


High rate of bacterial respiratory tract co-infections upon admission amongst moderate to severe COVID-19 patients

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ABSTRACT

Background: The role of bacterial and viral co-infection in the current COVID-19 pandemic remains elusive. The aim of this study was to describe the rates and features of co-infection on admission of COVID-19 patients, based on molecular and routine laboratory methods.

Methods: A retrospective study of COVID-19 and non-COVID-19 patients undergoing Biofire[®], FilmArray[®] Pneumonia Panel, bioMérieux, and routine cultures during the first 3 days from admission, between June 2019 and March 2021.

Results: FilmArray tests were performed in 115 COVID-19 and in 61 non-COVID-19 patients. Most (>99%) COVID-19 patients had moderate-critical illness, 37% required mechanical ventilation. Sputa and endotracheal aspirates were the main samples analyzed. Positive FilmArray tests were found in 60% (70/116) of the tests amongst COVID-19 patients and 62.5% (40/64) amongst non-COVID-19 patients. All 70 cases were positive for bacterial targets, while one concomitant virus (*Rhinovirus/Enterovirus*) and one *Legionella* spp. were detected. The most common bacterial targets were *Haemophilus influenzae* (36%), *Staphylococcus aureus* (23%), *Streptococcus pneumoniae* (10%) and *Enterobacter cloacae* (10%). Correlation between FilmArray and cultures was found in 81% and 44% of negative and positive FA tests, respectively. Positive FilmArray results typically (81%) triggered the administration of antibiotic therapy and negative results resulted in antimicrobials to be withheld in 56% of cases and stopped in 8%. Bacterial cultures of COVID-19 patients were positive in 30/88 (34%) of cases.

Conclusions: Bacterial co-infection is common amongst moderate-critical COVID-19 patients on admission while viral and atypical bacteria were exceedingly rare. Positive FilmArray results could trigger potentially unnecessary antibiotic treatment.

KEY POINT

- We found high rates of on-admission bacterial co-infection amongst hospitalized moderate to severe COVID-19 patients. Molecular tests (Biofire, FilmArray) and routine microbiological tests revealed 60% and 34% bacterial co-infection, respectively, while viral and fungal co-infections were rare.



KEYWORDS

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Introduction

During 2020, the world saw the widespread dissemination of the novel coronavirus (COVID-19). While the contribution of respiratory bacterial or fungal co- or super-infections to the morbidity and mortality of influenza is well known [1], their role in the severity and outcome of COVID-19 infections remains elusive.

Respiratory infections accompanying COVID-19 may be divided into co-infections (evident on COVID-19 diagnosis or admission) and superinfections occurring during hospitalization. Adequate and timely initiation of antibiotic treatment is crucial in these patients, but the clinical and radiological picture of COVID-19 can often be misleading, and early accurate detection of co-infections or superinfections can be challenging. Diagnostic methods (culture- or molecular-based) may affect the rates of reported co-infections [2].

Studies from the early stages of the COVID-19 pandemic have reported low rates of bacterial co-infections [3]. Similarly, a retrospective cohort study from Spain found that only 3.1% of COVID-19 patients hospitalized for >48 h had bacterial co-infection, with *Streptococcus pneumoniae* and *Staphylococcus aureus* being the principal pathogens [4]. A systematic review and meta-analysis that included 30 studies and 3834 patients with COVID-19 reported a 7% rate of bacterial co-infections, with a higher rate of 14% amongst patients in the intensive care units. The most common bacteria reported were *Mycoplasma pneumoniae*, *Pseudomonas aeruginosa* and *Haemophilus influenzae*. They also reported 3% with viral infections (mainly Respiratory Syncytial Virus and Influenza A). Very few data on the timing of co-pathogen detection were present in these studies [5]. Another review and meta-analysis regarding 558 COVID-19 patients in the intensive care unit, reported detection rates of bacterial co-infection reaching 33% when molecular methods were used [2]. A recent retrospective cohort study reviewed 254 patients from 7 intensive care units in the United Kingdom and found that in 33% there were significant bacterial infections, but only 5.5% were reported in the first 48 h from admission to the unit, with *S. aureus* and *S. pneumoniae* the most common pathogens [6]. Several reports described higher early co-infection rates. Hughes et al. reported 12.5% bacterial co-infections during the first 5 days from admission, with *S. aureus* being most prevalent (28%) as community-acquired co-infection [7]. Silva et al. reported increased length of hospitalization and higher mortality amongst COVID-19 patients when bacterial or fungal were detected [8].

Israel faced three waves of COVID-19 during the study period, the latter subsided during April 2021. Although the Israeli Ministry of Health issued a plethora of guidelines [9], no recommendation regarding the use of antimicrobials was issued. Data are lacking from Israel regarding co-infections at presentation and during hospitalization of severe COVID-19.

In Sanz Medical Center, we introduced the use of a rapid multiplex kit for the molecular identification of lower respiratory tract pathogens (Biofire[®], FilmArray[®] Pneumonia Panel, bioMérieux, Marcy-l'Étoile, France) in June 2019. This panel provides rapid identification of respiratory pathogens that could be followed by rapid response of either prescribing or withholding antibiotic therapy. In this study, we aimed to describe the rates and features of co-infections present at admission amongst patients admitted to our COVID-19 wards. In addition, we evaluated the effects the use of a rapid multiplex assay had on antimicrobial decisions.

Materials and methods

Setting

The study was conducted in Sanz Medical Center, a university affiliated, 400-bed regional hospital, located in the city of Netanya, Israel. In response to the pandemic, a separate ward of up to 45 beds was allocated to treat COVID-19 patients and included 6–12 beds for critically ill COVID-19 patients.

