samples were 74 ETA and 45 BAL. Characteristics and ICU data are presented in **Table 1**. Bacteriological results were presented in **Tables 2** and **3**. The rate of clinically confirmed CAP and VAP were 5.0% and 40.3%, respectively. Bacterial pathogens were detected by FAPP (45.4%) and/or by CC (38.7%) in 41 and 34 ETA and in 13 and 12 BAL, respectively. The adequacy between FAPP and CC in pathogen detection was better ($p=0.017$) for BAL (95.6%) than for ETA (79.7%). *Staphylococcus aureus* and *Pseudomonas aeruginosa* were the most prevalent pathogens. Two cases of negative FAPP (no detection of *Morganella morganii*) have led to inappropriate discontinuation of empirical antibiotic therapy. Two Extended-Spectrum β -lactamase ESBL (not-CTX-M)-producing *Enterobacter cloacae* and one 3GC-resistant *P. aeruginosa* were

isolated by CC without detection of resistance gene by FAPP. Regarding the six samples with methicillin-resistance of *S. aureus* (MRSA) detected in FAPP, only three have a *S. aureus*-positive culture and all were methicillin-susceptible (MSSA). According to MEC analysis, FAPP-based therapeutic decision was concordant with CC-based therapeutic decision in 91% for BAL compared with 69% for ETA ($p=0.009$). The most contribution of FAPP regarding antibiotic prescription was antibiotic spare (**Table 2**). However, we observed that intensivists considered FAPP for treatment only in 42.0% (50/119) of cases.

These results confirmed the usefulness of FAPP to rapidly diagnose bacterial coinfection. However, there is a room for improvement of its use and interpretation. Herein, we suggest four tips for a tailored use of FAPP in critically ill ventilated patients:

1. Training for mastering FAPP by the intensivists is required for successful utilization in the daily routine practice. We believe that an appropriate knowledge about FAPP performance and results interpretation should lead to a better antibiotic use. Therefore, a collaboration between microbiologists and intensivists is mandatory.

2. FAPP should be performed on BAL to avoid over-diagnosis of bacterial coinfection. Lower relevance of FAPP results from ETA compared to BAL for treatment could be explained by detection of not significant bacteria from the tracheobronchial colonization. However, if BAL could not be performed, ETA could be used with cautious interpretation of FAPP results.

3. Conventional culture should be systematically performed in parallel. To detect bacteria not included in the FAPP [5] and to confirm resistance gene detection. For Gram-negative bacilli, FAPP detects only CTX-M ESBL. Moreover, as previously described [8], FAPP led to over-detection of MRSA that could lead to an overuse of anti-MRSA antibiotics, especially in case of local ecology with low prevalence of MRSA. Indeed,

among the 6 samples with MRSA detected in FAPP (5 ETA and 1 BAL), only three (2 ETA and 1 BAL) had a *S. aureus*-positive culture and all were MSSA. Such discordance could be explained either by the co-occurrence of a *S. aureus* with an empty *SCCmec* cassette and methicillin-resistant negative coagulase staphylococci, or by a mixed specimen of MSSA and MRSA, respectively above and below the threshold of culture detection.

4. Therapeutic decision must be re-evaluated with the result of 2-days conventional culture. The delay of 2 days for definitive CC interpretation should cover slowing growing bacteria (low bacterial load or prior antimicrobial treatment) as well as drug susceptibility testing results. Moreover, of 65 negative-FAPP, 62 (95.4%) showed 5-days negative culture and 3 (4.6%) were positive (for outside-panel bacteria) but within 2 days of culture. Consequently, in absence of i) severity criteria, namely septic shock or severe ARDS (according to Berlin criteria), and of ii) Gram-negative bacilli at Gram stain, empirical antibiotic therapy could be stopped based on a negative-FAPP result.

In the present study, a FAPP use based on these tips would allow 65.6% of antibiotic spare in bacterial coinfection and a better adequacy of empirical antibiotic treatment. Regarding VAP, FAPP should consider local ecology for optimal interpretation, especially for resistance detection (i.e. *P. aeruginosa* with non-enzymatic resistance). Based on our results, we propose an algorithm to improve the use of FAPP for antibiotic stewardship at the bedside (**Figure 1**). Further studies are now warranted to demonstrate that rational use of FAPP will also improve patient outcome.

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Figure legend.

Fig. 1. Clinical algorithm for initiating antibiotics using FAPP in bacterial coinfection of critically ill COVID-19 patients. IMV, invasive mechanical ventilation; BAL, bronchoalveolar lavage; FAPP, FilmArray® Pneumonia Panel; ATB, antibiotics; GNB, Gram-negative bacilli.

