Multidrug-Resistant Bacterial Colonization and Infections in Large Retrospective Cohort of Mechanically Ventilated COVID-19 Patients[1]

Davide Mangioni, Liliane Chatenoud, Jacopo Colombo, Emanuele Palomba, Fernando A. Guerrero, Matteo Bolis, Nicola Bottino, Giuseppe Breda, Maria V. Chiaruttini, Gabriele Fior, Manuela Marotta, Giovanni Massobrio, Caterina Matinato, Antonio Muscatello, Paola Previtali, Sara Santambrogio, Francesca Tardini, Gianluca Zuglian, Giacomo Grasselli, Roberto Fumagalli, Andrea Gori, Nino Stocchetti, Gianpaola Monti,[2] Alessandra Bandera,[2] and the MDR in FIERA Study Group[3]

Few data are available on incidence of multidrug-resistant organism (MDRO) colonization and infections in mechanically ventilated patients, particularly during the COVID-19 pandemic. We retrospectively evaluated all patients admitted to the COVID-19 intensive care unit (ICU) of Hub Hospital in Milan, Italy, during October 2020–May 2021. Microbiologic surveillance was standardized with active screening at admission and weekly during ICU stay. Of 435 patients, 88 (20.2%) had MDROs isolated ≤48 h after admission. Of the remaining patients, MDRO colonization was diagnosed in 173 (51.2%), MDRO infections in 95 (28.1%), and non-MDRO infections in 212 (62.7%). Non-MDRO infections occurred earlier than MDRO infections (6 days vs. 10 days; p<0.001). Previous exposure to antimicrobial drugs within the ICU was higher in MDRO patients than in non-MDRO patients (116/197 [58.9%] vs. 18/140 [12.9%]; p<0.001). Our findings might serve as warnings for future respiratory viral pandemics and call for increased measures of antimicrobial stewardship and infection control.

Bacterial superinfections represent a major threat for patients in intensive care units (ICUs), severely affecting clinical course and length of hospital stay. The COVID-19 pandemic caused an unprecedented rate of ICU admissions and drastically changed ICU care itself, in terms of infection control measures and therapeutic usage of steroids and immunomodulating drugs. The percentages of hospital-acquired infections (HAIs) in COVID-19 patients vary widely, ranging from 7% to 13% in nonintensive hospital wards and up to 45% in ICUs (*1*–*3*).

Several studies have assessed the burden of multidrug-resistant organisms (MDROs) in COVID-19 patients admitted to ICUs, reporting heterogeneous results with prevalence ranging from 11% to 50% and incidence rate from 4.5 cases/1,000 patient-days to 30 cases/1,000 patient-days (*4*–*21*). However, studies published so far have relevant limitations, often not clearly discriminating between colonization and infection (*8*,*9*,*11*,*12*), and either including small

Author affiliations: Foundation IRCCS Ca' Granda Ospedale Maggiore Policlinico di Milano, Milan, Italy (D. Mangioni, E. Palomba, M. Bolis, N. Bottino, G. Breda, G. Massobrio, C. Matinato, A. Muscatello, G. Zuglian, G. Grasselli, A. Gori, N. Stocchetti, A. Bandera); University of Milan, Milan (D. Mangioni, E. Palomba, G. Grasselli, A. Gori, N. Stocchetti, A, Bandera); Istituto di Ricerche Farmacologiche Mario Negri IRCCS, Milan (L. Chatenoud, M.V. Chiaruttini); Harvard Medical School, Boston, Massachusetts, USA (J. Colombo); ASST Grande Ospedale Metropolitano Niguarda, Milan (J. Colombo, F.A. Guerrero, G. Fior, M. Marotta, P. Previtali, S. Santambrogio, F. Tardini, R. Fumagalli, G. Monti); ULSS 3 Serenissima, Venice, Italy (G. Zuglian); University of Milano-Bicocca, Milan (R. Fumagalli)

DOI: https://doi.org/10.3201/eid2908.230115

[1]Preliminary results were presented at the European Congress of Clinical Microbiology and Infectious Diseases, 2022, Lisbon, Portugal, April 23–26, 2022 (abstract no. 02641).

[2]These senior authors contributed equally to this article.

[3]Study group members are listed at the end of this article.

populations or showing heterogeneity in clinical settings and microbiologic surveillance procedures when describing larger pool of persons, such as in multicentric studies (*18*–*20*).

Our study was conducted to address the need for further evidence on incidence and etiology of MDRO colonization and infections in mechanically ventilated COVID-19 patients. We analyzed clinical and microbiologic data systematically collected in a large ICU in northern Italy.

Methods

Study Design and Setting

We conducted a retrospective cohort study on routinely collected data of COVID-19 patients admitted to the Milano Fiera ICU during October 23, 2020–May 31, 2021. This ICU was a large COVID-19 ICU developed in Milan, Italy, to face the effect of the pandemic. It admitted patients who had SARS-CoV-2 infection requiring mechanical ventilation from different healthcare settings: emergency department, nonintensive hospital wards, and other ICUs. This ICU could accommodate up to 100 patients divided into distinct units (modules) managed by ICU staff from different hospitals. Microbiologic surveillance was standardized and consisted of perineal and nasal swab specimens for MDROs and endotracheal aspirate cultures obtained at ICU admission and then once (perineal and nasal swab specimens) or twice (endotracheal aspirate) a week. All modules referred to the IRCCS Ca' Granda Ospedale Maggiore Policlinico Foundation for laboratory and microbiologic analyses and for infectious diseases specialist consultation.

Study Participants and Data Collection

All consecutive patients who had laboratory-confirmed SARS-CoV-2 infection and were admitted to the ICU were considered for inclusion. Exclusion criteria were age <18 years, length of mechanical ventilation <48 h, and lack of comprehensive clinical documentation. We collected demographic, clinical, laboratory, and outcome data from clinical records and microbiologic and therapeutic data from dedicated hospital databases (Appendix, https://wwwnc.cdc.gov/EID/article/29/8/23-0115-App1.pdf). The study was registered by the Milan Area 2 Ethical Committee (#701_2021) and was conducted in accordance with standards of the Helsinki Declaration. Written informed consent was waived because of the retrospective nature of the analysis. The study was retrospectively registered at clinicaltrials.gov on March 24, 2022 (identifier: NCT05293418).

Microbiologic Data Processing

For each patient, we retrieved bacterial isolates from a microbiology database, which were independently reviewed by dedicated intensivists and infectious disease specialists and classified as contamination, colonization, or infection, according to international guidelines (Appendix) (*22*,*23*). In brief, infections were defined by the presence of a major bacterial load associated with clinical manifestations within the infection window period (±3 days from specimen collection) (*22*,*23*), Isolates were classified as colonization when no adverse clinical signs or symptoms were documented. We defined contamination as all microbiologic isolates that did not meet the criteria of infection or colonization and that were listed in the US Centers for Disease Control and Prevention National Healthcare Safety Network (https://www.cdc.gov/nhsn/index.html) list of common commensals. We retained only the first species-specific MDRO colonization of each patient for further analysis.

We distinguished new infectious episodes from persistent infections according to the European Centre for Disease Prevention and Control definitions (*23*). We stratified infection episodes as infection without sepsis, sepsis or septic shock according to Sepsis-3 criteria (*24*). We defined secondary bloodstream infections (BSIs) by using the secondary BSI attribution period according to the Centers for Diseases Control and Prevention National Healthcare Safety Network (*22*). We also defined isolates as MDROs when they were nonsusceptible to ≥1 agents in ≥3 antimicrobial drug categories (*25*) or when harboring specific antimicrobial drug resistance mechanisms (e.g., methicillin-resistant *Staphylococcus* spp., vancomycin-resistant *Enterococcus* spp., extended-spectrum β-lactamase/AmpC/carbapenemases–producing Enterobacterales) by using rapid detection methods (*4*).

Statistical Analysis

We reported patient characteristics overall and for selected groups of interest, such as MDROs acquired before/after ICU admittance and MDRO infection/colonization. Medians (interquartile range [IQRs]) are reported for continuous variables and numbers (percentages) for categorical variables. We calculated crude incidence rates (IRs) per 1,000 patient-days and relative 95% CIs, considering for each patient any first species-specific MDRO colonization or each new MDRO/non-MDRO HAI (*26*). We used SAS version 9.4 software (SAS Institute, https://www.sas.com) for statistical analysis (Appendix).

Results

Population Description

A total of 451 patients from 46 different hospitals were admitted to ICUs during October 2020–May 2021. Of those, 435 were included in the analysis. We provide details of the patient selection process (Figure 1) and trends of patient admission by referring hospital per month (Appendix Figure 1).

Only 12/435 patients (2.7%) were reported to have MDRO colonization/infection before ICU admission. In 88/435 patients (20.2%), MDRO were isolated within 48 h upon entry to the ICU ($MDR_{\leq 48h}$), and those patients were similarly distributed between referring hospitals (Appendix Figure 2). This group was composed of 78 colonizations and 10 infections; 35/78 (44.9%) colonized patients subsequently had MDRO infections develop. Compared with the 347 patients who had no evidence of MDRO during the first 48 hours of ICU stay (no-MDR+$MDR_{>48h}$), the $MDR_{\leq 48h}$ group was characterized by higher admittance from other ICUs and lower admittances from emergency departments (ICU 31/88 [35.2%] in $MDR_{\leq 48h}$ vs. 86/347 [24.8%]) in no-MDR+$MDR_{>48h}$;

