Brazilian Journal of Microbiology
https://doi.org/10.1007/s42770-022-00818-x

CLINICAL MICROBIOLOGY - RESEARCH PAPER

The impact of COVID-19 on antimicrobial prescription and drug resistance in fungi and bacteria

Junya L. Singulani[1] · Danielle L. Silva[1] · Caroline M. Lima[1] · Vanessa C. R. Magalhães[1,2] · Ludmila M. Baltazar[1] · Nalu T. A. Peres[1] · Rachel B. Caligiorne[3] · Alexandre S. Moura[2,3] · Ana Raquel O. Santos[1] · Tatiani Fereguetti[2] · Juliana C. Martins[2] · Lívia F. Rabelo[2] · Ana C. Lyon[2] · Susana Johann[1] · Juliana P. Falcão[4] · Daniel A. Santos[1]

Received: 30 May 2022 / Accepted: 22 August 2022

Abstract

Secondary infections are one of the complications in COVID-19 patients. We aimed to analyze the antimicrobial prescriptions and their influence on drug resistance in fungi and bacteria isolated from severely ill COVID-19 patients. Seventy-nine severely ill COVID-19 hospitalized patients with secondary bacterial or fungal infections were included. We analyzed the prescribed antimicrobial regimen for these patients and the resistance profiles of bacterial and fungal isolates. In addition, the association between drug resistance and patients' outcome was analyzed using correlation tests. The most prescribed antibacterial were ceftriaxone (90.7% of patients), vancomycin (86.0%), polymyxin B (74.4%), azithromycin (69.8%), and meropenem (67.4%). Micafungin and fluconazole were used by 22.2 and 11.1% of patients, respectively. Multidrug-resistant (MDR) infections were a common complication in severely ill COVID-19 patients in our cohort since resistant bacteria strains were isolated from 76.7% of the patients. Oxacillin resistance was observed in most Gram-positive bacteria, whereas carbapenem and cephalosporin resistance was detected in most Gram-negative strains. Azole resistance was identified among *C. glabrata* and *C. tropicalis* isolates. Patients who used more antimicrobials stayed hospitalized longer than the others. The patient's age and the number of antibacterial agents used were associated with the resistance phenotype. The susceptibility profile of isolates obtained from severely ill COVID-19 patients highlighted the importance of taking microbial resistance into account when managing these patients. The continuous surveillance of resistant/MDR infection and the rational use of antimicrobials are of utmost importance, especially for long-term hospitalized patients with COVID-19.

Keywords Antimicrobial resistance · COVID-19 · Secondary infections · Antimicrobial prescription

Junya L. Singulani and Danielle L. Silva contributed equally to this work.

Responsible Editor: Luiz Henrique Rosa

✉ Daniel A. Santos
das@ufmg.br; dasufmg@gmail.com

[1] Department of Microbiology, Institute of Biological Sciences, Universidade Federal de Minas Gerais, Av. Antonio Carlos, 6627, Pampulha, Belo Horizonte, Minas Gerais 31270-901, Brazil

[2] Hospital Eduardo de Menezes, Fundação Hospitalar Do Estado de Minas Gerais (FHEMIG), Belo Horizonte, Minas Gerais, Brazil

[3] Center of Post-Graduation and Research - IEP, Hospital Santa Casa de Belo Horizonte, Belo Horizonte, Minas Gerais, Brazil

[4] Faculdade de Ciências Farmacêuticas de Ribeirão Preto, Universidade de São Paulo-USP, Ribeirao Preto, Sao Paulo, Brazil

Introduction

The coronavirus disease 2019 (COVID-19), caused by the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), has impacted the lives of people worldwide. As of March 31, 2022, there have been more than 480 million infected people globally and more than 6 million deaths [1].

Most COVID-19 patients present mild or moderate disease, but some need to be hospitalized due to severe respiratory complications. In these cases, secondary bacterial and fungal infections have been commonly identified and associated with increased morbidity and mortality in severely ill COVID-19 patients [2, 3]. Previous studies have shown that secondary infections occur in 10–15% of COVID-19 patients, whereas the rate of antimicrobial prescription for COVID-19 patients was much higher (94–100%) [4].

Published online: 10 September 2022

Antimicrobials are essential in treating suspected or confirmed bacterial or fungal respiratory, blood, and urinary infections [5]. However, especially during the pandemic first wave, most COVID-19 hospitalized patients (~ 70%) received empirical antimicrobial treatment before they were diagnosed with a secondary infection [5–7]. The rate of antibacterial use by COVID-19 patients from lower and middle-income countries was even higher compared with high-income countries (89% *vs* 58%) [7]. Furthermore, antibiotics with alleged antiviral and anti-inflammatory properties (e.g., azithromycin) were used in the initial treatment of SARS-CoV-2 [8]. As a consequence, antimicrobial resistance (AMR) has been exacerbated, and an increase in multidrug-resistant (MDR) pathogens is alarming [2, 9–11].

A recent review estimated 4.95 million deaths associated with AMR bacteria in 2019, mainly caused by six leading pathogens (*Escherichia coli*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Streptococcus pneumoniae*, *Acinetobacter baumannii*, and *Pseudomonas aeruginosa*) [12]. The World Health Organization (WHO) highlighted that AMR would probably become an even more significant challenge in the post-pandemic era. The estimative is that the increased use of antimicrobials in the COVID-19 pandemic may result in 10 million deaths annually by 2050 due to infections by resistant bacteria to various classes of antimicrobials, reinforcing the need for the rational use of these drugs [6, 13]. Unlike antibacterials, antifungals have not been widely used in patients with COVID-19. However, issues such as toxicity and drug interactions should be considered [14]. On the other hand, although the diagnosis of secondary fungal infections is challenging, it must be assumed that these patients are also more likely to develop opportunistic fungal infections that can lead to an unfavorable outcome [15]. Therefore, the rational prescription of antifungals is vital to reduce the emergence of resistant isolates [16, 17].

Several studies have demonstrated the high occurrence of secondary infections and antimicrobial stewardship in COVID-19 patients [3–5]. However, the risk factors of MDR isolation and the profile of antifungal agents used in these patients is underexplored. Thus, the present study aimed to provide information about the antimicrobial administration and susceptibility profile of bacterial and fungal isolates, as well as the clinical features of antimicrobial resistance in hospitalized patients diagnosed with COVID-19 in Brazil. The results are very important for establishing strategies to treat COVID-19 and prevent infection by MDRs.

Methods

Study population

This single center, cohort retrospective study was performed at Eduardo de Menezes Hospital (Fundação Hospitalar do Estado de Minas Gerais) and Universidade Federal de Minas Gerais, Belo Horizonte, Brazil. We obtained medical record data and microbiological clinical specimens from 79 COVID-19 hospitalized patients with secondary bacterial/fungal infections (from May to November 2020). The diagnosis of COVID-19 was confirmed by a positive real-time polymerase chain reaction (RT-PCR) for SARS-CoV-2 in the nasopharyngeal swab, associated with suggestive signs, symptoms, and radiological findings. Patients included in the study were severely ill, and most of them required critical care unit admission. The impact of secondary infection in this cohort was previously published [3].

