Secondary Infection in Severe and Critical COVID-19 Patients in China: A Multicenter Retrospective Study

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Research

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Abstract

Background. Since 2020 COVID-19 pandemic became an emergent public sanitary incident. The epidemiology data and the impact on prognosis of secondary infection in severe and critical COVID-19 patients in China remained largely unclear.

Methods. We retrospectively reviewed medical records of all adult patients with laboratory-confirmed COVID-19 who were admitted to ICUs from January 18th 2020 to April 26th 2020 at two hospitals in Wuhan, China and one hospital in Guangzhou, China. We measured the frequency of bacteria and fungi cultured from respiratory tract, blood and other body fluid specimens. The risk factors for and impact of secondary infection on clinical outcomes were also assessed.

Results. Secondary infections were very common (86.6%) when patients were admitted to ICU for >72 hours. The majority of infections were respiratory, with the most common organisms being *Klebsiella pneumoniae* (24.5%), *Acinetobacter baumannii* (21.8%), *Stenotrophomonas maltophilia* (9.9%), *Candida albicans* (6.8%), and *Pseudomonas spp.* (4.8%). Furthermore, the proportions of multidrug resistant (MDR) bacteria and carbapenem resistant Enterobacteriaceae (CRE) were high. We also found that age ≥60 years and mechanical ventilation ≥13days independently increased the likelihood of secondary infection. Finally, patients with positive cultures had reduced ventilator free days in 28 days and patients with CRE and/or MDR bacteria positivity showed lower 28 day survival rate.

Conclusions. In a retrospective cohort of severe and critical COVID-19 patients admitted to ICUs in China, the prevalence of secondary infection was high, especially with CRE and MDR bacteria, resulting in poor clinical outcomes.

Introduction

Since December 2019, the coronavirus disease 2019 (COVID-19) pandemic has resulted in 45968799 laboratory-confirmed cases and 1192911 death cases worldwide up to November 1st 2020[1]. According to existing data, approximately 14–26% of hospitalized COVID-19 patients have needed treatment in an intensive care unit (ICU)[2, 3], and the clinical outcomes of these cases has generally been poor, with the mortality rate reaching 61.5% within 28 days[4].

Secondary infection, including those caused by bacteria and fungi, may occur during the course of respiratory viral infection. The incidence of secondary infections during the 2009 influenza A (H1N1) pandemic was as high as 23%[5], and was found to cause poor clinical outcomes in critically ill patients[6]. Over the past 20 years, although coronaviruses including severe acute respiratory syndrome coronavirus (SARS-CoV), Middle East respiratory syndrome coronavirus (MERS-CoV), and SARS-CoV-2, all of which mainly attack the lungs, have caused several outbreaks worldwide, the epidemiology of secondary infection during coronavirus epidemics still remains unknown. Only one single center retrospective study demonstrated that the presence of SARS-CoV in the ICU led to changes in the pattern of pathogens detected and increased the acquisition rate of multi-drug resistant *Staphylococcus aureus* (MRSA)[7]. The long course of the disease and the immuno-suppressed state of severe and critical COVID-19 patients place them at high risk of secondary infection[8]. Descriptive data found that 64.4% of hospitalized COVID-19 patients were prescribed antibiotics[9] and this proportion in critically ill patients was as high as 97.5%[10]. Unfortunately, until now the epidemiology of secondary infections in severe and critical COVID-19 patients admitted to the ICU remained largely unclear. Therefore, we performed a retrospective study to evaluate the incidence, risk factors and prognosis of secondary infection in severe and critical COVID-19 patients.

Methods

Patients

We retrospectively reviewed medical records of all adult patients (> 18 years) with laboratory-confirmed COVID-19 who were admitted to the ICU between January 18th 2020 and April 26th 2020 at two hospitals in Wuhan, China (Jinyintan Hospital and Union West Hospital) and one hospital in Guangzhou, China (The First Affiliated Hospital of Guangzhou Medical University). All three hospitals were designated hospitals for COVID-19 patients. All patients met the criteria of having severe or critical COVID-19 according to the Chinese guidelines [11]. The Ethics Committees of all hospitals approved the study protocol. Informed consent was waived due to the retrospective nature of the study.

Data extraction

The clinical data, including patient demographics, comorbidities, laboratory findings, treatment, pathogen culture results, and clinical outcomes were extracted from the electronic records by two independent intensivists in each hospital (Ling Sang and Bin Song in Jinyintan Hospital; Yin Xi and Ying Pan in Union West Hospital; Zhimin Lin and Chang-an Li in The First Affiliated Hospital of Guangzhou Medical University) who subsequently cross-checked for data accuracy. All data were entered into the computerized database for further statistical analyses.

Study definitions

The management of each patient in the ICU was decided upon by the attending physicians, and was mainly in accordance with the Chinese guidelines[11]. The decision to conduct microbiological cultivation was also based on the judgment of the attending physician. Specimens

included sputum for non-intubated patients and endotracheal aspirates or bronchoscopically obtained samples for intubated or tracheotomy patients, blood, urine, pleural effusion and any other samples acquired from suspected sites of infection. Secondary infection was defined as at least one or more positive culture result among a patient's specimens after ICU admission of more than 72 hours duration.

Study outcomes

The primary outcome measure was the frequency of bacterial and fungal organisms cultured from respiratory tract, blood and other body fluid specimens. We also assessed the risk factors for secondary infection, and the impact of this on clinical outcomes of COVID-19 patients.

Statistical analysis

Descriptive statistics included frequency analysis (percentages) for categorical variables and means (SD) for normally distributed continuous variables, or medians (interquartile range [IQR]) for skewed data. To test for differences, we used two-sample *t* tests for continuous variables and the χ^2 test for discrete variables. The Kaplan-Meier method was used to depict the probability of survival and to generate survival curves. Variables identified as significant ($p < 0.2$) were entered into a multivariate logistic regression model to investigate the relationship between explanatory variables and the occurrence of secondary infection. All analyses were carried out using SPSS version 10 (IBM Corp, Armonk, NY). A two-sided P value of 0.05 was considered to be significant.

