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ORIGINAL ARTICLE

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Carbapenemase-producing *Enterobacterales* infections in COVID-19 patients

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ABSTRACT

Background: Carbapenemase-producing *Enterobacterales* (CPE) infections have been occasionally described in patients with coronavirus disease-19 (COVID-19). We assess the clinical features and outcome of these infections.

Methods: In this retrospective single-centre, case-control study, we included 54 patients with CPE infection: 30 case-patients (COVID-19) and 24 controls (non-COVID-19), collected between March and May 2020. We compared the epidemiological, clinical features, and outcome between cases and controls.

Results: CPE infection was more frequent in COVID-19 patients than in controls (1.1 *vs.* 0.5%, $p = .005$). COVID-19 patients were younger, had a lower frequency of underlying diseases ($p = .01$), and a lower median Charlson score ($p = .002$). Predisposing factors such as antimicrobial use, mechanical ventilation, or ICU admission, were more frequent in COVID-19 patients ($p < .05$). There were 73 episodes of infection (42 cases and 31 controls) that were more frequently hospital-acquired and diagnosed at the ICU in COVID-19 patients ($p < .001$). Urinary tract was the most common source of infection (47.9%), followed by pneumonia (23.3%). The frequency of severe sepsis or shock ($p = .01$) as well as the median SOFA score ($p = .04$) was higher in cases than in controls. *Klebsiella pneumoniae* (80.8%), *Serratia marcescens* (11%) and *Enterobacter cloacae* (4.1%) were the most common bacteria in both groups (KPC 56.2%, OXA-48 26% and VIM 17.8%). Overall 30-d mortality rate of COVID-19 patients and controls was 30 and 16.7%, respectively ($p = .25$).

Conclusions: COVID-19 patients have an increased risk of CPE infections, which usually present as severe, nosocomial infections, appearing in critically-ill patients and associated with a high mortality.

KEYWORDS
COVID-19
SARS-CoV-2
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Supplemental data for this article can be accessed here.

Introduction

Co-infections have been reported in patients with severe acute respiratory syndrome (SARS) and Middle East respiratory syndrome, but there is limited knowledge on co-infection among patients with SARS-coronavirus 2 (SARS-CoV-2) infection. The reported prevalence of bacterial or fungal infections among patients with coronavirus disease 2019 (COVID-19) has been variable and ranges between 3.5 and 15.5% [1–4].

A potential consequence of the COVID-19 pandemic is the propagation of antimicrobial resistance in the acute care setting, resulting from increasing use of antimicrobials. The impact of the pandemic on the prevalence of multidrug-resistant bacteria (MDR) is still unclear at present, but recent reports have described a high use of broad-spectrum antibiotics, a recognized risk factor for MDR bacteria [5,6]. In addition, due to the severity of COVID-19 pneumonia, other risk factors for antimicrobial resistance [7,8], such as intensive care unit (ICU) admission, mechanical ventilation,, and other invasive devices are frequently reported in patients with COVID-19.

Carbapenem-resistant *Enterobacterales* have spread to all regions of the world and infections due to these MDR bacteria are associated with an unfavourable outcome [9]. In Spain, the number of reports on carbapenemase-producing *Enterobacterales* (CPE) has increased in recent years, and many hospitals have reported outbreaks of CPE. The impact of CPE in Spain is primarily due to OXA-48-producing and VIM-1-producing *Klebsiella pneumoniae* isolates, although other species such as *Escherichia coli* and *Enterobacter cloacae* are also increasing [10,11]. An endemic situation of both, CPE infection and colonization, has been established in our hospital, mainly related with the isolation of OXA-48, as well as KPC and VIM-producing *Enterobacterales* [12,13]. To our knowledge, although secondary infections due to MDR *Enterobacterales* have been occasionally reported among COVID-19 patients [14–19], no large series on infections due to CPE have been published.

In this study, we investigated the clinical features of 30 patients with CPE infection associated with COVID-19, and 24 SARS-CoV-2 negative patients with CPE infection, to describe the characteristics and outcome of CPE infections associated with COVID-19.

Methods

Study design and setting

This retrospective case-control study was performed at the Hospital Ramón y Cajal, a 1100-bed general teaching hospital, serving a population of 525,000 inhabitants in Madrid, Spain. The study was approved by the Ethics Committee of our institution. Informed consent was waived because no intervention was involved and no identifying patient information was included.

Study population

Adult patients (≥16 years) presenting to the hospital between 1 March and 31 May 2020 in whom a CPE infection was diagnosed were analysed. Case-patients (COVID-19) were defined as individuals who had positive SARS-CoV-2 PCR test, and controls (non-COVID-19) were defined as individuals admitted to the hospital without clinical or microbiological evidence of SARS-CoV-2 infection (for whom PCR were negative). Laboratory confirmation of SARS-CoV-2 infection was done by qualitative real-time RT-PCR assay of nasopharyngeal swabs, sputum or lower respiratory tract aspirates. Patients were prospectively identified from microbiology laboratory records. All cases were reviewed by a senior Infectious Diseases specialist to determine the presence of true clinical infection and source. Patients were classified as having a CPE infection if they had a positive culture from a significant clinical sample and clinical signs of infection. Patients were excluded from the analysis if the positive culture was not associated with clinical signs of infection. Patients with incomplete data were also excluded from the analysis.

