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Detection of bacteria via multiplex PCR in respiratory samples of critically ill COVID-19 patients with suspected HAP/VAP in the ICU

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Summary

Background Critically ill Coronavirus disease 2019 (COVID-19) patients have high rates of bacterial superinfection. Multiplex polymerase chain reaction panels may be able to provide useful information about the incidence and spectrum of bacteria causing superinfections.

Methods In this retrospective observational study we included all COVID-19 positive patients admitted to our intensive care unit with suspected hospital-acquired pneumonia/ventilator-associated pneumonia (HAP/VAP) in whom the BioFire® Pneumonia Panel (PP) was performed from tracheal aspirate or bronchoalveolar lavage fluid for diagnostic purposes. The aim of our study was to analyze the spectrum of pathogens detected with the PP.

Results In this study 60 patients with a median age of 62.5 years were included. Suspected VAP was the most frequent (48/60, 80%) indication for performing the PP. Tracheal aspirate was the predominant sample type (50/60, 83.3%).

The PP led to a negative, monomicrobial and polymicrobial result in 36.7%, 35% and 28.3% of the patients, respectively. The three most detected bacteria were *Staphylococcus aureus* (13/60, 21.7%), *Klebsiella pneumoniae* (12/60, 20%) and *Haemophilus influenzae* (9/60, 15%). Neither atypical bacteria nor resistance genes were detected.

Microbiological culture of respiratory specimens was performed in 36 (60%) patients concomitantly. The PP and microbiological culture yielded a nonconcordant, partial concordant and completely concordant result in 13.9% (5/36), 30.6% (11/36) and 55.6% (20/36) of the analyzed samples, respectively.

Conclusion In critically ill COVID-19 patients with suspected HAP/VAP results of the PP and microbiological culture methods were largely consistent. In our cohort, *S. aureus* and *K. pneumoniae* were the most frequently detected organisms. A higher diagnostic yield may be achieved if both methods are combined.

Keywords Superinfection · Mortality · Biofire · Pneumonia panel · Intensive care unit

Introduction

The clinical spectrum of coronavirus disease 2019 (COVID-19) ranges from completely asymptomatic or mild manifestations to severe and life-threatening forms requiring treatment in an intensive care unit [1–5]. Early studies have suggested that approximately 7% of patients are affected by bacterial co/superinfections [6, 7]. One meta-analysis showed that 3.5% of COVID-19 patients had a bacterial coinfection on admission and 14.3% developed a bacterial superinfection during hospital stay but 72% of all patients received empirical antibiotic treatment, mainly with third generation cephalosporins and respiratory fluoroquinolones (e.g. levofloxacin) [8]. In contrast, rates of bacterial superinfections are higher in influenza positive patients and range from 20% to 30% [9].

According to observational data, COVID-19 patients treated in the intensive care unit (ICU) have higher rates of bacterial superinfections than patients

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on normal wards [8, 10]. Interestingly, the ventilator-associated pneumonia rates are higher in COVID-19 patients ranging from 29% to 57% [11–14] compared to non-COVID-19 patients, where the incidence is approximately 10% (range 5–40%, depending on the underlying population) [11, 15, 16].

While multiplex polymerase chain reaction (PCR) based panels from cerebrospinal fluid on top of standard diagnostic procedures for suspected meningitis/encephalitis have been shown to have excellent diagnostic accuracy in two meta-analyses [17, 18], data for respiratory tract infections are conflicting, with some studies indicating a decrease in length of stay and antibiotic prescriptions [19, 20], while others failed to demonstrate any impact [21–23].

The aim of our retrospective, observational study was to analyze the spectrum of pathogens detected with multiplex PCR and culture from respiratory samples in critically ill COVID-19 patients with suspected hospital-acquired pneumonia (HAP) or ventilator-associated pneumonia (VAP).