Results

During the study period, a total of 190 patients were enrolled. Of the study cohort, 62 patients (32.6%) were female, and the mean age was 62.7 (SD 13.3) years. The majority of patients had underlying comorbidities (140/190, 73.7%). Eleven patients (5.8%) were classified as "severe", while 179 patients (94.2%) were classified as "critical". However, all of them needed respiratory support upon admission to the ICU. Table 1 describes the patient characteristics in detail.

Table 1
Baseline patient characteristics

Patient Characteristics	No.	Data
Age, mean (SD), years	190	62.68(13.3)
Gender, female	190	62(32.6)
Any comorbidity, yes	190	140(73.7)
Major comorbidities		
Hypertension	190	95(50.0)
Diabetes	190	40(21.1)
Cardiopathy	190	30(15.8)
Malignancy	190	12(6.32)
Liver cirrhosis	190	3(1.58)
Cerebrovascular	190	23(12.1)
Tuberculosis	190	1(0.5)
Chronic respiratory disease	190	15(7.9)
Chronic kidney diseases	190	1(0.5)
Others	190	29(15.3)
Eligibility criteria for COVID-19		
Severe	190	11(5.8)
Critical	190	179(94.2)
Respiratory support on ICU admission		
[#]COT	190	2(1.1)
[$]HFNC	190	65(34.2)
[&]NIV	190	52(27.4)
[*]IMV	190	71(37.4)

Data are presented as No. (%) unless otherwise noted. SD, standard deviation; COVID-19, coronavirus disease 2019; ICU, intensive care unit; [#]COT, conventional oxygen therapy; [$]HFNC, high flow nasal cannular; [&]NIV, noninvasive ventilation; [*]IMV, invasive mechanical ventilation.

Among the 190 patients, 1929 specimens were collected and 1104 positive cultures (57.2%) were obtained. Of these positive cultures, 935 (935/1104, 84.7%) were from the lower respiratory tract, 131 (131/1104, 11.9%) were from blood, and 38 (38/1104, 3.4%) were from other body fluid specimens. The distribution of cultures in the three hospitals is shown in Table 2.

Table 2
Distribution of cultures in three hospitals

	Total	Jinyintan hospital	Union west hospital	The 1st affiliated hospital of Guangzhou Medical University
	(n = 190)	(n = 97)	(n = 74)	(n = 19)
Inspection frequency	1929	1072	440	417
Positive cultures	1104(57.2%)	525(49%)	335(76.1%)	244(58.5%)
From lower respiratory tract	935(84.7%)	414(78.9%)	289(86.3%)	232(95.1%)
From blood	131(11.9%)	90(17.1%)	40(11.9%)	1(0.4%)
#From other body fluid specimens	38(3.4%)	21(4%)	6(1.8%)	11(4.5%)

#Other body fluid specimens included urine, pleural effusion and any other samples acquired from suspected sites of infection.

Of the study cohort, 165 patients (86.6%) had positive cultures (including bacteria and fungi) after ICU admission of more than 72 hours duration. Of these, 150 patients showed positive sputum/lower respiratory tract aspirate cultures, followed by 64 patients showing positive blood cultures, and 24 patients with positive cultures from other sites (Table 3).

Table 3
Characteristics of positive bacterial cultures

Characteristics of Positive Cultures	No.	Data
Any positive culture after ICU Admission > 72 hours, yes	190	165(86.8)
Number of positive sputum/lower respiratory tract aspirates cultures per patient, median (IQR)	190	3.0(1.0–6.0)
Frequency of positive sputum/lower respiratory tract aspirates culture among all positive cultures	150	
1 Positive culture		30(20.0)
2 Positive cultures		19(12.7)
≥ 3 Positive cultures		101(67.3)
Number of positive blood cultures per patient, median (IQR)	190	0(0–1.0)
Frequency of positive blood culture among all positive cultures	64	
1 Positive culture		32(50.0)
2 Positive cultures		20(31.2)
≥ 3 Positive cultures		12(18.8)
[#]Number of other positive cultures per patient, median (IQR)	190	0(0–0)
Frequency of other positive culture among all positive cultures	24	
1 Positive culture		15(62.5)
2 Positive cultures		5(20.9)
≥ 3 Positive cultures		4 (16.7)

Data are presented as No. (%) unless otherwise noted. [#]Other positive cultures were obtained from urine, pleural effusion and any other samples acquired from suspected sites of infection. ICU, intensive care unit; IQR, interquartile range.

Of the 1104 positive cultures obtained from 165 patients, the most common organisms overall were *Klebsiella pneumoniae* (24.5%), *Acinetobacter baumannii* (21.8%), *Stenotrophomonas maltophilia* (9.9%), *Candida albicans* (6.8%), and *Pseudomonas spp.* (4.8%). It was noteworthy that the proportions of multidrug resistant bacteria and carbapenem resistant Enterobacteriaceae (CRE) were very high (94.5% in *K. pneumoniae*, 98.3% in *A. baumannii* and 92.5% in *Pseudomonas spp.*). The positive cultures were mainly obtained from sputum and/or lower respiratory tract aspirates. Conversely, only four patients showed six instances of MRSA positivity, all of which were obtained from sputum and/or lower respiratory tract aspirates. More details are shown in Table 4. Of the total positive cultures, 525 were from Jinyintan Hospital (47.6%), with the most common organisms being *K. pneumoniae* (29.3%), *A. baumannii* (22.7%), *C. albicans* (10.5%), *Pseudomonas spp.* (5.9%) and *S. maltophilia* (3.6%). A total of 335 were from Union West Hospital, with the most common organisms being (36.1%), *K. pneumoniae* (32.5%), *S. maltophilia* (10.5%), *Pseudomonas spp.* (6.6%) and *Escherichia coli* (3%). The remaining 244 positive samples were from The First Affiliated Hospital of Guangzhou Medical University, and the most common organisms were *S. maltophilia* (22.5%), *Burkholderia cepacia* (12.7%), *Ralstonia pickettii* (4.9%), *K. pneumoniae* (3.3%) and *C. albicans* (3.3%) (Fig. 1).