Patients

We included all hospitalized and ambulatory patients (COVID-19 and non-COVID-19) for which FilmArray[®] (FA) was performed within 72 h of hospital admission (i.e. early FA result) between June 2019 and March 2021. This panel includes 18 bacterial targets, 7 antimicrobial resistance genes and 9 viruses, not including Severe Acute Respiratory Syndrome Coronavirus-2 (SARS-CoV-2) [10]. Basic demographic data (age, sex, residence place), highest COVID-19 severity status according to the National Institutes of Health guidelines [11], admission and discharge dates, dates on which FA and respiratory cultures were obtained and dates of published results were documented. We also recorded the date of first diagnosis of COVID-19 by polymerase chain reaction (PCR) for positive cases and patients' outcome (need for mechanical ventilation and mortality during the index hospitalization). Antimicrobial therapy that was administered prior to and during the hospitalization was

extracted. COVID-19 was detected using PCR platforms (Xpert[®] Xpress SARS-CoV-2, Cepheid, Sunnyvale, CA; BD SARS-CoV-2, Beckton Dickinson, Franklin Lakes, NJ; Allplex[™] 2019-nCoV Assay, Seegene Inc., Korea).

FA tests for COVID-19 patients were performed, after approval from an infectious diseases expert to patients who were suspected of having bacterial co-infection, those that were defined as moderate to severe COVID-19, and those who developed signs of sepsis or suspected ventilator-associated pneumonia during their hospitalization. FA has been used more sparingly amongst non-COVID-19 patients: generally reserved for more severe cases and for those with a questionable diagnosis despite routine diagnostic efforts. Microbiology cultures were requested for each case in which FA was approved.

Biofire FilmArray and microbiology samples

FA and microbiologic cultures were performed on sputa, endotracheal aspirates, bronchoalveolar lavage fluid obtained during bronchoscopy performed by a pulmonologist, and on non-bronchoscopic lavage samples (in which ~10 ml of saline 0.9% were injected into the endotracheal tube and then aspirated) performed by the ICU nursing team. Results of FA tests, including the bacteria and viruses identified, semi-quantitative burden (genome copies/ml), and related resistance genes were recorded. Routine respiratory samples were cultured on blood (trypticase soy agar enriched with 5% sheep blood), chocolate blood, columbia naladixic acid, MacConkey, CHROMagar[™] Orientation agars (Hy Laboratories, Rehovot, Israel). Bacterial identification and antibiotic susceptibility testing of suspected colonies were performed using a VITEK[®]2 system (Biomérieux, Marcy-l'Étoile, France). Susceptibility was interpreted according to the current CLSI guidelines [12]. 'Normal respiratory flora' and non-pulmonary pathogens such as *Candida* spp. and *Enterococcus faecalis* were considered as negative. An early respiratory secretion was considered as a sample received and cultured within 72 h of hospital admission.

Co-infection definition

Co-infection was defined as the identification of a respiratory tract pathogen using FA or microbiological cultures from respiratory secretions that were obtained during the first three calendar days from admission.

Antimicrobial treatment decisions

For each FA result, we scanned the medical record for the clinical implications, i.e. alterations made to the antimicrobial treatment. The options were – triggering antimicrobial treatment, withholding treatment, stopping a previously administered therapy, expanding/narrowing the antimicrobial coverage range, or no change. There were no established guidelines regarding the interpretation of FA results, and antimicrobial decisions were made by an infectious diseases specialist.

Statistical analysis

Categorical variables were expressed as frequencies and percentages and compared between COVID-19 and non-COVID-19 patients using Fisher's exact test. Continuous variables were presented as means and standard deviations (SD) and compared using Student's *t*-test. Calculations were performed using GraphPad Prism[®] 7.0 (GraphPad Software, La Jolla, CA) for Windows. Statistical significance was defined when $p < .05$.

The study was approved by the Institutional Review Board (IRB) of Sanz Medical Center, 0014-21-LND.

Results

FilmArray tests

Between June 2019 and March 2021, 418 FA tests were performed on 336 patients: 257 FA tests (61%) performed on 198 COVID-19 patients and 161 tests (39%) on 138 non-COVID-19 patients. Of these, in 176 patients, FA tests performed during the first 3 days from admission: 115 COVID-19 and 61 non-COVID-19 patients. The sources for the FA tests (for both patients' groups) were sputa (66%), followed by endotracheal aspirates (25%), bronchoalveolar lavage (7%) and non-bronchoscopic lavage (2%). Sputa was more commonly used for FA tests amongst COVID-19 patients than for non-COVID-19 patients (75% versus 51%, $p = .002$). In 75% of cases, we had a concurrent microbiologic culture obtained within 72 h from admission (Table 1 and Figure 1).

