



Multiplex PCR in the empirical antibiotic treatment of patients with SARS-CoV-2 and bacterial respiratory superinfection

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SUMMARY

Background: The severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) pandemic led to overuse of antimicrobials, which increased concerns regarding antimicrobial resistance.

Objective: To measure the impact of a multiplex polymerase chain reaction (PCR) pneumonia panel on empirical antibiotic treatment for patients with critical coronavirus disease 2019 (COVID-19) with suspected bacterial respiratory superinfection.

Methods: This descriptive, prospective study was undertaken in a 36-bed intensive care unit from June 2020 to July 2021. Patients with severe COVID-19 who were ventilated and under suspicion of bacterial respiratory superinfection were included in the study. The intervention was a semi-quantitative multiplex PCR alongside concurrent standard cultures. When PCR panel results were expected to be obtained within 3 h of sampling, empirical antibiotic treatment was not administered while awaiting the results. Otherwise, empirical treatment was initiated. Patients classified as 'avoided empirical treatment' avoided 48–72 h of empirical antibiotic therapy. For those patients who received empirical treatment, the PCR panel results were used to decide whether treatment should be escalated, de-escalated, maintained or stopped. Positive and negative predictive values, and 'avoided empirical treatment' were calculated. Medical conduct and panel results were analysed for patients who received empirical treatment.

Results: Eighty-two patients (71% male, 29% female) were included in this study. The mean age was 57.5 years, and the mean APACHE II score was 16. Ninety PCR panels were performed, and the negative and positive predictive values were 99.9% and 66.7%, respectively. Empirical treatment was avoided in 61% of episodes. Of those patients who were receiving antibiotics when the PCR panel was performed, treatment was de-escalated in 71%, escalated in 14%, stopped in 9% and maintained in 6%. A diagnosis of bacterial respiratory superinfection was ruled out in 19% of cases.

Conclusions: PCR panels prevented the initiation of empirical antibiotic treatment in two-thirds of patients, and led to de-escalation in more than two-thirds of those who had started empirical antibiotic treatment. The high negative predictive value of the PCR panel allowed the diagnosis of bacterial respiratory superinfection to be ruled out. This tool represents a significant contribution to diagnostic stewardship in order to avoid the unnecessary use of antibiotics.

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Introduction

The severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) pandemic had a devastating effect on society, the economy and the health system globally [1]. Hospitalized patients overwhelmed the healthcare infrastructure in many parts of the world [2], with innumerable consequences. Health centres experienced high consumption of analgesic drugs, muscle relaxants, anaesthetics, heparin and antibiotics [3]. The impact of the high use of antibiotics on antimicrobial resistance is a matter of concern. Molecular diagnostic tools could be introduced as diagnostic stewardship tools to make more rational use of antibiotics for patients with coronavirus disease 2019 (COVID-19) with bacterial co-infection. The objective of this study was to measure the impact of a multiplex polymerase chain reaction (PCR) pneumonia panel (PP) on empirical antibiotic treatment for patients with critical COVID-19 and suspected bacterial respiratory superinfection. This study also analysed whether the antibiotic treatment based on the PP results matched the final treatment defined by the antibiogram.

Methods

This descriptive, prospective study was conducted in a 36-bed intensive care unit (ICU) from June 2020 to July 2021. The cohort was formed of patients with severe COVID-19 with suspected bacterial respiratory superinfection under mechanical ventilation at diagnosis. The ICU has the following infection control schemes in place: healthcare-associated infection surveillance programme, handwashing programme, multi-resistant micro-organism surveillance programme, antimicrobial stewardship programme, bundle implementation, a hospital-environment hygiene nurse, an infection control link nurse, exclusive cleaning staff for biomedical equipment, and an exclusive infection specialist.