Methods

Study design and population

This retrospective observational study took place at the Department for Infectious Diseases, Klinik Favoriten, in Vienna, Austria. All patients with PCR confirmed COVID-19 infections treated in our ICU between March and October 2020 with suspected HAP or VAP in whom the BioFire® Pneumonia Panel (PP) (bioMérieux SA RCS, Lyon, France) was performed were included in the study. The PP was carried out by trained medical staff at our point-of-care laboratory from respiratory samples (tracheal aspirate or bronchoalveolar lavage fluid) collected immediately before the test. The decision to utilize the PP was based on clinical grounds (e.g., deterioration, fever, rise in inflammatory markers, purulent secretion, new consolidations on chest X-ray) by the treating physicians.

The PP is a multiplex PCR designed to detect the most important pathogens of viral (*adenovirus, coronavirus, human metapneumovirus, human rhinovirus/enterovirus, influenza A/B, parainfluenza virus, respiratory syncytial virus*) and bacterial (*Acinetobacter calcoaceticus-baumannii, Enterobacter cloacae complex, Escherichia coli, Haemophilus influenzae, Klebsiella aerogenes, Klebsiella oxytoca, Klebsiella pneumoniae group, Moraxella catarrhalis, Proteus spp, Pseudomonas aeruginosa, Serratia marcescens, Staphylococcus aureus, Streptococcus agalactiae, Streptococcus pneumoniae, Streptococcus pyogenes, Chlamydia pneumoniae, Legionella pneumophila, Mycoplasma pneumoniae*) pneumonia within less than 2 h. Additionally, it is capable of detecting the most common antibiotic resistance genes (mecA/C and MREJ, CTX-M, KPC, NDM, VIM, IMP, OXA-48-like) [24].

If a respiratory sample for conventional micro-biological culture was obtained concomitantly, we compared the results with the PP and classified them as completely concordant (all detected pathogens matched in both tests or no pathogens was found), partially concordant (both tests detected the same pathogen, but an additional pathogen was detected in either the PP or culture) or non-concordant (pathogen detected in either PP or culture differed from each other).

For example, if *S. aureus* plus *H. influenzae* were detected as the only pathogens in PP and culture this was classified as completely concordant. If the PP result was *S. aureus* and *H. influenzae* but culture grew only *S. aureus* this was classified as partially concordant. If the PP result was *P. aeruginosa* and the culture result was negative or the PP result was *H. influenzae* and the culture result was *E. coli*, this was classified as non-concordant. Clinically irrelevant organisms that are generally regarded as contaminants or typical colonizers of the respiratory tract were excluded from the analysis.

Definition of variables

Hospital-acquired pneumonia and VAP were defined as pneumonia occurring ≥48 h after hospital admission and ≥48 h after intubation, respectively. A combination of the following clinical criteria led to the suspicion of HAP/VAP: new onset of fever, increased purulent sputum, worsening of respiratory function, detection of a new pulmonary consolidations [25].

Statistical analysis and data collection

Data were collected from patient medical records, entered in a MS Excel sheet (Microsoft, Redmond, WA, USA) and anonymized before statistical analysis. All analyses were made with SPSS 25 (IBM, Armonk, NY, USA) for Mac OS (Apple, Cupertino, CA, USA). Categorial variables were described by counts and percentages. For metric, non-normally distributed variables the median (Md) and interquartile range (IQR) were used. Significance tests for categorial variables were made via cross-tables and χ^2-tests or Fisher's exact test where applicable. A two-sided alpha < 0.05 was considered statistically significant.

The study was approved by the ethics committee of the City of Vienna (EK 20-079). All methods were carried out in accordance with the ethical principles of the declaration of Helsinki.

Results

Demographics

A total of 60 patients with a median age of 62.5 years (IQR 52–71.75 years) admitted to the ICU between March and October 2020 were included in the study.

