Performance of a multiplex polymerase chain reaction panel for identifying bacterial pathogens causing pneumonia in critically ill patients with COVID-19

François Caméléna [a,b,1], Anne-Clotilde Moy [c,1], Emmanuel Dudoignon [c,d], Thibaut Poncin [a,b], Benjamin Deniau [c,d], Lucie Guillemet [c], Jérôme Le Goff [a], Mélissa Budoo [a], Mourad Benyamina [c], Maïté Chaussard [c], Maxime Coutrot [c], Matthieu Lafaurie [e], Benoît Plaud [c,d], Alexandre Mebazaa [c,d], François Depret [c,d], Béatrice Berçot [a,b,*]

[a] Department of Microbiology, Saint-Louis-Lariboisière Hospital Group, Assistance Publique-Hôpitaux de Paris, Paris, France
[b] University of Paris, INSERM 1137, IAME, Paris, France
[c] Department of Anesthesiology and Critical Care and Burn Unit, Saint-Louis-Lariboisière Hospital Group, Assistance Publique-Hôpitaux de Paris, Paris, France
[d] University of Paris, INSERM 942, INI-CRCT network, Paris, France
[e] Department of Infectious Disease, Saint-Louis-Lariboisière Hospital Group, Assistance Publique-Hôpitaux de Paris, Paris, France

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ABSTRACT

The FilmArray® Pneumonia Plus (FA-PP) panel can provide rapid identifications and semiquantitative results for many pathogens. We performed a prospective single-center study in 43 critically ill patients with coronavirus disease 2019 (COVID-19) in which we performed 96 FA-PP tests and cultures of blind bronchoalveolar lavage (BBAL). FA-PP detected 1 or more pathogens in 32% (31/96 of samples), whereas culture methods detected at least 1 pathogen in 35% (34/96 of samples). The most prevalent bacteria detected were *Pseudomonas aeruginosa* (*n* = 14) and *Staphylococcus aureus* (*n* = 11) on both FA-PP and culture. The FA-PP results from BBAL in critically ill patients with COVID-19 were consistent with bacterial culture findings for bacteria present in the FA-PP panel, showing sensitivity, specificity, and positive and negative predictive value of 95%, 99%, 82%, and 100%, respectively. Median turnaround time for FA-PP was 5.5 h, which was significantly shorter than for standard culture (26 h) and antimicrobial susceptibility testing results (57 h).

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1. Introduction

Severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2), which causes coronavirus disease 2019 (COVID-19), has rapidly spread worldwide. Several studies have reported complications of COVID-19, such as bacterial pneumonia, acute respiratory distress syndrome (ARDS), and multiple organ failure syndromes (Lescure et al., 2020; Rodriguez-Morales et al., 2020; Shen et al., 2020). Recent guidelines for the management of adults critically ill with COVID-19 have suggested the empiric use of antimicrobial agents in patients with respiratory failure (Alhazzani et al., 2020). The accurate and timely diagnosis of bacterial pneumonia, particularly in cases of hospital-acquired pneumonia (HAP) and ventilator-associated pneumonia (VAP), is particularly challenging, and this condition remains a major cause of morbidity and mortality (Nair and Niederman, 2015; Papazian et al., 2020).

Molecular tests provide a rapid turnaround time (TAT), together with identifications and semiquantitative results for many pathogens responsive to antimicrobial therapy. Multiplex testing may provide additional information concerning the presence of antibiotic resistance genes, thereby improving antibiotic management (Lee et al., 2019; Yoo et al., 2020). We performed a prospective single-center study on critically ill patients with COVID-19 in which we conducted parallel tests of blind bronchoalveolar lavage (BBAL) by conventional culture and FilmArray® Pneumonia Plus (FA-PP) panel (BioFire, Salt Lake City, UT). The aim of this study was to evaluate the performance of FA-PP and to compare its TAT with that of conventional cultures.

2. Materials and methods

2.1. Study population

We conducted a prospective single-center study of consecutive patients admitted to the intensive care unit (ICU) at the Saint-Louis Hospital (Assistance Publique-Hôpitaux de Paris, Paris, France) between

* Corresponding author. Tel.: +33-1-42-38-50-96; fax: +33-1-42-49-92-00.
 E-mail address: beatrice.bercot@aphp.fr (B. Berçot).
[1] These authors contributed equally to this work.