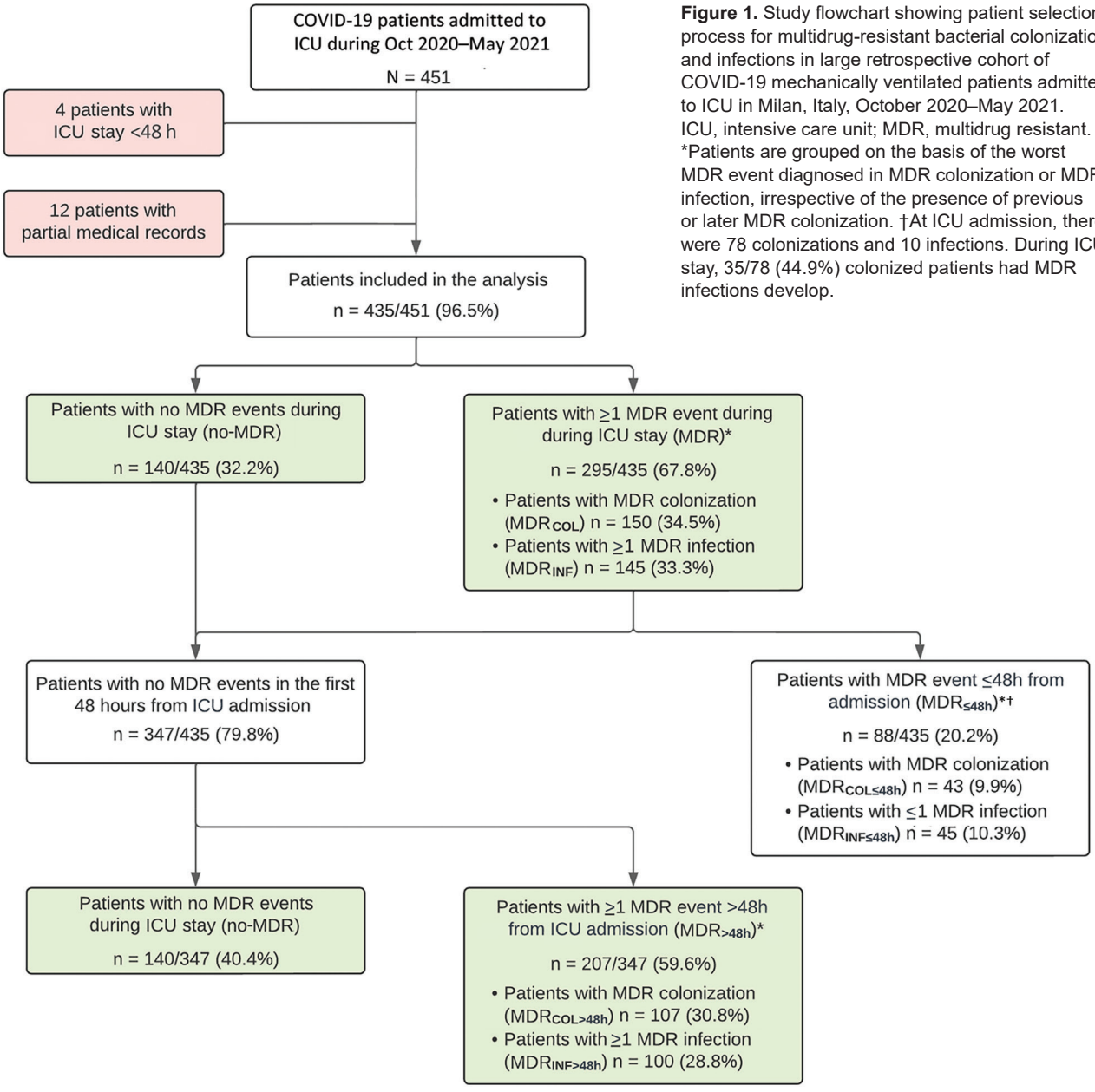


Figure 1. Study flowchart showing patient selection process for multidrug-resistant bacterial colonization and infections in large retrospective cohort of COVID-19 mechanically ventilated patients admitted to ICU in Milan, Italy, October 2020–May 2021. ICU, intensive care unit; MDR, multidrug resistant. *Patients are grouped on the basis of the worst MDR event diagnosed in MDR colonization or MDR infection, irrespective of the presence of previous or later MDR colonization. †At ICU admission, there were 78 colonizations and 10 infections. During ICU stay, 35/78 (44.9%) colonized patients had MDR infections develop.

emergency department 15/88 [17.1%] in $MDR_{\leq 48h}$ vs. 102/347 [29.4%] in no-MDR+$MDR_{>48h}$). The $MDR_{\leq 48h}$ group showed slightly longer (although not significantly) length of stay in the ICU of origin than patients who developed MDRO events later during their stay and to no-MDR patients (medians 11.5, 9, and 7 days, respectively; p = 0.09). The $MDR_{\leq 48h}$ group was also characterized by a larger amount of antimicrobial drug intake before ICU admission (no antimicrobial drug in 25/88 [28%] of $MDR_{\leq 48h}$ vs. 126/327 [36.3%] of no-MDR+$MDR_{>48h}$; ≥3 classes of antimicrobial drugs in 12/88 [13.6%] of $MDR_{\leq 48h}$ vs. 23/347 [6.6%] of no-MDR+$MDR_{>48h}$). We compiled demographic and clinical characteristics by groups (Appendix Table 1) and duration between hospitalization and transfer to the ICU on the basis of patients' setting of provenance (Appendix Table 2).

Of the 347 patients who had no MDRO isolates within the first 48 hours from ICU admission, 207 (67.5%) had ≥1 MDRO event ($MDR_{>48h}$); 107 (30.8%) patients had MDRO colonization only ($MDR_{COL>48h}$) and 100 (28.8%) had ≥1 MDRO infection ($MDR_{INF>48h}$) (Figure 1). We compiled patient characteristics and outcomes (Table 1, https://wwwnc.cdc.gov/EID/article/29/8/23-0115-T1.htm) overall and for no-MDR and $MDR_{>48h}$ patients, further stratified as $MDR_{COL>48h}$ and $MDR_{INF>48h}$. Median age was 65 years (IQR 59–71 years); 95/347 (27.4%) patients were female. More than 80% of patients had ≥1 concurrent condition, and hypertension was the most common (181/347, 52.2%). Patients who had ever smoked were more frequent in the $MDR_{INF>48h}$ group (26/100, 26%) than in the $MDR_{COL>48h}$ group (11/107, 10.3%; p = 0.003). Transfer to the ICU occurred mostly from nonintensive hospital wards (159/347, 45.8%), but relevant proportions were transferred directly from the emergency department (102/347, 29.4%) or from other ICUs (86/347, 24.8%). Patients were transferred to ICU early during hospitalization, a median time of 5 days from first hospital admittance.

Groups did not differ for steroid use or antimicrobial drug therapies received before ICU admission. According to clinical practice, steroids had been administered for SARS-CoV-2 infection management in 252/347 (72.6%) patients, mostly (228/347, 65.7%) with only a standard dose (dexamethasone 6 mg/d). Most patients (221/347, 63.7%) had previously received antimicrobial drugs before ICU admission. MDRO events before ICU admission were reported in only 4 patients (1.2%). During ICU stay, 118 patients (34%) died, but there were no significant differences between groups. When compared with no-MDR patients, we found that $MDR_{>48h}$ patients had a longer duration of mechanical ventilation (median 18 vs. 14 days; p = 0.001) and of ICU stay (median 25 vs. 15.5 days; p = 0.001). Those differences were largely caused by the $MDR_{INF>48h}$ group (Table 1).

Bacterial Isolate Description and Incidence

Complete microbiologic reports were available for 426/435 patients, including 338/347 patients (97.4%) with no MDRO isolates within the first 48 hours of ICU admission. We describe the selection process conducted to assess incidences of HAIs and of MDRO events distinguishing between colonization and infection (Figure 2). We identified 801 bacterial isolates from 271 patients that correspond to first MDRO colonization (255 isolates in 173/338 patients, 51.2%) and new episodes of bacterial superinfections, either by MDRO (130 isolates in 95/338 patients, 28.1%) or antimicrobial drug–susceptible bacteria (non-MDRO, 416 isolates in 212/338 patients, 62.7%). A total of 73 (21.6%) patients had both MDRO colonization and MDRO infection develop during ICU stay, and infections were caused by the same colonizing bacterial species in nearly one third of them (24/73, 32.9%) (Appendix Table 3). Clinical interpretation of bacterial isolates as colonization/infection by attending physicians at the time of arrival of microbiologic results was found to be highly concordant with the retrospective evaluation conducted according to international guidelines (κ coefficient 0.902, 95% CI 0.890–0.913) (Appendix Table 4).

Overall, 546 bacterial HAIs were recorded, 130 (23.8%) caused by MDRO. Gram-negative bacteria accounted for 59.7% (326/546) of all HAIs and for 60% (78/130) of infections caused by MDROs. Bacterial species responsible for HAIs varied by infection site and severity of infection (Appendix Tables 5, 6). Ventilator-associated lower respiratory tract infections (VALRTIs) represented most infectious episodes (359/546, 65.7%), followed by BSI (141, 25.8%) and urinary tract infections (40, 7.3%). Among BSIs, 31/141 (22%) were associated with a central line, 43 (30.5%) were secondary to VALRTI or urinary tract infections, and the remaining 67 (47.5%) were classified as primary BSI without a known bacteremic focus (Appendix Figure 3).

Among MDRO colonization, *Enterococcus faecium* (112/255 isolates, 43.9%) was the most frequent isolate, followed by *Klebsiella* spp. (34, 13.3%), *Escherichia coli* (26, 10.2%), *Staphylococcus aureus* (25, 9.8%), *Pseudomonas aeruginosa* (15, 5.9%) and *Acinetobacter baumannii* (13, 5.1%). We compiled the percentages of MDRO colonization, MDRO HAIs, and non-MDRO