Informed consent was obtained from all participants. This study was approved by the National Ethics Committee (Comissão Nacional de Ética em Pesquisa — CONEP) and the hospital's Ethics Committee (CAAE: 30627320.6.0000.0008).

Definitions

COVID-19 patients were classified as severely ill if they have SpO2 < 94% on room air at sea level, a ratio of arterial partial pressure of oxygen to fraction of inspired oxygen (PaO_2/FiO_2) < 300 mmHg, a respiratory rate > 30 breaths/min, or lung infiltrates > 50% [18]. The secondary infection was considered when patients presented signs and symptoms and positive cultures during the hospital stay [19].

The pathogen resistance to at least one antimicrobial agent from three or more classes was defined as multidrug-resistant (MDR) [20].

Pathogen detection and antimicrobial susceptibility tests

Bacterial and fungal isolates (78 and 84, respectively) were obtained from blood, urine, catheter tip, and respiratory samples from 79 patients included in this study. In some of these patients, bacterial and fungal samples were isolated from different anatomic sites several times sequentially sampled. The identification of pathogens was performed with standard microbiological procedures (culture and biochemical tests) at the hospital laboratory. In some cases, the identification of the bacteria occurred only at the genus level. Furthermore, yeasts were isolated and presumptively identified in Chromagar *Candida*. After that, yeast isolates were identified by polymerase chain reaction (PCR). Species-specific primers were used for *Candida albicans* (5′-TGTTGCTCTCTCGGGGGCGGCCG-3′), *Candida glabrata* (5′-TGGGCTTGGGACTCTCGCAGCTC-3′), *Candida tropicalis* (5′-TGGGCGGTAGGAGAATTGCGTTA-3′), and *Candida parapsilosis* (5′-GCATCAGTTTGAGCGGTAGGATAAGC-3′).

The universal reverse primers used were NL4 (5′-CGTCCG TGTTTCAAGACGG-3′). The yeast isolates that were not identified by the PCR were submitted to sequencing reactions using the Big Dye kit version 3.1 (Applied Biosystems, USA) in combination with the ABI 3730 automated system.

The microdilution susceptibility test for bacterial isolates was performed as described in the document M100 from the Clinical and Laboratory Standards Institute, CLSI [21]. Interpretative criteria from the CLSI guidelines were used to determine the minimum inhibitory concentrations (MIC) of ampicillin, azithromycin, cefazolin, ceftriaxone, clindamycin, gentamicin, meropenem, oxacillin, polymyxin, and vancomycin. All antibacterial agents were tested at concentrations from 0.125 to 64 mg/L. *Enterococcus faecalis* ATCC 7080, *E. coli* ATCC 8739, and *P. aeruginosa* ATCC 9027 were used as control strains. For fungal isolates, the microdilution susceptibility test to antifungal agents (amphotericin B, fluconazole, itraconazole, voriconazole, and caspofungin) was evaluated according to CLSI guidelines [22, 23]. Itraconazole, voriconazole, and amphotericin B were used at concentrations from 0.03 to 16 mg/L; fluconazole and caspofungin were used at concentrations from 0.125 to 64 and 0.015 to 8.0 mg/L, respectively. *C. albicans* ATCC 18804, *C. tropicalis* ATCC 750, *C. krusei* ATCC 20298, *C. glabrata* ATCC 2001, *C. parapsilosis* ATCC 22019, *Aspergillus fumigatus* ATCC 16913, and *Aspergillus flavus* IMI 190443 were used as control strains.

Clinical data

Clinical data such as age, gender, comorbidities, length of hospital stay, number, and type of prescribed antimicrobial agents were obtained from the patient's medical record.

Statistical analysis

Given the MIC values, the geometric means, MIC_{50} and MIC_{90} (MIC values that inhibited 50 and 90% of the strains, respectively), were calculated for each bacterial and fungal species. Specific clinical parameters were described as percentage, mean, and standard deviation. Continuous variables were tested for normality using the D'Agostino and Pearson test and analyzed using the unpaired *t*-test or Mann–Whitney *U* test, depending on each case. We also evaluated risk factors related to the occurrence of resistant isolates and MDR in adjusted and unadjusted logistic regression analyses. Univariate and multivariate regression analyzes were performed to identify factors associated with these isolates. Variables with a p-value < 0.2 in unadjusted analyses were included in a step-by-step backward and forward elimination approach. Statistical significance was set at $p < 0.05$. Odds ratios (OR) and 95% CI were calculated for each factor in the final model. Pearson's correlation coefficient (r) was determined between selected MICs. Data were analyzed using EpiInfo 7.2, GraphPad Prism 5.0, and Microsoft Excel 2007.

Results

We obtained 78 positive bacterial cultures from 43 severely ill patients hospitalized with SARS-CoV-2. *Acinetobacter* spp. (22%), negative-coagulase staphylococci (20%), *Klebsiella* spp. (18%), *S. aureus* (17%), *Pseudomonas* spp. (13%), *Enterobacter* spp. (5%), *Enterococcus* spp. (4%), and *E. coli* (1%) were the bacterial isolates (Fig. 1A). Seventy-four fungal strains were isolated from 54 patients: 72 yeasts and two filamentous fungi. Among these, we found the following prevalence (Fig. 1B): *C. albicans* (54%), *C. tropicalis* (23%), *C. glabrata* (14%), *C. parapsilosis* (3%), *C. kefyr* (3%), *C. lusitaniae* (1%), *A. nomius* (1%), and *A. flavus* (1%).

The clinical sources for bacterial isolates were endotracheal aspirate (54% of samples), catheter tip (23%), blood (19%), and urine (4%). Otherwise, fungi were isolated from endotracheal aspirate (54%), urine (34%), catheter tip (5%), blood (5%), and bronchoalveolar lavage (BAL; 1%) (Fig. 1C).

Notably, 97.7% of the patients were treated with at least one antibacterial, mostly treated with 4 to 6 different agents during the hospital stay (Fig. 2A). The higher use of antibacterials was also related to a longer length of hospital stay: (i) patients who were treated with 0 to 3 agents were hospitalized for 12 to 19 days, (ii) those who were treated with 4 to 6 stayed hospitalized from 18 to 21 days, and (iii) those who were treated with 7 to 9 agents were hospitalized from 28 to 36 days. The most prescribed antibacterial agents were (1) ceftriaxone (90.7% of patients), (2) vancomycin (86.0%), (3) polymyxin B (74.4%), (4) azithromycin (69.8%), and (5) meropenem (67.4%) (Fig. 2B).

Antifungals were prescribed to 18 (33.33%) of the 54 patients with positive cultures for fungi, with micafungin prescribed to 12 (22.22%) and fluconazole to six (11.11%) of them (Fig. 2C). Overall, the mortality rate was 81.48% ($n = 44$) for patients with positive cultures for fungi, with no prescription of antifungal drugs for 61.36% (27) of the patients who died. No multidrug resistance was observed in the fungal isolates. Therefore, antibacterial agents were prescribed for all patients who also received an antifungal agent.

