The significance of monitoring respiratory sample cultures and polymerase chain reaction tests for detecting bacterial pathogens in severely and critically ill patients with COVID-19

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Background. Bacterial superinfection is one of the most common and potentially lethal complications in severely and critically ill patients with COVID-19.
Objectives. To determine the colonisation time frame and the spectrum of potential bacterial pathogens in respiratory samples from patients with severe and critical COVID-19, using routine culture and polymerase chain reaction (PCR) tests.
Methods. A prospective observational study was conducted on patients aged ≥18 years with confirmed severe and critical COVID-19 who were admitted to or transferred to the intensive care unit (ICU). Respiratory samples were collected for microbial culture and PCR testing within the first 2 days after ICU admission/transfer, between days 3 and 6, and after 7 days of ICU stay.
Results. A total of 82 patients, with a median (interquartile range) age of 74.5 (67.3 - 81.0) years and a median Charlson comorbidity index of 4 (3 - 5), were enrolled in the study. Colonisation with any pathogen was observed in 67% of patients, after a median of 4 (2 - 6) days in the ICU. On days 0 - 2 of the ICU stay, micro-organisms were detected in 18% of patients, with *Klebsiella pneumoniae* (without acquired antibiotic resistance) and methicillin-susceptible *Staphylococcus aureus* being most frequently identified. Later, *Acinetobacter baumannii* and carbapenem-resistant *K. pneumoniae* became the predominant micro-organisms, identified in nearly half of the patients. In 74% of the samples, the results of microbial culture and PCR tests were identical. In 17%, PCR revealed bacterial pathogens not identified by culture.
Conclusion. Our study confirms that colonisation of the respiratory tract occurs early in the course of ICU stay. Superinfections are predominantly caused by multidrug-resistant Gram-negative bacteria.
Keywords. COVID-19, SARS-CoV-2, superinfection, colonisation, polymerase chain reaction, PCR.

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Study synopsis
What the study adds. This real-world study provides valuable insights into the significance of microbiological monitoring of critically ill COVID-19 patients. It confirms that bacterial colonisation of the respiratory tract occurs early in the course of ICU stay, with nosocomial superinfections caused predominantly by multidrug-resistant Gram-negative pathogens. Polymerase chain reaction (PCR) testing can assist in ruling out colonisation and in early detection of potential bacterial superinfections.
Implications of the findings. Bacterial superinfections present a major challenge in critically ill COVID-19 patients, owing to their high prevalence and mortality rates. Their early detection, determination of causative agents, and antibiotic susceptibility profiling are therefore of paramount importance. PCR testing of clinical specimens appears to be a valuable supplement to respiratory culture, enhancing the precision of diagnosis of lower respiratory tract infections.

Despite a decline in the number of COVID-19 cases, the pandemic remains the most significant healthcare crisis of the 21st century.[1] Many researchers continue to investigate the role of bacterial infections in COVID-19.[2,3] On the one hand, there is sufficient evidence suggesting that coinfection as opposed to superinfection is infrequent. It would therefore be advisable to restrict the routine use of antibiotics

for COVID-19 unless specific indications are present.[2,4] Superinfections, on the other hand, develop in a substantial proportion of hospitalised patients with severe disease, often affecting the outcome. According to a meta-analysis of 118 studies, the incidence of superinfection in COVID-19 was 24%, and patients with superinfection had longer hospital stays and an increased risk of death.[3] In a multicentre study by He *et al*.,[5] it was found that the risk of death in hospitalised patients with COVID-19 and bacterial superinfection increased by 8.2-fold (95% confidence interval 4.5 - 15.1). In another study, Buehler *et al*.[6] found that bacterial superinfections in patients with severe or critical COVID-19 were linked to fewer days without mechanical ventilation, despite a high rate of empirical antibiotic therapy. In addition to the traditional predisposing factors, the risk of secondary bacterial complications in COVID-19 patients may be increased as a result of the widespread use of immunosuppressive therapy.[7]

Lower respiratory tract infections (LRTIs), including ventilator-associated pneumonia, frequently occur in mechanically ventilated patients.[8] Among intubated patients, the rate of superinfections can be as high as 42 - 61%.[6,9-11] Regular testing of respiratory specimens for the presence of potential bacterial pathogens may therefore be considered so that antibacterial therapy can be prescribed timeously.