Table 1 Patient characteristics

	All patients N = 60
Sex (male)	48/60 (80%)
Age in years (median, IQR)	62.5 (52–71.75)
BMI (median, IQR)	29 (26–36.75)
Hypertension	40/60 (66.7%)
Diabetes mellitus, type 2	18/60 (30%)
Coronary heart disease	12/60 (20%)
Chronic kidney disease	6/60 (10%)
Time from symptom onset before hospital admission (n = 58) in days (median, IQR)	5 days (3–8)
Time from symptom onset before ICU admission (n = 58) in days (median, IQR)	7 days (5–10)
Type of pneumonia	
HAP	12/60 (20%)
VAP	48/60 (80%)
Type of sample for PP	
Tracheal aspirate	50/60 (83.3%)
BAL	8/60 (13.3%)
Unknown	2/60 (3.3%)
Patients on antibiotics at the time of PP	44/60 (73%)
Time from hospitalization to PP (median, IQR)	10 days (6–15.75)
Time from ICU admission to PP (median, IQR)	7.5 days (3–12)

IQR interquartile range, *BMI* body mass index, *ICU* intensive care unit, *HAP* hospital-acquired pneumonia, *VAP* ventilator-associated pneumonia, *BAL* bronchoalveolar lavage, *PP* pneumonia panel

The majority of the patients were male (48/60, 80%). Hypertension (66.8%), type 2 diabetes mellitus (30%) and coronary heart disease (20%) were the most common comorbidities. Time from symptom onset to hospitalization and ICU admission was 5 days (IQR 3–8 days) and 7 days (IQR 5–10 days) respectively.

Table 2 Specific results from the pneumonia panel and microbiological culture

	Pneumonia panel N = 60	Microbiological culture N = 36
Staphylococcus aureus	13 (21.7%)	10 (27.8%)
Klebsiella pneumoniae	12 (20%)	9 (25%)
Haemophilus influenzae	9 (15%)	2 (5.6%)
Echeria coli	5 (8.3%)	2 (5.6%)
Streptococcus pneumoniae	5 (8.3%)	0 (0%)
Pseudomonas aeruginosa	3 (5%)	3 (8.3%)
Serratia marcesens	3 (5%)	2 (5.6%)
Klebsiella aerogenes	3 (5%)	3 (8.3%)
Streptococcus agalactiae	2 (3.3%)	2 (5.6%)
Klebsiella oxytoca	2 (3.3%)	1 (2.8%)
Acinetobacter baumannii	2 (3.3%)	0 (0%)
Enterobacter cloacae	2 (3.3%)	2 (5.6%)
Proteus spp	2 (3.3%)	0 (0%)
Other bacteria[1]	0 (0%)	10 (27.7%)

[1]Other bacteria detected: *Burkholderia cepacian* (1), *Citrobacter koserii* (1), *Enterococcus faecalis* (1), *Roseateles aquatilis* (1), *Raoultella ornithinolytica* (1), *Raoultella planticola* (1), *Serratia liquefaciens* (2), *Staphylococcus lugdunensis* (1), *Viridans group streptococci* (1)

Of the patients 22 (36.7%) died during the hospital stay. Median length of stay in the ICU of survivors was 24.5 days (IQR 18–30.75 days) and total hospital length of stay of survivors was 41 days (IQR 30–62.5 days). For details see Table 1.

Results of the PP and microbiological culture

Of the PPs 80% were performed in patients with suspected VAP and in 20% with suspected HAP. Tracheal aspirates and bronchoalveolar lavages (BAL) were analyzed in 83% and 17% of the patients, respectively. The PP was performed on average 7.5 days [3–10, 17, 18] after ICU admission. Most patients (73%) received an antibiotic at the time when the PP was performed (Table 1).

