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Respiratory bacterial co-infections in intensive care unit-hospitalized COVID-19 patients: Conventional culture vs BioFire FilmArray pneumonia *Plus* panel

Claudio Foschi [a,b,*], Anna Zignoli [a], Paolo Gaibani [b], Caterina Vocale [b], Giada Rossini [b], Silvia Lafratta [a], Andrea Liberatore [a], Gabriele Turello [a], Tiziana Lazzarotto [a,b], Simone Ambretti [b]

[a] Microbiology, DIMES, University of Bologna, via Massarenti 9, Bologna, Italy
[b] Microbiology Unit, IRCCS S.Orsola-Malpighi Hospital, Via Massarenti n 9, Bologna, Italy

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ABSTRACT

The prevalence and microbiology of concomitant respiratory bacterial infections in patients with SARS-CoV-2 infection are not yet fully understood. In this retrospective study, we assessed respiratory bacterial co-infections in lower respiratory tract samples taken from intensive care unit-hospitalized COVID-19 patients, by comparing the conventional culture approach to an innovative molecular diagnostic technology.

A total of 230 lower respiratory tract samples (i.e., bronchial aspirates or bronchoalveolar lavages) were taken from 178 critically ill COVID-19 patients. Each sample was processed by a semi-quantitative culture and by a multiplex PCR panel (FilmArray Pneumonia *Plus* panel), allowing rapid detection of a wide range of clinically relevant pathogens and a limited number of antimicrobial resistance markers.

More than 30% of samples showed a positive bacterial culture, with *Pseudomonas aeruginosa*, *Klebsiella pneumoniae* and *Staphylococcus aureus* the most detected pathogens.

FilmArray showed an overall sensitivity and specificity of 89.6% and 98.3%, respectively, with a negative predictive value of 99.7%. The molecular test significantly reduced the turn-around-time (TAT) and increased the rates of microbial detection. Most cases missed by culture were characterized by low bacterial loads (10^4–10^5 copies/mL). FilmArray missed a list of pathogens not included in the molecular panel, especially *Stenotrophomonas maltophilia* (8 cases).

FilmArray can be useful to detect bacterial pathogens in lower respiratory tract specimens of COVID-19 patients, with a significant decrease of TAT. The test is particularly useful to rule out bacterial co-infections and avoid the inappropriate prescription of antibiotics.

1. Introduction

The pandemic of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2)-associated coronavirus disease 2019 (COVID-19) is a major threat for global health, worldwide. Approximately 15% of patients develop a severe respiratory failure requiring hospitalization in intensive care units (ICUs) (Kolenda et al., 2020).

In view of the poor prognosis of these severe forms, respiratory bacterial co-infections may be of importance, with increased rates of shock, mechanical ventilation, and mortality (Li et al., 2020).

Studying the prevalence and the aetiology of bacterial co-infections in patients with viral respiratory infections can be very helpful to initiate an early and appropriate antimicrobial treatment and improve the prognosis.

The prevalence and microbiology of concomitant bacterial infections in patients with SARS-CoV-2 infection are not yet fully understood (Thaden and Maskarinec, 2020). Recent works, mainly based on conventional culture approaches, showed that respiratory secondary bacterial infections occur in many COVID-19 patients, with different prevalence and aetiology depending on the setting, patients, and severity of the disease (Thaden and Maskarinec, 2020; Vaillancourt and Jorth, 2020).

* Corresponding author at: Microbiology, DIMES, University of Bologna, St. Orsola Hospital, Via Massarenti, 9, 40138 Bologna, Italy.
E-mail address: claudio.foschi2@unibo.it (C. Foschi).

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Besides culture, only a few data are available about the use of molecular techniques for the identification of bacterial pathogens in the respiratory tract of COVID-19 patients (Kolenda et al., 2020; Caméléna et al., 2021).

Molecular tests provide a rapid turnaround time (TAT), together with identifications and semi-quantitative results for many pathogens responsive to antibiotic therapy. Moreover, multiplex testing may provide information about the presence of the most clinically relevant antibiotic resistance markers, thereby improving antimicrobial stewardship.