The diagnosis of superinfections in COVID-19 poses specific challenges. New infiltrates on chest computed tomography (CT) scans are often poorly visible in patients with extensive lung disease, and the presence of leucocytosis and neutrophilia may be linked to the use of corticosteroids.[12] Moreover, the use of dexamethasone and/or interleukin-6 antagonists can reduce the diagnostic value of procalcitonin as a marker for bacterial superinfection, because they may suppress its production.[13] Elevated procalcitonin and C-reactive protein levels are also associated with a severe course and poor prognosis in COVID-19, making it challenging to establish precise threshold values for these biomarkers.[14,15]

The present study aimed to determine the time frame for colonisation and identify the potential range of bacterial pathogens in respiratory samples from patients with severe and critical COVID-19, using routine culture and polymerase chain reaction (PCR) testing.

Methods

A prospective observational study was conducted in a multidisciplinary hospital in Moscow between December 2021 and February 2022. The study included patients aged ≥18 years with confirmed severe and critical COVID-19, who were either admitted directly or transferred to the ICU.

A respiratory sample (expectorated sputum or endotracheal aspirate (ETA) for intubated patients or bronchoalveolar lavage (BAL) when clinically indicated) was collected for Gram staining and culture within the first 2 days of ICU admission/transfer, between days 3 and 6, and after 7 days of ICU stay.

Sputum quality was assessed according to standard criteria. To isolate aerobic and facultative anaerobic micro-organisms, selective and differential media were used in accordance with standard methods and procedures. Micro-organisms were identified using a BD Phoenix M50 automatic analyser (Becton Dickinson, USA). Antibiotic susceptibility was evaluated according to national guidelines[16] closely aligned with the European Committee on Antimicrobial Susceptibility Testing (EUCAST) methodology and EUCAST clinical breakpoints from 2021. The micro-organisms isolated were considered potentially significant if the sputum contained ≥10^5 colony-forming units (CFUs)/mL (≥10^4 CFUs/mL for ETA and ≥10^3 CFUs/mL for BAL).

In a subset of patients, real-time PCR was performed concurrently with culture to detect common bacterial pathogens and markers of antibiotic resistance. DNA from *Klebsiella pneumoniae*, *Acinetobacter baumannii*, *Pseudomonas aeruginosa*, *Escherichia coli*, *Staphylococcus aureus* and the *MecA* gene indicating *S. aureus* resistance to methicillin was detected and quantified using the AmpliTest K.p./A.b./P.a./ E.c. (research use only (RUO), Centre for Strategic Planning and Management of Biomedical Health Risk of the Federal Medical Biological Agency (CSP), Russia) and AmpliSens MRSA-screen-titre-FRT (*in vitro* diagnostics (IVD), Central Research Institute of Epidemiology (CRIE), Russia). *Stenotrophomonas maltophilia* DNA was detected using the AmpliTest SM (RUO, CSP, Russia). Identification of carbapenemase genes belonging to the NDM, OXA-48-like and KPC groups was performed using AmpliSens MDR MBL-FRT (IVD) and AmpliSens MDR KPC/OXA-48-FRT (IVD) reagent kits (CRIE, Russia).

The initial sample was also tested for RNA/DNA of respiratory viruses, including influenza viruses A and B, human respiratory syncytial virus, human adenovirus, human metapneumovirus, human coronaviruses (229E, HKUI, OC43, NL63), human parainfluenza viruses 1 - 4, human rhinovirus, human bocavirus, *Mycoplasma pneumoniae* and *Chlamydia pneumoniae*, as well as *Streptococcus pneumoniae* and *Haemophilus influenzae* DNA. These tests were carried out using real-time PCR kits (AmpliSens Influenza virus A/B-FRT (IVD), AmpliSens ARVI-screen-FRT (IVD), AmpliSens *Mycoplasma pneumoniae*/*Chlamydophila pneumoniae*-FRT (IVD) and AmpliSens Pneumo-quantum-FRT (IVD) (CRIE, Russia)) to reveal potential cases of co-infection. Real-time PCR was performed using a CFX96 system (Bio-Rad, USA). RNA/DNA extraction from the samples was done using the AmpliSens RIBO-prep reagent kit, and reverse transcription was performed using the AmpliSens REVERTA-L RT reagent kit (CRIE, Russia). A positive PCR result was defined as a bacterial DNA load ≥10^4 genome equivalents per mL.