March 22, 2020, and April 15, 2020, for COVID-19 complicated by respiratory failure. We included all patients on invasive mechanical ventilation for whom BBAL was performed. The diagnosis of COVID-19 was based on the detection of SARS-CoV-2 from nasopharyngeal samples. This detection relies on viral RNA amplification by using reverse-transcription polymerase chain reaction (PCR) (RealStar Altona®) to amplify SARSCoV2 E gene. Patients with the following criteria were excluded from the analysis: aged under 18 years, pregnant, or dying within 48 h of admission. For each patient, BBAL was performed immediately after intubation (or on admission to the ICU if the patient was already intubated) and in cases in which VAP was suspected. We recorded clinical, laboratory, and antimicrobial therapy data. The study was approved by the local ethics committee (the ethics committee of *Société Française d'Anesthésie Réanimation* IRB 00010254-2019-203).

2.2. Conventional microbiological analysis

BBAL was performed with a sterile catheter and a specimen trap kit. The suction tube was introduced blindly through the endotracheal tube and wedged into the tracheobronchial tree before suction; 50 mL of saline was successively injected and aspirated twice, and the contents of the second aspiration were analyzed, the contents of the first being discarded (Mentec et al., 2004). Samples were transported immediately to the laboratory. Samples received from 6 PM to 8 AM were conserved at +4 °C and tested the following morning. We tested the BBAL samples in parallel by FA-PP and conventional culture, and the results were released as soon as they become available. The decision to use FA-PP results for the management of antimicrobial therapy was left to the discretion of the treating physician.

We plated 10 µL of BBAL on agar media (BioMérieux, Marcy l'Etoile, France) including blood, colistin–nalidixic acid blood and chocolate agars, and incubated the plates in ambient air or under an atmosphere enriched with 5% CO_2 at 35 °C. No Gram staining was performed on the BBAL to protect laboratory staff, as this procedure requires centrifugation, which may generate aerosols. Bacteria were semiquantified by calculating colony-forming units (CFU) per mL. Culture results were interpreted according to the recommendations of the European Society for Clinical Microbiology and Infectious Diseases (Cornaglia et al., 2012), after observation at 18–24 h, and negative plates were incubated for a further 2 days, to ensure an absence of bacterial growth, before being classified definitively as negative. A cutoff of 10^4 CFU/mL for significant growth of potential pathogens for BBAL was used. Bacteria were identified by using MALDI-TOF MS (VITEK-MS, BioMérieux, Marcy-l'Étoile, France) according to the manufacturer's instructions. Antimicrobial susceptibility testing (AST) was performed by the disk diffusion method or with the VITEK 2 (BioMérieux, Marcy-l'Étoile, France) in accordance with the recommendations of the European Committee on Antimicrobial Susceptibility Testing.

2.3. Molecular investigation

As recommended by the manufacturer, a dry swab was dipped into the BBAL and then immersed in dilution buffer before injection of the fluid into the FA-PP cartridge. The analysis was carried out in 67 min on the Filmarray® apparatus (version 2.1.336.0) with the Pneumoplus v2.0 pouch system (version 2.1.0.5).

2.4. Statistical analysis

Sensitivity (Se), specificity (Sp), positive and negative predictive values (PPV and NPV), likelihood ratio, and κ index were calculated by comparing the results for conventional culture with those of FA-PP only for bacterial pathogens present in the FA-PP panel. Performance was measured only for bacterial analysis, with bacterial culture used as the gold standard reference method. Two-tailed Mann–Whitney tests were used to compare TAT between the 2 methods.

3. Results

In total, 96 samples from 43 patients were tested in parallel by FA-PP and conventional BBAL culture. The median age of the patients was 64 years, 86% were male, 27% were obese, and 72% had hypertension (Table 1). On admission to the ICU, 37 (86%) patients were already on invasive mechanical ventilation. BBAL was performed under antibiotic treatment in 55% (53/96) of samples.

FA-PP detected 1 or more pathogens in 32% (31/96) of samples (corresponding to 18 patients), whereas culture methods detected at least 1 pathogen in 35% (34/96) of samples (corresponding to 20 patients). At first BBAL, 25% (10/43) of FA-PP tests and 21% (9/43) of cultures gave positive results. The most prevalent bacteria detected were

Table 1
Main characteristics of patients on admission to the ICU.