COVID-19 was diagnosed by PCR assay using nasopharyngeal swabs. Clinical diagnoses of healthcare-associated respiratory infections were made according to the definitions of the National Hospital Infection Surveillance System [4], and community-acquired pneumonia (CAP) was diagnosed according to the guidelines of the local infectious diseases society [5]. A healthcare-associated infection was diagnosed when symptoms commenced >48 h after patient admission. Bronchoalveolar lavage by fibrobronchoscopy (BAL), mini-bronchoalveolar lavage (mini-BAL) and endotracheal aspirate were conducted for the diagnosis of bacterial respiratory superinfection. All samples were transported to the laboratory immediately.

The microbiological diagnosis was conducted using an automated PCR method: BioFire FilmArray Pneumonia Panel (bioMérieux, Marcy l'Etoile, France). This tool expresses the results in a semi-quantitative manner (copies/mL), except for *Legionella pneumophila*, *Chlamydia pneumoniae*, *Mycoplasma pneumoniae* and viruses which are expressed qualitatively (detected/not detected). The PP identifies 26 micro-organisms

which cause pneumonia (*Acinetobacter calcoaceticus-baumannii* complex, *Enterobacter cloacae*, *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*, *Legionella pneumophila*, *Mycoplasma pneumoniae*, *Chlamydia pneumoniae*, influenza A, influenza B, adenovirus, coronavirus, parainfluenza, respiratory syncytial virus, rhinovirus/enterovirus and metapneumovirus) and seven antimicrobial resistant genes (*mecA/C* and *MREJ*, *KPC*, *NDM*, *Oxa-48*-like, *VIM*, *IMP* and *CTX-M*). Specimens were processed according to the manufacturer's instructions. The interpretation of results was performed by ICU infection specialists with previous experience in the use of this tool. PPs were only conducted on representative respiratory samples, defined as <1% of epithelial cells/hpf and >10% polymorphonuclear leukocytes/hpf in the case of BAL and mini-BAL samples; and <10 scaly epithelial cells/hpf and >25 polymorphonuclear leukocytes/hpf for the endotracheal aspirate. Regarding immunocompromised patients, the presence of an inflammatory reaction was not considered necessary to perform the PP. In the context of the pandemic, Gram staining was suspended in order to protect the laboratory personnel from the risk of aerosolization from respiratory samples, although direct microscopy was sustained. Additionally, conventional culture and sensitivity testing were conducted. Quantitative cultures were performed by direct inoculation and 1/100, 1/1000, 1/10,000 and 1/100,000 dilutions on to sheep blood agar, CLED agar in aerobiosis and chocolate agar in micro-aerophilic conditions at 35°C. Organism identification was performed by mass spectrometry (MALDI-ToF, Bruker BD, Billerica, MA, USA), and sensitivity was assessed by diffusion and automated Phoenix BD. Confirmation of extended-spectrum beta lactamases was achieved through synergy testing of discs of cefotaxime and ceftazidime, with discs of amoxicillin-clavulanic acid. Confirmation of carbapenemases was achieved through synergy testing of discs of boronic acid and EDTA, with discs of imipenem and meropenem, or by chromatographic methods. Plates were incubated for 72 h before they were reported as culture negative.

The decision regarding whether or not to initiate empirical antibiotic treatment was made when the respiratory samples were taken. For patients whose PP results were expected to be available within 3 h of sampling, empiric antibiotic treatment was not administered until the results had been reviewed. In this context, the definition of 'avoided empirical treatment' was that empirical antimicrobials would have been administered for 48–72 h if the PP had not been conducted. Alternatively, if respiratory samples were taken between 8 pm and 8 am, they were preserved at +4°C and processed the next morning. In these cases, empiric antibiotic treatment was started, which consisted of meropenem, colistin, fosfomycin and linezolid for healthcare-associated pneumonia (HAP); and ceftriaxone, clarithromycin and linezolid for CAP. Based on the PP results, treatment of those

patients who had received tailored antibiotic treatment was: (i) escalated, defined as an increase in the antibiotic spectrum; (ii) de-escalated, defined as a decrease in the antibiotic spectrum or the suspension of some of the antibiotics the patient was receiving; (iii) maintained, defined as no modifications; or (iv) stopped. As a secondary objective, this study analysed whether the antibiotic treatment informed by the PP results matched the treatment determined by the results of culture and sensitivity testing. In this sense, if the treatment determined by the PP was maintained after receiving the culture and sensitivity results, the treatment was defined as 'not modified treatment'. A treatment which was escalated or de-escalated based on the culture and sensitivity results was defined as 'modified treatment' (Fig. 1).