Patients' characteristics

Between March 2020 and March 2021, 887 COVID-19 patients were hospitalized in our facility. Significant differences were observed in the COVID-19 group when compared to the non-COVID group. COVID-19 patients were younger (mean age of 58.5 years versus 64.4, $p = .015$) and hospitalized for longer periods (13.7 days

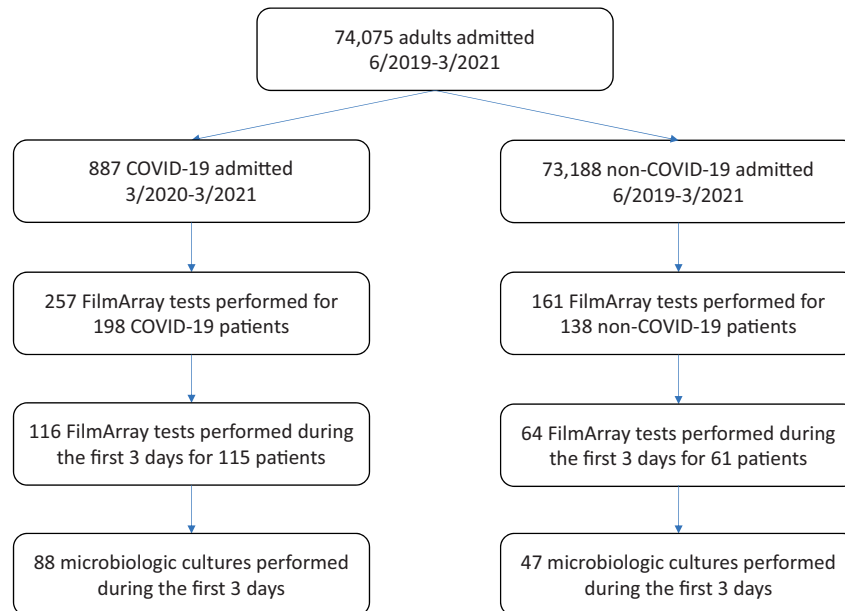
Table 1. Characteristics of patients that underwent Biofire FilmArray Pneumonia panel test within the first 3 days from admission.

| | COVID-19 patients, <i>n</i> = 115 | Non-COVID patients, <i>n</i> = 61 | Fisher exact test, <i>p</i> |
|--|-----------------------------------|-----------------------------------|-----------------------------|
| Age, years – mean ± SD (range) | 58.5 ± 15 (20–92) | 64.4 ± 15.7 (27–96) | .015 |
| Male sex (%) | 84 (73) | 40 (65) | .3 |
| LTCF (%) | 3 (2.6) | 4 (6.5) | .23 |
| Hospitalization (%) | 115 ^b (100) | 54 (88) | .0005 |
| LOS, days, mean (range) | 13.7 ± 13.8 (0–90) | 8.4 ± 10.5 (0–60) | .0096 |
| COVID-19 severity (%) | | | |
| Mild | 1 (<1) | NA | |
| Moderate | 13 (11) | | |
| Severe | 58 (50) | | |
| Critical | 43 (37) | | |
| Mechanical ventilation (%) | 43 (37) | 22 (36) | .99 |
| Death during the index hospitalization (%) | 12 (10) | 13 (21) | .06 |
| Source of sample (%) | | | |
| Sputum, <i>n</i> (%) | 86 (75) | 31 (51) | .002 |
| ETA, <i>n</i> (%) | 23 (20) | 21 (34) | .04 |
| BAL, <i>n</i> (%) | 4 (3) | 8 (13) | .025 |
| NBL, <i>n</i> (%) | 2 (2) | 1 (2) | .6 |
| Concurrent ^a microbiologic culture performed/total FA tests (%) | 82/116 (70) | 47/64 (73) | .35 |

SD: standard deviation; LTCF: long-term care facility; LOS: length of stay; ETA: Endotracheal aspirate; BAL: Bronchoalveolar lavage; NBL: non-bronchoscopic lavage; FA: FilmArray.

^aWithin 3 days from admission.

^bOne patient with severe COVID-19 was transferred to another hospital from the emergency department.

**Figure 1.** FilmArray and routine cultures from COVID-19 and non-COVID-19 patients that were included in the study.

versus 8.4, $p = .0095$). Mechanical ventilation and mortality rates were not significantly different between the groups, although a trend for lower mortality amongst COVID-19 patients was seen (10% versus 21%, $p = .06$). All the COVID-19 patients were hospitalized versus 88% of the non-COVID patients ($p = .0005$) (Table 1).

Early co-infection

Molecular-based data

Of the 198 COVID-19 patients, 115 (58%) had FA results. One patient had two FA tests performed within 3 days, hence altogether 116 FA results were analyzed. Most FA tests (89%) were ordered and obtained during

hospitalization days 2 and 3 (Table 2). In 46/116 (40%), the FA test was negative for all the targets tested, and a positive result was found in 70 cases (60%) – all were bacterial targets. In one case, a viral (*Rhinovirus/Enterovirus*) pathogen and in another case an atypical bacterial spp. (*Legionella pneumophila*) were detected in addition to the bacteria. In 27/116 cases (23%), there was one bacterial species and in 43 (37%) two or more bacterial species were detected. The detailed FA results are depicted in Table 3. The positive FA results according to the source of sampling (sputa versus lower respiratory tract samples) are presented in Figure 2.

Of the 138 non-COVID-19 patients, 61 patients (44%) had a FA test taken during the first three days of

Table 2. Characteristics of FilmArray tests and respiratory cultures taken during the first 3 days of hospitalization among COVID-19 and non-COVID-19 patients.