In this retrospective study, we assessed respiratory bacterial co-infections in lower respiratory tract samples taken from ICU-hospitalized COVID-19 patients, by comparing the conventional culture approach to an innovative molecular diagnostic technology (i.e., BioFire FilmArray Pneumonia *Plus* panel).

2. Materials and methods

2.1. Study setting

We performed a retrospective study of all consecutive patients with laboratory-confirmed SARS-CoV-2 virus infection, hospitalized from 10 March through 30 December 2020 in ICUs from Hospitals in Bologna, Italy.

Inclusion criteria were: (i) age $\geq$ 18 years; (ii) patients requiring ICU admission and assisted ventilation; (iii) subjects who underwent a lower respiratory tract sampling for conventional bacterial culture and a multiplex PCR, targeting respiratory pathogens and antibiotic resistance genes (i.e., BioFire FilmArray Pneumonia *Plus* panel). Exclusion criteria included: (i) early (<48 h) ICU discharge; (ii) age < 18 years.

The decision to perform the multiplex PCR test in addition to the standard bacterial culture was taken according to the clinical judgment in selected cases (e.g., evidence of clinical disease progression, increases in respiratory secretions or deterioration in respiratory status after a period of clinical stability).

For all the patients, the respiratory samples (i.e., bronchial aspirates or bronchoalveolar lavages) were processed at the Microbiology Unit of S. Orsola-Malpighi Hospital in Bologna following routine diagnostic procedures. Each respiratory specimen was analysed both by conventional culture and by a multiplex PCR panel (BioFire FilmArray Pneumonia *Plus* panel; BioFire Diagnostics, Salt Lake City, UT, USA) the same day of arrival at the laboratory (see specific paragraph).

The study was conducted according to the regulations of the S. Orsola-Malpighi Hospital Ethical Committee and to the 1964 Helsinki declaration and its later amendments.

2.2. COVID-19 detection

The presence of SARS-Cov2 was detected by RT-PCR assay, starting from nasopharyngeal swabs (UTM-RT swab, Copan, Italy) or bronchoalveolar lavages (BAL). Nucleic acids were extracted from 280 μL of the clinical samples by Nuclisens EasyMag (BioMérieux, Marcy l'Etoile, France) following the manufacturer's instructions. Detection of SARS-CoV-2 virus was performed by real time RT-PCR following the WHO and/or CDC protocol in a QuantStudio S5 Real-time PCR system (ThermoFisher, Waltham, MA, USA). Microbiological diagnosis of SARS-CoV2 infection was defined as a positive RT-PCR test on respiratory specimens.

2.3. Conventional culture

Respiratory samples were analysed by a semi-quantitative culture, as follows. A ten-microliter volume of BAL or bronchial aspirates was seeded on different media (horse blood agar, salt-mannitol agar, Herellea agar, *Haemophilus* chocolate agar) and incubated at 37 °C for up to 48 h. Afterwards, microorganisms that grew in significant amounts according to the guidelines of standard laboratory procedures were quantified (expressed as 10^4, 10^5, 10^6 and $> 10^6$ colony forming units/mL) and sub-cultured on non-selective plates.

Bacterial identification at the species level was achieved by matrix-assisted laser desorption/ionisation time-of-flight mass spectrometry (MALDI TOF MS; Maldi Biotyper, Bruker Daltonics, Bremen, Germany). An antimicrobial susceptibility testing (AST) was performed by a broth microdilution-based method (WalkAway Microscan, Beckman Coulter, Milano, Italy), following the manufacturer's instructions.

2.4. BioFire FilmArray Pneumonia Plus panel

The BioFire FilmArray Pneumonia *plus* panel is a fully automated and multiplex PCR assay that allows rapid detection (approximately 1 h) of a wide range of clinically relevant pathogens and a limited number of antimicrobial resistance markers (Crémet et al., 2020). The test was performed according to the manufacturer's instructions starting from 200 μL of respiratory sample.

2.5. Data analysis

Results obtained from the two approaches were compared for detection of bacteria and antibiotic resistance. Sensitivity, specificity, positive and negative predictive values (PPV and NPV), were calculated by comparing the results for conventional culture with those of FilmArray only for bacterial pathogens present in the molecular panel. Performance was measured considering bacterial culture as the gold standard reference method.