Subsequently, we evaluated the resistance pattern of bacterial and fungal isolates. The bacteria were divided into two large groups to analyze the antibacterial susceptibility profile: (1) Gram-negative bacteria and (2) Gram-positive cocci, and MIC values are presented in Table 1. Resistant bacteria strains were isolated from 76.7% of the patients. 100% of *Acinetobacter* spp. and 50% of *Pseudomonas* spp. strains were resistant to carbapenem

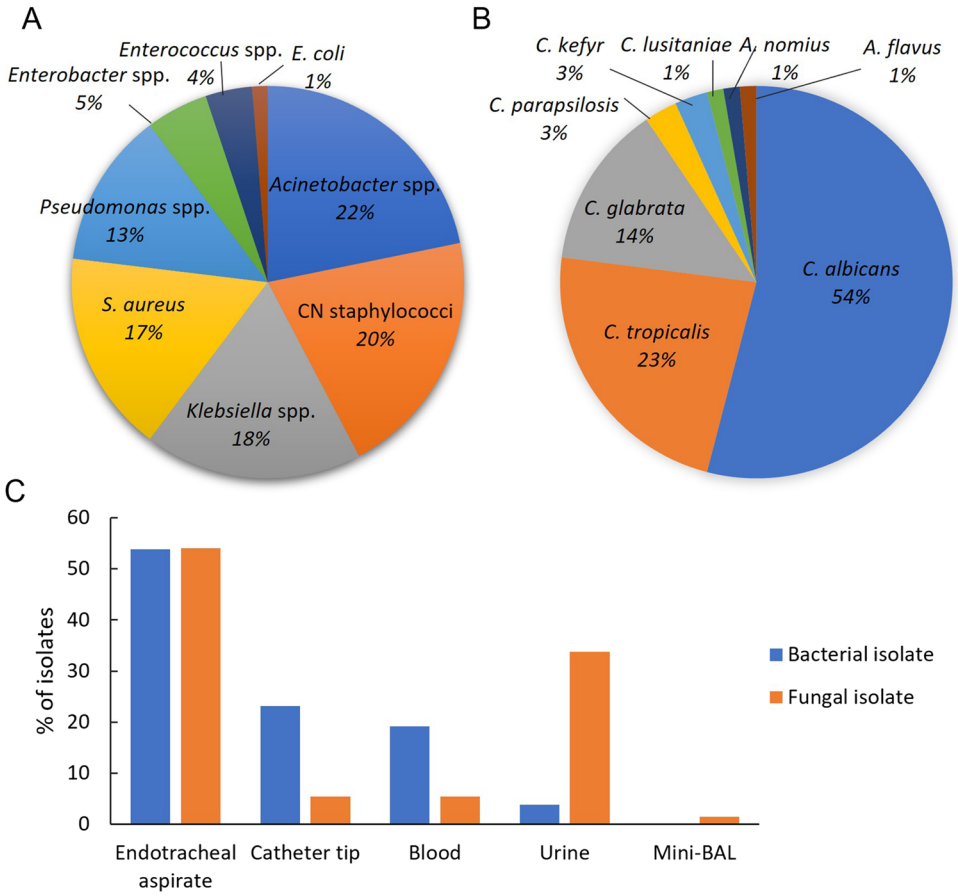

Fig. 1 Species distribution of bacterial (**A**) and fungal (**B**) isolates and clinical sources of secondary bacterial and fungal infections (**C**) from patients with COVID-19. CN staphylococci, negative-coagulase staphylococci; BAL, bronchoalveolar lavage

(meropenem) (Table 1). Considering *Enterobacterales*, the resistance to a third-generation cephalosporin (ceftriaxone) was observed in 50% of *Enterobacter* spp. and 100% of *Klebsiella*. The rates of oxacillin resistance were 62% and 81% for *S. aureus* and negative-coagulase staphylococci. The resistance to vancomycin was detected in 15 and 25% of *S. aureus* and negative-coagulase staphylococci, respectively (Table 1). Ampicillin resistance in *Enterococcus* spp. was found in 67% of isolates.

Furthermore, an MDR bacterial infection occurred in 65% of the COVID-19 patients during the hospital stay. Overall, 38 MDR strains were isolated from these patients, in which a single MDR pathogen was isolated from 21 patients, two pathogens in five patients, and three or four MDR pathogens in one patient each. The MDR Gram-negative bacteria were *K. pneumoniae* (11 isolates), *A. baumannii* (6), and *Enterobacter* spp. (2). Among Gram-positive bacteria, there were negative-coagulase staphylococci (13) and *S. aureus* (6).

Most of the fungal isolates were susceptible to the tested antifungals. The exceptions were 5% (two strains) *C. albicans* and 20% (two strains) *C. glabrata* isolates resistant to caspofungin. In addition, 5.88% (one strain) of *C. tropicalis* and 70% (seven strains) of *C. glabrata* were resistant to voriconazole, and 20% (two strains) of *C. glabrata* resistant to fluconazole. In addition, fluconazole MIC $\geq$ 4.0 was found for all voriconazole-resistant *C. glabrata*, and a strong correlation was observed between these MICs ($r > 0.99$, $p < 0.0001$). Therefore, the high prevalence of resistance in *C. glabrata* isolates is noteworthy (Table 2).

The patients with resistant isolate or MDR bacterial infections were stratified by gender, age, comorbidities (diabetes, cardiovascular disease, hypertension, and HIV), length of hospital stay, and the number of antibacterial agents used per patient (Table 3). Resistance was found mainly for strains obtained from patients older than 60 years old. However, the mean age of COVID-19 patients was lower for those who showed resistant isolates (60.90 years old) or MDR (59.07 years old; $p = 0.03$) than those without bacterial resistance (67.40 and 68.67), respectively. The prevalence of resistant isolate and MDR was higher among males when compared to females (18% and 16% *vs* 15% and 12%, respectively; $p = 0.01$). Regarding comorbidities, diabetes and cardiovascular disease were associated with the occurrence of resistant isolate ($p < 0.05$). Although the length of hospital stay was not statistically associated with antimicrobial resistance, we observed a longer length of hospitalization for patients with resistant bacterial isolate or MDR compared to those without resistance or MDR (22.42 and 23.08 *vs*. 17.7