Table 3 General results PP and microbiological culture

	All patients	On antibiotics when tested		*p*-value
		No	Yes	
Results of PP (n = 60)				0.151
Negative	22/60 (36.7%)	3/16 (18.8%)	19/44 (43.2%)	
Monomicrobial	21/60 (35%)	6/16 (37.5%)	15/44 (34.1%)	
Polymicrobial	17/60 (28.3%)	7/16 (43.8%)	10/44 (22.7%)	
Results from culture (n = 36)				0.710
Negative	12/36 (33.3%)	2/9 (22.2%)	10/27 (37.1%)	
Monomicrobial	15/36 (41.7%)	4/9 (44.4%)	11/27 (40.7%)	
Polymicrobial	9/36 (25%)	3/9 (33.3%)	6/27 (22.2%)	
Comparison PP and culture (n = 36)				–
Non-concordant	5/36 (13.9%)	–	–	
Partially concordant	11/36 (30.6%)	–	–	
Completely concordant	20/36 (55.6%)	–	–	

PP pneumonia panel

Overall, the PP led to a negative, monomicrobial and polymicrobial result in 36.7%, 35% and 28.3% of the patients, respectively. The five most commonly detected pathogens were *S. aureus* (13/60, 21.7%), *K. pneumoniae* (12/60, 20%), *H. influenzae* (9/60, 15%), *E. coli* (5/60, 8.3%) and *S. pneumoniae* (5/60, 8.3%) (see Table 2).

No resistance genes, and no viruses or atypical bacteria were detected.

In 36 patients a microbiological culture of a respiratory specimen was performed concomitantly with the PP. The microbiological culture led to a negative, monomicrobial and polymicrobial result in 33.3%, 41.7% and 25% of the patients, respectively. The four most commonly detected pathogens were *S. aureus* (10/36, 27.8%), *K. pneumoniae* (9/36, 25%), *K. aerogenes* (3/36, 8.3%) and *P. aeruginosa* (3/36, 8.3%). Other detected bacteria are listed in Table 2.

Of the patients 75% (27/36) had a positive PP and/or culture result. Antibiotic administration had no statistically significant effect on the results of the PP ($p=0.151$) and culture ($p=0.710$) but patients on antibiotics had a numerically higher rate of negative PP and culture results, as shown in Table 3.

The PP and microbiological culture yielded a non-concordant, partial concordant and completely concordant result in 13.9% (5/36), 30.6% (11/36) and 55.6% (20/36) of the analyzed samples, respectively. For details see Table 3.

Discussion

In patients with COVID-19 treated in our ICU with suspected HAP/VAP, the rate of positive PP results was high. Monomicrobial, polymicrobial and negative results were found in approximately 30% each. The most frequently detected bacteria were *S. aureus, K. pneumoniae* and *H. influenzae.* Microbiological culture led to similar results in our study population, where *S. aureus* and *K. pneumoniae* have been detected most frequently.

In other studies with COVID-19 patients *S. aureus* und *S. pneumoniae* have been identified as the most important pathogens in community-acquired pulmonary superinfections, while in hospital-acquired superinfections *S. aureus, H. influenzae, K. pneumoniae* and, on some occasions, non-fermenting bacteria such as *P. aeruginosa* and *A. baumannii* predominated [6–8, 10, 26, 27]. In our study non-fermenting bacteria were only detected occasionally. One study found a high rate of atypical bacteria in COVID-19 co-infections but these diagnoses were based on serological tests, which can generally not be regarded as a reliable tool [7]. In contrast, we detected neither any atypical bacteria nor viruses other than severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). Empirical antibiotic coverage for atypical bacteria in COVID-19 is not recommended in the guidelines [28].

A major advantage of multiplex PCRs and their use in point-of-care laboratories is the fast turn-around time permitting the treating physicians to establish a targeted anti-infective therapy more rapidly than with conventional methods. The PP delivers results of the most common bacteria and resistance genes within less than 2 h with very little hands-on time; however, to interpret the results properly and to draw the correct conclusions expert knowledge on infectious diseases as well as antimicrobial therapy is necessary, especially when it comes to interpreting polymicrobial results. Furthermore, interpretation is complicated if results from the PP and microbiological culture differ.