^a Endotracheal aspirate samples could be used but need cautious interpretation regarding the risk of over-diagnosis due to tracheobronchial colonization

^b Septic shock (according to SEPSIS-3) or severe ARDS (according to Berlin criteria)

Ethics consideration

The institutional ethics committee approved this study (Comité d'éthique du CHRU de Nancy, N°CO-20). All experiments were performed in accordance with guidelines and regulations. Informed consent was obtained from all participants and/or their legal guardians.

Authors' contributions: EN and CA participated equally in this work. EN, CA and TG contributed substantially to the study design and the writing of the manuscript. EN, CA, TG, CT, AG contributed to the acquisition, analysis and interpretation of data. CT and AG made critical revision of the manuscript.

210 **Table 1.** Clinical characteristics of patients

Variables	Patients N=90
Male Sex	72 (80.0)
Age (years)	65 [58.3-70.0]
Obesity (BMI >40 kg/m²)	6 (6.6)
Comorbidities:	
Hypertension	50 (55.5)
Diabetes	27 (30.0)
Immune deficiency	18 (20)
Chronic respiratory failure	10 (11.0)
Chronic hemodialysis	1 (1.0)
Cirrhosis	1 (1.0)
ICU data:	
SAPS 2 score	44 [36-61]
ICU LOS (days)	23 [14-37]
IMV duration (days)	17 [11-27]
ECMO	10 (11)
ICU mortality	25 (28)

211 Data are presented as: n (%) – median [IQR]

212 Immune deficiency: diabetes, neoplasia, transplant, neutropenia/aplasia, immunosuppressive
 213 therapy / SAPS 2 score: Simplified Acute Physiology Score II / LOS: Length of stay / IMV:
 214 invasive mechanical ventilation.

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217 **Table 2.** Bacteriological results according to the type of pneumonia and contribution of the
 218 panel FAPP on antibiotic prescription

Samples N=119		
	CAP	VAP
Type of suspected pneumonia	27 (22.7)	92 (77.3)
Confirmed diagnostic of pneumonia	6	48
(% among suspected / % among total)	(22.2 / 5.0)	(52.2 / 40.3)
Type of samples:		
ETA	15	59
BAL	12	33
Antibiotics 48h prior to samples	15 (55.6)	40 (43.5)
Bacterial copathogens:	FAPP / CC	FAPP / CC
<i>Staphylococcus aureus</i>	0 / 0	17 / 12
<i>Pseudomonas aeruginosa</i>	0 / 0	11 / 11
<i>Haemophilus influenzae</i>	4 / 0	6 / 3
<i>Escherichia coli</i>	0 / 0	9 / 5
<i>Klebsiella pneumoniae</i>	1 / 1	6 / 6
<i>Enterobacter cloacae</i>	0 / 0	5 / 5
<i>Klebsiella aerogenes</i>	0 / 0	4 / 2
<i>Proteus spp.</i>	0 / 0	4 / 3
<i>Serratia marcescens</i>	0 / 0	3 / 3
<i>Streptococcus agalactiae</i>	0 / 0	3 / 1
<i>Moraxella catarrhalis</i>	1 / 0	1 / 1
<i>Mycoplasma pneumoniae</i>	1 / NA	0 / NA

<i>Morganella morganii</i>	NA / 0	NA / 2
<i>Hafnia alvei</i>	NA / 0	NA / 1 ^a
<i>Providencia stuartii</i>	NA / 0	NA / 1 ^a
Resistance detection:	FAPP / AST	FAPP / AST
MRSA	0 / 0	6 / 0
3GC-R Gram-negative bacilli	0 / 0	5 / 6 ^b
Type of pneumonia	CAP	VAP
Contribution of FAPP at first intensivist decision^c		
No modification of empirical antibiotics	0	3 (7.5)
Speeded-up adequate antibiotic	2 (20.0)	9 (22.5)
Antibiotic spare ^d	8 (80.0)	20 (50.0)
Inappropriate antibiotic treatment	0	7 (17.5)
Inappropriate stopped antibiotic	0	1 (2.5)
Contribution of FAPP based on MEC analysis^e		
No modification of empirical antibiotics	1 (3.7)	11 (12.0)
Speeded-up adequate antibiotic	4 (14.8)	13 (14.1)
Antibiotic spare ^d	22 (81.5)	56 (60.9)
Inappropriate antibiotic treatment	0	10 (10.9)
Inappropriate stopped antibiotic	0	2 (2.2)

Data are presented as: n (%)

ETA: endotracheal aspirate / BAL: bronchoalveolar lavage / CAP: community-acquired pneumonia (defined as infections occurring during the first 48h of ICU admission) / VAP: ventilator-associated pneumonia / FAPP: FilmArray® Pneumonia Panel / CC: conventional culture / AST: antimicrobial susceptibility testing / MRSA: methicillino-resistant *Staphylococcus aureus* / 3GC-R: third generation cephalosporins resistance / NA: not applicable (species not detected either by the FAPP or by the CC) / MEC: multidisciplinary expert committee.