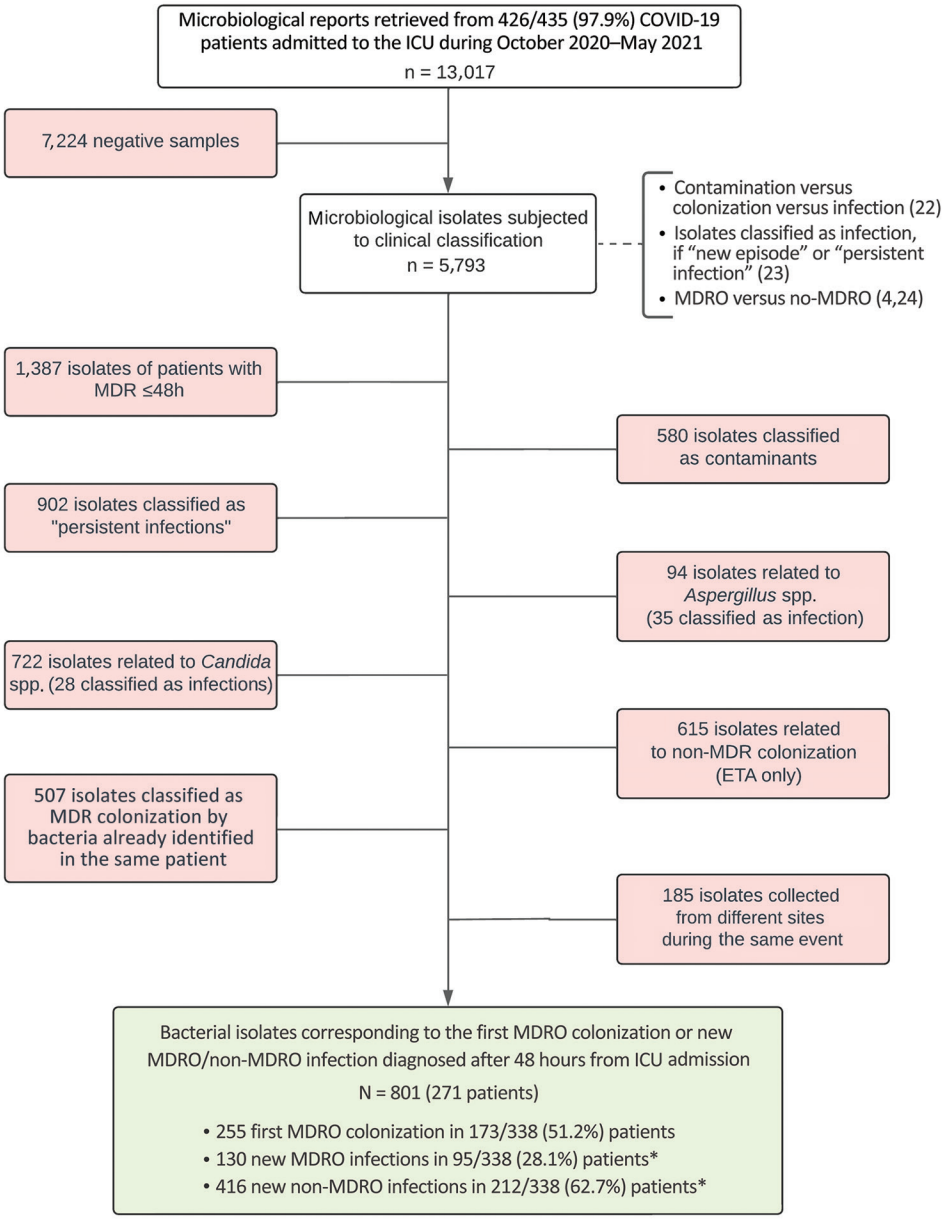


Figure 2. Study flowchart showing microbial isolates selection process for multidrug-resistant bacterial colonization and infections in large retrospective cohort of COVID-19 mechanically ventilated patients admitted to ICU in Milan, Italy, October 2020–May 2021. ETA, emergency treatment area; ICU, intensive care unit; MDR, multidrug resistant; MDRO, MDR organism. *Of 338 patients, 159 (47.0%) had either MDRO or non-MDRO infections; 74/338 (21.9%) had both MDRO and non-MDRO infections.

HAIs for the most frequently isolated bacteria of the World Health Organization priority pathogens list (27) (Appendix Figure 4).

First MDRO colonization occurred at a median time of 13 (IQR 8–12) days after ICU admission. HAIs caused by antimicrobial drug–susceptible bacteria occurred earlier than in those caused by MDROs at 6 (IQR 3–10) and 10 (IQR 6–17) days from admission ($p<0.001$) (Figure 3). The incidence rates for MDRO colonization was 29.97 cases/1,000 patient-days (95% CI 26.34–34.10), for MDRO infection was 14.99 cases/1,000 patient-days (95% CI 12.36–18.19), and for non-MDRO infection, was 50.12 cases/1,000 patient-days (95% CI 44.59–56.32). Infection rates varied substantially by infection site (Table 2).

Association of Antimicrobial Drugs and Steroids to MDRO Events

We investigated possible associations between MDRO events and previous steroid and antimicrobial drug therapies (Appendix Tables 7, 8). Because steroids were included in the management of COVID-19 pneumonia from the early stage of the disease, we evaluated their intake before and during ICU stay. Almost the entire population had received steroid therapy (313/338, 92.6%), without major differences

between no-MDR (132/140, 94.3%), $MDR_{COL>48h}$ (98/103, 95.1%) and $MDR_{INF>48h}$ (83/95, 87.4%) (Appendix Table 7).

To assess possible association between MDRO events and previous antimicrobial drug use, we focused on therapies administered during the first 10 days of ICU stay. This timeline was set to balance observation time between no-MDR and $MDR_{>48h}$ groups because three fourths of MDRO events occurred within this timeframe. Also, three fourths of patients in no-MDR group stayed in ICU ≥10 days (Appendix Table 8). Previous exposure to antimicrobial drugs was notably higher in patients who developed MDRO events than in patients who did not (116/197 [58.9%] in $MDR_{>48h}$ vs 18/140 [12.9%] in no-MDR; p<0.001) (Appendix Table 8).

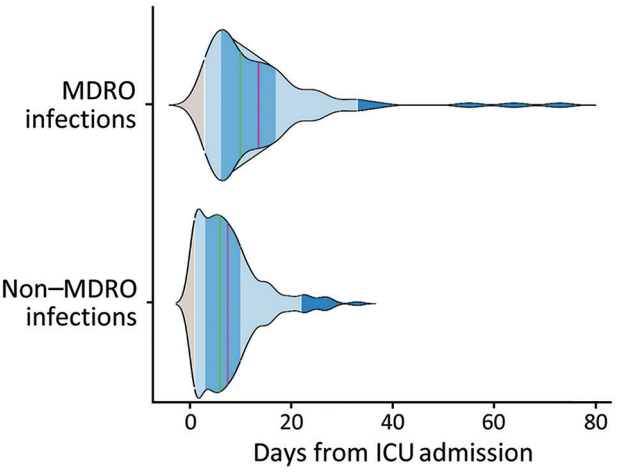


Figure 3. Multidrug-resistant bacterial colonization and infections in large retrospective cohort of COVID-19 mechanically ventilated patients admitted to ICU in Milan, Italy, October 2020–May 2021. Kernel density plot (violin plot) shows healthcare-associated infections by onset time comparing MDRO with non-MDRO. Red lines indicate mean and green lines median onset times; medium blue shading indicates interquartile ranges, and the light blue shading indicates 95% CIs of the mean (p<0.001 by Wilcoxon rank-sum test). ICU, intensive care unit; MDRO, multidrug-resistant organism.

Discussion

We describe incidences and clinical characteristics of HAIs and MDRO events, distinguishing between colonization and infection, in a large cohort of ICU COVID-19 patients from a country with high prevalence of MDRO (*28*). Despite being composed of patients admitted from >45 different hospitals, our cohort is homogeneous for concurrent conditions and risk factors for MDRO acquisition, clinical severity of COVID-19, management of antimicrobial drug therapy, and infection prevention and control strategies within the ICU, including surveillance sampling.

Antimicrobial drug resistance represents a major challenge in the ICU. Its occurrence is the result of the influx of previously colonized patients and acquisition of MDROs during ICU stay, as a consequence of antimicrobial drug overexposure and interpatient transmission, as well as contact with colonized healthcare workers, fomites, or the environment. The incidence of MDROs is strongly influenced by pandemic periods, such as during COVID-19, when unprecedented patient loads in ICUs resulted in breaches in IPC, such as gaps in microbiologic surveillance, lack of communication between clinicians, and reduced attention to environmental measures and contact precautions among healthcare workers (*29*). In addition, ICU admissions caused by viral pandemics place a strain on ICU resources, requiring the reallocation of non-ICU beds, along with the use of non-ICU staff to meet the urgent demand. In this setting, strengthening measures, such as active surveillance with prompt recognition of outbreaks, staff training, increased environmental disinfection and cohorting, become essential to reducing MDRO circulation (*30*).

In the pre–COVID-19 pandemic era, the prevalence of infections caused by MDROs in ICU patients varied from a reported rate of 14.1% in VALRTIs acquired in ICUs in North America (*31*) to an average

Table 2. Incidence rate of MDRO events, overall and divided by infection site, of COVID-19 patients admitted to ICU in Milan, Italy, October 2020–May 2021, who had no MDRO isolates within the first 48 h of admission*

Characteristic	Infections			
	VALRTIs	BSIs	UTIs	Total
MDRO events, first colonization plus new infections	NA	NA	NA	41.68 (36.98–46.99)
First MDRO colonization	NA	NA	NA	29.97 (26.34–34.1)
New MDRO infection	9.44 (7.58–11.74)	4.89 (3.55–6.75)	0.47 (0.14–1.08)	14.99 (12.36–18.19)
New non-MDRO infection	33.25 (29.04–38.07)	11.62 (9.23–14.64)	4.19 (2.97–5.72)	50.12 (44.59–56.32)
Overall new infections, MDRO plus non-MDRO	42.41 (37.81–47.58)	16.57 (13.51–20.31)	5.15 (3.36–6.26)	65.13 (58.76–72.2)

*Values are IR/1,000 person-days (95% CIs). The time considered for IRs was set from ICU admission to discharge, except for VALRTI, where total intubation time was considered. BSIs, bloodstream infections; ICU, intensive care unit; IR, incidence rate; MDRO, multidrug-resistant organism; NA, not applicable (MDRO colonization refers to patients and not infection sites); UTIs, urinary tract infections; VALRTIs, ventilator-associated lower respiratory tract infections.

of >40% in 2 large multicentric worldwide studies of nosocomial BSIs (*32*,*33*). Variability exists between participating countries, ranging from 8% (Australia) to >75%–80% in Asia, eastern Europe, and southern Europe. Carbapenem resistance was present in more than one third of gram-negative bacteria, and 36% of all gram-positive bacteria were MDR (*32*,*33*).

Several studies have been published on MDRO incidence, etiology and source of HAIs in ICU COVID-19 patients (*4*–*21*) (Table 3, https://wwwnc.cdc.gov/EID/article/29/8/23-0115-T3.htm). Most of those studies evaluated overall MDRO infections or specific HAIs, such as BSI or VALRTIs (*7*,*15*–*17*,*19*,*21*), whereas colonization events were assessed in only a few studies (*8*–*12*,*14*). Incidence measures of MDRO events varied widely; cumulative incidence of the first MDRO event was 5%–57% (*7*,*17*) and incidence rate 2.6–31.48 cases/1,000 patient-days (*11*,*16*). The percentage of MDRO was 27%–100% for all recorded events (*15*,*17*). Compared with the amount of literature evaluating MDRO events during ICU stay, we found that few data are available on MDRO proportions among COVID-19 patients at ICU admission. In recent work of the multicenter HAI-ICU surveillance network in France, the percentage of MDR gram-negative bacteria among >4,000 COVID-19 patients admitted was 11.7% (*34*).