3. Results

3.1. Study population

During the study period, a total of 230 lower respiratory tract samples (178 bronchial aspirates and 52 BAL) from 178 patients were tested (29 patients were tested twice, 3 three times, 4 four times and 1 five times). For patients undergoing multiple sampling, the mean time between the tests was about 7–10 days.

The sampling for bacterial culture and FilmArray was performed within 3 weeks after ICU admission/COVID-19 diagnosis.

3.2. Bacterial culture

Cultures were positive for at least 1 bacterial pathogen in 79 samples (79/230; 34.3%). Of them, 23 showed a contemporary positivity for two or more microorganisms.

The commonest isolated bacteria included *Pseudomonas aeruginosa* (*n* = 34; 14.7%), *Klebsiella pneumoniae* (*n* = 16; 6.9%), *Staphylococcus aureus* (*n* = 15; 6.5%), *Acinetobacter baumannii* (*n* = 8; 3.4%) and *Stenotrophomonas maltophilia* (n = 8; 3.4%). Less frequently, cultures were positive for *Klebsiella aerogenes* (*n* = 6; 2.6%), *Escherichia coli* (*n* = 5; 2.1%), *Serratia marcescens* (n = 5; 2.1%), *Streptococcus pneumoniae* (*n* = 2; 0.8%), *Burkholderia* spp. (n = 2; 0.8%), *Citrobacter koseri* (n = 2; 0.8%), *Enterobacter cloacae* (n = 2; 0.8%), *Haemophilus influenzae* (n = 2; 0.8%), *Proteus mirabilis* (*n* = 1; 0.4%), *Corynebacterium striatum* (n = 1; 0.4%) and *Enterobacter asburiae* (n = 1; 0.4%).

Considering antimicrobial resistance, we found 6 methicillin-resistant *S. aureus* (MRSA) strains, and 3 Enterobacterales (2 *K. pneumoniae* and 1 *E. coli*) resistant to 3rd-generation cephalosporins, producing extended-spectrum beta-lactamases (ESBL).

3.3. FilmArray Pneumonia Plus panel

Overall, 92 samples tested positive (40%) by FilmArray for at least 1 bacterial pathogen. In 38 cases (41%) two or more microorganisms were identified. Only 1 case positive for atypical bacteria was found (i.e.,

Mycoplasma pneumoniae), whereas no viruses were detected.

As for culture, the commonest bacteria detected by FilmArray were *Pseudomonas aeruginosa* ($n = 37$; 16.0%), *Staphylococcus aureus* ($n = 25$; 10.8%), *Klebsiella pneumoniae* ($n = 23$; 10.0%) and *Acinetobacter calcoaceticus-Acinetobacter baumannii* complex ($n = 16$; 6.9%), followed by *Escherichia coli* ($n = 8$; 3.4%), *Serratia marcescens* (n = 8; 3.4%), *Haemophilus influenzae* ($n = 7$; 3.0%), *Klebsiella aerogenes* ($n = 6$; 2.6%) and *Streptococcus pneumoniae* (n = 6; 2.6%). In addition, 4 cases of *Enterobacter cloacae complex* (1.7%), 2 of *Klebsiella oxytoca* (0.8%), 1 of *Streptococcus pyogenes* (0.4%) were found.

Detection of antimicrobial resistance markers was as follows: 6 CTX-M and 8 MecA/C (methicillin resistance genes) and MREJ (staphylococcal cassette chromosome Mec-orfX right-extremity junction) from *S. aureus*.

3.4. Comparison between culture and FilmArray

Table 1 shows the performance of FilmArray compared to conventional culture. Considering only the pathogens available in the molecular panel, FilmArray showed an overall sensitivity of 89.6% with a specificity of 98.3%. PPV and NPV were 60.1% and 99.7%, respectively. The best performances were reached for *A. baumannii*, *E. cloacae complex*, *H. influenzae*, *S. aureus* and *S. pneumoniae*.

The pathogens most frequently detected by FilmArray in the absence of positive cultures were *K. pneumoniae* ($n = 10$) and *S. aureus* (n = 10).

Most cases (about 70%) missed by culture in presence of a positive FilmArray were characterized by low bacterial loads (10^4 - 10^5 copies/mL) (Table 2). Most pathogens (90%) not detected by FilmArray showed a polymicrobial culture with bacterial loads ranging between 10^4 and 10^5 cfu/mL (Table 2).