Data collection and definitions

We collected the following data from the electronic medical records: demographic characteristics, medical comorbidities, predisposing factors to CPE infection, source of infection, as well as information on response to treatment and outcome. We only included in the study cases with confirmed infection, defined by the presence of a positive culture of a significant clinical sample, associated with clinical signs of infection and/or worsening organ failure. Conventional microbiological testing (tracheal aspirate, blood and urine cultures) was requested by the treating physician when infection was suspected, and was not protocolized. All infections were defined according to the Centres for Disease Control and Prevention criteria [20]. Early-onset and late-onset ventilator-associated pneumonia (VAP) were defined as pneumonia diagnosed before and after 5 d of mechanical ventilation, respectively [21]. The definitions of hospital-acquired, healthcare-associated or community-acquired infection are described

elsewhere [22]: hospital acquisition was considered when symptoms of infection started >48 h after hospital admission or within 48 h of hospital discharge. Healthcare acquisition was considered if patients had attended haemodialysis or received intravenous chemotherapy in the past 30 d, had been admitted to an acute-care hospital for at least 2 d or had surgery in the past 90 d, or resided in a nursing home or long-term care facility. Other infections were considered community-acquired. Other variables collected were hospital ward of acquisition, Charlson comorbidity index score [23] and sequential organ failure assessment (SOFA) score [24], severity of systemic inflammatory response [25], *Enterobacterales* species, carbapenemase type and antimicrobial susceptibility testing.

Bacterial identification was performed by MALDI-TOF MS (Bruker-Daltonics, Bremen, Germany). Antibiotic susceptibility testing was assessed by standard microdilution (MicroScan, Beckman Coulter, Brea, CA) and results were interpreted according to EUCAST-2020 criteria (http://www.eucast.org). Carbapenemase production was screened using the included the ROSCO KPC/Metallo-beta-lactamase (MBL) and OXA-48 Confirm Kit (ROSCO Diagnostica, Taastrup, Denmark). The presence of carbapenemase genes was detected initially by the Eazyplex®-Superbug-CRE system (Amplex-Biosystems GmbH, Giessen, Germany) and later confirmed by PCR and sequencing [26].

Therapy administered before and after the susceptibility data became available was considered empirical or targeted, respectively. Empirical antimicrobial therapy was considered to be appropriate if the initial antibiotics included at least one active drug. Drugs were considered active when the isolate was susceptible according to EUCAST-2020 criteria. We defined combination therapy as a regimen including more than one *in vitro* active antimicrobial during at least half of the treatment duration, and monotherapy as including one active drug.

The main outcome variable was 30-d all-cause mortality, measured from the day of diagnosis of CPE infection. In patients with more than one episode of CPE infection, we evaluated the final outcome of the patient at the end of the follow-up period (30 June 2020). For the assessment of the risk of mortality of the CPE infection, we used the INCREMENT-CPE score (ICS) [27].

Statistical analysis

Study variables from the two groups were analysed for differences between COVID-19 and non-COVID-19 subjects using Mann–Whitney–Wilcoxon test or Yates' corrected chi-square test. All tests were two-tailed, and $p < .05$ was considered to be statistically significant.

Results

Demographic and epidemiological data

A total of 7048 patients were admitted to our institution during the study period and 2615 (37.1%) of them were diagnosed with SARS-CoV-2 infection. CPE were isolated in 30 patients with SARS-CoV-2 infection and in 24 patients without SARS-CoV-2 infection. CPE infection was therefore diagnosed in 1.1% (30/2615) of COVID-19 patients and in 0.5% (24/4433) of controls ($p = .005$). We excluded four case-patients whose CPE isolates were considered as a colonization (two respiratory and two urinary), three potential episodes of infection in two case-patients (with another CPE confirmed infection) whose urine isolates were considered as a colonization, and three controls (urinary isolates) with unavailable clinical records. Of the 54 patients included in the analysis, 30 were case-patients and 24 were controls.

The main demographic and epidemiological data of both groups are shown in Table 1. The median age was 65 years (range, 35–95 years, interquartile range [IQR], 57–74) and the majority were male (66.7%). Case-patients were more likely to be younger compared with controls ($p = .011$). The most common comorbidities were hypertension (27, 50%), malignancy (17, 31.5%), cardiovascular disease (15, 27.8%) and diabetes (14, 25.9%). Comorbidities were significantly less frequent ($p = .011$) in case-patients, due a lower frequency of malignancy ($p = .009$) and other medical conditions ($p = .034$). The median score on the Charlson comorbidity index was two points (IQR, 0–5), and was significantly lower in cases than in controls ($p = .002$).

The clinical features and therapy of SARS-CoV-2 infection of the 30 case-patients are described in Supplementary Table S1. All of them had SARS-CoV-2 pneumonia, that was complicated with ARDS in 23 patients (76.7%) and with shock in 3 (10%), requiring ICU admission (25, 83.3%) and mechanical ventilation (25, 83.3%) in most of them. COVID-19 was community-acquired in 27 cases (90%) and hospital-acquired in 3 (Supplementary Table S2, patients 13, 20 and 28). The diagnosis of COVID-19 was prior to the CPE infection in 93.3% (28/30) of the patients. COVID-19 and CPE infection were simultaneously diagnosed in two patients (Table S2, cases 23 and 24). The median time from

Table 1. Baseline characteristics of COVID-19 and non-COVID-19 patients.