In order to determine predictive values, and taking culture as the reference method, the following were defined: (i) 'true positive', when the PP result agreed with the culture result, and the latter showed a recount of colony-forming units (CFU) $\geq 10^4$ for mini-BAL and BAL, and $\geq 10^5$ CFU for endotracheal aspirate; (ii) 'true negative', when both methods showed negative results; (iii) 'false positive', when the PP result was positive and the culture result was negative; and (iv) 'false negative', when the PP result was negative and the culture result was positive with $\geq 10^4$ CFU for mini-BAL and BAL, and $\geq 10^5$ CFU for endotracheal aspirate. General characteristics of the population were analysed. Positive predictive value, negative predictive value and 'avoided empiric treatment' were calculated. In terms of patients who received empiric antibiotic treatment, data were analysed along with the PP result. Data was analysed using Excel 2016 (Microsoft Corp., Redmond, WA, USA).

Results

Eighty-two patients were included in this study (71% male, 29% female). The median age was 57.5 years [interquartile range (IQR) 49–67], and the median APACHE II score was 16 (IQR 12–18.5). In total, 90 PPs were performed, which showed a negative predictive value of 99.9% and a positive predictive value of 66.7% (Table I). Of the respiratory samples used, 88% (79/90) were mini-BAL, 10% (9/90) were BAL, and 2% (2/90) were endotracheal aspirates. Diagnoses included mechanical-ventilation-associated pneumonia (VAP) in 66% (59/90) of cases, CAP in 12% (11/90) of cases, HAP unrelated to mechanical ventilation in 2% (2/90) of cases, and tracheobronchitis related to mechanical ventilation in 1% (1/90) of cases. Sixty-one percent of cases (55/90) 'avoided empiric treatment'. Of the patients who were receiving antibiotic treatment when the PP was performed, treatment was de-escalated in 71% (25/35), escalated in 14% (5/35), stopped in 9% (3/35) and maintained in 6% (2/35) (Table II). Bacterial respiratory superinfection was ruled out in 19% (17/90) of cases. According to the culture and sensitivity results, 80% of cases were classed as 'not modified treatment' (72/90); the remaining 20% of cases were classed as 'modified treatment' (18/90), all of which were de-escalated. The number of micro-organisms documented between the PP and culture results was 142, of which 65% (94/142) demonstrated concordant identification between methods. The most common micro-organism was *S. aureus* (32%, 46/142; of which 28% were oxacillin resistant), followed by *K. pneumoniae* (15%, 21/142) and *H. influenzae* (13%, 18/142) (Table I). No cases of *Stenotrophomonas maltophilia* or fungi were identified via culture. No viruses were detected by the PP.