Characteristics	Patients (n = 43)
Median age, years (IQR)	64 [56–70]
Sex, male, no. (%)	37 (86)
Median BMI, kg/m² (IQR)	27 [26–30]
Comorbidities, no. (%)	
Hypertension	31 (72)
Heart failure	1 (2.3)
ARBS	17 (39.5)
Coronary disease	5 (11.6)
Diabetes mellitus	16 (37.0)
Pulmonary disease	7 (16.0)
Immunosuppression	3 (7.0)
Severity of illness	
Duration from onset of symptoms to ICU admission, days (IQR)	8.5 [5.3–11.6]
SAPS II (IQR)	37 [28–49]
SOFA score on day 1 (IQR)	5 [4–7]
Acute kidney injury, no. (%)	28 (65.1)
KDIGO 1	6 (13.9)
KDIGO 2	6 (13.9)
KDIGO 3	16 (34.9)
Renal replacement therapy, no. (%)	8 (18.6)
Mechanical ventilation on ICU admission, no. (%)	37 (86)
ARDS, no. (%)	43 (100)
Ratio of PaO_2 to FiO_2, mm Hg (PaO_2/FiO_2) at admission (IQR)	136 [82.5–174.5]
Ratio of PaO_2 to FiO_2 nadir, mm Hg (IQR)	82 [70–103]
Median number of prone position ventilation sessions (IQR)	1 [0–2]
Duration of mechanical ventilation, days (IQR)	10 [7–12]
Use of noradrenaline during the first 48 h, no. (%)	36 (83.7)
Length of stay in ICU, days (IQR)	11 [8–13]
Death, no. (%)	12 (27.9)
Admission biological data	
WBC count, ×10⁹ cells/L (IQR)	9.52 [5.2–12.4]
Lymphocyte count, ×10⁹ cells/L (IQR)	0.79 [0.5–1.1]
Serum creatinine concentration, µmol/L (IQR)	75 [57–100.5]
D-dimers, ng/mL (IQR)	2610 [1490–4820]
Radiological data	
X-ray, number of dials affected at admission	4 [2–4]
CT during hospitalization, no. (%)	29 (67.4)
10–25[a]	2 (6.9)
25–50[a]	9 (31.0)
50–75[a]	10 (34.5)
>75[a]	8 (27.6)
Antimicrobial treatment, no. (%)	
Antibiotic therapy before admission	29 (67.4)
Third-generation cephalosporin	22 (51.1)
Macrolide	21 (48.3)
Other antibiotics	11 (25.6)

BMI = body mass index; ICU = intensive care unit; SAPS II = Simplified Acute Physiology Score II; SOFA = Sepsis-Related Organ Failure Assessment; KDIGO = Kidney Disease Improving Global Outcomes; ARDS = acute respiratory distress syndrome; ARBS = angiotensin–renin–aldosterone blocker system; WBC = white blood cells; CT = computerized tomography.

[a] Specific COVID-19 pulmonary lesions.

Staphylococcus aureus ($n = 5$ on both FA-PP and culture) followed by *Haemophilus influenzae* ($n = 4$ and $n = 2$ on FA-PP and culture, respectively) (Table 2). In cases in which other BBALs were performed, 40% (21/53) of samples tested positive by FA-PP, whereas 47% (25/53) tested positive on culture. The most prevalent bacteria detected was *Pseudomonas aeruginosa* ($n = 11$ on both FA-PP and culture) followed by *S. aureus* ($n = 6$ on both FA-PP and culture) (Table 2). The performance of FA-PP for each pathogen is shown in Table 2. Of the 43 patients, 11/43 (25.5%) had only 1 sampling, and in each case, a negative result was found for both FA-PP tests and cultures. For the remaining 32/43 (74.5%) patients, iterative sampling was performed; all FA-PP test and culture results for these patients are presented in Supplementary Table S1. Neither atypical bacteria nor viruses were detected by FA-PP. The pathogen most frequently detected by FA-PP in the absence of positive cultures was *H. influenzae* ($n = 3$). Positive cultures were obtained for 8 samples that tested negative by FA-PP. In 6 of these 8 samples, the pathogens present were not included in the FA-PP panel (*Citrobacter koseri* in 4 cases, *Stenotrophomonas maltophilia* in 1 case, and *Enterococcus faecium* in 1 case). *S. aureus* and *Klebsiella aerogenes* were recovered from single culture from 2 samples that tested also negative by FA-PP. Bacterial load was 10^3 CFU/mL of culture for both these pathogens, corresponding to a low organism burden. We found that 28 of the bacteria of the FA-PP panel grew to significant levels (i.e., $\geq 10^4$ CFU/mL) in culture, with 100% (28/28) presenting $\geq 10^4$ copies of bacterial nucleic acid/mL on FA-PP. Another 10 bacteria grew to nonsignificant levels (i.e., $< 10^4$ CFU/mL), and 80% (8/10) of these bacteria were found to be present at a level $\geq 10^4$ copies/mL by FA-PP. FA-PP detected 2 bla_{CTX-M} extended-spectrum β-lactamases (ESBLs), 1 bla_{VIM} carbapenemase, and 2 *S. aureus* methicillin-resistance genes. AST detected the presence of an ESBL encoded by a bla_{CTX-M} gene in *Escherichia coli* and methicillin-resistant *S. aureus* (MRSA) in 2 isolates corresponding to 2 cases of *mecA/C* detection by FA-PP. The detection of the other resistance genes, 1 bla_{CTX-M} and 1 bla_{VIM}, remained unexplained because