Table 4
Frequency of organisms isolated according to date of acquisition

Pathogens Isolated	Total positive (No = 1104)	Patients (No = 165)	Frequency of organisms per patients
Gram negative bacteria			
Acinetobacter baumannii	241(21.8)	78(47.3)	3.09
MDR-Acinetobacter baumannii	237(98.3)	76(97.4)	3.12
Sputum/lower respiratory tract aspirates	225(94.9)	75(98.7)	3.00
Blood	9(3.8)	9(11.8)	1.00
Other places	2(0.8)	2(2.6)	1.00
nonMDR-Acinetobacter baumannii	4(1.7)	3(3.8)	1.33
Sputum/lower respiratory tract aspirates	2(50.0)	2(66.7)	1.00
Blood	2(50.0)	1(33.3)	2.00
Other places	0(0)	0(0)	-
Pseudomonas species	53(4.8)	17(10.3)	3.31
MDR -Pseudomonas species	49(92.5)	16(94.1)	3.06
Sputum/lower respiratory tract aspirates	49(100)	16(100.0)	3.06
Blood	0(0)	0(0)	-
Other places	0(0)	0(0)	-
non MDR -Pseudomonas species	4(7.5)	2(11.8)	2.00
Sputum/lower respiratory tract aspirates	4(100)	2(100.0)	2.00
Blood	0(0)	0(0)	-
Other places	0(0)	0(0)	-
Klebsiella pneumoniae	271(24.5)	77(46.7)	3.52
CRE-Klebsiella pneumoniae	256(94.5)	74(96.1)	3.46
Sputum/lower respiratory tract aspirates	221(86.3)	73(98.6)	3.03
Blood	34(13.3)	22(29.7)	1.55
Other places	1(0.4)	1(1.4)	1.00
nonCRE-Klebsiella pneumoniae	15(5.5)	4(5.2)	3.75
Sputum/lower respiratory tract aspirates	9(60.0)	2(50.0)	4.50
Blood	3(20.0)	1(25.0)	3.00
Other places	3(20.0)	1(25.0)	3.00
Escherichia coli	19(1.7)	10(6.1)	1.90
CRE-Escherichia coli	3(15.8)	2(20.0)	1.50
Sputum/lower respiratory tract aspirates	2(66.7)	1(50.0)	2.00
Blood	0(0)	0(0)	-
Other places	1(33.3)	1(50.0)	1.00
nonCRE-Escherichia coli	16(84.2)	8(80.0)	2.00

Data are presented as No. (%). MRSA, methicillin-resistant *Staphylococcus aureus*; CRE, carbapenem resistant Enterobacteriaceae; MDR, multidrug resistant. Other places included urine, pleural effusion and any other samples acquired from suspected sites of infection.

Pathogens Isolated	Total positive (No = 1104)	Patients (No = 165)	Frequency of organisms per patients
Sputum/lower respiratory tract aspirates	15(93.8)	7(87.5)	2.14
Blood	0(0)	0(0)	-
Other places	1(6.2)	1(12.5)	1.00
Stenotrophomonas maltophilia	109(9.9)	33(20.0)	3.30
Sputum/lower respiratory tract aspirates	104(95..4)	32(97.0)	3.25
Blood	2(1.8)	2(6.1)	1.00
Other places	3(2.8)	3(9.1)	1.00
Burkholderia cepacia	38(3.4)	8(4.8)	4.75
Sputum/lower respiratory tract aspirates	38(100.0)	8(100.0)	4.75
Blood	0(0)	0(0)	-
Other places	0(0)	0(0)	-
Elizabethan enterotoxicosis of meninges	1(0.1)	1(0.6)	1.00
Sputum/lower respiratory tract aspirates	1(100.0)	1(100.0)	1.00
Blood	0(0)	0(0)	-
Other places	0(0)	0(0)	-
Ralstonia pickettii	12	3(1.8)	4
Sputum/lower respiratory tract aspirates	12(100.0)	3(100.0)	4
Blood	0(0)	0(0)	-
Other places	0(0)	0(0)	-
Corynebacterium striatum	7(0.6)	6(3.6)	1.17
Sputum/lower respiratory tract aspirates	4(57.1)	4(66.7)	1.00
Blood	3(42.9)	2(33.3)	1.50
Other places	0(0)	0(0)	-
Gram positive bacteria			
MRSA	6(0.5)	4(2.4)	1.50
Sputum/lower respiratory tract aspirates	6(100)	4(100.0)	1.50
Blood	0	0(0)	-
Other places	0	0(0)	-
Coagulase-negative staphylococci	43(3.9)	31(18.8)	1.39
Sputum/lower respiratory tract aspirates	9(20.9)	6(19.4)	1.50
Blood	33(76.7)	26(83.9)	1.27
Other places	1(2.3)	1(3.2)	1.00
Enterococcus faecium	20(1.8)	15(9.1)	1.33
Sputum/lower respiratory tract aspirates	3(15.0)	2(13.3)	1.50
Blood	9(45.0)	8(53.3)	1.13
Other places	8(40.0)	7(46.7)	1.14
Enterococcus faecalis	3(0.3)	3(1.8)	1.00
Sputum/lower respiratory tract aspirates	1(33.3)	1(33.3)	1.00

Data are presented as No. (%). MRSA, methicillin-resistant *Staphylococcus aureus*; CRE, carbapenem resistant Enterobacteriaceae; MDR, multidrug resistant. Other places included urine, pleural effusion and any other samples acquired from suspected sites of infection.