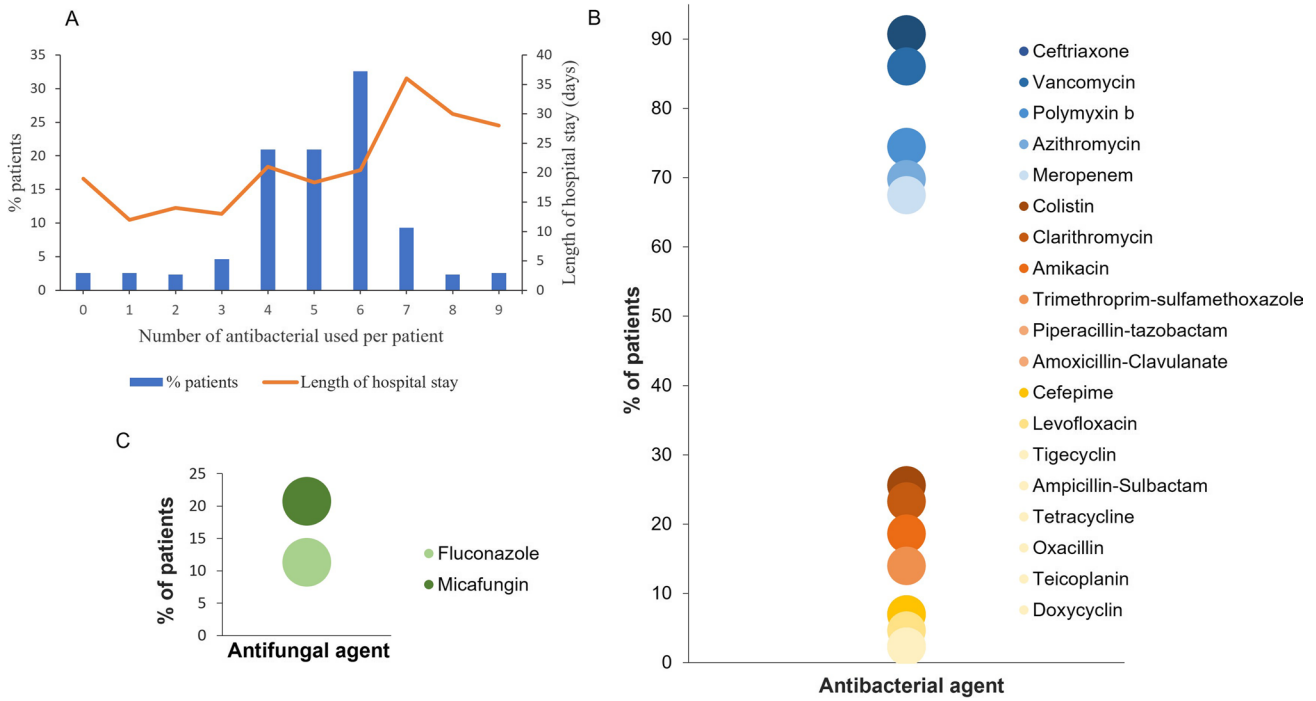

Fig. 2 **A** Number of antibacterial agents used per patient during the hospital stay (blue bars) and length of hospital stay (orange line) of patients with COVID-19 and secondary infection. **B** Antibacterial agents. **C** Antifungal agents used by patients with COVID-19 and secondary infection during the hospital stay. Blue circles represent the most commonly prescribed antibacterial agents; orange circles represent the least used ones, and green circles show the antifungals prescribed for the patients

and 18.13, respectively). Finally, a higher number of used antibacterial per patient was associated with MDR isolation when compared to those without MDR (5.46 *vs* 4.40; $p=0.04$). The characteristics of patients with resistant fungal isolates were not evaluated since the sample size was small, and the cutoff for the definition of resistance is not described for some fungal species.

Discussion

Previously, our group performed a cohort study focused on the outcomes of patients with severe COVID-19 in Brazil. Among these patients, 41.8% had secondary bacterial and fungal infections, which increased the death risk compared to those without secondary infection [3]. Here, we obtained bacterial and fungal cultures from the original study with 79 patients severely ill hospitalized with SARS-CoV-2 and secondary bacterial and fungal infection. *Acinetobacter* spp., negative-coagulase staphylococci, *Klebsiella* spp., *S. aureus*, and *Pseudomonas* spp. were the main bacterial isolates from these patients. Although previous studies have demonstrated a diverse species distribution depending on the studied site and sample selection, our findings are similar to those of several other reports. For example, Du et al. [24] described secondary bacterial infections at a late stage of COVID-19 caused mainly by *K. pneumoniae*, *Staphylococcus* spp., *A. baumannii*, and *E. coli*. On the other hand, *A. baumannii* and *K. pneumoniae* were the main pathogens found by Li et al. [25] Another study showed the distribution of positive cultures represented mainly by *K. pneumoniae* (53%), *A. baumannii* (37%), and *S. aureus* (10%) [4].

According to the clinical samples for bacterial isolates, endotracheal aspirate was the leading infection site, followed by blood and catheter tip samples. The bacteria isolated from endotracheal aspirates and blood were mainly Gram-negative. On the other hand, the rate of Gram-positive bacterial isolates was higher in catheter tips. Thus, our results suggested that bloodstream infections with Gram-negative bacteria can be related to lung infections. In contrast, the bloodstream infection of patients with Gram-positive bacteria was associated with central venous catheter implantation during hospitalization. Similarly, Li et al. [25] found that 80.0% of the patients with bloodstream infections caused by Gram-positive bacteria were due to central venous catheter. In addition, they analyzed 21 patients in which the lung infections occurred first, followed by bloodstream infections, and found that 21 patients had the same Gram-negative bacteria in both sites, including *K. pneumoniae* and *A. baumannii*.

Regarding fungal species, we found *C. albicans*, *C. tropicalis*, and *C. glabrata* as the main agents of secondary yeast infections in hospitalized COVID-19 patients. Furthermore,

Table 1 Minimal inhibitory concentration (MIC) of antibacterial agents against bacterial isolates from COVID-19 patients

Gram-negative bacterial species	Antibacterial agent	MIC (mg/L)				Isolates (%)[¶]		
		Range	MIC_{50}	MIC_{90}	Geometric mean	Susceptible	Intermediate	Resistant
Acinetobacter spp.*(n = 17)*	Ceftriaxone	64–> 64	> 64	> 64	64			100
	Gentamicin	0.25–> 64	16	> 64	9.8	41	6	53
	Meropenem	32–64	64	64	53.8			100
	Polymyxin b	0.25–> 64	1	4.4	1.2		88	12
Pseudomonas spp.*(n = 10)*	Gentamicin	< 0.125–32	0.25	32	0.7	80		20
	Meropenem	0.25–64	4	64	6.3	20	30	50
	Polymyxin b	1–4	2	2.4	1.9		80	20
Enterobacter spp.*(n = 4)*	Azithromycin	1–8	1	5.9	1.7	100		
	Cefazolin	> 64–> 64	> 64	> 64	> 64			100
	Ceftriaxone	< 0.125–> 64	32.1	64	3.4	50		50
	Gentamicin	< 0.125–< 0.125	< 0.125	< 0.125	< 0.125	100		
	Meropenem	< 0.125–16	8.1	16	1.4	50		50
	Polymyxin b	0.5–> 64	2.5	46	3.4		50	50
Klebsiella spp. *(n = 14)*	Azithromycin	1–> 64	48	> 64	18.6	36		64
	Cefazolin	> 64–> 64	> 64	> 64	> 64			100
	Ceftriaxone	> 64–> 64	> 64	> 64	> 64			100
	Gentamicin	< 0.125–> 64	16	57.6	3.6	43		57
	Meropenem	< 0.125–> 64	> 64	> 64	41	7		93
	Polymyxin b	1.0–> 64	4	27.2	4.9		21	79
Escherichia coli (n = 1)*	Ampicillin	> 64						100
	Azithromycin	4				100		
	Cefazolin	32						100
	Ceftriaxone	< 0.125				100		
	Gentamicin	< 0.125				100		
	Meropenem	< 0.125				100		
	Polymyxin b	4						100
Staphylococcus aureus (n = 13)	Azithromycin	> 64–> 64	> 64	> 64	> 64			100
	Clindamycin	< 0.125–> 64	> 64	> 64	15.2	23		77
	Oxacillin	0.5–> 64	> 64	> 64	11.6	38		62
	Vancomycin	0.5–> 64	0.5	51.4	1.1	85		15
CN *Staphylococcus (n = 16)*	Azithromycin	> 64–> 64	> 64	> 64	> 64			100
	Clindamycin	< 0.125–> 64	> 64	> 64	24.3	13		87
	Oxacillin	0.25–> 64	> 64	> 64	21.7	19		81
	Vancomycin	0.5–> 64	2	> 64	4	69	6	25
Enterococcus spp.*(n = 3)*	Ampicillin	0.25–> 64	> 64	> 64	12.7	33		67
	Vancomycin	32–> 64	> 64	> 64	50.8			100