| | Early FA tests | | | Early respiratory cultures | | |
|---------------------------------------|---|--|----------------------|----------------------------|-------------------------------|----------------------|
| | COVID-19 patients, n = 116 ^a | Non-COVID-19 patients, n = 64 ^b | Fisher exact test, p | COVID-19 patients, n = 88 | Non-COVID-19 patients, n = 52 | Fisher exact test, p |
| Number of samples, n (%) | | | | | | |
| HD-1 | 13 (11) | 17 (27) | .011 | 12 (14) | 21 (40.5) | .0005 |
| HD-2 | 53 (46) | 28 (44) | NS | 43 (49) | 20 (38.5) | NS |
| HD-3 | 50 (43) | 19 (29) | NS | 33 (37) | 11 (21) | .059 |
| Negative results ^c , n (%) | 46 (40) | 24 (37.5) | NS | 58 (66) | 34 (65) | NS |
| Positive FA test or culture results | | | | | | |
| Total, n (%) | 70 (60) | 40 (62.5) | NS | 30 (34) | 18 (35) | NS |
| Bacterial species (1 or more) | 70 (100) | 33 (82.5) | .0006 | 30 (100) | 17 (94) | NS |
| Viruses | 1 (1.4) | 12 (30) | <.0001 | NA | NA | NA |
| Atypical bacteria | 1 (1.4) | 3 (7.5) | NS | NA | NA | NA |
| Moulds | NA | NA | NA | 0 | 2 (11) | NS |

FA: FilmArray; HD: hospitalization day; NA: not applicable; NS: non-significant.

^aOut of 115 patients with early FA samples.

^bOut of 61 patients with early FA samples.

^cIn Biofire FilmArray tests: negative for all targets; in microbiological cultures: sterile/normal flora/yeasts.

hospitalization. Three patients had two tests in this period (altogether 64 FA results). In 24/64 (37.5%) the FA test was negative for all the targets tested. A positive result was found in 40 cases (62.5%) – 33 tests detected bacterial targets, 12 – viral targets and 3 atypical bacteria (all *L. pneumophila*). Viruses were significantly more commonly encountered amongst non-COVID patients, in comparison to COVID-19 patients (30% versus 1.4%, $p < .0001$). Data are detailed in Tables 2 and 3.

The most common bacterial targets found in early FA tests from COVID-19 patients were *H. influenzae* (36%), *S. aureus* (23%, 70% of which were methicillin-sensitive *S. aureus* (MSSA), *S. pneumoniae* (10%) and *E. cloacae* (10%). Enterobacteriaceae constituted 16% of the positive FA tests.

Haemophilus influenzae and MSSA were also the most common bacteria identified by early FA tests amongst non-COVID patients (22% and 17%, respectively), and Enterobacteriaceae were documented in 13% of the positive tests (Table 3).

Resistance genes identification and correlation to standard cultures are described in Supplementary 1.

Culture-based data

Of the 198 COVID-19 patients, 88 (44%) had an early respiratory secretion cultured. Most were performed on sputa (72%) and endotracheal aspirate samples (22%). In 58/88 (66%) the culture was negative or grew 'normal flora' or other non-respiratory pathogens such as yeasts and *E. faecalis*. A positive culture was found in 30 cases (34%) of which 22 had one bacteria species, 7 cases had two bacteria and one patient had three bacteria. Details are shown in Table 4.

Of the 138 non-COVID-19 patients, 52 patients (37%) had a respiratory culture taken during the first three days of hospitalization. Most tests were done on sputa (38%) and endotracheal aspirate samples (42%). In 34/52 (65%), the culture results were negative. A positive result was found in 18 cases (35%) of which 12 patients had 1 bacteria species, 3 patients had two bacteria, 2 patients had 3 bacteria and 1 patient had four. The detailed bacteria species are depicted in Table 4.

Enterobacteriaceae, Staphylococci and respiratory pathogens including Streptococci were the main bacteria found in early cultures of COVID-19 patients – together comprising 82% of the bacteria found amongst positive cultures. The findings were similar amongst non-COVID patients, with no statistically significant differences in rates for each group of bacteria (Table 4). *Aspergillus* spp. were found in early cultures of two non-COVID patients, but not amongst COVID-19 patients.

While the results for the FA tests were available within the same day of obtaining the samples, the average time interval for the routine culture results was 3.3 days.

FilmArray tests and microbiological culture correlation in the COVID-19 patient population

For 50 (out of 70, (71%)) positive FA tests, we had concomitant microbiological cultures. Of these 50 cases, in 24 (48%), bacteria were cultured, and, of these, 22 were concordant with the FA tests (44%). In the remaining 26, 'normal flora' or yeasts were reported. Only 2/26 discordant negative cultures received prior antibiotic treatment, as far as we are aware.

Table 3. Biofire FilmArray Pneumonia panel detailed results of tests performed during the first 3 days from hospitalization amongst COVID-19 and non-COVID-19 patients.