Pathogens Isolated	Total positive (No = 1104)	Patients (No = 165)	Frequency of organisms per patients
Blood	1(33.3)□	1(33.3)	1.00
Other places	1(33.3)□	1(33.3)	1.00
Enterococcus casseliflavus	7(0.6)	6(3.6)	1.17
Sputum/lower respiratory tract aspirates	0(0)	0(0)	-
Blood	7(100.0)	6(100.0)	1.17
Other places	0(0)	0(0)	-
Fungus			
Candida albicans	75(6.8)	60(36.4)	1.25
Sputum/lower respiratory tract aspirates	69(92.0)	59(98.3)	1.17
Blood	5(6.7)	1(1.7)	5.00
Other places	1(1.3)	1(1.7)	1.00
Non-candida albicans	52(4.7)	38(23.0)	1.37
Sputum/lower respiratory tract aspirates	40(76.9)	32(84.2)	1.25
Blood	7(13.5)	5(13.2)	1.40
Other places	5(9.6)	4(10.5)	1.25
Aspergillus	7(0.6)	4(2.4)	1.25
Sputum/lower respiratory tract aspirates	7(100.0)	4(100.0)	1.75
Blood	0(0)	0(0)	-
Other places	0(0)	0	-

Data are presented as No. (%). MRSA, methicillin-resistant *Staphylococcus aureus*; CRE, carbapenem resistant Enterobacteriaceae; MDR, multidrug resistant. Other places included urine, pleural effusion and any other samples acquired from suspected sites of infection.

On comparison, patients with positive cultures were older (63.9 ± 12.9 vs 54.6 ± 14.1 years, $p = 0.001$), had a higher proportion of cardiopathy (18.2% vs 0, $p = 0.04$), and had a longer duration of mechanical ventilation (15.0 [IQR, 7.5–27.0] vs 4.0 [IQR, 2.5–5.5], $p = 0.03$) compared with those without secondary infections. Other baseline characteristics were similar. It seemed that more patients with positive cultures received glucocorticoid exceeding 1 mg/kg/day for more than 3 days (73.4% vs 54.5%, $p = 0.07$) and carbapenem for more than 3 days (49.7% vs 33.3%, $p = 0.16$) in the week preceding ICU admission. Additionally, more patients with positive cultures received invasive mechanical ventilation upon ICU admission (40% vs 20%, $p = 0.05$) compared with those patients with negative cultures. However, these difference were not statistically significant (Table 5). Finally, the variables identified as significant ($p < 0.2$) were entered into a multivariate logistic regression model to investigate the relationship between explanatory variables and the occurrence of secondary infection, and the result showed that age ≥ 60 years (odds ratio [OR] 4.885; 95% confidence interval [CI], 1.813–13.158) and mechanical ventilation ≥ 13 days (OR 24.759; 95% CI, 3.044–201.383) independently increased the likelihood of secondary infection in severe and critical COVID-19 patients (Table 6).

Table 5
Characteristics, treatments, and outcomes of patients with positive cultures compared with those without

Patient Variable	[a]Overall (N = 190)	No Positive Culture. (n = 25)	Positive Culture(s). (n = 165)	*p* Value
Age, mean (SD), y	**62.7(13.3)**	**54.6(14.1)**	**63.9(12.9)**	**0.001**
Gender, Female, No. (%)	62(32.6)	7(28.0)	55(33.3)	0.59
Any comorbidity, yes, No. (%)	140(73.7)	17(68.0)	123(74.6)	0.49
Total No. major comorbidities, mean (SD)	1.36(1.3)	1.04(1.0)	1.4(1.2)	0.13
Hypertension, No. (%)	95(50.0)	10(40.0)	85(51.5)	0.51
Diabetes, No. (%)	40(21.1)	4(16.0)	36(21.8)	0.13
Cardiopathy, No. (%)	**30(15.8)**	**0(0.0)**	**30(18.2)**	**0.04**
Malignancy, No. (%)	12(6.3)	1(4.0)	11(6.7)	0.94
Liver cirrhosis, No. (%)	3(1.6)	1(4.0)	2(1.2)	0.86
Cerebrovascular, No. (%)	23(12.1)	2(8.0)	21(12.7)	0.73
Tuberculosis, No. (%)	1(0.5)	0(0.0)	1(0.6)	0.27
Chronic respiratory disease, No. (%)	15(7.9)	1(4.0)	14(8.5)	0.71
Chronic kidney diseases , No. (%)	1(0.5)	1(4.0)	0(0.0)	0.27
Others, No. (%)	29(15.3)	5(20.0)	24(14.6)	0.68
Lymphocyte, mean (SD),10^9	0.73(0.98)	0.60(0.41)	0.76(1.04)	0.45
Neutrophil / Lymphocyte, mean (SD)	23.16(20.82)	23.15(15.28)	23.16(21.58)	0.99
Creatinine, mean (SD),umol/L	83.71(71.97)	96.78(117.97)	80.70(62.55)	0.30
ALT, mean (SD),U/L	64.65(148.51)	56.98(55.78)	64.90(156.42)	0.80
AST, mean (SD),U/L	62.47(170.85)	51.68(40.91)	64.12(182.75)	0.74
Duration of mechanical ventilation(IQR)	**13.0(6.0-26.3) (n = 154)**	**4.0(2.5–5.5) (n = 15)**	**15.0(7.5–27.0) (n = 139)**	**0.003**
Severe COVID-19 ,No. (%)	11(5.8)	3(12.0)	8(4.8)	0.84
Critical COVID-19 ,No. (%)	179(94.2)	22(88.0)	157(95.2)	0.33
Respiratory support on ICU admission				
[#]COT	2(1.1)	1(4.0)	1(0.6)	0.62
[$]HFNC	65(34.2)	11(44.0)	54(32.7)	0.27
[&]NIV	52(27.4)	8(32.0)	44(26.7)	0.58
[*]IMV	**71(37.4)**	**5(20.0)**	**66(40.0)**	**0.05**
Glucocorticoid use exceeds 1 mg/kg/day for more than 3 days before ICU admission within 1 week, yes ,No. (%)	**117(70.9) (n = 165)**	**12(54.5) (n = 22)**	**105(73.4) (n = 143)**	**0.07**
Antibiotic use > 3 days before ICU admission within 1 week,yes ,No. (%)	151(93.2) (n = 162)	9(81.8) (n = 11)	132(87.4) (n = 151)	0.95
Carbapenem ,No. (%)	**117(47.5) (n = 162)**	**7(33.3) (n = 21)**	**70(49.7) (n = 141)**	**0.16**

Data are presented as No. (%) unless otherwise noted. [a]Total sample size is 190. Where data are unavailable, sample size for data is provided. SD, standard deviation; IQR, interquartile range; COVID-19, coronavirus disease 2019; ICU, intensive care unit; [#]COT, conventional oxygen therapy; [$]HFNC, high flow nasal cannular; [&]NIV, noninvasive ventilation; [*]IMV: invasive mechanical ventilation.