We documented clinical, demographic and laboratory data, chest CT scan results, comorbidities, and pharmacological and non-pharmacological treatments, as well as all documented cases of nosocomial LRTI and outcomes.

The decision about the significance of the micro-organisms identified and the presence of infection was made by two clinicians based on the following criteria: the presence of symptoms indicating secondary infection (such as increasing respiratory failure and haemodynamic disorders, relapse of fever and the development of purulent respiratory secretions); observed laboratory changes (severe leucocytosis and increased procalcitonin level); and the emergence of new lung infiltrates that could be interpreted as indicators of bacterial superinfection.

Descriptive statistics were performed using SPSS version 26.0 (IBM, USA). We used the Shapiro-Wilk test to check continuous data for normality. Continuous data are shown as means with standard deviations or medians with interquartile ranges (IQRs), while categorical data are presented as frequencies and percentages. To compare PCR with culture, we provided the number (and percentage) of concordant positive results (indicating the same micro-organism detected), concordant negative results and discordant results. The study was approved by the Local Ethics Committee of City Clinical

Hospital named after S. S. Yudin (ethics approval letter no. 1, dated 11 January 2021).

Results

A total of 82 patients with an median (IQR) age of 74.5 (67.3 - 81.0) years were enrolled in the study (Table 1). The median Charlson comorbidity index was 4 (3 - 5), with arterial hypertension and diabetes mellitus being the most common concomitant conditions. Most of the patients (77%) required invasive mechanical ventilation.

Initially, during the first 2 days of ICU admission, respiratory samples were obtained from 55 (67%) of the patients. The remaining 27 patients (33%) did not have a productive cough and were not intubated. Initial culture of the respiratory samples identified a microorganism in only 15 patients (18%). The most frequently detected potential bacterial pathogens were *K. pneumoniae* without acquired antibiotic resistance, and methicillin-susceptible *S. aureus* (MSSA). All samples tested with PCR were negative for *M. pneumoniae* and *Chlamydia pneumoniae* DNA; however, 3 cases of *S. pneumoniae* and 1 case of *H. influenzae* were detected. Additionally, 2 cases of co-infection with human adenovirus were identified among the viruses.

Between days 3 and 6 of the ICU stay, 30 patients had positive culture results. Using both culture and PCR, we identified *K. pneumoniae* and *A. baumannii* as the most common microorganisms, with 27% of *K. pneumoniae* being carbapenem resistant. After day 7 in the ICU, *A. baumannii* and *K. pneumoniae* continued to be the most frequently isolated pathogens, and the proportion

Table 1. Characteristics of patients with severe/critical COVID-19 (*N*=82)

	n* (%)
Demographic data	
Age (years), median (IQR)	74.5 (67.3 - 81.0)
Women	56 (68.3)
Recent previous hospitalisation	10 (12.2)
Hospital stay before admission to ICU (days), median (IQR)	3.5 (2.0 - 5.0)
ICU stay (days), median (IQR)	8 (5.0 - 12.0)
COVID-19 severity	
Chest CT stage	
CT-2	2 (2.4)
CT-3	44 (53.7)
CT-4	36 (43.9)
Invasive mechanical ventilation	63 (76.8)
Main comorbidities	
Arterial hypertension	77 (93.9)
Type 2 diabetes mellitus	26 (31.7)
Congestive heart failure	16 (19.5)
Stroke/TIA	11 (13.4)
Active cancer	9 (11.0)
Laboratory data†	
Lymphopenia (lymphocytes <1.26 × 10^9/L)	53 (64.6)
Anemia (haemoglobin <12 g/dL in females and <13 g/dL in males)	36 (43.9)
Thrombocytopenia (platelets <180 × 10^9/L)	27 (32.9)
Leucopenia (leucocytes <4 × 10^9/L)	6 (7.3)
Serum CRP (mg/L), median (IQR)	56.4 (18.7 - 103.4)
Elevated procalcitonin (procalcitonin >0.5 ng/mL)	15 (18.3)
Treatment	
Glucocorticosteroids	82 (100)
Monoclonal antibodies	56 (68.3)
Levilimab	52 (63.4)
Olokizumab	4 (4.9)
Tofacitinib	2 (2.4)
Outcomes	
Death	62 (75.6)
Transfer to rehabilitation facility	4 (4.9)
Discharge	16 (19.5)