| FA target | COVID-19 patients with 70 positive FA results of 116 early FA tests | | | | | Non-COVID-19 patients with 40 positive FA results of 64 early FA tests | | | | |
|--|---|---------------------------------|-----------------|-----------------|------------------|--|---------------------------------|-----------------|-----------------|--|
| | Number detected (%) | Quantitative result (copies/ml) | | | | Number detected (%) | Quantitative result (copies/ml) | | | |
| | | 10 ⁴ | 10 ⁵ | 10 ⁶ | >10 ⁷ | | 10 ⁴ | 10 ⁵ | 10 ⁶ | >10 ⁷ |
| Bacterial targets | | | | | | | | | | |
| <i>H. influenzae</i> | 42 (36) | 15 | 9 | 9 | 9 | 14 (22) | 5 | 3 | 3 | 3 |
| <i>S. aureus</i> | | | | | | | | | | |
| Methicillin sensitive | 22 (19) | 9 | 6 | 6 | 1 | 11 (17) | 4 | 3 | 2 | 2 |
| Methicillin resistant | 5 (4) | 2 | 1 | 1 | 1 | 1 (1) | | | 1 | |
| <i>E. cloacae</i> | 12 (10) | 8 | 3 | 1 | | 2 (3) | 2 | | | |
| <i>S. pneumoniae</i> | 12 (10) | | 3 | 3 | 6 | 4 (6) | | 1 | | 3 |
| <i>M. catarrhalis</i> | 8 (7) | 2 | 1 | 2 | 3 | 2 (3) | 1 | 1 | | |
| <i>P. aeruginosa</i> | 9 (7) | 3 | 1 | 3 | 2 | 8 (12) | 4 | 2 | | 2 |
| <i>S. agalactiae</i> | 6 (5) | 2 | | 4 | | 1 (1) | | 1 | | |
| <i>E. coli</i> | 3 (2) | | 3 | | | 0 | | | | |
| <i>K. aerogenes</i> | 3 (2) | 2 | 1 | | | 1 (1) | | | | 1 |
| <i>K. pneumoniae</i> | 3 (2) | 3 | | | | 6 (9) | 2 | 1 | 2 | 1 |
| <i>S. pyogenes</i> | 2 (1) | 2 | | | | 1 (1) | | | | 1 |
| <i>A. baumannii</i> | 2 (1) | | 2 | | | 0 | | | | |
| <i>Serratia</i> spp. | 2 (1) | 2 | | | | 0 | | | | |
| <i>Proteus</i> spp. | 1 (<1) | | | | 1 | 2 (3) | 1 | 1 | | |
| <i>K. oxytoca</i> | 0 | | | | | 0 | | | | |
| Total bacterial targets | 132 | 50 | 30 | 29 | 23 | 53 | 19 | 13 | 8 | 13 |
| Resistance targets | | | | | | | | | | |
| CTX-M | | | 7 | | | | | 4 | | |
| IMP | | | | | | | | | | |
| KPC | | | | | | | | | | |
| mecA/C & MREJ | | | 5 | | | | | 1 | | |
| NDM | | | | | | | | 1 | | |
| OXA-48-like | | | | | | | | | | |
| VIM | | | | | | | | | | |
| Total resistance targets | | | 12 | | | | | 6 | | |
| Atypical bacterial and viral targets | | | | | | | | | | |
| <i>C. pneumoniae</i> | | | | | | | | | | |
| <i>L. pneumophila</i> | | | 1 | | | | | 3 | | |
| <i>M. pneumoniae</i> | | | | | | | | | | |
| Adenovirus | | | | | | | | | | |
| Human Coronavirus | | | | | | | | | | 2 (1 co-infected with Respiratory Syncytial Virus) |
| Human Metapneumovirus | | | | | | | | | | 2 (1 co-infected with Rhinovirus/Enterovirus) |
| Human Rhinovirus/Enterovirus | | | 1 | | | | | | | 9 (1 co-infected with Human Metapneumovirus) |
| Influenza A | | | | | | | | | | |
| Influenza B | | | | | | | | | | |
| Middle East Respiratory Syndrome-Coronavirus | | | | | | | | | | |
| Parainfluenza virus | | | | | | | | | | |
| Respiratory Syncytial Virus | | | | | | | | | | 1 (co-infected with human Coronavirus) |
| Total atypical and viral targets | | | 2 | | | | | 17 | | |

For 32 (out of 46) negative FA tests (70%), there were concurrent culture results. Of these, there were concordant negative cultures in 26 (81%). Of the 6 discordant results, 3 grew bacteria that are featured in the FA profile (two *E. cloacae* and one *K. oxytoca*) and 3 non-target organisms, specifically – Streptococci (*S. constellatus* and *S. dysgalactiae*) and *Citrobacter* spp.

Effect of prior antimicrobial therapy on FA and culture results amongst COVID-19 patients

Amongst the 115 COVID-19 patients with FA tests, 25 were treated with antibiotics prior to testing and 90 were not reported to be treated. Positive FA tests were

found in 36% (9/25) and 67% (60/90) of these groups, respectively ($p = .01$).

Amongst the 88 COVID-19 patients with cultures taken, 18 were treated with antibiotics prior to testing and 70 were not. Positive culture results were 16% (3/18) and 38% (27/70), respectively ($p = .09$).

Positive versus negative early co-infection groups

The demographics, as well as the hospitalization characteristics of patients with positive versus negative early FA and microbiology cultures were similar, with a few exceptions. One exception was that more critical