no other viable bacteria with a phenotype corresponding to the presence of these genes were obtained in culture.

Median TAT was significantly shorter for FA-PP tests than for standard culture and AST results [5.5 h (IQR: 3.4–12.4) versus 25.9 h (IQR: 22.5–35.7) and 57 h (IQR: 47.7–70.8), $P < 0.001$ and $P < 0.001$, respectively].

4. Discussion

We studied 43 critically ill patients with COVID-19 who underwent BBAL at least once for suspected bacterial pneumonia. A few cases of bacterial pneumonia have been reported in critically ill patients with COVID-19. The diagnosis of bacterial pneumonia in such patients remains challenging, as obtaining distal respiratory samples by bronchoscopy exposes intensivists to a high risk of contamination (Bhatraju et al., 2020; Lescure et al., 2020; Rodriguez-Morales et al., 2020). We, therefore, performed BBAL, which is simple, is suitable for bacterial culture, and does not require bronchoscopy (Mentec et al., 2004). Multiplex syndromic panels for the detection of bacterial pathogens responsible for pneumonia have recently been developed and shown to yield more sensitive results more rapidly than conventional culture, particularly in cases in which antibiotics were administered before sampling (Lee et al., 2019; Papazian et al., 2020; Yoo et al., 2020). However, there are currently no recommendations for routine molecular testing, and multiplex PCR is rarely used for detection of the bacteria typically responsible for pneumonia. Questions about the clinical usefulness of this approach remain, particularly in the context of critically ill patients with COVID-19.

This is, to our knowledge, the first study to evaluate, in real-time, FA-PP relative to culture using exclusively distal respiratory samples for the diagnosis of bacterial pneumonia in critically ill patients. It shows that the results of FA-PP are consistent (Se 95%, Sp 99%, PPV 82%, and NPV 100%) with those of conventional culture for the bacterial pathogens

Table 2
Performance of FA-PP relative to conventional culture.