IQR = interquartile range; ICU = intensive care unit; CT = computed tomography; CT-2 = 25 - 50% of lung involvement on CT, CT-3 = 50 - 75% of lung involvement on CT, CT-4 = >75% of lung involvement on CT;[17] TIA = transient ischaemic attack; CRP = C-reactive protein.
*Except where otherwise indicated.
†All thresholds determined according to the local laboratory reference range.

of carbapenem-resistant *K. pneumoniae* had risen to 86%. All *A. baumannii* isolates, regardless of the time period, were multidrug resistant, including resistance to carbapenems. Additionally, we identified genes from the following carbapenemase groups in samples from 10 patients: NDM + OXA-48 in 6/10 cases, OXA-48 in 3/10 cases, and KPC + NDM + OXA-48 in 1/10 cases. The *MecA* gene was found in one sample containing *S. aureus* DNA, which corresponded to the culture results. Details of the monitoring process and pathogen findings are shown in Fig. 1.

The median time to colonisation of respiratory samples by various bacterial pathogens is presented in Table 2. Colonisation with any pathogen occurred in 55 patients (67%). The median (IQR) time to colonisation was 7 (5 - 11) days of hospital stay and 4 (2 - 6) days of ICU stay. In 37 patients, *A. baumannii*, carbapenem-resistant *K. pneumoniae* or both were detected.

Comparison of microbiological and PCR findings showed a 74% overlap of the results. PCR revealed additional micro-organisms in 17% of samples, which were not identified by culture (Fig. 2).

Clinical, laboratory and radiological data supported a diagnosis of LRTI (nosocomial pneumonia or ventilator-associated tracheobronchitis) in 49 patients (60%). Among the causative agents of LRTI, *A. baumannii* and carbapenem-resistant *K. pneumoniae* were the predominant pathogens, responsible for 69% of cases, when considering mixed infections. A detailed description of the pathogens identified is presented in Table 3.

During the observation period, 78 patients (95%) received a median (IQR) of 3 (2 - 3.75) courses of antibiotics. The antibiotics administered at different stages of hospital stay are detailed in Table 4. Ampicillin + sulbactam and cefepime were the most frequently prescribed antibiotics before transfer to the ICU. Cefepime + sulbactam and meropenem