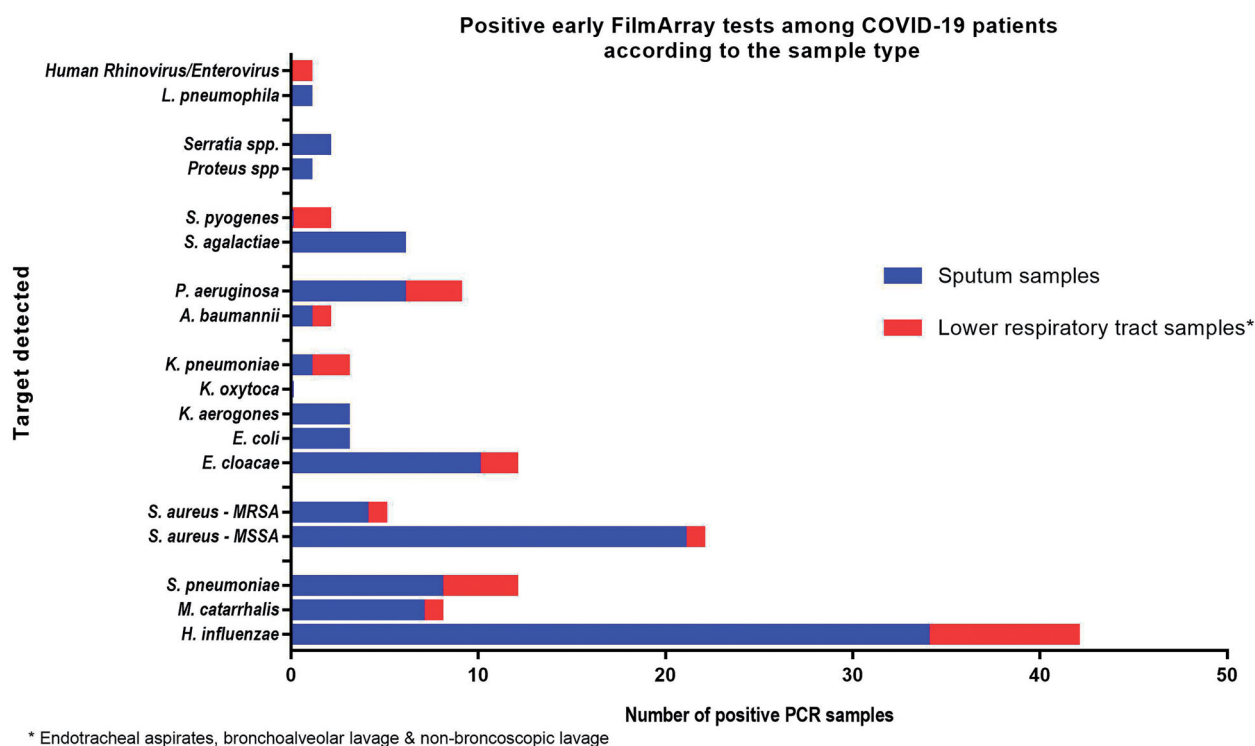


Figure 2. Positive early FilmArray tests amongst COVID-19 patients according to the sample type.

COVID-19 patients had a negative early FA result (52% versus 27.5%, $p = .01$), and another was that patients treated with antimicrobials before obtaining FA had higher rates of negative results ($p = .01$) (Table 5).

Antimicrobial therapy decisions following FA results in the COVID-19 patient population

FA testing influenced antibiotic stewardship decisions of COVID-19 patients. Of the 69 positive FA results, in 56 patients (81%), antimicrobial treatment was started in response. Of these 56, in 11 (16%), the treatment included the use of expanded spectrum coverage (to cover methicillin-resistant *S. aureus* (MRSA), *P. aeruginosa*, *L. pneumophila* and extended spectrum beta-lactamase bacteria), and in one patient it included narrowing the spectrum (for better coverage of MSSA). In 9/69 (13%), antibiotics were avoided because of the low quantitative FA result and, in 4 patients (6%), no changes were made to antimicrobial treatment, as they were already on presumed appropriate empiric treatment.

Of the 46 negative FA, in 26 (56%), antibiotic treatment was withheld, and, in 4 (8%), pre-administered antibiotic treatment was stopped. In 16 patients (34%), there was no change in the treatment and 6 of them were not treated with antibiotics at the time.

Discussion

The main finding of this study is the relatively high rate of bacterial co-infection evident upon admission of hospitalized COVID-19 patients. All patients that were tested with FA had a high clinical suspicion of bacterial co-infection and most had severe or critical COVID-19. Conventional cultures and FA tests yielded rates of 34% and 60% bacterial co-infection in COVID-19 patients, respectively. All tests were performed in the first 3 days of admission on mainly sputa and endotracheal aspirate samples. Viral, atypical bacterial and fungal co-infections were rarely found or absent. These co-infection rates were not statistically different compared with non-COVID hospitalized patients with severe pulmonary disease undergoing the same diagnostic procedures. The most common bacterial pathogens identified, by both methods, were *H. influenzae* (and to a significant lesser extent, other respiratory pathogens as *S. pneumoniae* and *M. catarrhalis*); *S. aureus* (mainly methicillin-sensitive strains), and Enterobacteriaceae.

A prospective study conducted in three French ICUs has found 15% bacterial co-infection using conventional cultures [13]. They also reported *S. aureus* and *H. influenzae* as the most common bacteria found. Concurrent FA tests identified 60% more bacterial targets than were successfully cultured, and discordance was attributed (in 77%) to cases with low bacterial nucleic acid loads

Table 4. Detailed respiratory culture results obtained from COVID-19 and non-COVID-19 patients during the first 3 days of hospitalization.

