Research Square

Preprints are preliminary reports that have not undergone peer review.
They should not be considered conclusive, used to inform clinical practice,
or referenced by the media as validated information.

Diagnostic Concordance Between Multiplex PCR FilmArray® Pneumonia Panel and Culture in Patients with COVID-19 Pneumonia Admitted to Intensive Care Units: The Experience of the Third Wave in Eight Hospitals in Colombia.

Francisco José Molina (francisco.molina@upb.edu.co)
Escuela de Ciencias de la Salud, Universidad Pontificia Bolivariana
Luz Elena Botero
Escuela de Ciencias de la Salud, Universidad Pontificia Bolivariana
Juan Pablo Isaza
Escuela de Ciencias de la Salud, Universidad Pontificia Bolivariana
Luz Elena Cano
Centro de Investigaciones Biológicas
Lucelly López
Escuela de Ciencias de la Salud, Universidad Pontificia Bolivariana
Leidy Tamayo
Escuela de Ciencias de la Salud, Universidad Pontificia Bolivariana
Antoni Torres
University of Barcelona

Research Article

Keywords: COVID-19, FilmArray, Bacterial co-infection, Bacterial pneumonia, Intensive Care Units

Posted Date: March 21st, 2022

DOI: https://doi.org/10.21203/rs.3.rs-1447836/v1

License: This work is licensed under a Creative Commons Attribution 4.0 International License. Read Full License

Abstract

Background

Detection of co-infections is important to initiate appropriate antimicrobial therapy. Molecular diagnostic testing identifies pathogens at a greater rate than conventional microbiology. We assessed both bacterial co-infections identified in culture or multiplex PCR FilmArray® Pneumonia Panel (FA-PNEU) in patients infected with SARS-CoV-2 in ICU and the concordance between these techniques.

Methods

Prospective study of patients with SARS-CoV-2 who were hospitalized for no more than 48 hours, in mechanical ventilation no longer than 24 hours in 8 ICUs in Medellín, Colombia. We studied mini-bronchoalveolar lavage or endotracheal aspirate samples processed in conventional culture and FA-PNEU. Co-infection was defined as the identification of a respiratory pathogen using FA-PNEU or cultures.

Results

Of 110 patients who underwent both methods, FA-PNEU and culture-positive samples comprised 24.54% vs 17.27%, respectively. 18 samples were positive for both techniques, 82 were negative, one was culture-positive with negative FA-PNEU, and 9 were FA-PNEU-positive with negative culture. The two bacteria most frequently detected by FA-PNEU were *Staphylococcus aureus* (37.5%) and *Streptococcus agalactiae* (20%) and by culture were *Staphylococcus aureus* (34.78%) and *Klebsiella pneumoniae* (26.08%). The overall concordance was 90.1% and by microorganism it was between 92.7% and 100%. Positive predictive values (PPV) were between 50% and 100%, being lower for *Enterobacter cloacae* and *Staphylococcus aureus*. Negative predictive values (NPV) were high (between 99.1% and 100%); MecA/C/MREJ had a specificity of 94.55% and a NPV of 100%.

Conclusions

The overall concordance was 90.1%, and it was between 92.7% and 100% by microorganisms. The positive qualitative agreement was between 50% and 100%, with a very high NPV.

1. Introduction

Critically-ill patients with COVID-19 had a high mortality rate (38.4%), mainly related to advanced age, the severity of illness on intensive care unit (ICU) admission, vasopressor support, and renal replacement therapy [1]. A recent meta-analysis reported an incidence of 7% of hospitalized COVID-19 patients with a bacterial co-infection; this proportion increased to 14% in studies that only included patients who required ICU admission, almost universally from studies utilizing culture-based methods[2]. Concern over bacterial co-infections has led to significant antimicrobial use in up to 80% of critically-ill COVID-19 patients [3], although early administration of antibiotics does not impact mortality in these subjects[4]; therefore, strategies must be established for improving antimicrobial stewardship in COVID-19.

Molecular tests provide a more rapid turnaround time, early isolations, semi-quantitative results for many pathogens, and antibiotic resistance markers, thus improving antimicrobial stewardship. Biofire® FilmArray® Pneumonia plus Panel (FA-PNEU) detects severe pneumonia pathogens at a greater rate than conventional microbiology tests. In a sub-study of the PROGRESS trial, sputum samples of 90 patients with sepsis and lower respiratory tract infection (LRTI) were retrospectively analyzed; FA-PNEU detection rate was 72.2% compared to 10% based on conventional microbiology ($p < 0.001$)[5]. Few data are available on the use of molecular techniques for the identification of bacterial pathogens in the respiratory tract of critically-ill COVID-19 patients[6–9]

Identification of co-infections could be very helpful to initiate an early and appropriate antimicrobial treatment; in contrast, in the absence of clinical or radiological evidence of bacterial co-infection and microorganisms in respiratory samples could preclude unnecessary antibiotics. In this prospective multicenter study, we assessed bacterial co-infections based on culture or FA-PNEU in the first low respiratory sample taken in SARS-CoV-2 patients hospitalized in an ICU. We also analyzed the concordance between conventional culture and FA-PNEU.