We were able to demonstrate that the results from the PP and concomitantly microbiological culture were completely concordant in approximately 55.6% and partially concordant in 30.6%, emphasizing the usefulness of point-of-care multiplex PCRs additionally to microbiological techniques, especially when classical microbiological culture methods are not available due to laboratory opening hours or delayed transportation time and regarding slower results from culture. Recently a study demonstrated high concordance between conventional microbiological culture and the PP in non-COVID-19 patients admitted to hospital for lower respiratory tract infections. Interestingly, the PP showed polymicrobial results in almost half of the patients, with *S. aureus* and *H. influenzae* being the most detected bacteria via the PP [29]. While the high incidence of *S. aureus* in the PP was confirmed by culture in our study, *H. influenzae* was more frequently found in the PP alone. This raises the question if the prevalence of *H. influenzae* may be overestimated by using PCR-based methods.

The detection of bacteria via multiplex PCR based methods may only reflect colonization and not infection on some occasions, which is one of the main shortcomings of such diagnostic tools. Furthermore, despite the potential detection of major resistance genes such methods lack the ability of generating detailed antimicrobial susceptibility profiles. Resistance rates in Austria are low compared to most other countries [30], which might explain why no resistance genes were identified in our patient cohort. Additionally, the rate of empirical antibiotic prescription is lower at our department compared to other hospitals which might contribute to low resistance gene detection in our ICU cohort [8, 31]. The majority of patients are transferred from our normal ward to the ICU and are not directly admitted to the ICU.

The administration of antibiotics had no statistically significant effect on the detection rate of bacteria in the PP and culture but patients on antibiotics had a numerically higher rate of negative results in both groups. While each method on its own detected any bacteria in approximately two thirds of the patients, 75% of the patients had either a positive PP and/or culture result. This supports the additional

use of both diagnostic methods. Very recently, studies have shown the potential of multiplex PCR to improve antibiotic treatment in bacterial pneumonia in non-COVID-19 and COVID-19 patients [32, 33].

Limitations of our study are the small sample size and the single center study design. Furthermore, the decision to perform the PP was not guided by specific criteria but was based on clinical judgement of the treating physician. Moreover, we neither performed a microbiological culture in all patients, nor did we test for fungal infections routinely, despite the increasing number of publications regarding COVID-19-associated pulmonary aspergillosis [34, 35]. We did not have a control group of patients with other viral disease like influenza to compare.

The strength of our study is that it reflects common practice in clinical routine. Little is known about the usability of multiplex PCR in the setting of a pandemic. Additionally, our results can give clinicians guidance on which pathogens should be covered empirically in COVID-19 patients at ICUs when diagnostic results are pending or not available. The local epidemiology and resistance rates have to be taken into account.

In summary, in critically ill COVID-19 patients the PP provides fast results with high detection rates and consistent results in comparison to culture. VAP is a very common complication of COVID-19 mechanically ventilated patients, with *S. aureus, K. pneumoniae* and *H. influenzae* being the most frequently detected organisms in our cohort. Due to a lack of stringent, evidence-based recommendations, the interpretation of PCR results, especially if they are polymicrobial, remains challenging. While many issues regarding the proper use of multiplex PCRs are yet unresolved, these diagnostic tools may help clinicians to obtain additional information faster in everyday clinical routine and should be implemented when possible. A higher diagnostic yield may be achieved if multiplex PCR based methods are combined with microbiological culture techniques. In our cohort 75% of the patients had either a positive PP and/or culture result, while each method on its own detected any bacteria in only approximately two thirds of the patients.

Author Contribution Mario Karolyi and Erich Pawelka had the idea of the study. Mario Karolyi wrote the manuscript. Julian Hind, Sebastian Baumgartner, Wolfgang Hoepler, Sara Omid and Tamara Seitz collected the data. Mario Karolyi, Emanuela Friese, Stephanie Neuhold and Marianna Traugott analyzed the data. Christoph Wenisch and Alexander Zoufaly supervised the study.

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