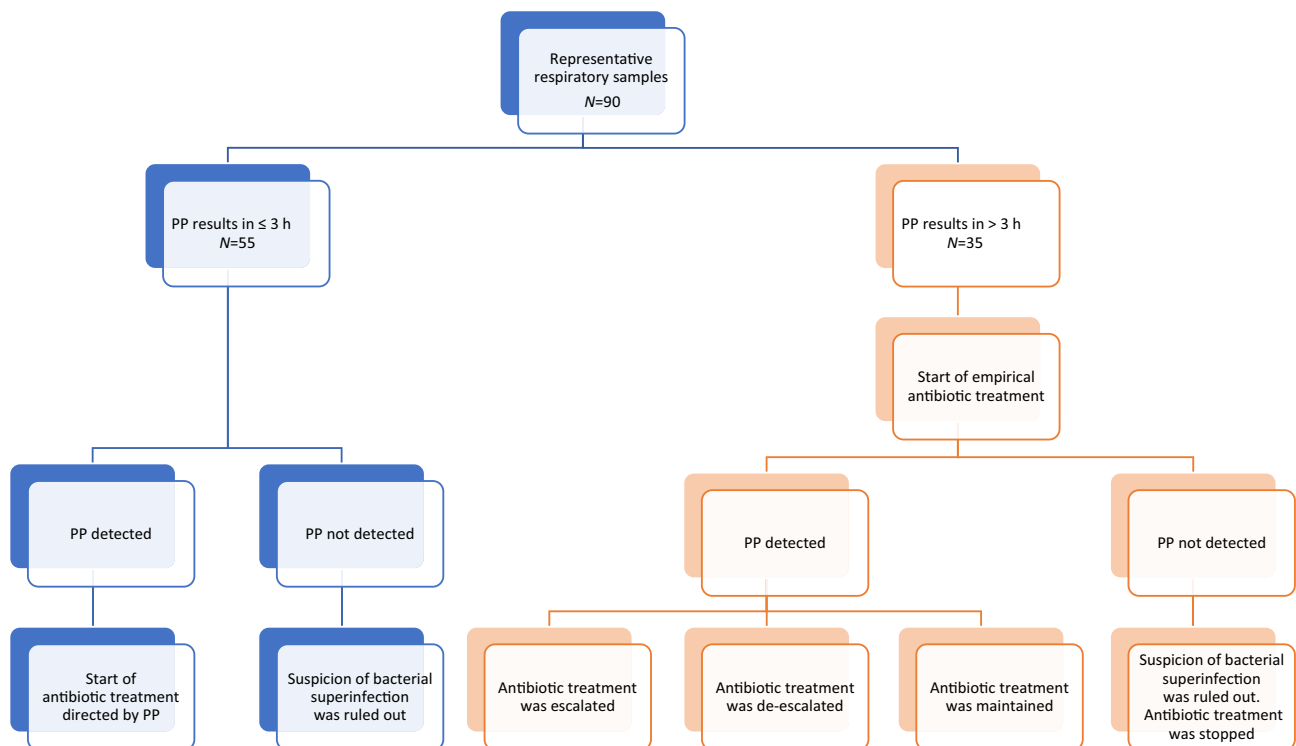


Figure 1. Decision-taking algorithm. PP, pneumonia panel.

Table I

Comparison between multiplex polymerase chain reaction and conventional culture on 90 respiratory samples

Micro-organisms	TP (N) (PP +/C +)	FP (N) (PP +/ C -)	FN (N) (PP-/C +)	TN (N) (PP -/C +)	PPV % (95% CI)	NPV % (95% CI)
<i>Staphylococcus aureus</i>	36	10	0	44	78.3	100
<i>Klebsiella pneumoniae</i> group	12	9	0	69	57.1	100
<i>Haemophilus influenzae</i>	9	9	0	72	50	100
<i>Pseudomonas aeruginosa</i>	13	3	0	74	81.3	100
<i>Escherichia coli</i>	6	3	0	81	66.7	100
<i>Streptococcus pneumoniae</i>	3	5	0	82	37.5	100
<i>Acinetobacter calcoaceticus-baumannii</i> complex	6	0	0	84	100	100
<i>Proteus</i> spp.	4	0	0	86	100	100
<i>Streptococcus agalactiae</i>	0	4	0	86	-	100
<i>Serratia marcescens</i>	2	1	0	87	66.7	100
<i>Klebsiella aerogenes</i>	2	1	0	87	66.7	100
<i>Enterobacter cloacae</i>	1	1	1	87	50	98.9
<i>Klebsiella oxytoca</i>	0	1	0	89	-	100
<i>Moraxella catarrhalis</i>	0	0	0	90	-	100
<i>Streptococcus pyogenes</i>	0	0	0	90	-	100
Total	94	47	1	1208	66.67 (60.15–72.60)	99.92 (99.42–99.99)

TP, true positive; PF, false positive; FN, false negative; TN, true negative; PPV, positive predictive value; NPV, negative predictive value; PP, pneumonia panel; C, culture; CI, confidence interval.