In our cohort, 20% of patients had MDRO isolation within the first 48 hours, indicating acquisition before ICU admittance. We found that patients who had MDROs isolated during the first 48 hours were more frequently transferred from other ICUs and exposed to a higher number of antimicrobial drugs before ICU admission. Both of those factors are well known to be associated with development of infections by antimicrobial drug–resistant pathogens (*6*). Only 2.7% of our cohort had MDRO colonization/infection before ICU admission. The marked difference between expected and observed MDRO prevalence at ICU admission probably reflects the major issues in IPC during the emergency situation of the pandemic mentioned beforehand.

Considering patients without MDRO isolation within the first 48 hours, we observed no differences in demographic characteristics or in clinical severity at admission between patients who showed or not showed development of MDRO events during ICU stay, underlying consistency between groups at ICU admission. In our cohort, we did not find direct association between MDRO infection and in-ICU deaths. However, length of ICU stay and duration of mechanical ventilation were longer for patients with MDRO events and, among them, longer for patients who had infections than for colonized patients. No causative effect can be drawn from these results because occurrence of MDRO events could be either responsible for longer ICU stay or its direct consequence because of longer exposure time (*35*,*36*).

Active surveillance screening coupled with the evaluation of all microbial isolates enabled us to precisely identify patients who had with MDRO events. Two thirds of the cohort showed development of MDRO colonization or infection during ICU stay. Half of our patients were given diagnoses of MDRO colonization during ICU stay, compared with 21% observed in a recent study analyzing a smaller population (*10*). Our results can be, in part, explained by strict routine microbiologic surveillance, which enabled prompt and precise recognition of such cases. Data from previous studies on bacterial superinfections in COVID-19 ICU patients are heterogenous and describe MDRO HAIs in 11%–250% of the population (*6*,*13*). Our results confirm the substantial risk for mechanically ventilated COVID-19 patients to have MDRO infections develop; such infections affected almost 30% of our cohort during ICU stays. Also, more than twice as many patients had antimicrobial drug–susceptible HAIs.

We found high concordance between clinical diagnosis and retrospective evaluation of HAIs according to literature criteria. We believe this result well demonstrates how implementation of structured antimicrobial stewardship and IPC measures, with collaboration of infectious disease consultants and intensivists, can strongly effect management of critically ill patients, favoring accurate diagnosis and therapeutic choices, according to international guidelines.

Patients who had MDRO events had greater exposure to antimicrobial drugs the first 10 days of ICU stay than patients who had no MDRO findings. This observation is consistent with results of recent studies conducted on large population of patients, which showed major associations between exposure to specific antimicrobial drug classes and drug resistance, and a decreasing pattern over time (*37*,*38*). However, accurate analysis of the association between antimicrobial drug exposure and MDRO events was beyond the scope of this study because other variables, such as average intake time of each antimicrobial drug class and infections with antimicrobial drug–susceptible bacteria during the observation time, should be considered.

The first limitation of this study is that it was a retrospective monocentric cohort and, therefore, had intrinsic risks of limited accuracy and generalizability. However, interpretation of all microbiologic findings has been conducted ex post on the basis of standardized literature criteria and independent from the

physicians' view. Also, even though the study was monocentric, patients were admitted from >45 hospitals and assisted by different hospital staff. Advantages to this study design derive from the standardized microbiologic surveillance, both in terms of timing and laboratory method, as well as from homogeneous antimicrobial stewardship and IPC strategies among ICU modules. This factor enabled us to provide precise and consistent data in terms of incidence of HAIs and MDRO events, not only infections but also colonization.

Second, this study was not conducted for evaluation of the effect of antimicrobial drugs on development of MDRO or the effect of MDRO events on ICU deaths and length of stay; the sample size was probably inadequate for these issues. Therefore, our findings on this issue should be interpreted with caution.

Third, patients' data before ICU admission were retrieved from information registered at ICU entry and not from hospital databases of the single referring centers. Accuracy of previous MDRO events and steroids and antimicrobial drug treatments might be limited, although these factors play a major role in routine management of ICU patients, and we do not expect major gaps in data acquisition.

In conclusion, our in-depth analysis of incidence measures of HAIs and MDRO events contributes to increase knowledge of MDRO colonization and infections in ICU COVID-19 patients. These findings should be a priority in contributing toward IPC and antimicrobial stewardship policies for ensuring the best clinical care.

MDR in FIERA study group: Valeria Pastore, Mara Tomasello, Lisa Cariani, Anna Grancini, Anna Maraschini (Foundation IRCCS Ca' Granda Ospedale Maggiore Policlinico, Milan, Italy); Teresio Arazzi (Rho Hospital, Rho, Italy); Alessandro Protti (Humanitas Clinical and Research Center IRCCS, Rozzano, Milan, Italy); Virginia Porta (Legnano Hospital, Legnano, Italy); Marco Dei Poli (San Donato Hospital, Milano, Italy); Paolo Severgnini (Varese Hospital, Varese, Italy); Egle Rondelli (San Gerardo Hospital, Monza, Italy).

This study was partially supported by the Italian Ministry of Health and projects STOP-COVID and PREP-COVID.

Deidentified patient data used for the results reported in this article, including data in text, tables, figures, and appendixes, will be available to researchers who provide a methodologically sound proposal to achieve their aims. Proposals should be addressed to andrea.gori@unimi.it and davide.mangioni@policlinico.mi.it. To gain access, data applicants will need to sign a data access agreement.

D.M., J.C., G.Ma., and A.B. designed the study; L.C. and M.C.V. performed methods and formal analysis; D.M., J.C., E.P., F.A.G., M.B., B.B., M.C., G.F., M.M., G.Mo, P.P., S.S., F.T., and G.Z. performed investigations; D.M. and E.P. wrote the original draft of the paper, wrote, reviewed, and edited the paper, and performed a literature review; L.C., J.C., F.A.G., M.B., N.B., B.B., M.C., G.F., M.M., G.Ma., C.M., A.M., P.P., S.S., F.T., G.Z., G.G., R.F., A.G., N.S., G.Mo., and A.B. wrote, reviewed, and edited the paper; and N.B., N,S., G.Ma., and A.B. supervised the study. All authors have read and agreed to the published version of the manuscript.

About the Author

Dr. Mangioni is an assistant professor at the University of Milan and Consultant in Infectious Diseases at the Foundation IRCCS Ca' Granda Ospedale Maggiore Policlinico di Milano, Milan, Italy. His primary research interests are infections in critically ill patients and infections caused by multidrug-resistant organisms.

References

1. Lansbury L, Lim B, Baskaran V, Lim WS. Co-infections in people with COVID-19: a systematic review and meta-analysis. J Infect. 2020;81:266–75. https://doi.org/10.1016/j.jinf.2020.05.046
2. Pasero D, Cossu AP, Terragni P. Multi-drug resistance bacterial infections in critically ill patients admitted with COVID-19. Microorganisms. 2021;9:1773. https://doi.org/10.3390/microorganisms9081773
3. Russell CD, Fairfield CJ, Drake TM, Turtle L, Seaton RA, Wootton DG, et al.; ISARIC4C investigators. Co-infections, secondary infections, and antimicrobial use in patients hospitalised with COVID-19 during the first pandemic wave from the ISARIC WHO CCP-UK study: a multicentre, prospective cohort study. Lancet Microbe. 2021;2:e354–65. https://doi.org/10.1016/S2666-5247(21)00090-2
4. Grasselli G, Scaravilli V, Mangioni D, Scudeller L, Alagna L, Bartoletti M, et al. Hospital-acquired infections in critically ill patients with COVID-19. Chest. 2021;160:454–65. https://doi.org/10.1016/j.chest.2021.04.002
5. Ramos R, de la Villa S, García-Ramos S, Padilla B, García-Olivares P, Piñero P, et al. COVID-19 associated infections in the ICU setting: a retrospective analysis in a tertiary-care hospital. Enferm Infecc Microbiol Clin. 2021.
6. Karruli A, Boccia F, Gagliardi M, Patauner F, Ursi MP, Sommese P, et al. Multidrug-resistant infections and outcome of critically ill patients with coronavirus disease 2019: a single center experience. Microb Drug Resist. 2021;27:1167–75. https://doi.org/10.1089/mdr.2020.0489
7. Palanisamy N, Vihari N, Meena DS, Kumar D, Midha N, Tak V, et al. Clinical profile of bloodstream infections in COVID-19 patients: a retrospective cohort study. BMC Infect Dis. 2021;21:933. https://doi.org/10.1186/s12879-021-06647-x
8. Baiou A, Elbuzidi AA, Bakdach D, Zaqout A, Alarbi KM, Bintaher AA, et al. Clinical characteristics and risk factors for the isolation of multi-drug-resistant Gram-negative bacteria from critically ill patients with COVID-19. J Hosp