[*] Only one *E. coli* isolate was found, and therefore, the MIC_{50}, MIC_{90}, and geometric mean data were not calculated

[¶] According to CLSI guideline M100 (CLSI, 2021)

risk factors such as more extended ICU stay, catheter placement, mechanical ventilation, broad-spectrum antibiotics, and corticosteroids are associated with candidiasis in COVID-19 patients [26]. Endotracheal aspirate and urine were the first and second most common clinical sources for fungal isolates, respectively. *Candida* colonization is common in mechanically ventilated patients, as long-term ventilation is associated with a significant increase in respiratory and urinary tract *Candida* populations [27].

Our study demonstrated that most of the patients with COVID-19 and secondary infection received from 4 to 6 antibacterial agents during the hospital stay. Some factors could have influenced this high rate: (1) Hospital Eduardo de Menezes is a tertiary hospital that only receives patients from other hospitals or care units; the antibacterial drugs can be prescribed before admission. (2) Brazilian guidelines indicate the prescription of empirical antibiotic therapy in suspected sepsis before identifying the pathogen,

Table 2 Minimal inhibitory concentration (MIC) of antifungal agents against fungal isolates from COVID-19 patients

Fungal species	Antifungal agent	MIC (mg/L)				Resistance[¶]
		Range	MIC_{50}	MIC_{90}	Geometric mean	% resistance (*n*)
C. albicans (n = 40)	Fluconazole	< 0.125–0.5	0.125	0.25	0.17	0 (0)
	Itraconazole	< 0.03–0.25	0.06	0.125	0.07	NA
	Voriconazole	0.06–0.5	0.125	0.25	0.14	0 (0)
	Caspofungin	< 0.015–2.0	0.06	0.125	0.05	5 (2)
	Amphotericin B[#]	0.125–2.0	1.0	2.0	0.92	0 (0)
C. tropicalis (n = 17)	Fluconazole	< 0.125–0.5	0.5	0.5	0.35	0 (17)
	Itraconazole[#]	< 0.03–0.25	0.06	0.125	0.06	0 (17)
	Voriconazole	< 0.125–2.0	0.25	0.5	0.26	5.88 (1)
	Caspofungin	< 0.015–0.125	0.06	0.125	0.05	0 (0)
	Amphotericin B[#]	0.25–2.0	1.0	2.0	1.13	0 (0)
C. glabrata (n = 10)	Fluconazole	0.5–64	4.0	64.0	5.28	20 (2)
	Itraconazole[#]	0.06–2.0	0.5	2.0	0.43	0 (0)
	Voriconazole[#]	0.125– > 16	1.0	13.6	1.00	70 (7)
	Caspofungin	< 0.015–2.0	0.05	0.65	0.07	20 (2)
	Amphotericin B[#]	0.5–2.0	1.0	1.1	1.0	0 (0)
C. parapsilosis (n = 02)	Fluconazole	0.25–1.0	0.63	0.93	0.5	0 (0)
	Itraconazole	0.125–0.25	0.09	0.12	0.09	NA
	Voriconazole	0.06–0.125	0.19	0.24	0.18	0 (0)
	Caspofungin	2.0–2.0	2.0	2.0	2.0	0 (0)
	Amphotericin B[#]	1.0–1.0	1.0	1.0	1.0	0 (0)
C. kefyr (n = 02)	Fluconazole	0.125–0.25	0.19	0.24	0.18	NA
	Itraconazole	0.125–0.25	0.19	0.24	0.18	NA
	Voriconazole	0.06–0.125	0.09	0.12	0.09	NA
	Caspofungin	< 0.015– < 0.015	0.02	0.02	0.02	NA
	Amphotericin B	2.0–2.0	2.0	2.0	2.0	NA
*C. lusitaniae (n = 01)**	Fluconazole[#]	0.25				0 (0)
	Itraconazole[#]	0.25				0 (0)
	Voriconazole	0.125				NA
	Caspofungin	0.5				NA
	Amphotericin B	0.5				NA
*Aspergillus flavus (n = 01)**	Fluconazole	-				-
	Itraconazole	0.125				NA
	Voriconazole	2.0				NA
	Caspofungin	1.0				NA
	Amphotericin B	1.0				NA
*Aspergillus nomius (n = 01)**	Fluconazole	-				-
	Itraconazole[#]	0.06				0 (0)
	Voriconazole[#]	4.0				0 (0)
	Caspofungin[#]	1.0				0 (0)
	Amphotericin B[#]	2.0				0 (0)

NA not available

[#]Epidemiological cutoff

[*]Only one isolate of each *C. lusitaniae*, *A. flavus*, and *A. nomius* species was found, and therefore, the MIC_{50}, MIC_{90}, and geometric mean data were not calculated

[¶]According to CLSI guidelines M60 ED4 (CLSI, 2017) and M38-A2 (CLSI, 2008)

Table 3 Clinical features of COVID-19 patients with resistant bacterial isolates

	Resistant isolate						*MDR*					
			Univariate analysis		*Multivariate analysis*				*Univariate analysis*		*Multivariate analysis*	
	Yes ($n=33$)	No ($n=10$)	OR (95% CI)	p value	OR (95% CI)	p value	Yes ($n=28$)	No ($n=15$)	OR (95% CI)	p value	OR (95% CI)	p value
Age (years)	60.9 ± 14.2	67.4 ± 12.3		0.20*			59.1 ± 13.6	68.7 ± 12.7		0.03*		
Gender (M/F)	18 (54.6%)/ 15 (45.5%)	5 (50.0%)/5 (50.0%)	0.83 (0.2–3.44)	**0.01**			16 (57.1%)/ 12 (42.9%)	7 (46.7%)/ 8 (53.3%)	0.67 (0.19–0.31)	**0.07**		
Comorbidity												
Asthma	5 (15.2%)	1 (10.0%)	1.60 (0.17–15.61)	< 0.01	2.79 (0.20–39.28)	0.45	5 (17.9%)	1 (6.7%)	3.04 (0.32–28.80)	0.14	2.91 (0.29–28.91)	0.36
Diabetes	15 (45.5%)	1 (10.0%)	7.45 (0.85–66.12)	0.09	21.52 (1.69–274.69)	**0.02**	12 (42.9%)	4 (26.7%)	2.06 (0.52–8.09)	0.34		
Cardiovascular disease	21 (63.6%)	8 (80.0%)	0.44 (0.08–2.41)	0.02	0.08 (0.01–0.91)	**0.04**	17 (60.7%)	12 (80.0%)	0.39 (0.09–1.69)	0.05	0.42 (0.08–2.10)	0.29
Arterial hypertension	8 (24.2%)	0	0.75 (0.16–3.59)	< 0.01	1.39 (0.16–12.15)	0.77	6 (21.4%)	5 (33.3%)	0.54 (0.13–2.218)	0.04	0.71 (0.14–3.46)	0.67
HIV	2 (6.1%)	1 (10.0%)	Undefined	< 0.01	Undefined	0.97	2 (7.1%)	15 (100.0%)	Undefined	0.09	Undefined	0.97
Obesity	17 (51.5%)	3 (30.0%)	2.48 (0.54–11.25)	0.07	5.52 (0.68–44.86)	0.11	14 (50.0%)	6 (40.0%)	1.5 (0.42–5.35)	0.30		
Length of hospital stay	22.4 ± 19.1	17.7 ± 8.0	1.02 (0.97–1.07)	0.45			23.1 ± 18.8	18.1 ± 13.7	1.20 (0.98–1.06)	0.38		
Number of antibacterials	4.9 ± 1.7	5.8 ± 1.8		0.14*			5.5 ± 1.3	4.4 ± 2.2		**0.04***		