| Pathogen cultured | | COVID-19 <i>n</i> = 88 early cultures obtained | Non-COVID-19 <i>n</i> = 52 early cultures obtained | Fisher exact test, <i>p</i> | |
|---|--|--|--|-----------------------------|--|
| Negative results | | 58/88 (66) | 34/52 (65) | NS | |
| Total patients with a positive culture/patients with early culture obtained, <i>n</i> (%) | | 30/88 (34) | 18/52 (35) | NS | |
| Total bacteria and fungi cultured, <i>n</i> (%) | | 39 (44) | 26 (50) | NS | |
| Respiratory pathogens and Streptococci | <i>H. influenzae</i> | 2 | 3 | | |
| | <i>M. catarrhalis</i> | 1 | 0 | | |
| | <i>S. pneumoniae</i> | 0 | 2 | | |
| | <i>S. constellatus</i> | 3 | 0 | | |
| | <i>S. dysgalactiae</i> | 1 | 1 | | |
| | <i>S. beta haemolytic non typeable</i> | 1 | 0 | | |
| | <i>S. pyogenes</i> | 0 | 0 | | |
| | Sum, <i>n</i> (%) | 8 (9) | 6 (11.5) | NS | |
| | <i>Staphylococcus aureus</i> | MSSA | 11 | 5 | |
| | | MRSA | 1 | 0 | |
| Sum, <i>n</i> (%) | | 12 (13.6) | 5 (9.5) | NS | |
| Enterobacterales | <i>E. cloacae</i> | 6 ^a | 0 | | |
| | <i>K. aerogenes</i> | 1 | 0 | | |
| | <i>K. oxytoca</i> | 1 | 0 | | |
| | <i>K. pneumoniae</i> | 1 | 1 ^c | | |
| | <i>E. coli</i> | 0 | 2 ^c | | |
| | <i>Kluyvera</i> spp. | 1 | 0 | | |
| | <i>Citrobacter</i> spp. | 2 | 2 | | |
| | Sum, <i>n</i> (%) | 12 (13.5) | 5 (9.5) | NS | |
| Non fermenters | <i>A. baumannii</i> | 2 ^b | 0 | | |
| | <i>S. maltophilia</i> | 0 | 1 | | |
| | <i>P. aeruginosa</i> | 3 | 5 | | |
| | <i>Achromobacter</i> spp. | 0 | 1 | | |
| | Sum, <i>n</i> (%) | 5 (5.5) | 7 (13) | NS | |
| Other bacteria | <i>Proteus</i> spp. | 1 | 0 | | |
| | <i>Serratia</i> spp. | 1 | 0 | | |
| | <i>Morganella</i> spp. | 0 | 1 | | |
| | Sum, <i>n</i> (%) | 2 (2) | 1 (2) | NS | |
| Fungi | <i>Aspergillus</i> spp., <i>n</i> (%) | 0 | 2 ^d (4) | NS | |

MSSA: methicillin-sensitive *Staphylococcus aureus*; MRSA: methicillin-resistant *Staphylococcus aureus*.

^aThree isolates were ceftriaxone resistant.

^bCarbapenem-susceptible *Acinetobacter baumannii*.

^cOne isolate was ceftriaxone resistant.

^dOne *A. niger* and one *A. terreus*.

(<10⁵ copies/ml) or to bacteria belonging to the oral flora. We have found similar results, although with higher rates of positive samples using both methods. It seems that overall, bacterial co-infection is not rare in COVID-19 patients as opposed to what was published at the beginning of the pandemic. These preliminary results could stem from underdiagnosis related to infection control issues concerning the difficulty in acquisition of respiratory samples from non-intubated, highly infectious patients. Additionally, the literature does not properly distinguish between co-infections and superinfections. The latter is probably much more prevalent than co-infection [14]. Since in many studies, as in this study, sputum was the principal substrate used for diagnosis of bacterial co-infection, dissecting colonization from infection remains a problem. A consistent finding in many reports is the high rate of *H. influenzae* and *S. aureus* cultivation, and the low rates of *S. pneumoniae* [7,15]. In our study, Enterobacteriaceae were surprisingly common, found in 13.5% of early cultures and in 16% of

FA tests. Amongst these Enterobacteriaceae, only three isolates cultured (3.5%) were ceftriaxone-resistant (all *E. cloacae*), and, in seven cases, CTX-M was detected by FA but with overall poor correlation to the corresponding culture results.

Similar bacteria were found in early cultures of both COVID-19 and non-COVID-19 patients. This may suggest that the varying co-infective pathogens are determined by what other infectious organisms are circulating in the general population at the time, and accounts for the differences between various studies conducted in different locations and seasons – as some studies report zero to very low rates of co-infections [15,16], while others report rates of 96% bacterial co-infections and viral co-infection as well [17]. As an example, no influenza co-infection was reported in our study since during the winter of 2020–2021 not a single case of Influenza was detected in Israel despite active surveillance by the Israeli Center for disease control (ICDC) [18]. Hence, the absence of co-infecting viruses may reflect the effects

Table 5. Positive versus negative molecular and microbiology tests groups characteristics and outcomes amongst COVID-19 patients with early samples.

| | Positive early FA result, n = 69 | Negative early FA result, n = 46 | Fisher exact test, p | Positive early culture result, n = 30 | Negative early culture result, n = 58 | Fisher exact test, p |
|--|----------------------------------|----------------------------------|----------------------|---------------------------------------|---------------------------------------|----------------------|
| Age years, mean ± SD (range) | 60 ± 14.1 (27–92) | 56.2 ± 16.1 (20–86) | NS | 61.7 ± 13.9 (40–92) | 56.3 ± 16.5 (20–91) | NS |
| Sex, male, n (%) | 50 (72) | 34 (74) | NS | 21 (70) | 44(75) | NS |
| LTCF, n (%) | 3 (4) | 0 | NS | 2 (7) | 1 (1) | NS |
| COVID-19 severity | | | | | | |
| Mild, n (%) | 1 (<1) | 0 | NS | 0 | 1 (1) | NS |
| Moderate, n (%) | 11 (15) | 2 (4) | NS .07 | 3 (10) | 4 (7) | NS |
| Severe, n (%) | 38 (55) | 20 (43) | NS | 18 (62) | 26 (45) | NS |
| Critical, n (%) | 19 (27.5) | 24 (52) | .01 | 8 (27) | 27 (46) | NS |
| LOS, mean ± SD (range) | 12.91 ± 14.71 (2–90) | 14.9 ± 12.5 (0–51) | NS | 16.1 ± 18.8 (2–90) | 14.2 ± 12.4 (2–51) | NS |
| In-hospital death (%) | 6 (8.6) | 6 (13) | NS | 4 (13) | 5 (8.6) | NS |
| Days from COVID-19 diagnosis to hospitalization, mean ± SD (range) | 4.7 ± 3.9 (0–16) | 5.5 ± 4.3 (0–19) ^a | NS | 4.9 ± 3.8 (0–16) | 5.3 ± 4.4 (0–19) | NS |
| Antimicrobials administered prior to FA test or culture, n (%) | 9 (13) | 16 (34) | .01 | 3 (10) | 15 (25) | NS .09 |
| FA result triggered antibiotics | | | | | | |
| Commence | 56 (81) | 0 | <.0001 | | | |
| With expanding coverage | 11 (16) ^b | 0 | .003 | | | |
| With narrowing coverage | 1 (1.5) | 0 | NS | | | |
| Avoidance | 9 (13) | 26 (56) | <.0001 | | | |
| Stopping | 0 | 4 (8) | .023 | | | |
| No change | 4 (6) | 16 (35) | <.0001 | | | |