Infect. 2021;110:165–71. https://doi.org/10.1016/j.jhin.2021.01.027
9. Bogossian EG, Taccone FS, Izzi A, Yin N, Garufi A, Hublet S, et al. The acquisition of multidrug-resistant bacteria in patients admitted to COVID-19 intensive care units: a monocentric retrospective case–control study. Microorganisms. 2020;8:1–11. https://doi.org/10.3390/microorganisms8111821
10. Fernández P, Moreno L, Yagüe G, Andreu E, Jara R, Segovia M. Colonization by multidrug-resistant microorganisms in ICU patients during the COVID-19 pandemic. Med Intensiva [English Ed]. 2021;45:313–5.
11. Pascale R, Bussini L, Gaibani P, Bovo F, Fornaro G, Lombardo D, et al. Carbapenem-resistant bacteria in an intensive care unit during the coronavirus disease 2019 (COVID-19) pandemic: a multicenter before-and-after cross-sectional study. Infect Control Hosp Epidemiol. 2022;43:461–6. https://doi.org/10.1017/ice.2021.144
12. Temperoni C, Caiazzo L, Barchiesi F. High prevalence of antibiotic resistance among opportunistic pathogens isolated from patients with COVID-19 under mechanical ventilation: results of a single-center study. Antibiotics (Basel). 2021;10:1080. https://doi.org/10.3390/antibiotics10091080
13. Pourajam S, Kalantari E, Talebzadeh H, Mellali H, Sami R, Soltaninejad F, et al. Secondary bacterial infection and clinical characteristics in patients with COVID-19 admitted to two intensive care units of an academic hospital in Iran during the first wave of the pandemic. Front Cell Infect Microbiol. 2022;12:784130. https://doi.org/10.3389/fcimb.2022.784130
14. Sathitakorn O, Jantarathaneewat K, Weber DJ, Warren DK, Nanthapisal S, Rutjanawech S, et al. The feasibility of procalcitonin and CPIS score to reduce inappropriate antibiotics use among severe-critically ill COVID-19 pneumonia patients: a pilot study. Am J Infect Control. 2022;50:581–4. https://doi.org/10.1016/j.ajic.2022.01.030
15. Bonazzetti C, Morena V, Giacomelli A, Oreni L, Casalini G, Galimberti LR, et al. Unexpectedly high frequency of enterococcal bloodstream infections in coronavirus disease 2019 patients admitted to an Italian ICU: an observational study. Crit Care Med. 2021;49:e31–40. https://doi.org/10.1097/CCM.0000000000004748
16. Scaravilli V, Guzzardella A, Madotto F, Beltrama V, Muscatello A, Bellani G, et al. Impact of dexamethasone on the incidence of ventilator-associated pneumonia in mechanically ventilated COVID-19 patients: a propensity-matched cohort study. Crit Care. 2022;26:176. https://doi.org/10.1186/s13054-022-04049-2
17. Mantzarlis K, Deskata K, Papaspyrou D, Leontopoulou V, Tsolaki V, Zakynthinos E, et al. Incidence and risk factors for blood stream infection in mechanically ventilated COVID-19 patients. Antibiotics (Basel). 2022;11:1053. https://doi.org/10.3390/antibiotics11081053
18. Falcone M, Suardi LR, Tiseo G, Galfo V, Occhineri S, Verdenelli S, et al. Superinfections caused by carbapenem-resistant Enterobacterales in hospitalized patient with COVID-19: a multicentre observational study from Italy (CREVID study). JAC Antimicrobial Resist. 2022;4:dlac064.
19. Yohannes S, Ahmed Z, Schelling R, Perinkulam Sathyanarayanan S, Pratt A, Schreiber MP. Incidence and impact of ventilator associated multidrug resistant pneumonia in patients with SARS-COV2. Crit Care Res Pract. 2022;2022:9730895. https://doi.org/10.1155/2022/9730895
20. Menekşe Ş, Deniz S. Secondary infections in COVID-19 patients: A two-centre retrospective observational study. J Infect Dev Ctries. 2022;16:1294–301. https://doi.org/10.3855/jidc.15637
21. Bansal S, Kalpakam H, Kumar A, Varsha A, Thorbole A, Mehta RM. Lower respiratory tract sampling in COVID-19 acute respiratory distress syndrome: a focus on microbiology, cellular morphology, cytology, and management impact. Lung India. 2022;39:139–44. https://doi.org/10.4103/lungindia.lungindia_532_21
22. Centers for Disease Control and Prevention. National Healthcare Safety Network (NHSN) Patient safety component manual; 2021.
23. European Centre for Disease Prevention and Control. Surveillance of healthcare-associated infections and prevention indicators in European intensive care units; 2017.
24. Singer M, Deutschman CS, Seymour CW, Shankar-Hari M, Annane D, Bauer M, et al. The third international consensus definitions for sepsis and septic shock (Sepsis-3). JAMA. 2016;315:801–10. https://doi.org/10.1001/jama.2016.0287
25. Magiorakos AP, Srinivasan A, Carey RB, Carmeli Y, Falagas ME, Giske CG, et al. Multidrug-resistant, extensively drug-resistant and pandrug-resistant bacteria: an international expert proposal for interim standard definitions for acquired resistance. Clin Microbiol Infect. 2012;18:268–81. https://doi.org/10.1111/j.1469-0691.2011.03570.x
26. Glynn RJ, Buring JE. Ways of measuring rates of recurrent events. BMJ. 1996;312:364–7. https://doi.org/10.1136/bmj.312.7027.364
27. World Health Organization. WHO publishes list of bacteria for which new antibiotics are urgently needed. 2017 [cited 2023 Jun 21]. https://www.who.int/news/item/27-02-2017-who-publishes-list-of-bacteria-for-which-new-antibiotics-are-urgently-needed
28. European Centre for Disease Prevention and Control. Antimicrobial resistance in the EU/EEA (EARS-Net). Annual epidemiological report 2020. Solna (Sweden): The Centre; 2022.
29. Witt LS, Howard-Anderson JR, Jacob JT, Gottlieb LB. The impact of COVID-19 on multidrug-resistant organisms causing healthcare-associated infections: a narrative review. JAC Antimicrob Resist. 2022;5:2dlac130.
30. Mangioni D, Fox V, Chatenoud L, Bolis M, Bottino N, Cariani L, et al. Genomic characterization of carbapenem-resistant *Acinetobacter baumannii* (CRAB) in mechanically ventilated COVID-19 patients and impact of infection control measures on reducing CRAB circulation during the second wave of the SARS-CoV-2 pandemic in Milan, Italy. Microbiol Spectr. 2023;11:e0020923. https://doi.org/10.1128/spectrum.00209-23
31. Lat I, Daley MJ, Shewale A, Pangrazzi MH, Hammond D, Olsen KM, et al.; DEFINE study group and the Discovery Research Network. A multicenter, prospective, observational study to determine predictive factors for multidrug-resistant pneumonia in critically ill adults: the DEFINEsStudy. Pharmacotherapy. 2019;39:253–60. https://doi.org/10.1002/phar.2171
32. Tabah A, Koulenti D, Laupland K, Misset B, Valles J, Bruzzi de Carvalho F, et al. Characteristics and determinants of outcome of hospital-acquired bloodstream infections in intensive care units: the EUROBACT International Cohort Study. Intensive Care Med. 2012;38:1930–45. https://doi.org/10.1007/s00134-012-2695-9
33. Tabah A, Buetti N, Staiquly Q, Ruckly S, Akova M, Aslan AT, et al.; EUROBACT-2 Study Group, ESICM, ESCMID ESGCIP and the OUTCOMEREA Network. Epidemiology and outcomes of hospital-acquired

bloodstream infections in intensive care unit patients: the EUROBACT-2 international cohort study. Intensive Care Med. 2023;49:178–90. https://doi.org/10.1007/s00134-022-06944-2
34. Lepape A, Machut A, Bretonniere C, Friggeri A, Vacheron CH, Savey A, et al. Effect of COVID-19 infection and pandemic period on healthcare-associated infections acquired in intensive care units. Clin Microbiol Infect. 2023;29:530–6. https://doi.org/10.1016/j.cmi.2022.10.023
35. Martin-Loeches I, Torres A, Rinaudo M, Terraneo S, de Rosa F, Ramirez P, et al. Resistance patterns and outcomes in intensive care unit (ICU)-acquired pneumonia. Validation of European Centre for Disease Prevention and Control (ECDC) and the Centers for Disease Control and Prevention (CDC) classification of multidrug resistant organisms. J Infect. 2015;70:213–22. https://doi.org/10.1016/j.jinf.2014.10.004
36. Liu P, Li X, Luo M, Xu X, Su K, Chen S, et al. Risk factors for carbapenem-resistant *Klebsiella pneumoniae* infection: a meta-analysis. Microb Drug Resist. 2018;24:190–8. https://doi.org/10.1089/mdr.2017.0061
37. Baraz A, Chowers M, Nevo D, Obolski U. The time-varying association between previous antibiotic use and antibiotic resistance. Clin Microbiol Infect. 2022;0.
38. Tacconelli E, Górska A, De Angelis G, Lammens C, Restuccia G, Schrenzel J, et al. Estimating the association between antibiotic exposure and colonization with extended-spectrum β-lactamase-producing Gram-negative bacteria using machine learning methods: a multicentre, prospective cohort study. Clin Microbiol Infect. 2020;26:87–94. https://doi.org/10.1016/j.cmi.2019.05.013

Address for correspondence: Andrea Gori, School of Medicine and Surgery, Department of Pathophysiology and Transplantation, University of Milan Infectious Diseases Unit, IRCCS Ca' Granda Ospedale Maggiore Policlinico Foundation, Via Francesco Sforza 35, Milan 20122, Italy; email: andrea.gori@unimi.

Article DOI: https://doi.org/10.3201/eid2908.230115

Multidrug-Resistant Bacterial Colonization and Infections in Large Retrospective Cohort of Mechanically Ventilated COVID-19 Patients

Appendix

Methods

Complete list of data collected and criteria for data classification

Demographic data

- Age
- Gender
- Weight, ideal weight
- Height
- Body mass index (BMI)
- Comorbidities/organ failure: Charlson Comorbidity Index, cardiac disease, lung disease, renal disease, hypertension, diabetes mellitus, smoking history, immunological deficit (at least 1 of: solid organ transplantation, bone marrow transplantation, active neoplastic disease, hematological tumors, rheumatological diseases, acquired immunodeficiency syndrome (AIDS), asplenia, chemotherapy within the previous 3 months, neutropenia (<500/microL), use of biologic drugs, use of corticosteroids (>10 mg/day prednisone or equivalent within the previous 3 months), other forms of immunosuppression including congenital/genetic immunodeficits)

Clinical Data before ICU Admittance

- date of COVID-19 symptoms onset

• date of hospital admittance

• date of ICU admittance

• antivirals (remdesivir) administration before ICU admittance

• antibiotics administration before ICU admittance: days of antibiotic intake, antibiotic class (betalactam/betalactamases inhibitors, oxacillin/cefazolin, 3–4th generation cephalosporins, 5th generation cephalosporins, cefiderocol, cerbapenems, macrolides, vancomycin/teicoplanin, daptomycin, linezolid, fluoroquinolones, aminoglycosides, colistin, fosfomycin, azoles, echinocandines, others)

• steroids administration before ICU admittance: days of steroids intake, steroids class (STANDARD prednisone or dexamethasone 6mg/day, HIGH DOSE methylprednisolone ≥1 mg/kg/day)

• MDROs colonization before ICU admittance: *E. coli* ESBL+, Klebsiella spp. ESBL+, Klebsiella spp. CARBA-R, P. aeruginosa CARBA R, Acinetobacter spp. CARBA-R, methicillin-resistant Staphylococcus aureus, Vancomycin-resistant Enterococcus faecium, others (any bacteria with resistance to at least 1 molecule in 3 or more antibiotic classes)

• MDRO infections before ICU Admittance

Clinical and laboratory data during ICU stay

• Date of ICU admittance

• Hospital of provenance

• Setting of provenance: Emergency room (ER) if ICU admission occurred within 48 hours from hospitalization; non-intensive hospital wards if ICU admission occurred after 48 hours from hospitalization; ICU if patients stayed in ICU for over 24 hours before ICU transferral.