^a The isolation of *H. alvei* and *P. stuartii* in CC had no impact on antibiotic therapy as they were covered by the antibiotics administered following the detection of other pathogens detected by FAPP.

^b Among 3GC-resistant Gram-negative bacilli, 3 CTX-M were detected by both FAPP and CC, 2 CTX-M were detected only by FAPP, 2 ESBL not belonging to CTX-M as well as one 3GC-resistant *P. aeruginosa* were detected only by CC.

^c A contribution of FAPP at first intensivist decision was noted in 50 samples (42.0%).

^d Decrease unnecessary antibiotic use (interruption or de-escalation).

^e Theoretical contribution of FAPP after MEC analysis of the 119 samples (100.0%).

Table 3. Bacteriological results according to the type of respiratory samples

Samples N=119		
	ETA (n=74)	BAL (n=45)
Type of pneumonia		
CAP : suspected/confirmed	15 / 5	12 / 1
VAP : suspected/confirmed	59 / 34	33 / 14
Antibiotics 48h prior to samples (n=55)	32 (58)	23 (42)
Positive direct examination	39 (53)	14 (33)
Presence of Gram +	6 (15)	3 (21)
Presence of Gram –	9 (23)	1 (7)
Polymicrobial	24 (62)	10 (72)
Infection polymicrobial		
FAPP (n=18)	14 (77)	4 (23)
Mean number of bacteria detected	2.2	2.25
CC (n=8)	5 (62)	3 (38)
Mean number of bacteria detected	2.2	2
Bacterial copathogens:	FAPP / CC	FAPP / CC
<i>Staphylococcus aureus</i>	12 / 6	5 / 6
<i>Pseudomonas aeruginosa</i>	10 / 10	1 / 1
<i>Haemophilus influenzae</i>	7 / 2	3 / 1
<i>Escherichia coli</i>	7 / 4	2 / 1
<i>Klebsiella pneumoniae</i>	6 / 6	1 / 1
<i>Enterobacter cloacae</i>	3 / 3	2 / 2
<i>Klebsiella aerogenes</i>	3 / 1	1 / 1
<i>Proteus spp.</i>	4 / 3	0 / 0
<i>Serratia marcescens</i>	2 / 2	1 / 1
<i>Streptococcus agalactiae</i>	2 / 1	1 / 0
<i>Moraxella catarrhalis</i>	2 / 1	0 / 0
<i>Mycoplasma pneumoniae</i>	1 / NA	0 / NA
<i>Morganella morganii</i>	NA / 1	NA / 1
<i>Hafnia alvei</i>	NA / 1 ^a	NA / 0
<i>Providencia stuartii</i>	NA / 1 ^a	NA / 0
Resistance detection:	FAPP / AST	FAPP / AST
MRSA	5 / 0	1 / 0
3GC-R Gram-negative bacilli ^b	4 / 3	1 / 3