2. Materials And Methods

2.1. Study setting

We performed a prospective study of 149 patients with laboratory-confirmed SARS-CoV-2 infection, hospitalized from March 1 to July 30, 2021, at eight ICUs in Medellín, Colombia. Inclusion criteria included: patients over 18 years of age with severe COVID-19 infection consecutively admitted to ICU according to NIH criteria [10] on mechanical ventilation. To meet co-infection criteria, patients could not have been hospitalized for more than 48 hours at the time of LRTI sampling. Patients who had received any dose of empiric antimicrobial therapy were excluded (Fig. 1). Sample size was calculated to estimate the sensitivity and specificity for *Staphylococcus aureus* based on the article by Kolenda et al., where the expected sensitivity for this same microorganism was 99.9%, 93.5% specificity, and absolute precision of 5%, obtaining a necessary sample of 96 patients [7].

The respiratory therapists of each ICU took samples of LRTI on the first day of intubation with mini- bronchoalveolar lavage fluid [mini-BAL] or endotracheal aspirate [ETA] for conventional culture and FA-PNEU. Of the total volume of samples, 5–10 milliliters (mL) were distributed for FA-PNEU, 5–10 mL for conventional culture, and another 5–10 mL stored for lung microbiome analysis with the extraction of deoxyribonucleic acid (DNA) and ribonucleic acid (RNA) for metagenomic and metatranscriptomic sample sequencing. According to microorganism identification through FA-PNEU, antibiotics were initiated,

considering the pathogen and resistance profile; consequently, with bacterial culture results and antibiogram, the definitive therapy was adjusted according to the treating physicians' criteria.

The study was conducted according to the regulations of the Universidad Pontificia Bolivariana ethics committee and the committees of each of the participating institutions. Informed consent was obtained from the patient or their legal representative for sample handling and the registered information in medical records.

2.2. COVID-19 detection

Detection of SARS- CoV-2 was performed by real-time polymerase chain reaction (RT-PCR) starting from nasopharyngeal swabs, following WHO and/or CDC protocol, in an Allplex SARS-CoV2 assay (Seegene, Inc. Korea) to amplify SARSCoV2 E, RdRp/s, and N genes.

2.3. Conventional culture

Respiratory samples were subjected to Gram staining and conventional semi-quantitative cultures. Ten microliter volume of ETA or mini-BAL was seeded on different media: chocolate, CNA (Colistin + Nalidixic Acid), and MacConkey. No sample dilution method was performed and the samples were incubated for up to 48 h (incubated at 35°C in an aerobic atmosphere and enriched with 5% CO2 for 2 days). Afterward, microorganisms that grew in significant amounts according to the guidelines of standard laboratory procedures were quantified (upon growth at or above 10^4 CFU/mL for a mini-BAL fluid sample or 10^5 CFU/mL for an ETA sample) and sub-cultured to isolate them if there were two or more microorganisms on non-selective plates. If they were negative, they were kept for further observation for up to 3 days. Cultures for viruses or atypical bacteria were not performed.

Bacterial species-level identification was conducted using Vitek R 2 identification (ID) cards. Antimicrobial susceptibility testing (AST) was performed by VITEK 2 (bioMériuex Marcy l'Etoile, France), following the manufacturer's instructions. The ASTs were interpreted according to current Clinical & Laboratory Standards Institute (CLSI) guidelines[11]. Normal respiratory microbiota and non-pulmonary pathogens such as *Candida spp.* and *Enterococcus faecalis* were considered negative.