Characteristic	All ($N = 54$)	Cases ($N = 30$)	Controls ($N = 24$)	p Value
Age, median (IQR), years	65 (57–74)	62.5 (56–71)	71 (63–76)	.011
Male sex	36 (66.7)	23 (76.7)	13 (54.2)	.081
Charlson comorbidity score, median (IQR)	2 (0–5)	1 (0–3)	4 (2–6)	.002
Comorbidities				
Any	47 (87.0)	23 (76.7)	24 (100)	.011
Hypertension	27 (50.0)	15 (50.0)	12 (50.0)	1.00
Malignancy	17 (31.5)	5 (16.7)	12 (50.0)	.009
Cardiovascular disease	15 (27.8)	7 (23.3)	8 (33.3)	.415
Diabetes	14 (25.9)	6 (20.0)	8 (33.3)	.267
Immunodeficiency state	10 (18.5)	4 (13.3)	6 (25.0)	.273
Obstructive pulmonary disease	7 (13.0)	3 (10.0)	4 (16.7)	.469
Chronic renal disease	7 (13.0)	4 (13.3)	3 (12.5)	.928
Cerebrovascular disease	6 (11.1)	2 (6.7)	4 (16.7)	.245
Chronic liver disease	6 (11.1)	2 (6.7)	4 (16.7)	.245
Other diseases	11 (20.4)	3 (10.0)	8 (33.3)	.034
Predisposing factors to CPE infection[a]				
Antimicrobials	48 (88.9)	30 (100)	18 (75.0)	.004
Urinary catheter	43 (77.8)	27 (90.0)	16 (66.7)	.034
Central venous catheter	39 (72.2)	27 (90.0)	12 (50.0)	.001
Mechanical ventilation	31 (57.4)	25 (83.3)	6 (25.0)	<.001
Parenteral nutrition	10 (18.5)	7 (23.3)	3 (12.5)	.309
Surgery (general anaesthesia)	6 (11.1)	0 (0.0)	6 (25.0)	.004
Renal replacement therapy	1 (1.9)	1 (3.3)	0 (0.0)	.367
Intensive care unit admission	32 (59.3)	25 (83.3)	7 (29.2)	<.001
Previous admission (<3 months)	19 (35.2)	5 (16.7)	14 (58.3)	.001
Transfer from another hospital	13 (24.1)	8 (26.7)	5 (20.8)	.618

Data are presented as *n* (%), unless otherwise indicated.
COVID-19: coronavirus disease 2019; CPE: carbapenemase-producing *Enterobacterales*; IQR: interquartile range.
[a]Risk factors present during ≥48 h in the previous 30 d to the first episode of CPE infection.

COVID-19 diagnosis to onset of CPE infection was 27 d (IQR, 13–41).

CPE infections

The main characteristics of CPE infections are described in Table 2. There were 42 episodes of infection among the 30 case-patients (six patients had two episodes and three had three), while 31 episodes were diagnosed among the 24 controls (three patients had two episodes and two patients had three). Supplementary Tables S2 and S3 show the source of infection, CPE isolate, antimicrobial therapy and outcome of cases and controls, respectively.

Most of the risk factors for nosocomial infection were significantly more frequent in cases than in controls, such as ICU admission ($p < .001$), mechanical ventilation ($p < .001$), central venous catheter ($p = .001$), previous antimicrobial use ($p = .004$) and urinary catheter ($p = .034$) (Table 1). Antimicrobials were more frequently prescribed in case-patients, due to a significantly higher use of azithromycin ($p < .001$), ceftriaxone ($p < .001$) and carbapenems ($p = .036$) (Supplementary Table S4).

Regarding the acquisition of infection, CPE infections were more frequently hospital-acquired in case-patients ($p < .001$), while healthcare-related and community-onset infections were more frequent in controls (Table 2). Similarly, patients with COVID-19 were more frequently admitted to the ICU at the time of diagnosis of CPE infection ($p < .001$), while infections among controls were diagnosed with higher frequency in the emergency department (ED) and medical/surgical wards. The median time from admission to onset of hospital-acquired infections was 21 d (IQR, 12–34).

Urinary tract infection (UTI) was the most common source of infection (35 episodes, 47.9%), followed by pneumonia (17, 23.3%), tracheobronchitis (6, 8.2%), skin and soft tissue (5, 6.8%) and intra-abdominal infection (4, 5.5%), with no significant differences between cases and controls ($p = .714$). Bacteraemia was documented in 35.1% (20/57) of the CPE infections (15 cases of bacteraemia secondary to urinary tract infection, pneumonia or intra-abdominal infection, and five cases of primary or catheter-associated bloodstream infection).

K. pneumoniae (59, 80.8%), *Serratia marcescens* (8, 11%), and *Enterobacter cloacae* (3, 4.1%) were the most common bacteria in both groups (Table 2). The most common type of carbapenemase was KPC (41, 56.2%), followed by OXA-48 (19, 26.0%) and VIM metallo-beta-lactamase (13, 17.8%). There were no significant differences either in the distribution of bacterial species or in the type of carbapenemase between cases and controls. Most isolates were resistant to piperacillin-tazobactam (100%), cefepime (93.2%), ceftazidime (93.2%), ertapenem (97.3%) and imipenem (75.3%), while the

Table 2. Clinical features of CPE infections of COVID-19 and non-COVID-19 patients.