Patient Variable	[a]Overall (N = 190)	No Positive Culture. (n = 25)	Positive Culture(s). (n = 165)	*p* Value
Beta-lactams,No. (%)	92(56.8) (n = 162)	10(47.6) (n = 21)	82(58.2) (n = 141)	0.36
Carbostyril No. (%)	102(63.0) (n = 162)	13(61.9) (n = 21)	89(63.1) (n = 141)	0.91
Aminoglycoside,No. (%)	2(1.2) (n = 162)	0(0) (n = 21)	2(1.4) (n = 141)	0.61
Glycopeptide,No. (%)	38(23.5) (n = 162)	5(26.3) (n = 21)	33(23.4) (n = 141)	0.81
Antifungal, No. (%)	24(14.8) (n = 162)	2(9.5) (n = 21)	22(15.6) (n = 141)	0.68

Data are presented as No. (%) unless otherwise noted. [a]Total sample size is 190. Where data are unavailable, sample size for data is provided. SD, standard deviation; IQR, interquartile range; COVID-19, coronavirus disease 2019; ICU, intensive care unit; [#]COT, conventional oxygen therapy; [$]HFNC, high flow nasal cannular; [&]NIV, noninvasive ventilation; [*]IMV: invasive mechanical ventilation.

Table 6
Independent high risk factors for secondary infection in severe and critical COVID-19 patients upon admission to ICU

	B	Wald	*p*	OR	95%CI
age ≥ 60 years	**1.436**	**9.841**	**0.002**	**4.885**	**1.813–13.158**
Glucocorticoid use exceeds 1 mg/kg/day for more than 3 days before ICU admission within 1 week	0.808	2.155	0.142	2..244	0.763-6.600
IMV	0.351	0.345	0.557	1.421	0.440–4.592
Carbapenem use > 3 days before ICU admission within 1 week	0.054	0.009	0.926	1.056	0.339–3.286
Comorbidity: cadiopathy	0.568	1.223	0.269	1.764	0.645–4.822
Mechanical ventilation ≥ 13days	**3.209**	**9.005**	**0.003**	**24.759**	**3.044-201.383**

OR, odds ratio; 95% CI, 95% confident interval

Patients with positive cultures had fewer ventilator free days in 28 days compared with those with negative cultures (2.2 ± 5.4 vs 6.1 ± 10.8 days, $p = 0.02$), while the mortality at 28 days ICU admission was similar (Table 7, Fig. 2). However, when the patients were divided into CRE and/or MDR bacteria-positive and non-CRE and/or MDR bacteria-positive groups, the patients with CRE and/or MDR bacteria positivity showed lower 28 days survival ($p = 0.02$; Fig. 3).

Table 7
Impact of secondary infection on clinical outcomes

Patient Variable	[a]Overall (n = 190)	No Positive Culture. (n = 25)	Positive Cultures. (n = 165)	*p* Value
Ventilator-free days in 28 days, mean (SD)	2.6(6.2) (n = 154)	6.1(10.8) (n = 15)	2.2(5.4) (n = 139)	0.02
28 days ICU mortality	95(51.4) (n = 185)	12(50.0) (n = 24)	83(51.6) (n = 161)	0.89

Data are presented as No. (%) unless otherwise noted. [a]Total sample size is 190. Where data are unavailable, sample size for data is provided. SD, standard deviation; ICU, intensive care unit.

Discussion

To date, this represents the first study to evaluate the epidemiology of secondary infections in severe and critical COVID-19 patients. We found that secondary infection was very common (86.6%) in this patient population when they were admitted to the ICU for more than 72 hours, and the majority were respiratory infections. The most common organisms isolated overall were *K. pneumoniae* (24.5%), *A. baumannii* (21.8%), *S. maltophilia* (9.9%), *C. albicans* (6.8%), and *Pseudomonas spp.* (4.8%). Furthermore, the proportions of MDR bacteria and CRE were surprisingly high (94.5% in *K. pneumoniae*, 98.3% in *A. baumannii* and 92.5% in *Pseudomonas spp.*). Interestingly, the distribution of organisms isolated was quite different between Wuhan and Guangzhou; the main organisms isolated in Wuhan were *K. pneumoniae* and *A. baumannii*, while *S. maltophilia* and *B. cepacia* were more common in Guangzhou. We also found that age ≥ 60 years and mechanical ventilation ≥ 13 days (OR 24.759; 95% CI, 3.044–201.383) independently increased the likelihood of secondary infection in severe and critical COVID-19 patients. Finally, patients with positive cultures had reduced ventilator free days in 28 days and those with CRE and/or MDR bacteria positivity showed a lower 28 day survival rate.