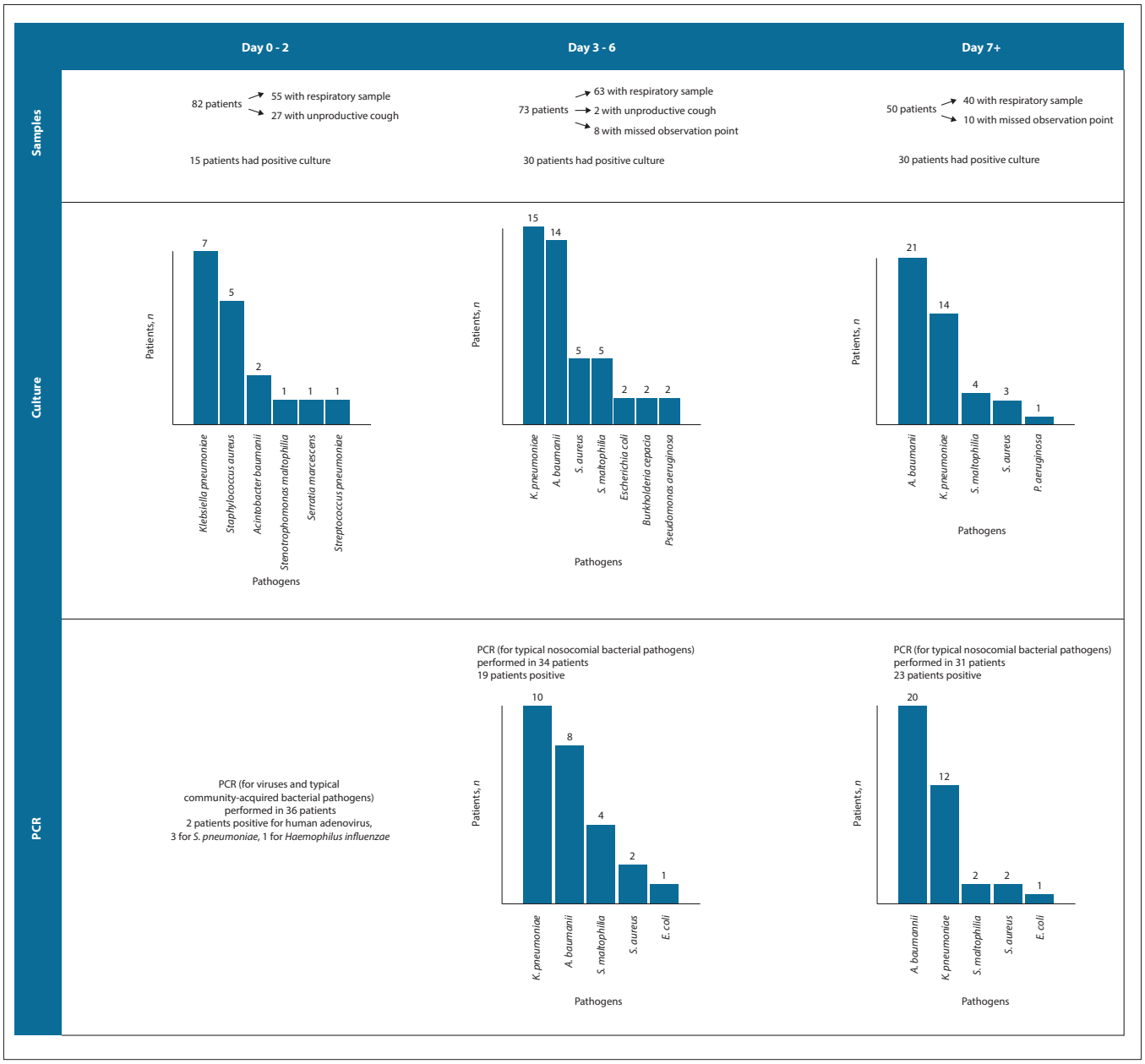

Fig. 1. Sample collection process and findings in patients with severe/critical COVID-19. (PCR = polymerase chain reaction.)

were used during the earlier ICU stay, whereas increases in the use of polymyxin B and tigecycline were noted during the later ICU stay.

Discussion

In the present study, clinically significant pathogens were detected in 18% of patients during the early days of their ICU stay. Inability to obtain sputum from some patients meant that it was not possible to perform cultures on all patients. Subsequently, the proportion of patients with positive findings on microbiological examination of respiratory specimens increased to 67%.

A systematic review found *Acinetobacter* spp., *P. aeruginosa*, *E. coli*, *K. pneumoniae*, *Enterococcus faecium* and *S. maltophilia* to be the