As expected, FilmArray missed a list of pathogens not included in the molecular panel but detected by culture, especially *Stenotrophomonas maltophilia* ($n = 8$) and *Citrobacter koseri* ($n = 2$).

For positive patients who underwent multiple testing, several cases were characterized by the reduction of the bacterial loads or by the missed detection of the pathogens, over time.

Regarding resistance markers, we found a 100%-concordance for the detection of ESBL (i.e., CTX-M vs resistance to 3rd generation cephalosporins), whereas a few discordant results were found for MRSA. In particular, 2 MRSA detected by culture tested negative for MecA/C and MREJ by FilmArray, and 3 cases of positivity for MecA/C by FilmArray were characterized by the growth of methicillin-sensitive *S. aureus* strains.

Table 2
Concordance of bacterial loads between culture and FilmArray Pneumonia *Plus*. Only bacterial pathogens present in the molecular panel were considered.

		FilmArray Pneumonia *Plus* (copies/mL)				
		$>10^7$/ 10^7	10^6	10^5	10^4	Not detected
Culture (cfu/mL)	$>10^6$	12	1	1	3	1
	10^6	7	2	0	1	0
	10^5	6	7	4	1	3
	10^4	9	12	9	4	6
	Not detected	7	10	17	23	

4. Discussion

We studied 230 lower respiratory tract samples (bronchial aspirates and BALs) from 178 ICU-hospitalized COVID-19 patients to assess the presence of bacterial co-infections. For this purpose, a conventional culture approach was compared to a molecular multiplex syndromic panel able to detect the most significant bacterial pathogens responsible for pneumonia, as well as some resistance markers.

In line with previous works, we found that respiratory bacterial co-infections are quite common in critically ill COVID-19 patients (more than 30% of positive cultures), with *Pseudomonas aeruginosa* (14.7%), *Klebsiella pneumoniae* (6.9%) and *Staphylococcus aureus* (6.5%) the most detected pathogens (Caméléna et al., 2021; Maes et al., 2021; Hughes et al., 2020).

The prevalence of bacterial infections found in our setting was twice as high as a recent study by Kolenda and colleagues (Kolenda et al., 2020), reporting a positive bacterial culture in 15% of COVID-patients. Differences in the time of sampling, in the presence and severity of comorbidities, and in the microbial ecology/epidemiology could explain this discrepancy.

Considering the time and criteria of sampling (up to 3 weeks after ICU admission, in presence of clinical disease progression) and the type of microorganisms detected (only a few cases of community acquired bacteria, with the predominance of hospital acquired pathogens), most of the respiratory bacterial infections should be considered 'secondary infections' rather than 'co-infections'.

FilmArray showed good performances (sensitivity: 89.6%, specificity: 98.3%), consistent with those of standard culture for the detection of pathogens available in the molecular panel.

Moreover, the molecular approach significantly decreased TAT (1 h vs about 48 h for bacterial identification and AST by culture) and increased the rates of bacterial detection in COVID-19 patients, being

Table 1
Performance of FilmArray Pneumonia *Plus* panel compared to conventional culture. Only bacterial pathogens present in the molecular panel were considered.

Pathogen	FA-PP + culture +	FA-PP + culture -	FA-PP - culture +	FA-PP - culture -	Sensitivity	Specificity	PPV	NPV
A. baumannii complex	8	8	0	214	100%	96.4%	50%	100%
E. cloacae complex	2	2	0	226	100%	99.1%	50%	100%
E. coli	4	4	1	221	80%	98.2%	50%	99.5%
H. influenzae	2	5	0	223	100%	97.8%	28.7%	100%
K. aerogenes	5	1	1	223	83.3%	99.6%	83.3%	99.5%
K. oxytoca	0	2	0	228	–	99.1%	–	100%
K. pneumoniae	13	10	3	204	81.3%	95.3%	56.5%	98.5%
M. catarrhalis	0	0	0	230	–	100%	–	100%
Proteus spp.	0	0	1	229	0%	100%	–	99.5%
P. aeruginosa	31	6	3	190	91.2%	96.9%	83.7%	98.4%
S. marcescens	4	4	1	221	80%	98.2%	50%	99.5%
S. aureus	15	10	0	205	100%	95.3%	60%	100%
S. agalactiae	0	0	0	230	–	100%	–	100%
S. pneumoniae	2	4	0	224	100%	98.2%	33.3%	100%
S. pyogenes	0	1	0	229	–	99.6%	–	100%
Total (per analysis)	86	57	10	3297	89.6%	98.3%	60.1%	99.7%