Secondary infection had always been a common concern in the field of critical care medicine worldwide. In 1995, the results of the EPIC study, which evaluated the prevalence of nosocomial infection in ICUs in Europe, demonstrated that 20.6% of ICU patients acquired nosocomial infection and this positively correlated with the mortality rate[12]. Even now, in the latest EPIC III study, the incidence of ICU acquired infection, which was with a substantial risk of in-hospital mortality, was 22%[13]. In another study that focused on secondary infection in 2009 influenza A (H1N1), positive cultures were obtained in 38% of critically ill patients who were admitted to the ICU[6]. However, the incidence of secondary infection in our study was surprisingly high (86.6%). We thought the main reasons were as follows. First, although a complete nosocomial infection prevention and control system was set up in China according to the guidelines[14, 15], medical staff wore extensive personal protective equipment, and the heavy workload contributed to the incomplete implementation of these measures, especially during the early stage of the pandemic in Wuhan. It is worth noting that only one patient acquired bloodstream infection in The First Affiliated Hospital of Guangzhou Medical University; this was significantly less than the other two hospitals in Wuhan, which might be due to insufficient medical resources in Wuhan. This phenomenon was consistent with the previous opinion that practices to prevent health care-acquired infections were generally absent in low-resource settings[16, 17]. Second, SARS-CoV-2 induces severe lung damage, damages the respiratory tract epithelium, and causes apoptosis of lung macrophages and neutrophils, thus weakening barrier function[18], ultimately increasing susceptibility to secondary infection in the lung.

Similar to the EPIC III study[13], the majority of the organisms isolated in our study were gram negative bacteria, particularly *K. pneumoniae* and *A. baumannii*. Additionally, we found that the proportions of MDR bacteria and CRE were surprisingly high. CRE and MDR *A. baumannii* emerged as a major worldwide human health threat, as CRE infections are associated with high mortality and morbidity[19–21]. In China, CRE and MDR *A. baumannii* represent a rapidly-emerging threat because the isolation rate has recently increased year by year[22], and this microbe reduces patients' clinical outcomes[23]. Interestingly, our study found that these pathogens mainly occurred in Wuhan, and the proportions were much lower in Guangzhou. We considered the possibility that many ICUs were improvised in response to a sudden outbreak of the pandemic in Wuhan with a serious shortage of experienced ICU medical staff, and this was likely to be a major contributing factor to the spread of drug-resistant bacteria.

Our study demonstrated that advanced age and long duration of mechanical ventilation were independent high risk factors for secondary infection in severe and critical COVID-19 patients, consistent with previous studies and guidelines[13, 14]. However, we failed to demonstrate that

glucocorticoid and antibiotic usage were independently associated with the occurrence of secondary infections. The main reasons behind this may be that the limited sample size did not have sufficient power to evaluate the true effect and the proportions of glucocorticoid and antibiotic usage in patients both with and without positive cultures were high, which made it more difficult to assess the real effects.

In the EPIC III study, no specific organism was independently associated with a higher risk of death when considering all patients with secondary infections. Elderly age, higher simplified acute physiology score II, and comorbidities of metastatic cancer, HIV infection, and heart failure were independently associated with a higher risk of death. However, this variation was associated with patient- and disease-specific factors, with the process of care and intercountry differences. When considering only antibiotic-resistant organisms, infections with *Klebsiella spp.* resistant to β-lactam antibiotics (including third-generation cephalosporins and carbapenems), and carbapenem-resistant *Acinetobacter spp.* were independently associated with an increased risk of death, highlighting the association of antibiotic resistance with mortality and the importance of good antibiotic stewardship[13]. The results of our study also demonstrated that secondary infections decreased ventilator-free days at 28 days, and CRE and MDR bacterial infection decreased the 28 day ICU survival rate, consistent with previous studies[24–27].

Conclusions

In a retrospective cohort of severe and critical COVID-19 patients admitted to ICUs in China, the prevalence of secondary infection was high, particularly CRE and MDR bacteria, resulting in poor clinical outcomes.

Abbreviations

Covid-19=coronavirus disease 2019

CRE =carbapenem resistant Enterobacteriaceae

ICU =intensive care unit

MERS-CoV = Middle East respiratory syndrome coronavirus

MRSA =multi-drug resistant Staphylococcus aureus

MDR= multi-drug resistant

SARS= Severe Acute Respiratory Syndrome

SARS-Cov2=severe acute respiratory syndrome coronavirus 2

Declarations

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Availability of data and materials

The datasets analysed during the current study were extracted from the electronic records by Jinyintan Hospital, Union West Hospital and The First Affiliated Hospital of Guangzhou Medical University.

Authors' contributions

Conception and design of the study: Yimin Li, Dingyu Zhang, Nuofu Zhang, Yuanda Xu; Data acquisition plan creation and raw data collection and accuracy conformation: Ling Sang ,Bin Song ,Yin Xi, Ying Pan, Zhimin Lin, Changan Li; Joined and assisted the data collection: Ming Zhong, Li Jiang, Chun Pan, Wei Zhang, Zheng Lv, Nanshan Chen, Dongdong Liu, Weibo Liang; Analysis or interpretation of the data: Xia Zheng, Jiaan Xia, Wenjuan Wu, Xuesong Liu; Statistical analysis: Changan Li, Yonghao Xu, Sibei Chen; Drafting of the manuscript: Ling Sang, Yin Xi, Zhimin Lin, Ying Pan, Bin Song, Changan Li; Critical revision of the manuscript for important intellectual content: Nanshan Zhong, Xiaoqing Liu, Shiyue Li, Dan Ye; Supervision: Yimin Li. All authors read and approved the final version of the manuscript.

Ethics approval and consent to participate

This study was approved separately by the Independence Ethics Committee of Union Hospital, Tongji Medical College, Huazhong University of Science and Technology(Approval No：2020-0307)；Medical Ethics Committee of Wuhan Infectious Disease Hospital(Approval No: KY-2020-56.01) and Ethics Committee of the First Affiliated Hospital of Guangzhou Medical University(2020-065). Informed consent was waived due to the retrospective nature of the study.

Consent for publication

Not applicable.

Declaration of interest

The authors report no conflict of interest. None of the authors has a financial relationship with a commercial entity that has an interest in the subject of this manuscript.