2.4. Biofire® FilmArray® Pneumonia Panel Plus (FA-PNEU)

FA-PNEU is a syndromic panel based on multiplex PCR targeting 18 bacterial pathogens (eleven Gram-negative, four Gram-positive, and three atypical), nine viruses, and seven determinants of resistance (namely, CTX-M, KPC, NDM, Oxa48-like, VIM, IMP, and MecA/C/MREJ). Viral, fungal, and atypical bacterial detections were reported as not detected or detected, and resistance genes were reported as positive. In the case of positive results, semiquantitative values expressed in DNA copies/mL were also reported for each pathogen detected. All steps from nucleic acid extraction to the final detection of pathogens were carried out in an automated manner. The sample swab included in the kit was used to dispense the appropriate amount of mini-BAL or ETA into the cartridge according to the manufacturer's instructions. Briefly, approximately 200 µL of the sample were collected using a flocked swab and transferred to a sample injection vial. It was then mixed with the provided sample buffer. This solution was then loaded into the FilmArray pouch, which in turn was loaded into the FilmArray platform. The preparation of each cartridge required approximately 2 min of hands-on time, while the run time was approximately 1 hour and 15 minutes[12, 13].

2.5 Co-infection definition

Co-infection was defined as the identification of a respiratory tract pathogen using FA-PNEU or microbiological cultures of respiratory samples from patients.

2.6. Data analysis

Categorical variables were expressed as frequencies and percentages. Continuous variables were presented as median and 25th and 75th percentiles. Overall concordance was calculated as [(true positive + true negative /total)] x 100, concordant positive was calculated as [(true positive/total)] x 100) and Cohen's kappa coefficient with 95% confidence intervals were estimated with FA-PNEU and conventional culture results. Sensitivity, specificity, positive and negative predictive values (PPV and NPV), along with 95% confidence intervals were calculated by comparing the results between conventional culture and FA-PNEU only for bacterial pathogens present in the molecular panel. Performance was measured considering bacterial culture as the gold standard reference method. We do not perform concordance analysis or qualitative agreements for atypical bacteria or viruses. The association between the presence of co-infection with inflammatory response was measured by Mann-Whitney U-test. Data were entered into a Microsoft Excel database and analyzed using SPSS software version 26.0.

3. Results.

During the study period, 682 patients admitted to the ICU with severe COVID-19 were screened, of which 149 met the inclusion criteria (Fig. 1). Study participant demographic characteristics are shown in Table 1. The median age was 58 years (P25 46 – P75 66); 57.7% were males; the APACHE score median was 10, and the most frequent comorbidity was arterial hypertension in 46.3%. In-hospital deaths occurred in 34.5% of the patients.

Table 1
Demographic characteristics of patients with COVID-19 pneumonia admitted to 8 Intensive Care Units in Colombia.

Characteristic (n = 149)	Frequency (%)
Male	57.7
Age (Me-P25 – P75-)	58 (46–66)
Hypertension	46.3
Diabetes	24.2
Chronic Kidney Disease	5.4
Rheumatologic Disease	2
Neoplasm	2
Chronic Obstructive Pulmonary Disease	1.3
HIV	1.3
Heart failure	0.7
Cirrhosis	0.7
Me: Median, P25 – P75: 25th and 75th percentiles	
HIV: Human immunodeficiency virus	

One hundred forty-nine samples were drawn (139 by ETA and 10 by mini-BAL) from 149 patients, of which 110 samples were processed through FA-PNEU and 149 through conventional culture. Of the 110 samples tested by both methods, a total of 27 samples (24.54%) were detected by FA-PNEU vs 19 samples (17.27%) detected by culture. In total FA-PNEU identified 40 microorganisms and culture 23. Table 2 details the summary of total, FA-PNEU, and microbiological cultures detections for all pathogens. The overall concordance was 90.1% and by microorganism it was between 92.7% and 100%. The highest concordant positive was for *Staphylococcus aureus* with 7.3%.

Table 2

Summary of total, FilmArray® Pneumonia Plus Panel, and microbiological cultures detections for all pathogens from patients with COVID-19 pneumonia (r

Microbial target	Number FA-PNEU (+) Microbiological culture (+)	Number FA-PNEU (+) Microbiological culture (-)	Number FA-PNEU (-) Microbiological culture (+)	Number Total (+)	Number FA-PNEU (+)	Number Microbiological culture (+)	Overall concordance	Concordant positive	Cohen's kappa ((95% CI
Aspergillus flavus	0	0	1	1	0	1	99.1	0.0	0
Enterobacter cloacae complex	2	2	0	4	4	2	98.2	1.8	65.8 (21.7 ; 1
Haemophilus influenzae	0	4	0	4	4	0	96.36	0.0	0
Klebsiella pneumoniae	5	0	1	6	5	6	99.1	4.5	90.4 (71.9 ; 1
Klebsiella oxytoca	1	0	0	1	1	1	100	0.9	100 (100 ; 1
Pseudomonas aeruginosa	1	0	1	2	1	2	99.1	0.9	66.26 (4.3 ; 12
Streptococcus agalactiae	0	8	0	8	8	0	92.7	0.0	0
Staphylococcus aureus	8	7	0	15	15	8	93.64	7.3	66.4 (43.7 ; 8
Streptococcus pneumoniae	2	0	1	3	2	3	99.1	1.8	79.51 (40.4 ; 1
Resistance									
MecA/C/M REJ	0	6	0	6	6	0	94.5	0.0	0
CTX-M	0	0	0	0	0	0	100.0	0.0	0
KPC	0	0	0	0	0	0	100.0	0.0	0
Global	18	9	1	28	27	19	90.1	16.4	72.7 (57.1 ; 8