p values < 0.05 are represented in bold.*p-value obtained by unpaired t-test or Mann–Whitney U test

MDR multidrug-resistant, *HIV* human immunodeficiency virus, *OR* odds ratio

considering patient risk factors and local epidemiology. (3) The third factor is the limited experience of health professionals and the lack of treatments in managing COVID-19 patients in the first pandemic wave. (4) The last factor is the challenge in differentiating COVID-19 pneumonia and bacterial pneumonia, or still distinguishing between sole SARS-CoV-2 infection and secondary bacterial/fungal infections [28, 29]. Consequently, we observed a more extended period of hospitalization in COVID-19 patients that used a higher number of antibacterial agents.

Ceftriaxone, vancomycin, polymyxin B, azithromycin, or meropenem were prescribed for more than 60% of patients. It is worth mentioning that polymyxin B belongs to the Reserve list of WHO, which points out that some antibiotics should be reserved for treatment of confirmed or suspected infections due to MDR organisms. The other four drugs are part of the Watch list, containing antibiotics with higher resistance potential [30]. Although azithromycin, ceftriaxone, and meropenem were the most frequent antibiotics prescribed among the hospitalized COVID-19 patients, [7, 31] their inclusion in 2019 WHO AWaRe Classification Database represents the need for caution in their use [30]. Fortunately, several studies have shown that antibiotic prescriptions decreased substantially after the first wave of COVID-19 [32–34]. However, assessing the long-term impact on antimicrobial resistance of the over-prescription of antibiotics from the first COVID-19 wave is required.

Bacterial resistance, including MDR infection, was common in COVID-19 patients, reaching more than half of them. In addition, oxacillin resistance was observed in most strains from the *Staphylococcus* genus, whereas carbapenem and cephalosporin resistance was mainly present in the Gram-negative isolates. This may be related to the prior selection due to early antibiotic treatment in the COVID-19 patients since meropenem and ceftriaxone were among the most used. Furthermore, the administration of azithromycin as an experimental therapy was a usual practice in Brazil's first wave of the pandemic. Therefore, it may have contributed to this drug's high percentage of bacterial resistance.

Bacterial resistance was increased in elderly patients and associated with comorbidities such as diabetes and cardiovascular disease. Similar to other studies, we also observed a higher proportion of resistant isolates and MDR in male patients [35, 36]. Interestingly, administering an increased number of antibacterial agents was associated with MDR infection. In this context, we also found that higher use of azithromycin, ceftriaxone, and meropenem was associated with bacterial resistance to each of these drugs.

Unlike secondary bacterial infections, fungal infections should be investigated in patients with COVID-19 with more than 7–10 days of hospitalization, exposed to multiple risk factors, and who develop signs of sepsis, despite antibacterial therapy. In our study, the diagnosis of secondary fungal infection and the treatment with antifungals was performed late in the hospitalization period (mean = 15.80 days after the patients' admission). Although nosocomial fungal infections may occur later in hospital admission and have a slower progression than bacterial infections, an earlier fungal diagnosis is necessary for the patient's adequate management. Regarding the antifungal prescribed against candidiasis, 34% of the patients received fluconazole or micafungin. This prescription was higher than that described by Seaton et al. (2020), in which antifungals (micafungin, fluconazole, and voriconazole) were prescribed to 9.8% of patients in intensive care. Like the antibacterial prescription during the pandemic, the antifungal has also increased. Bayona et al. [37] compared the use of antifungals between 2020 and 2019 and found an increase of 15%.

Among fungal species, we highlighted the occurrence of resistance of *C. glabrata* and *C. tropicalis* to azoles. This should be considered a concern due to the limited arsenal of antifungal agents [38]. Moreover, intrinsic and secondary resistance to azole has been extensively documented in *Candida* isolates [39]. Echinocandin resistance can also occur after exposure to members of this class and is mediated by point mutations in hot spot regions of the *FKS1* and *FKS2* genes, which encode the echinocandin target enzyme β-1,3-D-glucan synthase [16, 40]. In addition, previous exposure of *C. glabrata* to fluconazole has been linked to cross-resistance to voriconazole. As in our study, other authors report a significant positive correlation between the MIC values of these antifungals [17]. Together, these data reinforce the importance of correct antifungal management for effective antifungal therapy.

Our study has some limitations. First is the limited sample size, specifically during the first pandemic wave in Brazil. However, some important conclusions can add to many similar studies from other countries. Second, some of the COVID-19 patients may have used antibacterial before admission to the Eduardo de Menezes Hospital, and data regarding this use was unavailable. Thus, we could not describe the timing of an antibiotic prescription and link it to a microbiological diagnosis and resistance occurrence. Despite this, our study provides essential data on the susceptibility profile of secondary infection agents in patients with COVID-19.

In conclusion, our study draws attention to the need for the correct administration of antimicrobials, especially in intensive care units, where secondary infections occur more often. Finally, we emphasize the need for ongoing infection prevention and control and antimicrobial stewardship initiatives.

Author contribution All authors contributed to the study conception and design. Vanessa C. R. Magalhães, Rachel B. Caligiorne, Alexandre

S. Moura, Ana Raquel O. Santos, Tatiani Fereguetti, Juliana C. Martins, Lívia F. Rabelo, and Ana C. Lyon contributed to the patient enrollment, sample, and data collection. Junya L. Singulani, Danielle L. Silva, Caroline M. Lima, and Vanessa C. R. Magalhães were responsible for the microbiological identification and antimicrobial susceptibility tests. Daniel A. Santos performed the conceptualization, funding acquisition, project administration, resources, and supervision. The first draft of the manuscript was written by Junya L. Singulani and Danielle L. Silva, and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

Funding This work was supported by Fundação de Amparo à Pesquisado Estado de Minas Gerais — FAPEMIG (Grant APQ-00267–20) and Brazilian Ministry of Health and Conselho Nacional de Desenvolvimento Científico e Tecnológico — CNPq (440010/2018–7; 402200/2021–7). D. A. S. (303762/2020–9) and J. L. S. (163516/2020–0) are research fellows of the CNPq.