FA: FilmArray; SD: standard deviation; LTCF: long-term care facility; LOS: length of stay.

^aOne case was diagnosed with COVID-19 2 days after he was hospitalized.

^bFive MRSA, two *P. aeruginosa*, two extended spectrum beta-lactamase (ESBLs), one *Legionella* spp.

that facial masks and social distancing may have on other viruses circulating in the community [19,20].

Studies conducted prior to the COVID-19 pandemic that evaluated the FA pneumonia panel assay compared to standard methods and reported similar assay characteristics and similar bacterial targets detection diversity to those reported here [21,22]. Discrepancies can be attributed to the higher sensitivity of PCR, the over-detection of oral bacteria that do not represent actual pulmonary infection and pre-administered antimicrobial therapy that may hamper culturing of fastidious bacteria as *H. influenzae* and *S. pneumoniae*. We have found a correlation of 81% with negative FA results and 44% with positive FA results. Pre-administration of antibiotics was associated with lower rates of identification of bacterial co-infection, reducing the rates by half, with statistical significance on FA test results.

In an era of increasing multi-drug resistant bacterial and the awareness of antibiotic stewardship, rapid, multiplex, PCR-based detection kits for pneumonia confer many advantages. This is especially true during a pandemic of a respiratory infection. These tests have short turnaround times and are relatively safe for the

laboratory technicians, easy to use and extremely sensitive. In our study, comparing the cases for which concordance was evident between the FA and the cultures, the current organism was reported 3.3 days earlier by using the FA kit. Another important advantage of the multiplex tests is the identification of unexpected organisms, such as MRSA, *Legionella* spp., *P. aeruginosa* and others, triggering a critical change in the empiric therapy.

The limitations of PCR-based detection kits include insufficient correlation with cultures, especially with positive FA results; the absence of several important organisms (including moulds, *Stenotrophomonas maltophilia*, *Citrobacter* spp., etc.); and the over-diagnosis of low concentrations of bacteria (10^4 , 10^5 copies/ml) that unlikely to be clinically important and may result in unnecessary antibiotic use. In this study, a negative FA result was reliable and antibiotic initiation was avoided in 56% of cases and stopped in another 8% of cases. Positive FA results typically triggered antibiotic use and in retrospect this was maybe not always necessary. This study included high risk COVID-19 patients, with bilateral pulmonary infiltrates and hypoxaemia, for whom

bacterial co-infections were difficult to exclude. The presence of molecular evidence of bacteria, even if in low concentrations and even if not cultured eventually, can possibly mean early bacterial pulmonary infection, and early directed therapy could be clinically beneficial. Nevertheless, during the past year, we have developed increasing familiarity with this kit, and often learned to ignore low level of 10^4 – 10^5 copies/ml as clinically relevant. Kolenda et al. in their prospective study with the same FA kit, reached the same conclusions [13].

This study has limitations, including being a retrospective study conducted in a single centre, and a prospective randomized controlled trial would allow for reducing biases and ensuring similar pools of patients are available for a fair comparison. An additional limitation is the setup of the FA and microbiological cultures. In several cases, samples were not taken at the same time, possibly affecting the correlation between the two methods.

Conclusions

Bacterial co-infection in patients with severe-critical COVID-19 is not rare and rates may be substantially different between different locations and seasons. In this study, viruses other than SARS-CoV-2 were exceedingly rare possibly due to low rates in the community at the time relating to mask wearing and social distancing costumed. Moulds were absent altogether in the early days of hospitalization. *Haemophilus influenzae* and *S. aureus* were frequently found, in line with previously published studies, but ceftriaxone-susceptible Enterobacteriaceae were also frequently identified and cultured. Positive FA results should be carefully considered, taking into consideration the nucleic acid concentrations and the bacterial types, and caution should be applied with antimicrobial decisions triggered by positive FA results. It seems that the occurrence of bacterial co-infection diagnosed at admission does not have a significant effect on patients' outcomes and that bacterial or fungal superinfections are probably more influential determinants of morbidity and mortality in COVID-19 patients.

Institutional review board statement

The study was conducted according to the guidelines of the Declaration of Helsinki and approved by the Institutional Review Board (IRB) of Sanz Medical Center, 0014-21-LND.

Informed consent statement

Patient consent was waived since the study was a retrospective, observational, non-interventional study.

Disclosure statement

The authors declare no conflict of interests.

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Data availability statement

Raw data can be received from the authors per request.

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