CI: confidence intervals

Median, P25 – P75: 25th and 75th percentiles

Of the 110 samples that underwent both techniques, 18 samples were positive for both methods, 82 samples were negative for both techniques, one sample was culture-positive with negative FA-PNEU (*Aspergillus flavus*, which was assumed as contamination by medical staff), and 9 samples were FA-PNEU positive and culture-negative (six *Staphylococcus aureus*, two *Streptococcus agalactiae*, and one *Haemophilus influenzae)*. The two bacteria most frequently detected by FA-PNEU were *Staphylococcus aureus* (37.5%) and *Streptococcus agalactiae* (20%) and by culture were *Staphylococcus aureus* (34.78%) and *Klebsiella pneumoniae* (26.08%). Atypical bacteria were not detected by FA-PNEU. Two samples with *Rhinovirus/Enterovirus* were detected by FA-PNEU.

Among the 27 FE-PNEU positive samples, 12 (44.44%) were polymicrobial compared with 4/19 (21.05%) culture positive. The most common combination founded were *Staphylococcus aureus* and *Klebsiella pneumoniae*.

According to FA-PNEU, 40% of the detected *S. aureus* were methicillin-resistant (MRSA). In six samples, FA-PNEU detected the MecA/C/MREJ resistance mechanism in *Staphylococcus aureus*, which was not detected by conventional cultures. None of the *Klebsiella pneumoniae* had CTX-M, KPC, NDM, Oxa48-like, VIM or IMP by FA-PNEU or Extended-Spectrum Beta-Lactams (ESBL) nor carbapenemases by culture. In conventional cultures, one patient was positive for *Streptococcus pneumoniae* resistant to ceftriaxone, not detected by FA-PNEU.

Table 3 depicts the qualitative agreement of 11 targets (eight microorganisms, three resistance genes) that correspond to 46/110 samples of isolated microorganisms in total, detected by FA-PNEU and culture, representing the gold standard. Positive predictive values (PPV) were between 50% and 100%, being lower for *Enterobacter cloacae* and *Staphylococcus aureus*. Negative predictive values (NPV) were high (99.1%-100%). Regarding resistance mechanisms, MecA/C/MREJ had a specificity of 94.55% and a NPV of 100%.

Table 3
Comparison between FilmArray® Pneumonia Plus Panel and standard reference culture results in respiratory specimens from co-infected COVID-19 patients (n = 110)

Microbial target	Sensitivity% (95% CI)	Positive predictive value % (95% CI)	Specificity % (95% CI)	Negative predictive value %(95% CI)
Enterobacter cloacae complex	100 (34.24, 100)	50 (15,85)	98.15 (93.5, 99.49)	100 (96.5, 100)
Haemophilus influenza	-	0 (0-48.99)	96.36 (91.02,98.58)	100 (96.5, 100)
Klebsiella oxytoca	100 (20.65, 100)	100 (20.65, 100)	100 (96.6, 100)	100 (96.6, 100)
Klebsiella pneumoniae	83.3 (43.65, 96.99)	100 (56.55, 100)	100 (96.44, 100)	99.05 (94.8-99.83)
Pseudomonas aeruginosa	50 (9.45, 90.55)	100 (20.65, 100)	100 (96.57, 100)	99.08 (94.99, 99.84)
Streptococcus agalactiae	-	0 (0, 32.44)	92.73 (86.3, 96.7)	100 (96.37, 100)
Staphylococcus aureus	100 (67.56, 100)	53.33 (30.12, 75.19)	93.14 (86.51, 96.64)	100 (96.11, 100)
Streptococcus pneumoniae	66.67 (20.77, 93.85)	100 (34.24, 100)	100 (96.53, 100)	99.07 (94.94, 99.84)
Resistance				
MecA/C/MREJ	-	0 (0-39.03)	94.55 (86.61, 97.48)	100 (96.44, 100)
CTX-M	-	-	-	-
KPC	-	-	-	-

Regarding the quantitative agreement, of the microorganisms in the cultures of ETA samples with > 10^5 CFU, 84.21% had a count of ≥ 10^5 copies/mL in FA-PNEU; of the negative cultures, 40.9% had microorganisms with a count < 10^5 copies/mL in FA-PNEU. The 10 samples taken by mini-BAL were negative in both cultures and FA-PNEU.