Discussion

The overuse of antimicrobials in patients with COVID-19 at the beginning of the pandemic can be explained by various factors: (i) national and international recommendations which supported this practice due to the suspicion of bacterial coinfection, extrapolating the behaviour of SARS-CoV-2 to that of influenza H1N1 [1–6]; (ii) the high prevalence of VAP determined by ICU stay, mechanical ventilation and prone ventilation for unusually extended times [7]; (iii) diagnostic difficulty of VAP, for which signs and symptoms overlapped with SARS-CoV-2 infection [7,8]; (iv) mental and physical strain on health personnel, and working under pressure – conditions which led to lack of adhesion to the existing antimicrobial programmes [3]; (v) lack of specific and/or effective treatment and, on a more emotional level, the feeling that ‘we have to offer something’; and (vi) difficulty of taking respiratory samples, related to the safety of health personnel. Consequently,

at the beginning of the pandemic, 74.5% of patients admitted to the ICU received some form of antibiotic treatment [9].

A moderate proportion of patients with COVID-19 show a critical presentation which requires admission to the ICU [10,11], and these patients are more susceptible to bacterial respiratory superinfection [3]. The prevalence and aetiology of bacterial respiratory superinfections are not well understood, and depend on the scenario, comorbidities and severity of COVID-19 [12]. In the present study, VAP was the most common bacterial respiratory superinfection; in fact, the historical rate of VAP in the study ICU is 4.5% and this increased to 15% during the study period. The high prevalence of bacterial respiratory superinfections and the overuse of antibiotics in these critical patients precipitated the use of the PP.

According to the epidemiology of this ICU, the empirical antibiotic treatment for healthcare-associated respiratory infections is meropenem, colistin and fosfomycin. The empirical antibiotic treatment for CAP is ceftriaxone and clarithromycin. For both infections, linezolid was added to the aforementioned empirical antibiotic treatment from the advent of the COVID-19 pandemic. This decision was based on the high prevalence of infections produced by *S. aureus*, observed previously when treating patients with similar characteristics [13]. In this research, *S. aureus* was an aetiological pathogen in 59% of cases of VAP. Empiric linezolid was added to the empirical treatment of VAP, HAP and CAP to ensure prompt effective treatment when bacterial superinfection was suspected, and because it was known that the PP results would enable action to be taken in a few hours.

In the event of suspicion of respiratory bacterial superinfection, local recommendations were to start empiric antibiotic treatment immediately after taking the appropriate samples, while awaiting culture and sensitivity results. At the study institution, these results were obtained 48–72 h later.

After introducing the PP (and as established in other research [14,15]), access to rapid results enabled the authors

Table II

Therapeutic course of action for the 35 patients who started empirical antibiotic treatment [patients whose pneumonia panel (PP) results were received >3 h after sampling]

Therapeutic course of action with PP N of patients: 35	% (N)
De-escalated	71 (25)
Linezolid	92 (23)
Fosfomycin	88 (22)
Carbapenem group	80 (20)
Colistin	76 (19)
Ceftriaxone	8 (2)
Clarithromycin	8 (2)
Escalated	14 (5)
Stopped	9 (3)
Maintained	6 (2)

to stop prescribing empiric antibiotic treatment, and to prescribe directed antibiotics based on the PP results. This way, empirical treatment was avoided in 61% of cases.