Data availability The datasets generated during and/or analyzed during the current study are not publicly available to protect our participants' sensitive data but are available from the corresponding author on reasonable request.

Declarations

Ethics approval This study was approved by the National Ethics Committee (Comissão Nacional de Ética em Pesquisa — CONEP) and the hospital's Ethics Committee (CAAE: 30627320.6.0000.0008).

Consent to participate Each participant signed written informed participatory consent.

Competing interests The authors declare no competing interests.

References

1. COVID-19 Map (2022). COVID-19 data repository by the Center for Systems Science and Engineering (CSSE) at Johns Hopkins University. https://coronavirus.jhu.edu/map.html. Accessed March 16, 2022.
2. Hughes S, Troise O, Donaldson H, Mughal N, Moore LSP (2020) Bacterial and fungal coinfection among hospitalized patients with COVID-19: a retrospective cohort study in a UK secondary-care setting. Clin Microbiol Infect 26(10):1395–1399. https://doi.org/10.1016/j.cmi.2020.06.025
3. Silva DL, Lima CM, Magalhães VCR, Baltazar LM, Peres NTA, Caligiorne RB, Moura AS, Fereguetti T, Martins JC, Rabelo LF, Abrahão JS, Lyon AC, Johann S, Santos DA (2021) Fungal and bacterial coinfections increase mortality of severely ill COVID-19 patients. J Hosp Infect 113:145–154. https://doi.org/10.1016/j.jhin.2021.04.001
4. Gaspar GG, Ferreira LR, Feliciano CS, Campos Júnior CP, Molina FMR, Vendruscolo ACS, Bradan GMA, Lopes NAP, Martinez R, Bollela VR (2021) Pre- and post-COVID-19 evaluation of antimicrobial susceptibility for healthcare-associated infections in the intensive care unit of a tertiary hospital. Rev Soc Bras Med Trop 54:e00902021. https://doi.org/10.1590/0037-8682-0090-2021
5. Rawson TM, Moore LSP, Zhu N, Ranganathan N, Skolimowska K, Gilchrist M, Satta G, Cooke G, Holmes A (2020) Bacterial and fungal coinfection in individuals with coronavirus: a rapid review to support COVID-19 antimicrobial prescribing. Clin Infect Dis 71(9):2459–2468. https://doi.org/10.1093/cid/ciaa530
6. Mahoney AR, Safaee MM, Wuest WM, Furst AL (2021) The silent pandemic: Emergent antibiotic resistances following the global response to SARS-CoV-2. iScience 24 (4):102304. DOI: https://doi.org/10.1016/j.isci.2021.102304
7. Khan S, Hasan SS, Bond SE, Conway BR, Aldeyab MA (2021) Antimicrobial consumption in patients with COVID-19: a systematic review and meta-analysis. Expert Rev Anti Infect Ther:1–24. DOI: https://doi.org/10.1080/14787210.2022.2011719
8. Gautret P, Lagier JC, Parola P, Hoang VT, Meddeb L, Mailhe M, Doudier B, Courjon J, Giordanengo V, Vieira VE, Tissot Dupont H, Honoré S, Colson P, Chabrière E, La Scola B, Rolain JM, Brouqui P, Raoult D (2020) Hydroxychloroquine and azithromycin as a treatment of COVID-19: results of an open-label nonrandomized clinical trial. Int J Antimicrob Agents 56(1):105949. https://doi.org/10.1016/j.ijantimicag.2020.105949
9. Chowdhary A, Tarai B, Singh A, Sharma A (2020) Multidrug-resistant Candida auris infections in critically ill coronavirus disease patients, India, April-July 2020. Emerg Infect Dis 26(11):2694–2696. https://doi.org/10.3201/eid2611.203504
10. Nori P, Szymczak W, Puius Y, Sharma A, Cowman K, Gialanella P, Fleischner Z, Corpuz M, Torres-Isasiga J, Bartash R, Felsen U, Chen V, Guo Y (2020) Emerging co-pathogens: New Delhi Metallo-beta-lactamase producing Enterobacterales infections in New York City COVID-19 Patients. Int J Antimicrob Agents 56(6):106179. https://doi.org/10.1016/j.ijantimicag.2020.106179
11. Tiri B, Sensi E, Marsiliani V, Cantarini M, Priante G, Vernelli C, Martella LA, Costantini M, Mariottini A, Andreani P, Bruzzone P, Suadoni F, Francucci M, Cirocchi R, Cappanera S (2020) 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 9 (9). DOI: https://doi.org/10.3390/jcm9092744
12. Collaborators AR (2022) Global burden of bacterial antimicrobial resistance in 2019: a systematic analysis. Lancet 399(10325):629–655. https://doi.org/10.1016/S0140-6736(21)02724-0
13. Chibabhai V, Duse AG, Perovic O, Richards GA (2020) Collateral damage of the COVID-19 pandemic: exacerbation of antimicrobial resistance and disruptions to antimicrobial stewardship programmes? S Afr Med J 110(7):572–573. https://doi.org/10.7196/SAMJ.2020.v110i7.14917
14. Nestler M, Godbout E, Lee K, Kim J, Noda AJ, Taylor P, Pryor R, Markley JD, Doll M, Bearman G, Stevens MP (2021) Fungal superinfection in patients with COVID-19: role of antifungal stewardship? Am J Infect Control 49(2):279–280. https://doi.org/10.1016/j.ajic.2020.11.015
15. Kubin CJ, McConville TH, Dietz D, Zucker J, May M, Nelson B, Istorico E, Bartram L, Small-Saunders J, Sobieszczyk ME, Gomez-Simmonds A, Uhlemann AC (2021) Characterization of bacterial and fungal infections in hospitalized patients with coronavirus disease 2019 and factors associated with health care-associated infections. Open Forum Infect Dis 8 (6):ofab201. DOI: https://doi.org/10.1093/ofid/ofab201
16. Coste AT, Kritikos A, Li J, Khanna N, Goldenberger D, Garzoni C, Zehnder C, Boggian K, Neofytos D, Riat A, Bachmann D, Sanglard D, Lamoth F, (FUNGINOS) FINoS, (2020) Emerging echinocandin-resistant Candida albicans and glabrata in Switzerland. Infection 48(5):761–766. https://doi.org/10.1007/s15010-020-01475-8
17. Chen TC, Chen YH, Chen YC, Lu PL (2012) Fluconazole exposure rather than clonal spreading is correlated with the emergence of Candida glabrata with cross-resistance to triazole antifungal agents. Kaohsiung J Med Sci 28(6):306–315. https://doi.org/10.1016/j.kjms.2011.11.011