In patients on mechanical ventilation with SARS- CoV-2 severe pneumonia, co-infection was not associated with an increase in mortality, as opposed to FA-PNEU-positive patients (44.8% vs 32.1%; p = 0.219) and culture-positive individuals (40% vs. 35.2%; p = 0.652). Lastly, the inflammatory response tests showed no significant differences between patients with or without co-infection (Table 4).

Table 4
Results of laboratory tests of inflammatory response among patients with or without pulmonary co-infection.

Co-infection	Procalcitonin in ng/mL Me (P25-P75)	C-reactive protein in mg/dL Me (P25-75)	Leukocytes per 10^9/L Me (P25-75)
Yes	0.18 (0.07–0.74)	16.02 (6.7-21.86)	10.9 (7.9–13.5)
No	0.23 (0.1–0.6)	17 (10.68–24.87)	10.1 (7.4–13.5)
P value	0.41	0.20	0.57
Me: Median, P25 – P75: 25th and 75th percentiles			

4. Discussion

Our article has several take-home messages for the management of patients with COVID-19 pneumonia admitted to ICU: first, approximately a quarter of patients with COVID-19 pneumonia admitted to ICU have bacterial co-infection; second, a negative FA-PNEU result prevents the inappropriate empirical use of antibiotics in these patients as a stewardship strategy in COVID-19 and third the overall concordance was 90.1%, and it was between 92.7% and 100% by microorganisms.

Bacterial co-infection in critically-ill COVID-19 patients occurred in 24.54% and 17.27% by FA-PNEU and conventional cultures, respectively. In the most recent meta-analysis[14] on the identification of bacterial co-infections by FA-PNEU in ICU-hospitalized COVID-19 patients, four of the seven studies reported on the timing of specimen collection within the first 48 hours of ICU admission. In total, 221 patients were included and the pooled incidence of co-infections by FA-PNEU was 33% (95% CI 0.25 to 0.41) and 18% by conventional cultures (95% CI 0.02 to 0.45) [6–9]; the incidence is higher than the reported in inpatient services, which ranges between 3.5-8% [3, 15].The largest study by Kolenda et al [7] included 99 patients admitted to 3 ICUs from France and the samples were taken in absence of mechanical ventilation or within 48 hours after this was initiated; cultures identified 17 bacteria in 15 of 99 samples (15.1%).

The two most frequently detected bacteria were *Staphylococcus aureus* (37.5%) and *Streptococcus agalactiae* (20%) by FA-PNEU and *Staphylococcus aureus* (34.78%) and *Klebsiella pneumoniae* (26.08%) by culture. Verroken et al. [9], reported the results of 32 respiratory samples in 41 COVID 19 patients in the ICU; FA-PNEU identified 13/32 (40.6%) patients with a bacterial co-infection, where *Staphylococcus aureus* (38.46% -60% methicillin-sensitive-), *Haemophilus influenzae* (23.07%), and *Moraxella catarrhalis* (15.38%) were the main pathogens identified. Kreitmann et al.[8], documented bacterial co-infection in 13 of 47 subjects (27.7%) from samples taken within 24 hours of tracheal intubation, with three bacterial species representing ≥ 90% of those identified: *Staphylococcus aureus* 69.2% -all methicillin-sensitive-), *Haemophilus influenzae* (38.5%), and *Streptococcus pneumoniae* (23.1%). Kolenda et al. analyzed 99 patients with respiratory samples taken in the absence of mechanical ventilation or their first 48 hours; conventional cultures detected bacterial co-infection in

15%, being *Staphylococcus aureus* (46.6% -all methicillin-sensitive-), *Haemophilus influenzae* (26.66%), and *Streptococcus pneumoniae* (13.33%) the most prevalent pathogens.

When comparing the aforementioned studies with our work, three aspects are worth highlighting: firstly, in all the studies, including ours, *Staphylococcus aureus* was the most prevalent microorganism; secondly, in the present study, methicillin resistance was higher (40% of the FA-PNEU isolates had MecA / C / MREJ, while in the cultures no methicillin resistance was found); and lastly, unlike other studies, *Klebsiella pneumoniae* was the second most prevalent microorganism in the current study by culture, with no ESBL or KPC resistance mechanisms.