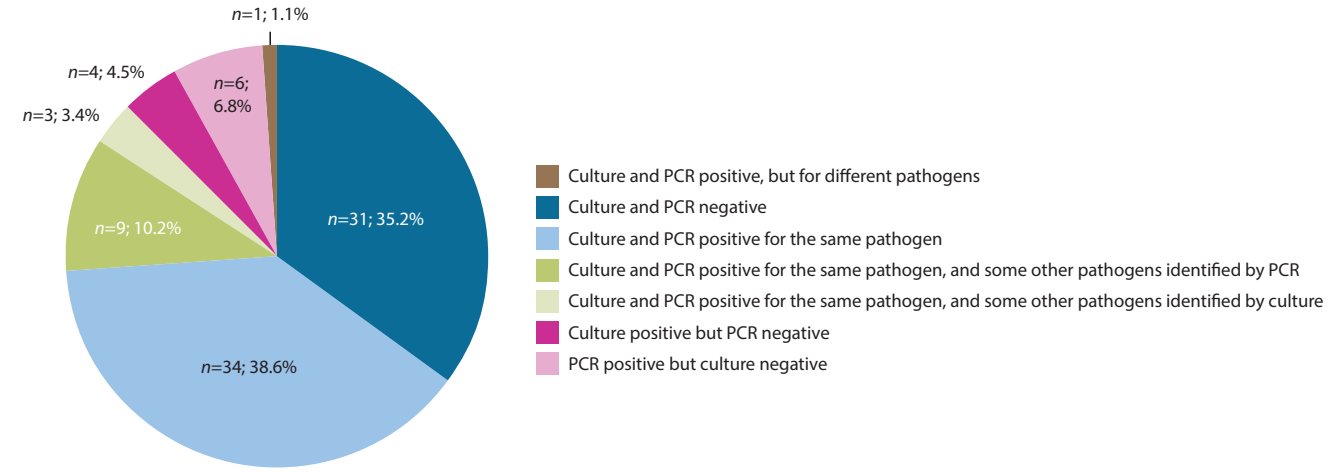

Fig. 2. Comparison of respiratory culture and PCR findings in patients with severe/critical COVID-19. (PCR = polymerase chain reaction.)

Table 2. Time to colonisation of respiratory specimens with various bacterial pathogens in patients with severe/critical COVID-19 (*N*=55)

Micro-organism	Patients, *n**	Hospital stay (days), median (IQR)	ICU stay (days), median (IQR)
Gram negative			
Non-fermenting bacteria	43	10 (7 - 13)	6 (4 - 8.75)
Acinetobacter baumannii, carbapenem resistant	34	10 (8 - 14)	7 (5 - 9)
Enterobacterales	30	9 (7 - 11.25)	4.5 (2.75 - 7)
Klebsiella pneumoniae, carbapenem resistant	15	10 (7 - 17)	7 (6 - 12)
Enterobacterales, carbapenem susceptible	20	7 (5 - 10)	3 (1 - 5)
K. pneumoniae, no acquired antibiotic resistance	16	8 (5 - 10)	3 (1 - 4)
Gram positive			
All Gram-positive bacteria	12	7 (5 - 10)	3 (1 - 5)
MSSA	11	7 (5 - 10)	3 (2 - 5)

IQR = interquartile range; ICU = intensive care unit; MSSA = methicillin-susceptible *Staphylococcus aureus.*
*Culture was performed in all patients and was negative in 27. Some patients were colonised with more than one pathogen.

Table 3. Spectrum of lower respiratory tract infection pathogens in patients with severe/critical COVID-19 (*N*=82)

Pathogen	Patients, *n**
Acinetobacter baumannii, carbapenem resistant	30
Klebsiella pneumoniae, carbapenem resistant	15
MSSA	6
Stenotrophomonas maltophilia	5
K. pneumoniae, no acquired antibiotic resistance	4
Pseudomonas aeruginosa	2
K. pneumoniae, third-generation cephalosporin resistant	1
Causative agent not identified	7

MSSA = methicillin-susceptible *Staphylococcus aureus.*
*Some patients were infected with more than one pathogen.

Table 4. Frequency of prescribing antibiotics to patients with severe/critical COVID-19 during different periods of hospitalisation (*N*=82)

	Patients, *n*
Before admission to ICU, new antibiotic prescribed to 28/75 patients	
Ampicillin + sulbactam	9
Cefepime	7
Cefepime + sulbactam	5
Meropenem	4
Moxifloxacin	3
Cefoperazone + sulbactam	2
Imipenem + cilastatin	1
Levofloxacin	1
Day 0 - 2 in ICU, new antibiotic prescribed to 35/82 patients	
Cefepime + sulbactam	10
Meropenem	9
Ampicillin + sulbactam	5
Levofloxacin	5
Cefoperazone + sulbactam	2
Cefotaxime + sulbactam	2
Fosfomycin	2
Cefepime	1
Imipenem + cilastatin	1
Moxifloxacin	1
Day 3 - 6 in ICU, new antibiotic prescribed to *n*=53/73 patients	
Meropenem	17
Imipenem + cilastatin	9
Cefepime + sulbactam	7
Polymyxin B	7
Tigecycline	7
Cefoperazone + sulbactam	5
Levofloxacin	2
Moxifloxacin	2
Fosfomycin	2
Ampicillin + sulbactam	1
Cefazolin	1
Amikacin	1
Linezolid	1
After day 7 in ICU, new antibiotic prescribed to *n*=33/50 patients	
Tigecycline	18
Polymyxin B	11
Meropenem	11
Co-trimoxazole	4
Imipenem + cilastatin	3
Fosfomycin	3
Linezolid	2
Ampicillin + sulbactam	1
Cefazolin	1
Cefepime + sulbactam	1
Amikacin	1