18. National Institutes of Health. Clinical spectrum of SARS-CoV-2 infection (2021). https://www.covid19treatmentguidelines.nih.gov/overview/clinical-spectrum. Accessed March 14, 2022.
19. Centers for Disease Control and Prevention/NHSN. Surveillance definitions for specific types of infections 2020 (2020). https://portaldeboaspraticas.iff.fiocruz.br/wp-content/uploads/2020/10/17pscNosInfDef_current.pdf. Accessed March 16, 2022.
20. Magiorakos AP, Srinivasan A, Carey RB, Carmeli Y, Falagas ME, Giske CG, Harbarth S, Hindler JF, Kahlmeter G, Olsson-Liljequist B, Paterson DL, Rice LB, Stelling J, Struelens MJ, Vatopoulos A, Weber JT, Monnet DL (2012) Multidrug-resistant, extensively drug-resistant and pandrug-resistant bacteria: an international expert proposal for interim standard definitions for acquired resistance. Clin Microbiol Infect 18(3):268–281. https://doi.org/10.1111/j.1469-0691.2011.03570.x
21. Clinical and Laboratory Standards Institute (2021). Performance standards for antimicrobial susceptibility testing. 31 st ed. CLSI suplement M-100. CLSI, Wayne, PA, USA.
22. Clinical and Laboratory Standards Institute (2008). Reference method for broth dilution antifungal susceptibility testing of filamentous fungi. 2nd ed. CLSI standardM38, CLSI, USA.
23. Clinical and Laboratory Standards Institute. Performance Standards for Antimicrobial Susceptibility Testing of Yeasts (2017). 1st ed. CLSI supplementM60. CLSI, USA.
24. Du RH, Liang LR, Yang CQ, Wang W, Cao TZ, Li M, Guo GY, Du J, Zheng CL, Zhu Q, Hu M, Li XY, Peng P, Shi HZ (2020) Predictors of mortality for patients with COVID-19 pneumonia caused by SARS-CoV-2: a prospective cohort study. Eur Respir J 55 (5). DOI: https://doi.org/10.1183/13993003.00524-2020
25. Li J, Wang J, Yang Y, Cai P, Cao J, Cai X, Zhang Y (2020) Etiology and antimicrobial resistance of secondary bacterial infections in patients hospitalized with COVID-19 in Wuhan, China: a retrospective analysis. Antimicrob Resist Infect Control 9(1):153. https://doi.org/10.1186/s13756-020-00819-1
26. Amin A, Vartanian A, Poladian N, Voloshko A, Yegiazaryan A, Al-Kassir AL, Venketaraman V (2021) Root causes of fungal coinfections in COVID-19 infected patients. Infect Dis Rep 13(4):1018–1035. https://doi.org/10.3390/idr13040093
27. Chakraborti A, Jaiswal A, Verma PK, Singhal R (2018) A prospective study of fungal colonization and invasive fungal disease in long-term mechanically ventilated patients in a respiratory intensive care unit. Indian J Crit Care Med 22(8):597–601. https://doi.org/10.4103/ijccm.IJCCM_181_18
28. Gysin M, Acevedo CT, Haldimann K, Bodendoerfer E, Imkamp F, Bulut K, Buehler PK, Brugger SD, Becker K, Hobbie SN (2021) Antimicrobial susceptibility patterns of respiratory Gram-negative bacterial isolates from COVID-19 patients in Switzerland. Ann Clin Microbiol Antimicrob 20(1):64. https://doi.org/10.1186/s12941-021-00468-1
29. Lai CC, Chen SY, Ko WC, Hsueh PR (2021) Increased antimicrobial resistance during the COVID-19 pandemic. Int J Antimicrob Agents 57(4):106324. https://doi.org/10.1016/j.ijantimicag.2021.106324
30. WHO 2021 AWaRe classification (2021). https://www.who.int/publications/i/item/2021-aware-classification. Accessed March 16, 2022.
31. Cong W, Poudel AN, Alhusein N, Wang H, Yao G, Lambert H (2021) Antimicrobial use in COVID-19 patients in the first phase of the SARS-CoV-2 pandemic: a scoping review. Antibiotics (Basel) 10 (6). DOI: https://doi.org/10.3390/antibiotics10060745
32. King LM, Lovegrove MC, Shehab N, Tsay S, Budnitz DS, Geller AI, Lind JN, Roberts RM, Hicks LA, Kabbani S (2021) Trends in US outpatient antibiotic prescriptions during the coronavirus disease 2019 pandemic. Clin Infect Dis 73(3):e652–e660. https://doi.org/10.1093/cid/ciaa1896
33. Kitano T, Brown KA, Daneman N, MacFadden DR, Langford BJ, Leung V, So M, Leung E, Burrows L, Manuel D, Bowdish DME, Maxwell CJ, Bronskill SE, Brooks JI, Schwartz KL (2021) The impact of COVID-19 on outpatient antibiotic prescriptions in Ontario, Canada: an interrupted time series analysis. Open Forum Infect Dis 8 (11):ofab533. DOI: https://doi.org/10.1093/ofid/ofab533
34. Zhu N, Aylin P, Rawson T, Gilchrist M, Majeed A, Holmes A (2021) Investigating the impact of COVID-19 on primary care antibiotic prescribing in North West London across two epidemic waves. Clin Microbiol Infect 27(5):762–768. https://doi.org/10.1016/j.cmi.2021.02.007
35. Humphreys H, Fitzpatick F, Harvey BJ (2015) Gender differences in rates of carriage and bloodstream infection caused by methicillin-resistant Staphylococcus aureus: are they real, do they matter and why? Clin Infect Dis 61(11):1708–1714. https://doi.org/10.1093/cid/civ576
36. Karruli A, Boccia F, Gagliardi M, Patauner F, Ursi MP, Sommese P, De Rosa R, Murino P, Ruocco G, Corcione A, Andini R, Zampino R, Durante-Mangoni E (2021) Multidrug-resistant infections and outcome of critically Ill patients with coronavirus disease 2019: a single center experience. Microb Drug Resist 27(9):1167–1175. https://doi.org/10.1089/mdr.2020.0489
37. Mulet Bayona JV, Tormo Palop N, Salvador García C, Fuster Escrivá B, Chanzá Aviñó M, Ortega García P, Gimeno Cardona C (2021) Impact of the SARS-CoV-2 pandemic in Candidaemia, invasive Aspergillosis and antifungal consumption in a tertiary hospital. J Fungi (Basel) 7 (6). DOI: https://doi.org/10.3390/jof7060440
38. Perfect JR (2017) The antifungal pipeline: a reality check. Nat Rev Drug Discov 16(9):603–616. https://doi.org/10.1038/nrd.2017.46
39. Pfaller MA, Diekema DJ, Gibbs DL, Newell VA, Ellis D, Tullio V, Rodloff A, Fu W, Ling TA, Group GAS (2010) Results from the ARTEMIS DISK Global antifungal surveillance study, 1997 to 2007: a 10.5-year analysis of susceptibilities of Candida species to fluconazole and voriconazole as determined by CLSI standardized disk diffusion. J Clin Microbiol 48 (4):1366–1377. DOI: https://doi.org/10.1128/JCM.02117-09
40. Shields RK, Nguyen MH, Press EG, Cumbie R, Driscoll E, Pasculle AW, Clancy CJ (2015) Rate of FKS mutations among consecutive Candida isolates causing bloodstream infection. Antimicrob Agents Chemother 59(12):7465–7470. https://doi.org/10.1128/AAC.01973-15