Regarding the qualitative agreements between FA-PNEU and conventional cultures, in our study we found that the PPV was between 50% and 100%, being lower for *Enterobacter cloacae* and Staphylococcus *aureus*; NPV were high (between 99.1% and 100%). Caméléna et al.[6] demonstrated that the results of FA-PNEU are consistent (sensitivity 95%, specificity 99%, PPV 82%, and NPV 100%) with those of conventional culture for bacterial pathogens of 96 samples from 43 intubated patients with suspected bacterial co-infection or superinfection; *Staphylococcus aureus*, as opposed to ours, did have a good PPV (91%). Kolenda et al.[7], reported a FA-PNEU sensitivity of 100%, since all isolated bacteria in culture were also detected using FA-PNEU, with a specificity of 98.7%, being the lowest for *Haemophilus influenza* (< 88.4%); the specificity for *Staphylococcus aureus* was 93.5%.

In our study, *Staphylococcus aureus* had a sensitivity of 100%, a PPV of 53.3%, a specificity of 93.1%, and a NPV of 100%, since 6 of the 9 patients with FA-PNEU positive and culture-negative microorganisms were *Staphylococcus aureus*. Moreover, in 6 FA-PNEU samples, the MecA/C/MREJ resistance mechanism was detected, not identified by conventional cultures. Fontana et al. [16] used FA-PNEU to assess co-infection in 152 respiratory specimens from inpatient COVID-19 individuals; 23 of them required assisted ventilation in the ICU. The most representative species was *Staphylococcus aureus* in both BAL (21; 16 mecA positive) and sputum (27; 14 mecA positive), with the majority being mecA positive (30/44, 62%). Although most of the patients were not in the ICU, their results are consistent with our findings.

Concerning the quantitative agreement, in our study, microorganisms in cultures of ETA samples with $> 10^5$ CFU, 84.21% had a count of $\geq 10^5$ copies/mL in FA-PNEU; of the culture-negative, 40.9% had microorganisms with a count $< 10^5$ copies/mL in FA-PNEU. In the study of Kolenda et al [7] among 16 bacteria reported in culture, 15 (93.8%) showed $\geq 10^6$ copies/mL using FA-PNEU; in contrast, amidst 26 bacteria detected using FA-PNEU yet culture-negative, 20 (76.9%) had $\leq 10^5$ copies/mL using FA-PNEU. We can conclude that most positive samples in FA-PNEU, with negative cultures, have low DNA copies/mL. These findings raise the following questions: is it possible that in these cases it is not strictly a coinfection and rather a contamination by the endogenous flora? What is the clinical impact of this finding? Could antibiotic treatment be discontinued in these cases? In future studies, we will try to give some answers by comparing the results of the cultures and FA-PNEU with the lung microbiome through the extraction of DNA and RNA for sequencing the same samples for metagenomics and metatranscriptomics, which will allow us to determine the functional profiles of the virulence and resistance genes of microorganisms.

We did not find that having co-infection by culture or by FA-PNEU, was associated with an increase in mortality. Another Latin American study, conducted by Soto et al. evaluated ninety-three hospitalized patients with a diagnosis of COVID-19 who were analyzed with FA-PNEU. Co-infection was evidenced in 38 (40.86%) cases and no association with mortality was found (OR 1.63; 95% CI 0.45–5.82)[17].

We acknowledge some limitations within our study: primarily, we did not perform an analysis of the empirical antibiotic therapies received by the patients, nor their modifications according to FA-PNEU or culture results. However, we established, as exclusion criteria, not having received an antibiotic before taking the samples of LRTI to not alter the results of FA-PNEU and cultures. Kolenda [7] found that the FA-PNEU positivity rate was 19.4% (14 of 72) and 51.9% (14 of 27) in patients with or without prior administration of antibiotics, respectively ($p = 0.001$), and the percentage of FA-PNEU positive results concordant with culture was not affected by antimicrobial administration. Besides, samples were collected either by endotracheal aspirate or mini-BAL and not through BAL; however, it is our usual practice due to the lack of availability of pulmonary professionals 24 hours a day.

There are several strengths in this study: first, the high NPV of FA-PNEU was demonstrated; therefore, we can conclude that if we find a negative result, bacterial co-infection is practically excluded. Novy et al. proposed an algorithm for rational use of FA-PNEU in critically-ill ventilated COVID-19 patients; this would allow 65.6% antibiotic spare in bacterial co-infection and better adequacy of empirical antibiotic therapy [18]. Secondly, as far as we know, this is the study with the largest number of patients included whose objective is the diagnostic concordance of FA-PNEU with culture, in subjects with COVID-19 pneumonia admitted to ICUs. Finally, this is the first Latin American study with this purpose; most of the studies have been carried out in Europe.