Bacterial pathogen	FA-PP+ & culture+	FA-PP+ & culture−	FA-PP− & culture+	FA-PP− & culture−	Se (95% CI)	Sp (95% CI)	PPV (95% CI)	NPV (95% CI)	LR+ (95% CI)	LR− (95% CI)	κ coefficient (95% CI)
Acinetobacter baumannii	0	0	0	96	-	1.00 (−)	-	1.00 (−)	-	-	1.00 (−)
Staphylococcus aureus	10	1	1	84	0.91 (0.58–1.0)	0.99 (0.94–1.0)	0.91 (0.59–0.98)	0.99 (0.93–1.0)	77 (11–547)	0.09 (0.01–0.06)	0.98 (0.93–1.0)
Streptococcus pneumoniae	3	1	0	92	1.00 (0.29–1.0)	0.99 (0.94–1.0)	0.75 (0.30–0.95)	1.00 (−)	93 (13–653)	0.00 (−)	0.99 (0.94–1.0)
Escherichia coli	3	0	0	93	1.00 (0.29–1.0)	1.00 (0.96–1.0)	1.00 (−)	1.00 (−)	-	0.00 (−)	1.00 (0.96–1.0)
Enterobacter cloacae	0	1	0	95	-	0.99 (0.97–1.0)	0.00 (−)	1.00 (−)	-	-	0.99 (0.94–1.0)
Klebsiella aerogenes	3	0	1	92	0.75 (0.19–0.99)	1.00 (0.96–1.0)	1.00 (−)	0.99 (0.94–1.0)	-	0.25 (0.06–1.36)	0.99 (0.94–1.0)
Klebsiella pneumoniae	0	1	0	95	-	0.99 (0.97–1.0)	0.00 (−)	1.00 (−)	-	-	0.99 (0.94–1.0)
Klebsiella oxytoca	0	0	0	96	-	1.00 (−)	-	1.00 (−)	-	-	1.00 (−)
Pseudomonas aeruginosa	14	0	0	82	1.00 (0.77–1.0)	1.00 (0.96–1.0)	1.00 (−)	1.00 (−)	-	0.00 (−)	1.00 (0.96–1.0)
Haemophilus influenzae	2	3	0	91	1.00 (0.16–1.0)	0.97 (0.91–0.99)	0.40 (0.18–0.67)	1.00 (−)	31 (10–95)	0.00 (−)	0.97 (0.91–0.99)
Streptococcus agalactiae	1	0	0	95	1.00 (0.03–1.0)	1.00 (0.96–1.0)	1.00 (−)	1.00 (−)	-	0.00 (−)	1.00 (0.96–1.0)
Streptococcus pyogenes	0	0	0	96	-	1.00 (−)	-	1.00 (−)	-	-	1.00 (−)
Serratia marcescens	0	0	0	96	-	1.00 (−)	-	1.00 (−)	-	-	1.00 (−)
Proteus spp.	0	1	0	95	-	0.99 (0.97–1.0)	0.00 (−)	1.00 (−)	-	-	0.99 (0.94–1.0)
Moraxella catarrhalis	0	0	0	96	-	1.00 (−)	-	1.00 (−)	-	-	1.00 (−)
Total (per analysis)	36	8	2	1394	0.95 (0.82–0.99)	0.99 (0.99–1.0)	0.82 (0.69–0.90)	1.00 (0.99–1.0)	166 (83–333)	0.05 (0.01–0.20)	0.99 (0.99–1.0)

FA-PP = FilmArray Pneumonia Plus; Se = sensitivity; Sp = specificity; PPV = positive predictive value; NPV = negative predictive value; LR = likelihood ratio.

present in the FA-PP panel. FA-PP significantly decreased TAT and increased the number of bacterial detections in critically ill patients with COVID-19. However, attention must be paid to bacteria not present in the FA-PP panel (e.g., *Citrobacter* spp.) that might affect antibiotic therapy and to calculations of FA-PP performance that were performed independently of the consideration of bacterial thresholds and antibiotic administration prior to sampling. Bacterial cultures of BBAL grew with significant amount of bacteria (i.e., $\geq 10^4$ CFU/mL) in 37% ($n = 16$) of patients. Among the 16 bacterial pneumonia, 4 (25%) were classified as community-acquired pneumonia, 1 (6%) as HAP, and 11 (69%) as VAP. In our study, 6% (6/96) of the samples were found to contain a bacterium not present in the FA-PP panel, and this bacterium was present at a high abundance (i.e. $\geq 10^4$ CFU/mL) in 4 of these samples. These bacteria were involved in 1 HAP and 3 VAPs.

Furthermore, FA-PP may provide a major argument for very early de-escalation and narrowing of antimicrobial therapy in specific situations (such as to withdraw anti-MRSA), if negative FA-PP results are obtained, or for the faster initiation of appropriate antimicrobial therapy, if positive FA-PP results are obtained. We also found that most of the positive FA-PP results from culture-negative samples corresponded to *H. influenzae*; the presence of this bacterium may reflect contamination by the oropharyngeal flora. Antimicrobial therapy is also known to impact bacterial growth, as 88% (7/8) of the false-positive FA-PP results were obtained from BBAL performed under antibiotic treatment. Similarly, 63% (5/8) of bacteria, present at a level $\geq 10^4$ copies/mL by FA-PP and which grew at a nonsignificant level in culture, were from BBAL performed under antimicrobial therapy. Furthermore, others studies have described that the bacterial burden could be overestimated by FA-PP compared to culture (Lee et al., 2019; Yoo et al., 2020). In addition, resistance genes detected by FA-PP were not confirmed by AST results in 2 samples, suggesting limitations to predict phenotypic susceptibility from molecular tests. These results raise important point that PCR can detect both dead and viable bacteria (Clavel et al., 2016; Lee et al., 2019; Yoo et al., 2020). It remains unclear for how long bacterial loads remain detectable after the initiation of appropriate antibiotic treatment in critically ill patients with COVID-19 and whether the monitoring of bacterial load by FA-PP would be useful in these patients and, more broadly, in all patients with suspected VAP. Further studies are required to investigate this issue. Additionally, FA-PP has a cost per test much greater than the costs of routine culture; future studies should focus on how best to use this test in a cost-effective manner.