Characteristic	All (*N* = 73)	Cases (*N* = 42)	Controls (*N* = 31)	*p* Value
Acquisition of infection				
Nosocomial	55 (75.3)	39 (92.9)	16 (51.6)	<.001
Healthcare	16 (21.9)	3 (7.1)	13 (41.9)	
Community	2 (2.7)	0 (0.0)	2 (6.5)	
Time from admission[a], median (IQR), days	21 (12–34)	26 (14–33)	12 (7–41)	.116
Ward of hospitalization				
Intensive care unit	37 (50.7)	30 (71.4)	7 (22.6)	<.001
Emergency department	17 (23.3)	4 (9.5)	13 (41.9)	
Medical ward	15 (20.5)	8 (19.0)	7 (22.6)	
Surgical ward	4 (5.5)	0 (0.0)	4 (12.9)	
Type of infection				
Urinary tract infection	35 (47.9)	18 (42.9)	17 (54.8)	.714
Pneumonia	17 (23.3)	12 (28.6)	5 (16.1)	
Tracheobronchitis	6 (8.2)	4 (9.5)	2 (6.5)	
Skin and soft tissue infection	5 (6.8)	3 (7.1)	2 (6.5)	
Intra-abdominal infection	4 (5.5)	1 (2.4)	3 (9.7)	
Primary bacteraemia	3 (4.1)	2 (4.8)	1 (3.2)	
Catheter-related bacteraemia	2 (2.7)	1 (2.4)	1 (3.2)	
Surgical site infection	1 (1.4)	1 (2.4)	0 (0.0)	
Polymicrobial infection[b]	16 (21.9)	8 (19.0)	8 (25.8)	.490
Bacteraemia	20/57 (35.1)	13/38 (34.2)	7/19 (36.8)	.844
Severity of infection				
Severe sepsis or septic shock	28 (38.4)	21 (50.0)	7 (22.6)	.017
No sepsis	45 (61.6)	21 (50.0)	24 (77.4)	
SOFA score, median (IQR)	3 (2–6)	4 (2–6)	2 (1–5)	.041
INCREMENT score				
High risk (0–7)	22 (30.1)	14 (33.3)	8 (25.8)	.488
Low risk (8–15)	51 (69.9)	28 (66.7)	23 (74.2)	
Enterobacterales				
Klebsiella pneumoniae	59 (80.8)	32 (76.2)	27 (87.2)	.188
Serratia marcescens	8 (11.0)	7 (16.6)	1 (3.2)	
Enterobacter cloacae	3 (4.1)	1 (2.4)	2 (6.4)	
Other species[c]	3 (4.1)	2 (4.8)	1 (3.2)	
Type of carbapenemase				
KPC	41 (56.2)	24 (57.1)	17 (54.8)	.145
OXA-48	19 (26.0)	8 (19.0)	11 (35.5)	
VIM	13 (17.8)	10 (23.8)	3 (9.7)	
Antimicrobial therapy				
Appropriate empirical therapy	24/59 (40.7)	13/29 (44.8)	11/30 (36.7)	.524
Combination targeted therapy	22 (30.1)	17 (40.5)	5 (16.1)	.025
Duration of therapy, median (IQR), days	11 (7–14)	11 (8–15)	9 (6–14)	.256

Data are presented as *n* (%), unless otherwise indicated.
COVID-19: coronavirus disease 2019; CPE: carbapenemase-producing *Enterobacterales*; IQR: interquartile range.
[a]Time from admission to the onset of the first CPE nosocomial infection.
[b]Polymicrobial infections were caused by carbapenem-susceptible *Enterobacterales* (7 cases), *Enterococcus* spp. (7), *P. aeruginosa* (5), *S. constellatus* (1), *Bacteroides* spp. (1), *Parvimonas micra* (1) and *C. albicans* (2).
[c]Cases: *K. oxytoca* (1), and *K. aerogenes* (1). Controls: *C. freundii* (1).

Table 3. Antimicrobial resistance of KPC-, OXA-48 and VIM-producing *Enterobacterales*.

Antimicrobial	All isolates (*N* = 73)	KPC (*N* = 41)	OXA-48 (*N* = 19)	VIM (*N* = 13)
Piperacillin-tazobactam	100% (73/73)	100% (41/41)	100% (19/19)	100% (13/13)
Ceftazidime-avibactam	0% (0/48)	0% (0/36)	0% (0/12)	—
Ceftazidime	93.2% (68/73)	100% (41/41)	73.7% (14/19)	100% (13/13)
Cefepime	93.2% (68/73)	100% (41/41)	73.7% (14/19)	100% (13/13)
Aztreonam	81.6% (40/49)	100% (30/30)	55.6% (5/9)	50% (5/10)
Ertapenem	97.3% (71/73)	100% (41/41)	100% (19/19)	84.6% (11/13)
Imipenem	75.3% (55/73)	100% (41/41)	21.1% (4/19)	76.9% (10/13)
Ciprofloxacin	87.7% (64/73)	97.6% (40/41)	94.7% (18/19)	46.2% (6/13)
Gentamicin	76.7% (56/73)	92.7% (38/41)	42.1% (8/19)	76.9% (10/13)
Amikacin	18.3% (11/60)	11.4% (4/35)	0% (0/14)	63.6% (7/11)
Cotrimoxazole	79.5% (58/73)	95.1% (39/41)	36.8% (7/19)	92.3% (12/13)
Colistin	27.4% (20/73)	24.4% (10/41)	10.5% (2/19)	61.5% (8/13)
Tigecycline	11.9% (7/59)	8.8% (3/34)	14.3% (2/14)	18.2% (2/11)
Fosfomycin	23.7% (9/38)	9.1% (2/22)	53.8% (7/13)	0% (0/3)

Data are presented as percentage (number of resistant isolates/number of tested isolates).