5. Conclusions

Bacterial co-infection in critically-ill COVID-19 patients was presented in 24.54% by FA-PNEU and in 17.27% by conventional cultures. The most frequently isolated microorganism was *Staphylococcus aureus.* Concerning qualitative agreements between FA-PNEU and conventional cultures, we found that the PPV ranges between 50% and 100%, being lower for *Enterobacter cloacae* and *Staphylococcus aureus*, with high NPV (between 99.1% and 100%). The overall concordance was 90.1%, and it was between 92.7% and 100% by microorganisms.

Abbreviations

FA-PNEU: Multiplex PCR FilmArray® Pneumonia Panel

RT-PCR: Real-time polymerase chain reaction

ICU: Intensive Care Unit

SRAS-CoV-2: Severe acute respiratory syndrome coronavirus 2

PPV: Positive predictive values

NPV: Negative predictive values

COVID-19: Infectious disease caused by a coronavirus-2019

LRTI: Lower respiratory tract infection

ETA: Endotracheal aspirate

mini-BAL: mini- bronchoalveolar lavage fluid

DNA: Deoxyribonucleic acid

RNA: Ribonucleic acid

AST: Antimicrobial susceptibility testing

CLSI: Clinical& Laboratory Standards Institute

MRSA:*Staphylococcus aureus methicillin-resistant*

ESBL: Extended-Spectrum Beta-Lactams

Declarations

Availability of data and materials

All data generated or analyzed during this study are included in this published article. The datasets generated or analyzed during the current study are available from the corresponding author on reasonable request

Acknowledgements

Acknowledgment to the 8 Institutions participating in the study with the respective collaborators:

Clínica Universitaria Bolivariana, Francisco Molina

Clínica El Rosario Tesoro, Álvaro Ochoa

Clínica CardioVid, Daniel Rodas

Clínica Sagrado Corazón, Nelson Fonseca

Clínica Las Américas Auna, Bladimir Gil

Clínica Medellín, Juan Echeverry

Hospital La María, Marco González

Hospital Manuel Uribe Ángel, Victoria Ángel

And Eleonora Bunsnow (Medical Advisor Iberia,bioMérieux) for her contributions in reviewing the article

Funding

Minciencias, Colombia, 121084468048

Contributions

All authors participated in project design and planning. FJM, LEB, LL and LT collected data , FJM,LL and LT performed the statistical analyses. FJMwrote the first draft of the manuscript. All authors revised the paper and approved the final version for publication.

Corresponding author

Correspondence to Francisco Molina

Ethics approval and consent to participate

All procedures performed in the present study were in accordance with the Declaration of Helsinki and the study was reviewed and approved by Ethics Committee of the Escuela de Ciencias de la Salud, Facultad de Medicina, Universidad Pontificia Bolivariana, Colombia. Informed consent to participate in the study was obtained from participants.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