Finally, the strengths of this study are the well-characterized population of patients (COVID-19 in ICU), the use of a single type of specimen (BBAL), and prospective testing by FA-PP and culture in parallel. Its limitations are that it was a single-center study and that the COVID-19 population may not be representative of the ventilated patients generally treated in the ICU.

5. Conclusion

In conclusion, in this prospective study of critically ill patient with COVID-19, FA-PP results were in agreement with standard culture for identifying bacterial pathogens in BBAL specimens, with a significant decrease of TAT. The use of FA-PP may enable a more complete and faster approach for the diagnosis of bacterial pneumonia in patient hospitalized in ICU. Further studies are required to address the clinical impact of FA-PP on the management of VAP in critically ill patients.

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References

Alhazzani W, Møller MH, Arabi YM, Loeb M, Gong MN, Fan E, et al. Surviving Sepsis Campaign: guidelines on the management of critically ill adults with coronavirus disease 2019 (COVID-19). Intensive Care Med 2020:1–41. https://doi.org/10.1007/s00134-020-06022-5.

Bhatraju PK, Ghassemieh BJ, Nichols M, Kim R, Jerome KR, Nalla AK, et al. Covid-19 in critically ill patients in the Seattle region—case series. N Engl J Med 2020:1–11. https://doi.org/10.1056/NEJMoa2004500.

Clavel M, Barraud O, Moucadel V, Meynier F, Karam E, Ploy M-C, et al. Molecular quantification of bacteria from respiratory samples in patients with suspected ventilator-associated pneumonia. Clin Microbiol Infect 2016;22:812.e1–812.e7. doi:https://doi.org/10.1016/j.cmi.2016.06.013.

Cornaglia G, Courcol R, Herrmann J. European manual of clinical microbiology. Basel: European Society for Clinical Microbiology and Infectious Diseases; 2012.

Lee SH, Ruan S-Y, Pan S-C, Lee T-F, Chien J-Y, Hsueh P-R. Performance of a multiplex PCR pneumonia panel for the identification of respiratory pathogens and the main determinants of resistance from the lower respiratory tract specimens of adult patients in intensive care units. J Microbiol Immunol Infect 2019;52:920–8. https://doi.org/10.1016/j.jmii.2019.10.009.

Lescure F-X, Bouadma L, Nguyen D, Parisey M, Wicky P-H, Behillil S, et al. Clinical and virological data of the first cases of COVID-19 in Europe: a case series. Lancet Infect Dis 2020;2:1–10. https://doi.org/10.1016/S1473-3099(20)30200-0.

Mentec H, May-Michelangeli L, Rabbat A, Varon E, Le Turdu F, Bleichner G. Blind and bronchoscopic sampling methods in suspected ventilator-associated pneumonia. A multicentre prospective study. Intensive Care Med 2004;30:1319–26. https://doi.org/10.1007/s00134-004-2284-7.

Nair GB, Niederman MS. Ventilator-associated pneumonia: present understanding and ongoing debates. Intensive Care Med 2015;41:34–48. https://doi.org/10.1007/s00134-014-3564-5.

Papazian L, Klompas M, Luyt C-E. Ventilator-associated pneumonia in adults: a narrative review. Intensive Care Med 2020:s00134. 20 https://doi.org/10.1007/s00134-020-05980-0.

Rodriguez-Morales AJ, Cardona-Ospina JA, Gutiérrez-Ocampo E, Villamizar-Peña R, Holguin-Rivera Y, Escalera-Antezana JP, et al. Clinical, laboratory and imaging features of COVID-19: a systematic review and meta-analysis. Travel Med Infect Dis 2020;101623. https://doi.org/10.1016/j.tmaid.2020.101623.

Shen C, Wang Z, Zhao F, Yang Y, Li J, Yuan J, et al. Treatment of 5 critically ill patients with COVID-19 with convalescent plasma. JAMA 2020:1–8. https://doi.org/10.1001/jama.2020.4783.

Yoo IY, Huh K, Shim HJ, Yun SA, Chung YN, Kang OK, et al. Evaluation of the BioFire® FilmArray® pneumonia panel for rapid detection of respiratory bacterial pathogens and antibiotic resistance genes in sputum and endotracheal aspirate specimens. Int J Infect Dis 2020;S1201–9712. https://doi.org/10.1016/j.ijid.2020.03.024.