• ICU module

• PaO2:FiO2 ratio at ICU admission

• SOFA score at ICU admission

• SAPS II score at ICU admission

• Date of mechanical ventilation start

• Date of mechanical ventilation end

Microbiological information during ICU stay

• Date of microbiological sample, type of microbiological sample

Microbiological sample	Classification_1	Classification_2
Sputum	ETA	respiratory
endotracheal aspirate	ETA	respiratory
endotracheal tube	ETA	respiratory
bronchoalveolar lavage	BAL	respiratory
right bronchoalveolar lavage	BAL	respiratory
left bronchoalveolar lavage	BAL	respiratory
pleural fluid	pleural fluid	respiratory
blood from venous catheter	central line	blood
blood from venous catheter (dialysis)	central line	blood
blood from arterial catheter	Peripheral line	blood
blood from peripheral vein	Peripheral line	blood
vascular catheter	catheter	catheter
arterial catheter	catheter	catheter
central venous catheter	catheter	catheter
central venous catheter (dialysis)	catheter	catheter
Urine	urine	urine
urine from urinary catheter	urine	urine
midstream urine sample	urine	urine
skin swab	surveillance	surveillance
pharyngeal swab	surveillance	surveillance
nasal swab	surveillance	surveillance
perianal swab	surveillance	surveillance
rectal swab	surveillance	surveillance
rectal/perianal swab	surveillance	surveillance
axillary swab	surveillance	surveillance
inguinal swab	surveillance	surveillance
fecal sample	other	other
abdominal drainage	other	other
thoracic drainage	other	other
Liquor	other	other
purulent material	other	other
vaginal secretion	other	other
wound swab	other	other
foreskin swab	other	other
tracheostomy swab	other	other
ulceral swab	other	other
eschar swab	other	other
labial swab	other	other

• Interpretation of resistance pattern of the identified microorganism (see below “MDR DEFINITIONS)

• Interpretation of microbiological sample according to attending physician: infection/colonization/contamination

• Interpretation of microbiological sample according to literature: infection/colonization/contamination. Infections were defined by the presence of significant bacterial load associated with clinical manifestations within the infection window period

(IWP,±3 days from specimen collection) (see [reference #22]: CDC. National Healthcare Safety Network (NHSN) Patient Safety Component Manual. January 2021, [reference #23]: European Centre for Disease Prevention and Control. Surveillance of healthcare-associated infections and prevention indicators in European intensive care units. Stockholm: ECDC; 2017). Isolates were classified as colonization when no adverse clinical signs or symptoms was documented. Isolates that did not meet the criteria of infection/colonization and were listed in the CDC-NHSN list of common commensals were interpreted as contaminants (Centers for Disease Control and Prevention (CDC). CDC/NHSN Common Commensals List [Internet]. 2021. Available from: https://www.cdc.gov/nhsn/xls/master-organism-com-commensals-lists.xlsx).

• For infections, interpretation of microbiological sample as new or persistent infection: the combination of a) new signs and symptoms and b) radiographic evidence (for pneumonia) or other diagnostic testing were required to consider an infection as a new infection episode (see [reference #23]: European Centre for Disease Prevention and Control. Surveillance of healthcare-associated infections and prevention indicators in European intensive care units. Stockholm: ECDC; 2017)

• For bloodstream infections, interpretation of the BSI episode as primary, secondary to another source of infection or catheter-related (see below "DIAGNOSTIC CRITERIA FOR INFECTIONS")

• For infections, interpretation of the severity of the episode as infection without sepsis, sepsis or septic shock based on clinical manifestations occurred during the infection window period (±3 days from specimen collection) (see [reference #24]: Singer M, Deutschman CS, Seymour CW, et al. The Third International Consensus Definitions for Sepsis and Septic Shock (Sepsis-3). JAMA. 2016;315(8):801–810. doi:10.1001/jama.2016.0287)

Therapeutic information during ICU stay

• Antibiotic therapy: date of start, date of end, date of change of posology, type of antibiotic therapy

Antibiotic (active ingredient)	Classification_1	Classification_2
amphotericin b	antifungal	AMB
liposomal amphotericin b	antifungal	AMB
Amikacin	no anaerobic activity	aminoglycosides
amoxicillin/ac. Clavulanic	anti-anaerobic activity	BL/BLIs
Ampicillin	anti-anaerobic activity	penicillins
Ampicillin/sulbactam	anti-anaerobic activity	BL/BLIs
Anidulafungin	antifungal	echinocandins

Azithromycin	no anaerobic activity	MLs
Caspofungin	antifungal	echinocandins
Cefazoline	no anaerobic activity	anti-Staph BLs
Cefepime	no anaerobic activity	3–4G cephalosporins
Cefiderocol	no anaerobic activity	novel anti G- cephalosporins
Cefotaxime	no anaerobic activity	3–4G cephalosporins
Ceftaroline	no anaerobic activity	novel anti G+ cephalosporins
Ceftazidime	no anaerobic activity	3–4G cephalosporins
ceftazidime/avibactam	no anaerobic activity	novel anti G- cephalosporins
ceftolozane/tazobactam	no anaerobic activity	novel anti G- cephalosporins
Ceftriaxone	no anaerobic activity	3–4G cephalosporins
Ciprofloxacin	no anaerobic activity	FQs
Clindamycin	anti-anaerobic activity	lincosamides
Colistin	no anaerobic activity	polymixins
Daptomycin	anti-anaerobic activity	glyco/lipopeptides
Fidaxomicin	other	other
Fluconazole	antifungal	azoles
Fosfomycin	no anaerobic activity	FOF
Gentamycin	no anaerobic activity	aminoglycosides
Imipenem	anti-anaerobic activity	carbapenems
isavuconazole	antifungal	azoles
Levofloxacin	no anaerobic activity	FQs
Linezolid	anti-anaerobic activity	oxazolidinones
Meropenem	anti-anaerobic activity	carbapenems
meropenem/vaborbactam	anti-anaerobic activity	novel carbapenems
metronidazole	anti-anaerobic activity	MTZ
Oxacillin	no anaerobic activity	anti-Staph BLs
penicillin G	anti-anaerobic activity	penicillins
Piperacillin	anti-anaerobic activity	penicillins
piperacillin/tazobactam	anti-anaerobic activity	BL/BLIs
Rifampin	no anaerobic activity	RIF
Rifaximin	other	other
trimethoprim/sulfamethoxazole	no anaerobic activity	SXT
Tigecycline	anti-anaerobic activity	tetracyclines
Tobramycin	no anaerobic activity	aminoglycosides
Vancomycin	anti-anaerobic activity	glyco/lipopeptides
Voriconazole	antifungal	azoles

- Steroid therapy: date of start, date of end, type of steroid therapy

Corticosteroid (active ingredient and posology)	Classification
Dexamethasone (any dosage)	STANDARD dose
Methylprednisolone <1mg/kg/die	STANDARD dose
Methylprednisolone ≥1mg/kg/die	HIGH dose

Outcome data

- Length of ICU stay
- Vital status at ICU discharge (alive/dead)

MDR definitions

Source	Definition criteria
Magiorakos et al, CMI 2012 [reference #25]	• MDR: non-susceptible to > = 1 agent in > = 3 antimicrobial categories + MRSA • XDR: non-susceptible to > = 1 agent in all but < = 2 categories • PDR: non-susceptible to all antimicrobial agents listed
Grasselli et al, CHEST 2021 [reference #4]	resistance to > = 1 agent in > = 3 antimicrobial categories + methicillin-resistant *Staphylococcus* spp, vancomycin-resistant *Enterococcus* spp, ESBL/AmpC/carbapenemases-producing *Enterobacterales*, carbapenem resistant gram-negative bacteria