References

1. Henríquez A, Accini J, Baquero H, Molina F, Rey A, Ángel VE, et al. Clinical features and prognostic factors of adults with COVID-19 admitted to intensive care units in Colombia: A multicentre retrospective study during the first wave of the pandemic. Acta Colomb Cuid Intensivo. 2021;S0122726221000203.
2. Lansbury L, Lim B, Baskaran V, Lim WS. Co-infections in people with COVID-19: a systematic review and meta-analysis. J Infect. 2020;81:266–75.
3. Langford BJ, So M, Raybardhan S, Leung V, Westwood D, MacFadden DR, et al. Bacterial co-infection and secondary infection in patients with COVID-19: a living rapid review and meta-analysis. Clin Microbiol Infect. 2020;26:1622–9.
4. Buetti N, Mazzuchelli T, Lo Priore E, Balmelli C, Llamas M, Pallanza M, et al. Early administered antibiotics do not impact mortality in critically ill patients with COVID-19. J Infect. 2020;81:e148–9.
5. Kyriazopoulou E, Karageorgos A, Liaskou-Antoniou L, Koufargyris P, Safarika A, Damoraki G, et al. BioFire® FilmArray® Pneumonia Panel for Severe Lower Respiratory Tract Infections: Subgroup Analysis of a Randomized Clinical Trial. Infect Dis Ther. 2021;10:1437–49.
6. Caméléna F, Moy A-C, Dudoignon E, Poncin T, Deniau B, Guillemet L, et al. Performance of a multiplex polymerase chain reaction panel for identifying bacterial pathogens causing pneumonia in critically ill patients with COVID-19. Diagn Microbiol Infect Dis. 2021;99:115183.
7. Kolenda C, Ranc A-G, Boisset S, Caspar Y, Carricajo A, Souche A, et al. Assessment of Respiratory Bacterial Coinfections Among Severe Acute Respiratory Syndrome Coronavirus 2-Positive Patients Hospitalized in Intensive Care Units Using Conventional Culture and BioFire, FilmArray Pneumonia Panel Plus Assay. Open Forum Infect Dis. 2020;7:ofaa484.
8. Kreitmann L, Monard C, Dauwalder O, Simon M, Argaud L. Early bacterial co-infection in ARDS related to COVID-19. Intensive Care Med. 2020;46:1787–9.
9. Verroken A, Scohy A, Gérard L, Wittebole X, Collienne C, Laterre P-F. Co-infections in COVID-19 critically ill and antibiotic management: a prospective cohort analysis. Crit Care. 2020;24:410.
10. Pletz MW, Blasi F, Chalmers JD, Dela Cruz CS, Feldman C, Luna CM, et al. International Perspective on the New 2019 American Thoracic Society/Infectious Diseases Society of America Community-Acquired Pneumonia Guideline. Chest. 2020;158:1912–8.
11. Humphries R, Bobenchik AM, Hindler JA, Schuetz AN. Overview of Changes to the Clinical and Laboratory Standards Institute *Performance Standards for Antimicrobial Susceptibility Testing,* M100, 31st Edition. McAdam AJ, editor. J Clin Microbiol. 2021;59:e00213-21.
12. 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.
13. Edin A, Eilers H, Allard A. Evaluation of the Biofire Filmarray Pneumonia panel plus for lower respiratory tract infections. Infect Dis. 2020;52:479–88.
14. Timbrook TT, Hueth KD, Ginocchio CC. Identification of bacterial co-detections in COVID-19 critically Ill patients by BioFire® FilmArray® pneumonia panel: a systematic review and meta-analysis. Diagn Microbiol Infect Dis. 2021;101:115476.
15. Rawson TM, Moore LSP, Zhu N, Ranganathan N, Skolimowska K, Gilchrist M, et al. Bacterial and Fungal Coinfection in Individuals With Coronavirus: A Rapid Review To Support COVID-19 Antimicrobial Prescribing. Clin Infect Dis. 2020;ciaa530.
16. Fontana C, Favaro M, Minelli S, Bossa MC, Altieri A. Co-infections observed in SARS-CoV-2 positive patients using a rapid diagnostic test. Sci Rep. 2021;11:16355.
17. Soto A, Quiñones-Laveriano DM, Valdivia F, Juscamayta-López E, Azañero-Haro J, Chambi L, et al. Detection of Viral and Bacterial Respiratory Pathogens Identified by Molecular Methods in COVID-19 Hospitalized Patients and Its Impact on Mortality and Unfavorable Outcomes. Infect Drug Resist. 2021;Volume 14:2795–807.
18. Novy E, Goury A, Thivilier C, Guillard T, Alauzet C. Algorithm for rational use of Film Array Pneumonia Panel in bacterial coinfections of critically ill ventilated COVID-19 patients. Diagn Microbiol Infect Dis. 2021;101:115507.

Figures

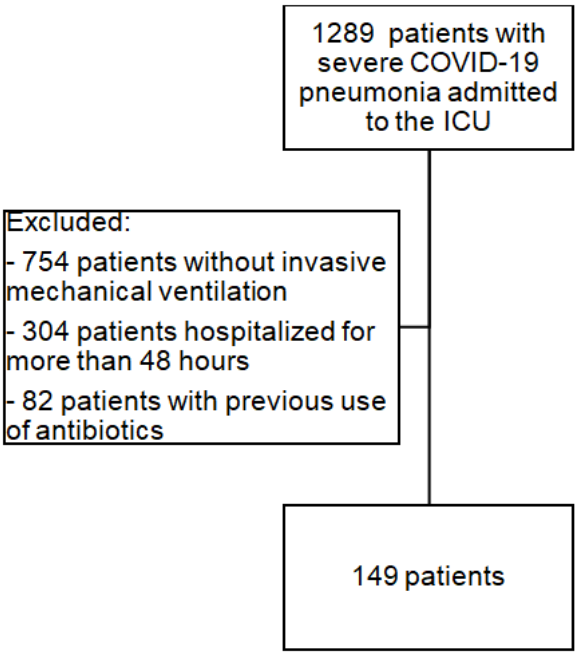

Figure 1

Trial profile. ICU, intensive care unit; FA-PNEU, multiplex PCR test FilmArray® Pneumonia Panel.