FF-PP = FilmArray Pneumonia *Plus* panel; PPV/NPV = positive and negative predictive values.

particularly suitable for rule out the presence of co-infections (NPV: 99.7%).

However, much attention must be paid to pathogens not included in the panel (especially to *Stenotrophomonas maltophilia*) that can reach significant positivity rates and might affect antibiotic therapy.

When looking at discordant results between the two methods, a few aspects should be mentioned.

At first, most discordant samples were characterized by low microbial loads by culture or by FilmArray, suggesting that the parallel use of both approaches can significantly improve the detection of bacteria present in low amounts. In this context, in line with our results, previous studies have described that the bacterial burden could be overestimated by FilmArray compared to culture (Yoo et al., 2020; Murphy et al., 2020).

Furthermore, antimicrobial therapy can impact bacterial growth, leading to negative cultures but to persistent positive PCR tests, not able to distinguish dead from viable bacteria (Yoo et al., 2020).

In our study, we cannot rule out that a significant number of respiratory samples were taken from patients under antimicrobial treatment, thus reducing/abolishing viable bacteria with discordant results between culture and FilmArray.

It remains unclear for how long bacterial loads remain detectable after the initiation of an appropriate antimicrobial therapy in COVID-19 patients and whether FilmArray would be useful to monitor bacterial loads in these patients (Caméléna et al., 2021).

Further studies with more detailed information about the subjects enrolled, including type and timing of antimicrobial regimen, are needed to better understand the role and clinical usefulness of FilmArray in the context of critically ill patients with COVID-19.

Considering the resistance markers, we noticed that resistance genes detected by FilmArray in 3 samples were not confirmed by AST, suggesting limitations to predict phenotypic susceptibility from molecular tests (Caméléna et al., 2021). Moreover, the presence of multiple and/or heteroresistant bacterial clones could explain these discordant results.

FilmArray missed two cases of MRSA detected by culture. As previously underlined, in particular for gram-negative pathogens, the detection of a resistance genetic marker cannot be definitively linked to the microorganism(s) detected, as there may be other organisms present that are not detectable with FilmArray or that are below the limit of detection of the assay (Yoo et al., 2020).

Further investigations, including the search of MecA/C genes in the isolated strains, are needed to clarify the discrepancies regarding the detection of methicillin resistance.

In conclusion, in this retrospective study of ICU-hospitalized COVID-19 patients we demonstrated that FilmArray can be useful to detect bacterial pathogens in low respiratory tract specimens, with a significant decrease of TAT (at least 48 h). Moreover, based on its excellent negative predictive value, the test is particularly useful to rule out bacterial co-infections and avoid the inappropriate prescription of antibiotics, and should be considered as an antimicrobial stewardship diagnostic tool.

However, positive tests should be interpreted with caution, considering bacterial loads and clinical signs, to distinguish dead from viable bacteria, as well as true infections from mere colonizations or from contaminations by the oropharyngeal flora. Moreover, the clinical role of low bacterial loads should be carefully assessed.

Future studies with detailed clinical information (e.g., comorbidities, antimicrobial therapy, biological markers) and a better stratification of subjects (disease severity) are required to address the clinical impact and the cost/effectiveness of FilmArray on the management of respiratory bacterial infections in COVID-19 patients.

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Author's contribution

CF, SA, and TL conceived and designed the study. PG, CV, GR performed the experiments. AZ, CF, GT, SL, and AL analysed the data. TL and SA contributed reagents/materials/analysis tools. CF and SA wrote the paper. All the authors read, reviewed, and approved the final manuscript.

Declaration of Competing Interest

This study was conducted in the absence of any commercial or financial relationships that could be construed as potential conflict of interest. All the authors declare the absence of any dual or conflicting interest.

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