Table 4. Outcome and causes of mortality of COVID-19 and non-COVID-19 patients.

Outcome	All ($N = 54$)	Cases ($N = 30$)	Controls ($N = 24$)	p Value
Mortality after first CPE infection (30 d)	13 (24.1)	9 (30.0)	4 (16.7)	.255
Final outcome				
Alive	33 (61.1)	17 (56.7)	16 (66.7)	.437
Dead (CPE infection)	11 (20.4)	8 (26.7)	3 (12.5)	
Dead (other causes)[a]	10 (18.5)	5 (16.7)	5 (20.8)	
Mortality (in-hospital)	21 (38.9)	13 (43.3)	8 (33.3)	.454
Time from diagnosis of CPE infection to discharge, median (IQR), days	16 (5–38)	25 (11–47)	7 (0–25)	.007

Data are presented as *n* (%).
COVID-19: coronavirus disease 2019; CPE: carbapenemase-producing *Enterobacterales*.
[a]Death was due to COVID-19 in 4 of the 13 patients with SARS-CoV-2 infection.

resistance rate to ceftazidime-avibactam (CZA) (0%), tigecycline (11.9%) and amikacin (18.3%), was significantly lower (Table 3). Around a half of VIM-producing isolates were susceptible to aztreonam (50%) and ciprofloxacin (53.8%). No significant differences were found in resistance rates between cases and controls. CPE infection was polymicrobial in 21.9% (16/73) of the cases, with a similar proportion of cases and controls (Table 2).

The proportion of patients with high-risk ICS was similar in cases and controls (33.3 *vs.* 25.8%, $p = .488$) while the frequency of severe sepsis or septic shock was higher in cases that in controls (50 *vs.* 22.6%, $p = .017$). The median SOFA score was 3 (IQR, 2–6), and was significantly higher in cases than in controls ($p = .041$).

Therapy and outcome

Empirical antimicrobial treatment was given in 59 episodes of CPE infection and was considered appropriate in 24 (40.7%) of them. Combination targeted therapy was more frequently used in cases than in controls (40.5 *vs.* 16.1%, $p = .025$) (Table 2). Most episodes of CPE infection were treated with CZA, either as monotherapy (24, 32.8%) or combined with amikacin (10, 13.6%) or other antimicrobials (8, 10.9%) (Tables S2 and S3). Ciprofloxacin and/or aztreonam were occasionally used in susceptible VIM-producing *S. marcenscens* infections. Eight episodes of mild UTI (seven of them among controls), were diagnosed in the ED and treated on an outpatient basis.

The outcome and causes of mortality of patients are presented in Table 4. Overall, 30-d mortality rate of the first episode of CPE infections was 24.1%. Although the mortality was higher in COVID-19 patients than in controls, the difference did not reach statistical significance (30 *vs.* 16.7%, $p = .255$). A higher 30-d mortality was found in patients with high-risk ICS than in patients with low-risk ICS (57.1 *vs.* 12.5%, $p = .001$).

At the end of the follow-up period, the final outcome of the patients was as follows: 33 (61.1%) were alive, 11 (20.4%) were dead from CPE infection and 10 (18.5%) were dead from other causes, with no significant differences between cases and controls ($p = .437$). Among COVID-19 patients, CPE infection was the main cause of mortality in eight (61.5%) of the 13 patients who dead, while respiratory failure secondary to SARS-CoV-2 pneumonia was the final cause of death in four of them (30.7%). The duration of the hospital stay after the diagnosis of CPE infection was significantly longer in COVID patients than in controls (Table 4).

Discussion

To our knowledge, the study presents the largest series that describes CPE infections in COVID-19 patients, thus allowing characterize the main epidemiological, clinical and prognostic factors of these new infections. We have observed that although CPE infections are uncommon in COVID-19 patients, their incidence is significantly higher than in patients without COVID-19. The most outstanding finding of the study is that CPE infections associated to COVID-19 generally present as severe nosocomial infections, which appear in critically ill patients admitted to the ICU and are associated with a high mortality rate.

Bacterial infections have been described in COVID-19 patients but the reported incidence has been variable, depending on the definition criteria (co-infection, as infection at presentation or secondary infection, emerging during the course of illness), the subset of patients (ICU *vs.* non-ICU patients) or the methods used for diagnosis. In contrast with the microorganisms found in other viral respiratory infections (mainly *Staphylococcus aureus* and *Streptococcus pneumoniae*), the most frequently isolated bacteria in COVID-19 patients have been *Mycoplasma* spp, *Pseudomonas aeruginosa*, *Haemophilus influenzae* and *K. pneumoniae* [3].