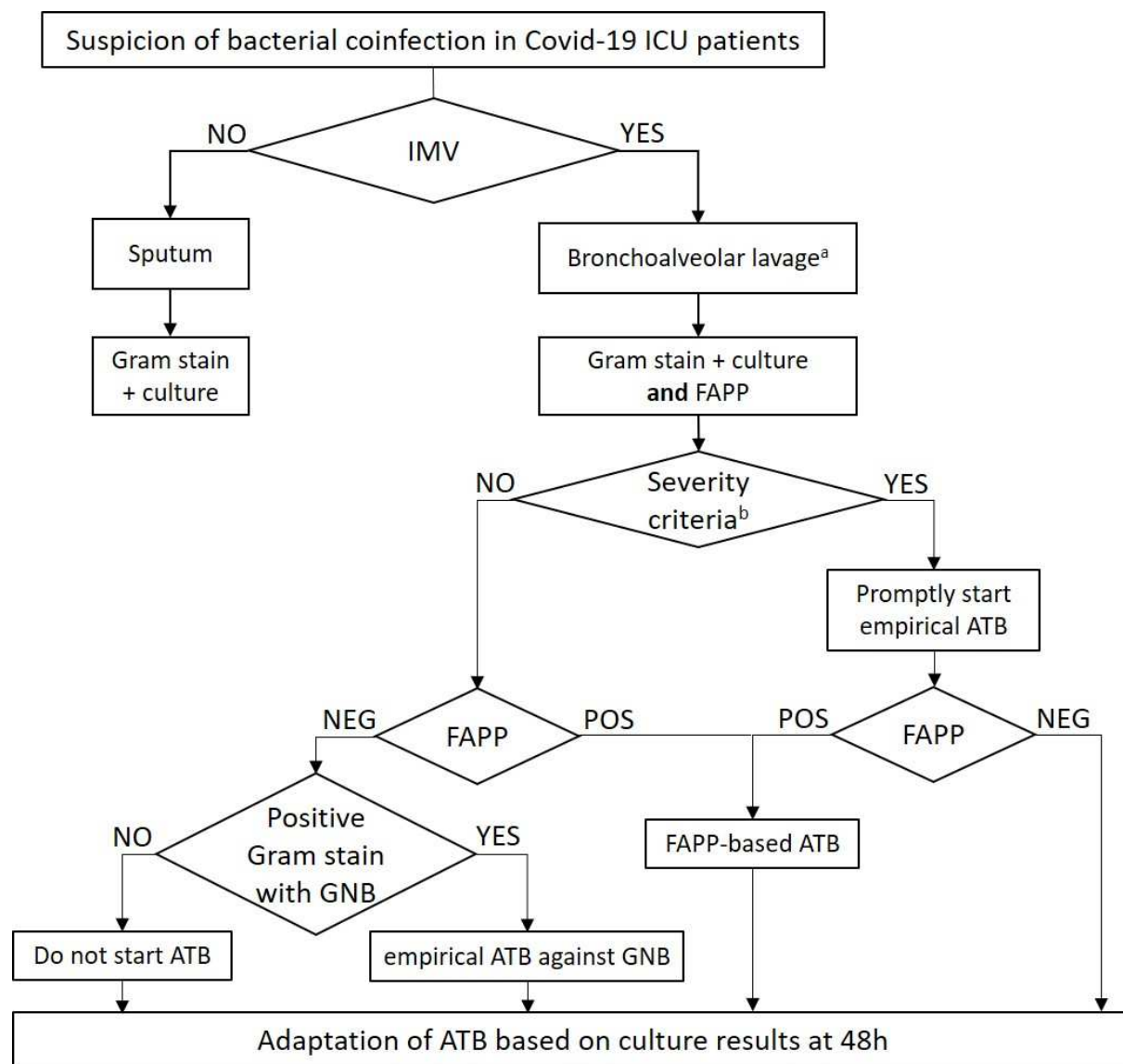
Data are presented as: n (%)

ETA: endotracheal aspirate / BAL: bronchoalveolar lavage / CAP: community-acquired pneumonia (defined as infections occurring during the first 48h of ICU admission) / VAP: ventilator-associated pneumonia / FAPP: FilmArray® Pneumonia Panel / CC: conventional culture / AST: antimicrobial susceptibility testing / MRSA: methicillino-resistant *Staphylococcus aureus* / 3GC-R: third generation cephalosporins resistance / NA: not applicable (species not detected either by the FAPP or by the CC).

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Figure 1. Clinical algorithm for initiating antibiotics using FAPP in bacterial coinfection of critically ill COVID-19 patients.



IMV, invasive mechanical ventilation; BAL, bronchoalveolar lavage; FAPP, FilmArray® Pneumonia Panel; ATB, antibiotics; GNB, Gram-negative bacilli.

^a Endotracheal aspirate samples could be used but need cautious interpretation regarding the risk of over-diagnosis due to tracheobronchial colonization

^b Septic shock (according to SEPSIS-3) or severe ARDS (according to Berlin criteria)

265 **Supplementary Table 1.** Targets identified by the FAPP 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
Atypical bacteria	qualitative	<i>Chlamydophila pneumoniae</i> , <i>Legionella pneumophila</i> , <i>Mycoplasma pneumoniae</i>
Bacteria	semi-quantitative*	<i>Acinetobacter calcoaceticus</i> - <i>Acinetobacter baumannii</i> complex, <i>Enterobacter cloacae</i> complex, <i>Escherichia coli</i> , <i>Haemophilus influenzae</i> , <i>Klebsiella aerogenes</i> , <i>Klebsiella oxytoca</i> , <i>Klebsiella pneumoniae</i> , <i>Moraxella catarrhalis</i> , <i>Proteus</i> spp., <i>Pseudomonas aeruginosa</i> , <i>Serratia marcescens</i> , <i>Staphylococcus aureus</i> , <i>Streptococcus agalactiae</i> , <i>Streptococcus pneumoniae</i> , <i>Streptococcus pyogenes</i>
Antimicrobial resistance markers	qualitative	CTX-M, KPC, NDM, IMP, VIM, OXA-48 like <i>mecA/mecC</i> and MREJ

266 * Reported as 10⁴, 10⁵, 10⁶, or ≥10⁷ copies/mL.

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