Diagnostic criteria for infections

Microbiologically defined infections (MDI) only

Infection	Site of Culture	Bacterial Load	Clinical Signs	Also
Primary Blood Stream Infection	2 percutaneous blood samples + eventual blood from catheters	—	Fever/chills/hypotension + No further sign of localized infection	No differential time to positivity between percutaneous and catheters
	If Common Commensal organisms (i.e., diphtheroids (*Corynebacterium* spp. not *C. diphtheria*), *Bacillus* spp. (not *B. anthracis*), *Propionibacterium* spp., coagulase-negative staphylococci (including *S. epidermidis*), viridans group streptococci, *Aerococcus* spp. *Micrococcus* spp. and *Rhodococcus* spp.): necessary two or more blood specimens drawn on separate occasions			
Central line associated Blood Stream Infection[1]	percutaneous blood samples + catheter blood or catheter tip	—	Fever/chills/hypotension + No further sign of localized infection. Eventual erythema, swelling, purulent drainage from catheter insertion-site.	Differential time to positivity >2 h or positive catheter tip
	If Common Commensal organisms (i.e., diphtheroids (*Corynebacterium* spp. not *C. diphtheria*), *Bacillus* spp. (not *B. anthracis*), *Propionibacterium* spp., coagulase-negative staphylococci (including *S. epidermidis*), viridans group streptococci, *Aerococcus* spp. *Micrococcus* spp. and *Rhodococcus* spp.): necessary two or more blood specimens drawn on separate occasions			
Ventilator-associated lower respiratory tract infections [2]	Bronchoalveolar lavage	$\geq 10^4$ CFU/mL	1 of: fever, leukocytosis/leucopenia + 1 of: worsening oxygenation, purulent secretions + New/progressive radiographic infiltrate (if available)	
	Endotracheal Aspirate	$\geq 10^5$ CFU/mL		
	secondary Blood Stream Infection[3]			
	Excluded organisms: “Normal respiratory flora,” “normal oral flora,” “mixed respiratory flora,” and, unless identified from lung tissue or pleural fluid (with specimen obtained during thoracentesis or initial placement of chest tube and NOT from an indwelling chest tube), *Candida* spp, coagulase-negative staphylococci, *Enterococcus* spp			
Catheter-associated Urinary Tract Infection[4]	urine culture[5]	$\geq 10^5$ CFU/mL	Fever/chills/hypotension + No further sign of localized infection	
	secondary Blood Stream Infection[3]			
	Excluded organisms: “mixed flora,” *Candida* spp, yeast, mold, dimorphic fungi, parasites			
Clostridioides difficile Colitis	Unformed stool culture	—	Fever/chills/hypotension + Unformed stool	Enzyme immunoassay positive for C. difficile GDH + toxins A/B or positive NAAT
COVID-Associated Pulmonary Aspergillosis	Proven Histopathological or direct microscopic detection of fungal hyphae, showing invasive growth with associated tissue damage or Aspergillus recovered by culture or microscopy or histology or PCR obtained by a sterile aspiration or biopsy from a pulmonary site, showing an infectious disease process			
	Probable – tracheobronchitis Tracheobronchial ulceration, nodule, pseudomembrane, plaque, or eschar seen on bronchoscopic analysis + One of the following: microscopic detection of fungal elements in bronchoalveolar lavage indicating a mold; positive bronchoalveolar lavage culture or PCR; serum galactomannan index >0.5; bronchoalveolar lavage galactomannan index ≥1.0			
	Probable – other pulmonary forms Pulmonary infiltrate or cavitating infiltrate, preferably documented by chest CT and not attributed to another cause + One of the following: microscopic detection of fungal elements in bronchoalveolar lavage indicating a mold; positive bronchoalveolar lavage culture or PCR; serum galactomannan index >0.5; bronchoalveolar lavage galactomannan index ≥1.0			
	Possible - other pulmonary forms Pulmonary infiltrate or cavitating infiltrate, preferably documented by chest CT and not attributed to another cause + One of the following: microscopic detection of fungal elements in non-bronchoalveolar lavage indicating a mold; positive non-bronchoalveolar lavage culture; single non-bronchoscopic lavage galactomannan index >4.5; non-bronchoscopic lavage galactomannan index >1.2 twice or more; non-bronchoscopic lavage galactomannan index >1.2 plus positive PCR			

Infection	Site of Culture	Bacterial Load	Clinical Signs	Also
Candidemia/ Invasive Candidiasis	Proven *Candida* spp. identified from one or more blood specimens obtained by culture or non-culture microbiologic testing methods			
	Presumptive Fever/chills/hypotension + risk factors (i.e., Candida score, Candida Colonization Index) + positive fungal biomarkers (i.e., 1,3-β-d-glucan BDG) + exclusion of alternative diagnoses			
CFU, Colony forming units. [1] at least 48 h after catheter positioning. Central line colonization: positive catheter blood or catheter tip and negative percutaneous blood samples. [2] at least 48 h after intubation. [3] positive blood specimen containing at least one eligible matching organism to the site-specific specimen o meeting the site-specific infection criteria. [4] at least 48 h after indwelling urinary catheter positioning. All the patients had urinary indwelling catheters. [5] if urinary catheter in place for more than 5 d, the catheter is removed, a new catheter is repositioned and a second specimen is collected.				

Statistical Analysis

Patients' characteristics were described overall and for selected groups of interest such ass MDROs acquired before/after ICU admittance, MDROs infection/colonization. Median (interquartile range, IQR) are reported for continuous variables, and number (percentages) for categorical variables. Groups were compared with parametric or nonparametric tests, according to data distribution, for continuous variables and with Pearson Chi-square test (or Fisher exact test when appropriate) for categorical variables. Crude incidence rates per 1000 patient-days ($IR/1000_{patient\text{-}days}$) and relative 95% confidence intervals (95%CIs) were calculated considering for each patient any first species-specific MDRO colonization and/or each new MDRO/non-MDRO HAI. Since we speculate that some patients have greater propensities for recurrent events than others, and thus events within a single patient may not be considered as independent observations, we calculated incidence rates considering the negative binomial distribution, as already proposed (see [reference #26]: Glynn RJ, Buring JE. Ways of measuring rates of recurrent events. BMJ Br Med J [Internet]. 1996 Feb 2 [cited 2022 Oct 20];312(7027):364. Available from: /pmc/articles/PMC2350293/?report = abstract) The time considered for IRs estimates was set from ICU admission to discharge, except for VALRTI where total intubation time was considered. To measure agreement between different methods for classifying microbiological isolates as infections, Cohen's kappa coefficient (κ) was applied. All tests were two-sided, and $p < 0.05$ was chosen to indicate statistical significance. Software SAS 9.4 (SAS Institute) was used for statistical analysis.

Literature Review

To identify relevant studies on MDRO events in COVID-19 ICU patients indexed on PubMed and/or Embase, we used the following string: ((“COVID-19”[MeSH Major Topic]) OR (COVID)) AND (“ICU”[Title/Abstract] OR “INTENSIVE CARE”[Title/Abstract] OR “CRITICAL*”[Title/Abstract]) AND (“MDR”[Title/Abstract] OR “multidrug-

resist*"[Title/Abstract] OR "multidrug resist*"[Title/Abstract] OR "carbapenem-resistant"[Title/Abstract]) AND ((COLONIZATION) OR (INFECTION) OR (EPIDEMIOLOGY)). The review was conducted based on the PRISMA guidelines for reviews (Appendix Figure 3). The last search was performed on September 19, 2022.

References

1. Centers for Disease Control and Prevention. National Healthcare Safety Network (NHSN) Patient Safety Component Manual. January 202 [cited 2023 Jun 6]. https://www.cdc.gov/nhsn/pdfs/pscmanual/pcsmanual_current.pdf

2. Koehler P, Bassetti M, Chakrabarti A, Chen SC, Colombo AL, Hoenigl M, et al.; European Confederation of Medical Mycology; International Society for Human Animal Mycology; Asia Fungal Working Group; INFOCUS LATAM/ISHAM Working Group; ISHAM Pan Africa Mycology Working Group; European Society for Clinical Microbiology; Infectious Diseases Fungal Infection Study Group; ESCMID Study Group for Infections in Critically Ill Patients; Interregional Association of Clinical Microbiology and Antimicrobial Chemotherapy; Medical Mycology Society of Nigeria; Medical Mycology Society of China Medicine Education Association; Infectious Diseases Working Party of the German Society for Haematology and Medical Oncology; Association of Medical Microbiology; Infectious Disease Canada. Defining and managing COVID-19-associated pulmonary aspergillosis: the 2020 ECMM/ISHAM consensus criteria for research and clinical guidance. Lancet Infect Dis. 2021;21:e149–62. PubMed https://doi.org/10.1016/S1473-3099(20)30847-1

Appendix Table 1. Characteristics of the 435 patients admitted to ICU, overall and for patients with and without MDRO isolates in the first 48 h (irrespective of MDRO developing (or not) during ICU stay)

PATIENTS CHARACTERISTICS	Total N = 435	$MDR_{\leq 48h}$ N = 88	No-MDR or $MDR_{>48h}$ N = 347
Age, years	65.0 (59.0–71.0)	65.0 (58.0–70.5)	65.0 (59.0–71.0)
Gender, female	117 (26.9)	22 (25.0)	95 (27.4)
BMI, kg/m2	28.0 (26.0–31.0)	28.0 (26.0–31.0)	28.0 (26.0–31.0)
Obesity (BMI >30)	284 (65.3)	59 (67.1)	225 (64.8)
Ever Smoker	87 (20.0)	21 (23.9)	66 (19.0)
Comorbidities			
Hypertension	225 (51.7)	44 (50.0)	181 (52.2)
Cardiovascular disease	115 (26.5)	23 (26.1)	92 (26.6)
Pneumopathy	62 (14.3)	14 (15.9)	48 (13.8)
Neuropathy	19 (4.4)	5 (5.7)	14 (4.0)
Diabetes	92 (21.2)	23 (26.1)	69 (19.9)
Immunological deficits*	29 (6.7)	7 (8.0)	22 (6.3)
Total no. of comorbidities			
0	83 (19.1)	17 (19.3)	66 (19.0)
1	141 (32.4)	26 (29.6)	115 (33.1)
2	109 (25.1)	22)25.0)	87 (25.1)

PATIENTS CHARACTERISTICS	Total N = 435	$MDR_{\leq 48h}$ N = 88	No-MDR or $MDR_{>48h}$ N = 347
>3	102 (23.9)	23 (26.1)	79 (22.8)
Setting characteristics	Total, n = 435	$MDR_{\leq 48h}$, n = 88	No-MDR or $MDR_{>48h}$, n = 347
Month of ICU_{FIERA} admission			
Oct 2020	20 (4.6)	2 (2.3)	18 (5.2)
Nov 2020	97 (22.3)	21 (23.9)	76. (21.9)
Dec 2020	53 (12.2)	19 (21.6)	34 (9.8)
Jan 2021	56 (12.9)	8 (9.1)	43 (13.8)
Feb 2021	53 (12.2)	12 (13.6)	41 (11.8)
Mar 2021	60 (13.8)	6 (6.8)	54 (15.6)
Apr 2021	17 (3.9)	5 (5.7)	12 (3.5)
Setting of provenance [α]			
ER	117 (26.9)	31 (35.2)	86 (24.8)
Non-intensive hospital wards	117 (26.9)	15 (17.1)	102 (29.4)
ICU	201 (46.2)	42 (47.7)	159 (45.8)
Center A	53 (12.2)	6 (6.8)	47 (13.5)
Center B	37 (8.5)	9 (10.2)	28 (8.1)
Center C	37 (8.5)	7 (8.0)	30 (8.7)
Center D	37 (8.5)	14 (15.9)	23 (6.6)
Center E	33 (7.6)	6 (6.8)	27 (7.8)
Center F	22 (5.1)	0 (0)	22 (6.3)
Center G	17 (3.9)	6 (6.8)	11 (3.2)
Center H	17 (3.9)	1 (1.1)	16 (4.6)
Center I	16 (3.7)	3 (3.4)	13 (13.8)
Center J	15 (3.6)	2 (2.3)	13 (13.8)
Center K	12 (2.8)	4 (4.6)	8 (2.3)
Other 36 centers with <10 patients	139 (32.0)	30 (34.1)	109 (31.4)
DISEASE CHARACTERISTICS PRIOR TO ICU ADMISSION			
time between first symptoms and hospitalization, days	5.0 (3.0–7.0)	6.0 (3.0–7.0)	5.0 (3.0–7.0)
time between hospitalization and ICU admission, days [β]	5.0 (2.0–9.0)	6.0 (3.0–12.0)	5.0 (2.0–8.0)
time between hospitalization and MV start, days	3.0 (1.0–6.5)	4.0 (2.0–7.0)	3.0 (1.0–6.0)
Steroid therapy, standard dose [#]	282 (64.8)	54 (61.4)	228 (65.7)
Steroid therapy, high dose [#γ]	56 (13.0)	17 (19.8)	39 (11.3)
Antibiotic therapy [δ]			
None	151 (34.7)	25 (28.4)	126 (36.3)
1 class	154 (35.4)	29 (33.0)	125 (36.0)
2 classes	95 (21.8)	22 (25.0)	73 (21.0)
>3 classes	35 (8.1)	12 (13.6)	23 (6.6)
MDROs infection/colonization	12 (2.8)	8 (9.1)	4 (1.2)
PaO2 to FIO2 ratio at ICU admission, mmHg	134.0 (105.0–180.0)	126.0 (100.5–179.0)	137.0 (106.0–180.0)
200	74 (17.0)	16 (18.2)	58 (16.7)
<100 and ≥200	268 (61.6)	50 (56.8)	50 (56.8)
<100	93 (21.4)	22 (25.0)	71 (20.5)
OUTCOME			
Alive at discharge	286 (65.8)	57 (64.8)	229 (66.0)
Deceased	149 (34.3)	31 (35.2)	118 (34.0)
Length of MV, days	17.0 (11.0–28.0)	19.0 (13.0–33.0)	16.0 (10.0–26.0)
ICU stay, days	20.0 (12.0–32.0)	17.0 (11.0–29.0)	21.0 (13.0–33.0)