COVID-19 pandemic has appeared at a time of great concern about antimicrobial resistance. Although it could be anticipated that a significant proportion of patients with severe COVID-19 would have infections

due to MDR bacteria [6,28], few studies have specifically assessed CPE infections in this population [14–19]. Nori et al. described 5 cases of NDM-producing *E. cloacae* infections that accounted for 3.2% of the cases of bacterial/fungal coinfections in a series of 4267 patients with COVID-19 [14]. A lower frequency of CPE infection (13/3152, 0.41%) has been described in a report from New York [16]. Other series from Italy and USA have also documented an increased risk of acquisition of CPE among COVID-19 patients, specially in the ICU setting, with the subsequent development of severe infections [15–19,29].

Although we found CPE infections in a only a minority of COVID-19 patients (1.1%), we have observed a higher incidence of CPE infections in COVID-19 patients than in controls. The increased exposure to multiple risk factors for nosocomial infection may explain this finding, despite the reinforcement of all infection control measures (patient isolation, universal personal protective equipment and cleaning regimens) introduced in our institution during the COVID-19 pandemic. Despite low rates of bacterial infections reported in COVID-19 patients, a high proportion of them (72–100%) received antibiotics during the course of disease, increasing the risk of antimicrobial resistance [1–4,28]. We have confirmed this finding, since broad-spectrum antimicrobials were more frequently prescribed in case-patients.

In addition, COVID-19 patients were more frequently exposed to other risk factors for MDR bacteria, such as mechanical ventilation, invasive devices or ICU admission [14–19,30], as an inevitable consequence of the critical care required by severe COVID-19 pneumonia. Other factors specifically related with the management of COVID-19 patients, such as increased patient transfer and cohorting on newly designed COVID-19 units, overwork due to the burden of disease (need for prone position in intubated patients), incomplete compliance for standard and contact precautions, lack of training of staff for the care of patients, or protective equipment limitations have been implicated in the increasing prevalence of MDR bacterial infections [14,18,31,32].

Main demographic characteristics are similar to those described in previous series of COVID-19 [33–35]. Compared to controls, case-patients were younger, had a lower frequency of underlying diseases, and a lower Charlson score. CPE infections in COVID-19 patients were a late complication of the disease (median time to onset, 26 d), were acquired almost exclusively in the hospital, and diagnosed in patients admitted to the ICU. These findings are a consequence of the specific care of COVID-19 patients, many of whom have severe pneumonia requiring mechanical ventilation [1–4]. In contrast, due to changes in the health system resources caused by the pandemic, many CPE infections in controls were diagnosed in the ED and managed in an outpatient basis. It is important to note that during the epidemic peak (April 2020), practically all hospital and ICU beds of the hospital were dedicated to the care of COVID-19 patients.

Microbiology and antimicrobial resistance patterns are likely to reflect institutional ecology. We have observed a higher prevalence of KPC-producing *K. pneumoniae* in COVID-19 patients and controls, as a manifestation of the current epidemiological situation of the hospital in 2020 [12,13]. Although unfortunately, a molecular investigation of the strains was not carried out, there was no epidemiological evidence of any outbreak of CPE during the study period. Other species of CPE, such as *E. coli* and *E. cloacae*, as well as different types of carbapenemase (NDM and OXA-48) have been described in series from Italy, Spain, France and USA [14–19]. Due to the variable prevalence of resistance among CPE isolates, therapy needs to be tailored according to the antimicrobial susceptibility test result. Different regimens of antimicrobials including CZA, carbapenems, aztreonam, tetracyclines, polymyxins, aminoglycosides, quinolones and fosfomycin have been used for therapy of these highly resistant isolates, with variable results [14–16]. CZA, amikacin, and tigecycline were the most active antimicrobials against KPC- and OXA-48-producing bacteria isolated in our patients, while aztreonam and ciprofloxacin were alternatives for some VIM-producing isolates [36]. We have found a high rate of resistance to colistin (27.4%) that can be explained in part by the significant proportion of *S. marcenscens* isolates (11%).

We have observed the usual distribution of CPE infections, with a predominance of UTI, followed by respiratory and intra-abdominal infections [8,36]. VAP and bacteraemia have been the most frequently reported CPE-associated infections among COVID-19 patients [14–19]. An increased incidence of bacterial pneumonia has been reported in these patients when more sensitive diagnostic methods are used. Nonfermenting gram-negative bacilli and *Enterobacterales* have been frequently isolated in patients with late-onset VAP [21]. In agreement with this finding, we found CPE as the cause of late-onset VAP and tracheobronchitis in 11 and 4 of the patients with COVID-19, respectively. Another outstanding finding of the cohort was the more severe clinical presentation of CPE infections in COVID-19

patients, with an increased frequency of severe sepsis or septic shock, and a higher SOFA score. This finding could be explained by the underlying severity of the clinical status of these patients, secondary to the presence of severe viral pneumonia and respiratory failure [33–35].

CPE infections are associated with a high mortality rate [37]. Regarding the prognostic impact of co-infections, a recent meta-analysis has shown that COVID-19 patients with a co-infection are more likely to die than patients without co-infection [2]. We have recently reported the increased risk of mortality associated with secondary infections in a series of 140 critically ill patients with COVID-19 and it is noteworthy that a high proportion of these infections (31%) were produced by MDR bacteria [38]. In previous reports, the mortality rate observed among COVID-19 patients with CPE infections was very high, ranging from 33.3 to 80% [14,16,17]. We have observed that CPE infections are associated with a high 30-d mortality rate in COVID-19 patients (30% during the first episode), although not significantly higher to that found in non-COVID-19 patients. The in-hospital mortality of COVID-19 patients was also very high (43.3%), and it is noteworthy that CPE infection was the main cause of mortality in around two thirds (61.5%) of them.