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Figures

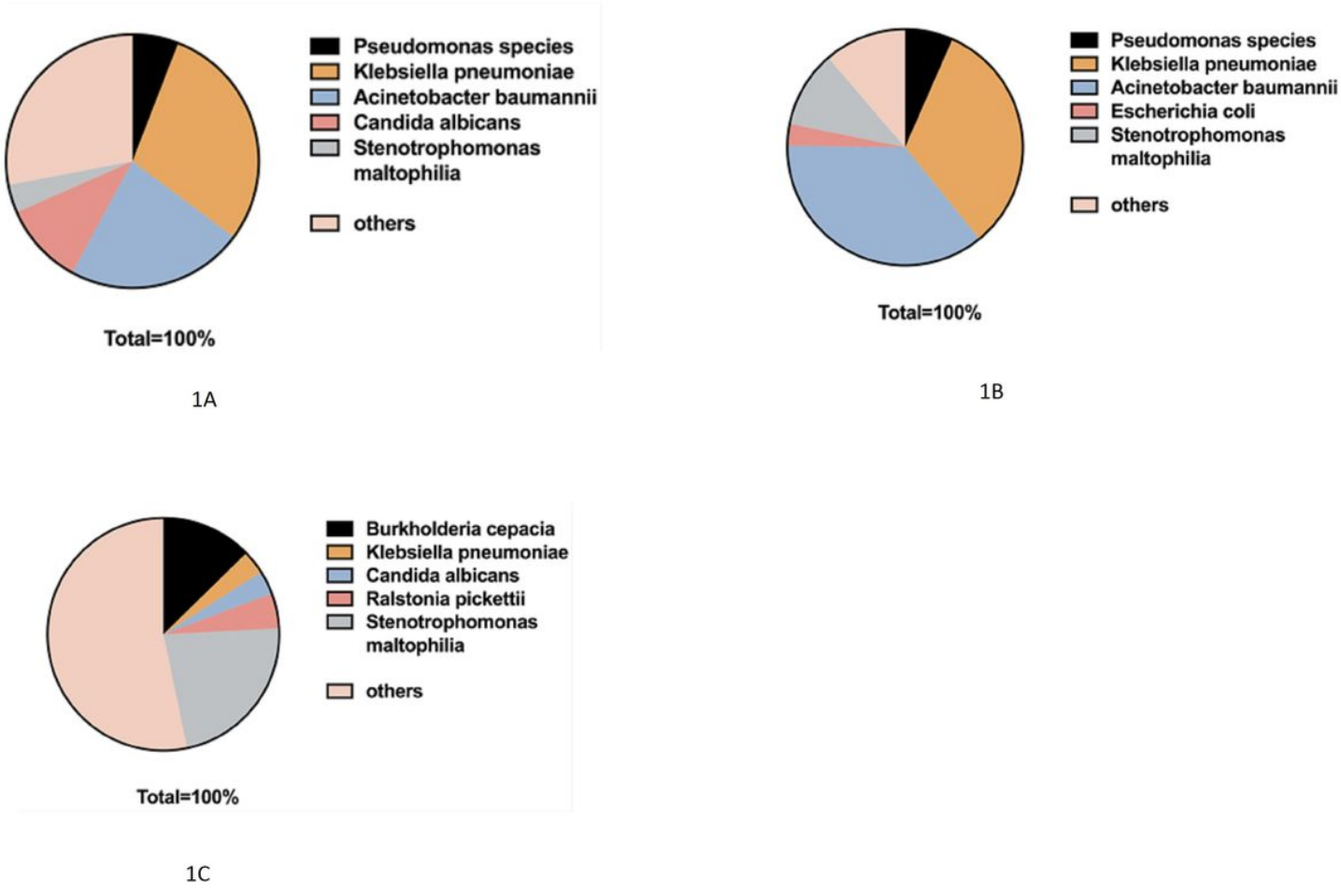

Figure 1

1A. Distribution of organisms isolated in Jinyintan Hospital. 1B. Distribution of organisms isolated in Union West Hospital. 1C. Distribution of organisms isolated in The First Affiliated Hospital of Guangzhou Medical University.