ICU = intensive care unit.

most common bacterial superinfections in COVID-19 patients.[3] The prevalence of multidrug-resistant bacterial pathogens in critically ill COVID-19 patients varies from 32% to 50%, with *A. baumannii* and carbapenem-resistant *K. pneumoniae* being particularly concerning.[18] In our study, *K. pneumoniae*, *A. baumannii* and *S. aureus* were detected most frequently. Colonisation was noted to occur relatively early, after a median of 4 days in the ICU. Importantly, the resistance profiles of organisms identified early v. those identified later were

significantly different. During the first days of ICU admission, *K. pneumoniae* without acquired antibiotic resistance and MSSA prevailed. As the ICU stay progressed, *A. baumannii* and carbapenem-resistant *K. pneumoniae* became the predominant isolates. These same micro-organisms were the most common causative agents of documented respiratory superinfections, affecting more than half of the patients.

In general, the resistance profile of the pathogens identified in the present study did not differ from the pattern observed prior to the pandemic. This trend is confirmed by the results of other Russian and international studies.[19-21] For instance, in a study by Maes *et al*.,[19] no significant differences in bacterial pathogens were found between patients with ventilator-associated pneumonia with and without COVID-19. However, the COVID-19 group had a significantly higher risk of developing ventilator-associated pneumonia, as well as isolated cases of invasive aspergillosis.

In our study, we employed both culture and PCR to identify potentially significant bacterial pathogens. Cohen *et al*.[22] used culture as the gold standard, and PCR showed high negative predictive values (99.6%) and moderate positive predictive values (~60%). Similar results were reported by Paz *et al*.[23] Pickens *et al*.[24] performed PCR on BAL specimens from intubated COVID-19 patients to determine whether antibiotics were necessary, and showed that they could be avoided in 75% of cases. Our study demonstrated generally good concordance between the results obtained by the two methods. Additionally, PCR appeared to detect the DNA of micro-organisms that often turned out to be clinically significant infectious agents somewhat earlier. Owing to the rapidity with which results may be obtained, PCR may offer a promising alternative to culture for identifying nosocomial pathogens. Furthermore, the additional detection of key resistance genes, especially the presence and type of carbapenemases among Enterobacterales, can enable earlier initiation of adequate antibiotic therapy in the presence of clinical signs of infection. Meanwhile, despite the advantages of PCR, it should be recognised that micro-organisms detected by this method may be colonisers and do not necessarily represent the true causative agents of LRTI.

Another important concern in patients with severe COVID-19 is the timing of administration of systemic antibiotics. On the one hand, as mentioned earlier, diagnosing nosocomial infections presents certain objective challenges. On the other hand, early antibiotic prescription for patients without clinical and laboratory signs of bacterial infection not only increases the risk of adverse drug reactions but can also foster colonisation by multidrug-resistant micro-organisms, which was found in our study. Consequently, at least one-third of COVID-19 patients received antibiotic therapy either before transfer to the ICU or during the initial days of their ICU stay, when the likelihood of superinfection was low. Although patterns of antibiotic use were beyond of the scope of the study, it is noteworthy that a substantial proportion of drugs fell within the Watch and Reserve groups according to the World Health Organization AWaRe (Access, Watch, Reserve) classification. This finding is consistent with the results of another Russian study, where the proportion of Watch group antibiotics administered to COVID-19 ICU patients reached as high as 70.4% and non-compliance with local guidelines reached 27%.[25]

Our study has some limitations. Firstly, ~9% of all observation points were missed, potentially resulting in failure to identify micro-organisms in some patients. Secondly, PCR was performed in only about half of the samples, reducing the value of comparing it with microbial culture. Thirdly, this single-centre study had a relatively small sample size, making it challenging to extrapolate the findings to the general population.

Conclusion

To the best of our knowledge, this is one of a few prospective studies that encompass clinical, microbiological and PCR monitoring of patients with severe and critical COVID-19. The study confirms the high prevalence of bacterial colonisation in the respiratory tract, which appears quite early during the ICU stay, along with superinfections predominantly caused by multidrug-resistant Gram-negative bacterial pathogens. PCR testing can be considered as a swift and reliable tool to rule out colonisation and facilitate the early detection of potential bacterial superinfection.

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