We should acknowledge some limitations to this study. The sample size was small and the retrospective design reduces control over multiple confounders and data collection. The clinical diagnosis of CPE infections was not established following a standardized protocol and therefore, some episodes may be missing. Finally, this study was limited to a single institution, with its own local epidemiology on antimicrobial resistance, which may limit the generalizability of the findings.

In conclusion, COVID-19 patients have an increased risk of infections due to highly resistant CPE, which usually appear in critically-ill patients. UTI and pneumonia were the most common infections and were associated with a severe clinical presentation and a high mortality. KPC-producing *K. pneumoniae* was the most frequently isolated bacteria, reflecting the current institutional ecology of the hospital. Given the progressive expansion of the COVID-19 pandemic, institutions and regions with a high prevalence of CPE should be prepared for a significant increase in the prevalence of these infections. Implementation of increased surveillance and antimicrobial stewardship programmes focussed on CPE infections should be an essential component of management strategies for COVID-19 patients.

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Ethics approval

The study was approved by the Ethics Committee of the Hospital Ramón y Cajal (Madrid, Spain). Informed consent was waived because no intervention was involved and no identifying patient information was included. The study complies with the principles of the 1964 Helsinki Declaration and its later amendments.

Disclosure statement

The authors declare that they have no conflict of interest.

Author contributions

All the authors participated sufficiently in the conception, analysis of the data, design and writing of the manuscript and take public responsibility for it. All the authors believe that the manuscript represents valid work and all have reviewed and approved the final version for publication.

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References

[1] Rawson TM, Moore LSP, Zhu N, et al. Bacterial and fungal co-infection in individuals with coronavirus: a rapid review to support COVID-19 antimicrobial prescribing. Clin Infect Dis. 2020;71:2459–2468.

[2] Lansbury L, Lim B, Baskaran V, et al. Co-infections in people with COVID-19: a systematic review and meta-analysis. J Infect. 2020;81(2):266–275.

[3] Langford BJ, So M, Raybhardan S, et al. Bacterial co-infection and secondary infection in patients with COVID-19: a living rapid review and meta-analysis. Clin Microbiol Infect. 2020;26:1622–1629.

[4] Clancy CJ, Nguyen MH. COVID-19, superinfections and antimicrobial development: what can we expect? Clin Infect Dis. 2020;71:2736–2743.