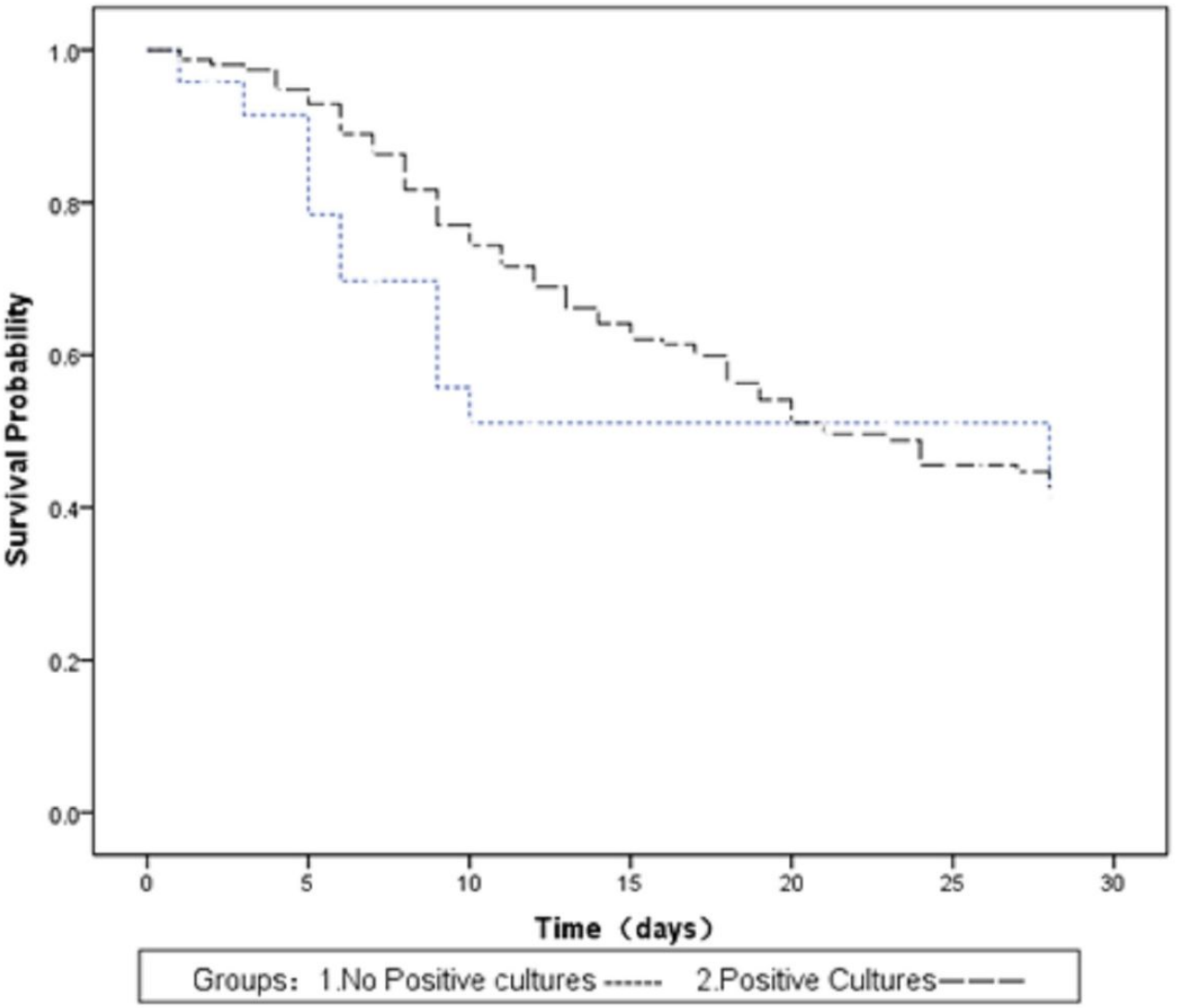

Figure 2

Twenty-eight day ICU Kaplan-Meier survival curve comparing patients without positive cultures to those with positive cultures. p=0.46

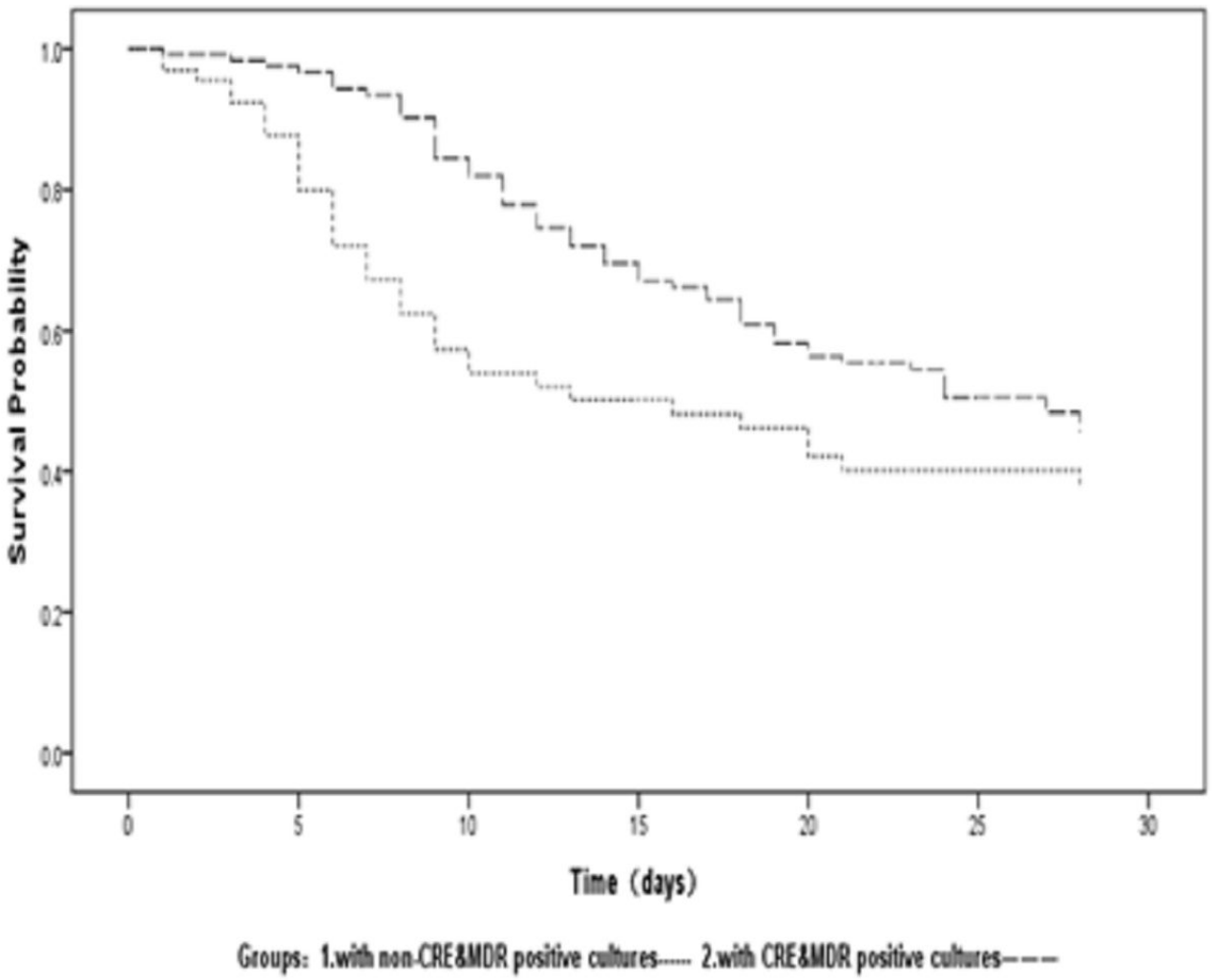

Figure 3

Twenty-eight day ICU Kaplan-Meier survival curve comparing patients with carbapenem resistant Enterobacteriaceae (CRE) and multidrug resistant (MDR) positive cultures with those with non-CRE and MDR positive cultures. p=0.02