In cases who were already receiving empirical antibiotic treatment, the PP enabled early escalation or de-escalation. De-escalation occurred in 71% of cases, which differs from the authors' previous studies where de-escalation occurred in 40% and 39% of cases [16,17]. The negative predictive value was high [8–15], which may make it possible to rule out bacterial superinfection and to avoid the inappropriate use of antibiotics [12]. In this study, the negative predictive value was 99.9%, which enabled the authors to decide not to administer antibiotics or to stop treatment in 15% of cases. However, it should be noted that the use of culture and sensitivity tests is an imperfect 'gold standard' against which to compare this PP. It has been demonstrated elsewhere that routine diagnostic cultures are less sensitive than multiplex PCR panels for microbiological diagnosis of nosocomial pneumonia in a UK ICU setting [18]. Therefore, calculations of PP test characteristics based upon the use of culture and sensitivity testing as a 'gold standard' should be viewed with caution.

Furthermore, molecular methods reduce the turnaround time of microbiological diagnostic tests and, together with antimicrobial programmes, significantly reduce the time required to start appropriate treatment, thus optimizing clinical and economic outcomes [16].

Concordance between antibiotic treatment determined by the PP results and the final treatment determined by culture and sensitivity tests was 80%. This includes those patients with a 'not detected' PP result, in which case bacterial respiratory superinfection was ruled out and the decision was made not to treat these patients; a decision which was subsequently confirmed by culture. Treatment was modified for the remaining 20% of cases, and de-escalated in all of these cases. It is noteworthy that, of the 20% of modified cases, 7% had *P. aeruginosa* detected in the PP, and received two antibiotics targeting this micro-organism to ensure its treatment. This prescription responded to the critical condition of the patients, as well as the variability that this micro-organism shows in terms of bacterial resistance in this ICU. However, at the time of receiving the culture and sensitivity results, patients continued with appropriate anti-pseudomonal monotherapy and, consequently, treatment was de-escalated. Finally, the high correlation between the PP results and the culture and sensitivity results determined that each patient was treated correctly from the moment that bacterial respiratory superinfection was suspected.

Although *S. maltophilia* is not included in the PP, it did not represent any difficulties in terms of this study as it is not an endemic bacterium in this ICU. In fact, there were no cases of *S. maltophilia* detected in the cultures, and its treatment is not included routinely in the habitual empirical antibiotic treatment of HAP.

According to previous studies, microbiological detection using multiplex PCR is significantly higher than the gold standard diagnosis [19]. It is known that this is an advantage in patients who receive antibiotic treatment before respiratory sampling, as PCR may detect micro-organisms that are not obtained in the culture. However, this diagnostic method could also detect airway-colonizing micro-organisms that are not involved in the respiratory superinfection under study [14]. Although there is no consensus about the clinically relevant

cut-off for this diagnostic method, some studies have suggested that positive tests with $\leq 10^5$ copies/mL should be interpreted with caution [10,12,20]. As such, it is considered that the multiplex PCR has limitations, and interpretation of its results must be the responsibility of multi-disciplinary teams formed by professionals with experience in the use of this diagnostic method. It is suggested that local epidemiology, characteristics of a direct observation examination of the sample, clinical presentation, and microbiological history of each patient should be considered, thus personalizing the therapeutic course of action in terms of the antibiotic treatment.

In conclusion, the PP prevented the start of empirical antibiotic treatment while waiting for results in two-thirds of patients in this study. It should be noted that treatment was de-escalated in more than two-thirds of those who had started empirical antibiotic treatment. As a result of the strong correlation between antibiotic treatment defined using the PP results and the final treatment determined by culture and sensitivity results, all patients were adequately treated from the moment that bacterial respiratory superinfection was suspected. In addition, the high negative predictive value of the PP enabled the authors to rule out bacterial respiratory superinfection. Consequently, this tool represents a useful contribution to diagnostic stewardship in order to avoid the unnecessary use of antibiotics.

Conflict of interest statement

None declared.

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