[5] Lai CC, Wang CY, Hsueh PR. Co-infections among patients with COVID-19: the need for combination therapy with non-anti-SARS-CoV-2 agents? J Microbiol Immunol Infect. 2020;53:505–512.
[6] Dona D, Chiara Sharland DC. M. Multidrug resistant infections in the COVID-19 era, a framework for considering the potential impact. J Hosp Infect. 2020;106:198–199.
[7] Safdar N, Maki DG. The commonality of risk factors for nosocomial colonization and infection with antimicrobial-resistant Staphylococcus aureus, enterococcus, gram-negative bacilli, Clostridium difficile, and candida. Ann Intern Med. 2002;136(11):834–844.
[8] Paño-Pardo JR, Serrano S, Ramos J, et al. Infections caused by carbapenemase-producing enterobacteriaceae: risk factors, clinical features and prognosis. Enferm Infecc Microbiol Clin. 2014;32(4):41–48.
[9] Martin A, Fahrbach K, Zhao Q, et al. Association between carbapenem resistance and mortality among adult, hospitalized patients with serious infections due to enterobacteriaceae: results of a systematic literature review and Meta-analysis. Open Forum Infect Dis. 2018;5:ofy150.
[10] Oteo J, Miró E, Pérez-Vázquez M, et al. Evolution of carbapenemase-producing enterobacteriaceae at the global and national level: what should be expected in the future? Enferm Infecc Microbiol Clin. 2014;32(4):17–23.
[11] Palacios-Baena ZR, Oteo J, Conejo C, et al. Comprehensive clinical and epidemiological assessment of colonisation and infection due to carbapenemase-producing enterobacteriaceae in Spain. J Infect. 2016;72(2):152–160.
[12] Tato M, Coque TM, Rucz-Garbajosa P, et al. Complex clonal and plasmid epidemiology in the first outbreak of enterobacteriaceae infection involving VIM-1 metallo-beta-lactamase in Spain: toward endemicity? Clin Infect Dis. 2007; 45(9):1171–1178.
[13] Ruiz-Garbajosa P, Curiao T, Tato M, et al. Multiclonal dispersal of KPC genes following the emergence of non-ST258 KPC-producing Klebsiella pneumoniae clones in Madrid, Spain. J Antimicrob Chemother. 2013;68(11):2487–2492.
[14] Nori P, Szymczak W, Puius Y, et al. Emerging co-pathogens: New Delhi metallo-beta-lactamase producing enterobacterales infections in New York city COVID-19 patients. Int J Antimicrob Agents. 2020;56(6):106179.
[15] Arcari G, Raponi G, Sacco F, et al. Klebsiella pneumoniae infections in COVID-19 patients: a 2-month retrospective analysis in an italian hospital. Int J Antimicrob Agents. 2021;57(1):106245.
[16] Gomez-Simmonds A, Annavajhala MK, McConville TH, et al. Carbapenemase-producing enterobacterales causing secondary infections during the COVID-19 crisis at a New York city hospital. J Antimicrob Chemother. 2020;76:380–384.
[17] Montrucchio G, Corcione S, Sales G, et al. Carbapenem-resistant Klebsiella pneumoniae in ICU-admitted COVID-19 patients: keep an eye on the ball. J Glob Antimicrob Resist. 2020;23:398–400.
[18] Farfour E, Lecuru M, Dortet L, et al. Carbapenemase-producing enterobacterales outbreak: another dark side of COVID-19. Am J Infect Control. 2020;48(12):1533–1536.
[19] García-Meniño I, Forcelledo L, Rosete Y, et al. Spread of OXA-48-producing Klebsiella pneumoniae among COVID-19-infected patients: the storm after the storm. J Infect Public Health. 2021;14(1):50–52.
[20] Horan TC, Andrus M, Dudeck MA. CDC/NHSN surveillance definition of health care-associated infection and criteria for specific types of infections in the acute care setting. Am J Infect Control. 2008;36(5):309–332.
[21] Dudoignon E, Caméléna F, Deniau B, et al. Bacterial pneumonia in COVID-19 critically ill patients: a case series. Clin Infect Dis. 2020;72:905–906.
[22] Russo A, Falcone M, Gutiérrez-Gutiérrez B, et al. Predictors of outcome in patients with severe sepsis or septic shock due to extended-spectrum β-lactamase-producing enterobacteriaceae. Int J Antimicrob Agents. 2018;52(5):577–585.
[23] Charlson ME, Pompei P, Ales KL, et al. A new method of classifying prognostic co-morbidity in longitudinal studies: development and validation. J Chronic Dis. 1987;40(5): 373–383.
[24] Vincent JL, Moreno R, Takala J, et al. The SOFA (sepsis-related organ failure assessment) score to describe organ dysfunction/failure. On behalf of the working group on sepsis-related problems of the European society of intensive care medicine. Intensive Care Med. 1996;22(7):707–710.
[25] Levy MM, Fink MP, Marshall JC, et al. 2001 SCCM/ESICM/ACCP/ATS/SIS international sepsis definition conference. Crit Care Med. 2003;31:1250–1256.
[26] Poirel L, Walsh TR, Cuvillier V, et al. Multiplex PCR for detection of acquired carbapenemase genes. Diagn Microbiol Infect Dis. 2011;70(1):119–123.
[27] Gutiérrez-Gutiérrez B, Salamanca E, De Cueto M, et al. A predictive model of mortality in patients with bloodstream infections due to carbapenemase-producing enterobacteriaceae. Mayo Clin Proc. 2016;91(10):1362–1371.
[28] Cantón R, Gijón D, Ruiz-Garbajosa P. Antimicrobial resistance in ICUs: an update in the light of the COVID-19 pandemic. Curr Opin Crit Care. 2020;26(5):433–441.
[29] Porretta AD, Baggiani A, Arzilli G, et al. Increased risk of acquisition of new Delhi metallo-beta-lactamase-producing carbapenem-resistant enterobacterales (NDM-CRE) among a cohort of COVID-19 patients in a teaching hospital in Tuscany, Italy. Pathogens. 2020;9:E365.
[30] Liu P, Li X, Luo M, et al. Risk factors for carbapenem-resistant Klebsiella pneumoniae infection: a meta-analysis. Microb Drug Resist. 2018;24(2):190–198.
[31] Tiri B, Sensi E, Marsiliani V, et al. Antimicrobial stewardship program, COVID-19, and infection control: spread of carbapenem-resistant Klebsiella pneumoniae colonization in ICU COVID-19 patients. What did not work? J Clin Med. 2020;9: 2744.
[32] Bork JT, Leekha S, Claeys K, et al. Change in hospital antibiotic use and acquisition of multidrug-resistant gram-negative organisms after the onset of coronavirus disease 2019. Infect Control Hosp Epidemiol. 2020. DOI:10.1017/ice.2020.1360
[33] Goyal P, Choi JJ, Pinheiro LC, et al. Clinical characteristics of COVID-19 in New York city. N Engl J Med. 2020;382(24): 2372–2374.

[34] Richardson S, Hirsch JS, Narasimhan M, et al. Presenting characteristics, comorbidities, and outcomes among 5700 patients hospitalized with COVID-19 in the New York city area. JAMA. 2020;323(20):2052–2059.

[35] Cummings MJ, Baldwin MR, Abrams D, et al. Epidemiology, clinical course, and outcomes of critically ill adults with COVID-19 in New York city: a prospective cohort study. Lancet. 2020;395(10239):1763–1770.

[36] Rodríguez-Baño J, Gutiérrez-Gutiérrez B, Machuca I, et al. Treatment of infections caused by extended-spectrum-beta-lactamase-, AmpC-, and carbapenemase-producing enterobacteriaceae. Clin Microbiol Rev. 2018;31(2): e00079–17.

[37] Agyeman AA, Bergen PJ, Rao GG, et al. A systematic review and meta-analysis of treatment outcomes following antibiotic therapy among patients with carbapenem-resistant Klebsiella pneumoniae infections. Int J Antimicrob Agents. 2020;55(1):105833.

[38] Bardi T, Pintado V, Gómez-Rojo M, et al. Nosocomial infections associated to COVID-19 in the intensive care unit: clinical characteristics and outcome. Eur J Clin Microbiol Infect Dis. 2021;40(3):495–502.