Categorical variables are expressed as frequency (percentages), continuous variables are expressed as median (interquartile range). [α] Chi-square test p-value = 0.032; [β] Mann–Whitney U test p-value = 0.006; [γ] Chi-square test p-value = 0.036; [δ] Chi-square for trend p-value = 0.021.
Legend: BMI body mass index, ER emergency room, ICU intensive care unit, MV mechanical ventilation, MDROs multidrug resistant organisms; * at least 1 of: solid organ transplantation, active neoplastic disease, hematological disease, rheumatological disease, AIDS, asplenia, chemotherapy in the past 3 mo, neutropenia (N < 500 / microL), use of biologics, use of corticosteroids (>10 mg / day prednisone or equivalent>3 mo pre-hospitalization), other forms of immunosuppression (including congenital / genetic forms); [#] standard dose in case of use of dexamethasone or methylprednisolone <1 mg / kg / day, high dose in case of use of methylprednisolone greater than or equal to 1 mg / kg / day or equivalent (patients could have received both standard and high dose of steroid.

Appendix Table 2. Duration between hospitalization and transfer to ICU based on the patients' setting of provenance

	Days between hospitalization and transfer to ICU by setting of provenance					
Setting of provenance		N	Median	Lower Quartile	Upper Quartile	p value*
ER	MDR = <48h	15	1.0	0.0	2.0	0.826
	MDR >48h	55	1.0	1.0	2.0	
	no-MDR	47	1.0	0.0	2.0	
non-intensive hospital wards	MDR = <48h	42	5.5	4.0	8.0	0.780
	MDR >48h	92	6.0	4.0	8.0	
	no-MDR	67	6.0	4.0	9.0	
ICU	MDR = <48h	31	11.5	8.0	18.0	0.091
	MDR >48h	60	9.0	6.0	13.0	
	no-MDR	26	7.0	4.0	12.0	

* Kruskal-Wallis test

Appendix Table 3. Details on bacterial species and time of acquisition of the subgroup of patients who developed MDRO infection from the same MDRO colonizing bacteria

Bacterial species	Days between ICU admission and colonization	Colonization sample	Days between ICU admission and Infection	Infection sample
Enterococcus faecalis	4	SURV SWAB	7	BLOOD
Enterococcus faecium	5	SURV SWAB	13	BLOOD
Enterococcus faecium	7	SURV SAWB	33	BLOOD
Enterococcus faecium	22	SURV SWAB	26	BLOOD
Escherichia coli	3	SURV SWAB	15	URINE
Klebsiella spp	6	ETA/BAL	9	ETA/BAL
Klebsiella spp	8	SURV SWAB	14	ETA/BAL
Klebsiella spp	9	ETA/BAL	16	ETA/BAL
Klebsiella spp	11	SURV SWAB	13	ETA/BAL
Klebsiella spp	11	SURV SWAB	15	ETA/BAL
Klebsiella spp	11	SURV SWAB	17	ETA/BAL
Klebsiella spp	15	SURV SWAB	27	ETA/BAL
Klebsiella spp	20	SURV SWAB	55	BLOOD
Klebsiella spp	30	SURV SWAB	33	BLOOD
Proteus mirabilis	21	ETA/BAL	24	ETA/BAL
Providencia stuartii	10	ETA/BAL	12	BLOOD
Pseudomonas aeruginosa	14	ETA/BAL	18	ETA/BAL
Pseudomonas aeruginosa	54	ETA/BAL	57	ETA/BAL
Staphylococcus aureus	3	SURV SWAB	6	ETA/BAL
Staphylococcus aureus	5	SURV SWAB	6	ETA/BAL
Staphylococcus aureus	8	SURV SWAB	9	ETA/BAL
Staphylococcus aureus	14	ETA/BAL	24	ETA/BAL
Staphylococcus aureus	25	SURV SWAB	30	ETA/BAL
Staphylococcus aureus	47	ETA/BAL	51	ETA/BAL

Appendix Table 4. Analysis of concordance in the interpretation of bacterial isolates as colonization or healthcare-associated infection (HAIs) between clinical criteria and retrospective evaluation according to international guidelines (N = 5213, isolates retrospectively classified as contaminants were excluded)

Literature criteria (retrospective evaluation)	Clinical diagnosis (real-life interpretation of microbiological reports)		
	colonization	HAI	Total
Colonization	2864 (93.9)	185 (6.1)	3049 (58.5)
HAI	66 (3.1)	2096 (96.9)	2162 (41.5)
Total	2930 (56.2)	2281 (43.8)	5211
simple kappa coefficient			
Estimate	Standard Error	95% Confidence Limits	
0.9016	0.0061	0.8897	0.9134

Numbers are expressed as frequency (percentages). Concordance between literature criteria and clinical diagnosis is reported in bold

Appendix Table 5. MDRO and antibiotic-susceptible (non-MDRO) bacterial isolates interpreted as healthcare-associated infections (HAIs), by bacterial species and infection site

		VALRTI (n = 359)		BLOOD (n = 141)		UTI (n = 40)		Other (n = 6)		Total HAIs	
Species	Total (n = 546)	MDRO (n = 82)	non-MDRO (n = 277)	MDRO (n = 42)	non-MDRO (n = 99)	MDRO (n = 4)	non-MDRO (n = 36)	MDRO (n = 2)	non-MDRO (n = 4)	MDRO (n = 130)	non-MDRO (n = 416)
Total Gram positive	220	21	82	27	64	2	20	2	2	52	168
Total Gram negative	326	61	195	15	35	2	16	0	2	78	248
Acinetobacter baumannii	9	7	0	2	0	0	0	0	0	9	0
Bacillus clausii	1	0	0	0	1	0	0	0	0	0	1
Citrobacter spp	10	1	9	0	0	0	0	0	0	1	9
Clostridioides difficile	2	0	0	0	0	0	0	0	2	0	2
coagulase negative Staphylococcus	20	0	0	16	3	0	1	0	0	16	4
Corynebacterium spp	7	0	3	0	4	0	0	0	0	0	7
Delftia acidovorans	3	0	2	0	1	0	0	0	0	0	3
Enterobacter spp	31	1	27	0	2	0	1	0	0	1	30
Enterococcus faecalis	62	0	7	2	36	0	16	0	1	2	60
Enterococcus faecium	16	1	1	3	9	0	2	0	0	4	12
Enterococcus spp	1	0	0	0	0	1	0	0	0	1	0
Escherichia coli	26	5	14	0	1	1	5	0	0	6	20
Fusobacterium necrophorum	1	0	0	0	1	0	0	0	0	0	1
Haemophilus influenzae	3	0	3	0	0	0	0	0	0	0	3
Hafnia alvei	8	1	4	0	3	0	0	0	0	1	7
Klebsiella spp	87	23	40	10	12	1	1	0	0	34	53
Legionella pneumophila	1	0	1	0	0	0	0	0	0	0	1
Morganella morganii	5	0	5	0	0	0	0	0	0	0	5
Proteus mirabilis	9	3	5	0	1	0	0	0	0	3	6
Providencia stuartii	1	0	0	1	0	0	0	0	0	1	0
Pseudomonas aeruginosa	101	20	62	2	9	0	8	0	0	22	79
Pseudomonas spp	1	0	1	0	0	0	0	0	0	0	1
Serratia marcescens	7	0	5	0	1	0	1	0	0	0	7
Staphylococcus aureus	102	19	63	6	10	1	1	2	0	28	74
Stenotrophomonas maltophilia	21	0	17	0	4	0	0	0	0	0	21
Streptococcus agalactiae	2	1	1	0	0	0	0	0	0	1	1
Streptococcus anginosus	2	0	0	0	1	0	0	0	1	0	2
Streptococcus pneumoniae	7	0	7	0	0	0	0	0	0	0	7

Appendix Table 6. MDRO and antibiotic-susceptible (non-MDRO) bacterial isolates interpreted as healthcare-associated infections (HAIs), by bacterial species and infection severity

Species	Total (n = 546)	no sepsis (n = 266)		sepsis (n = 194)		septic shock (n = 86)		Total HAIs	
		MDRO (n = 61)	non-MDRO (n = 205)	MDRO (n = 52)	non-MDRO (n = 142)	MDRO (n = 17)	non-MDRO (n = 69)	MDRO (n = 130)	non-MDRO (n = 416)
Total Gram positive	220	21	85	25	55	6	22	52	168
Total Gram negative	326	40	120	27	87	11	47	78	248
Acinetobacter baumannii	9	3	0	2	0	4	0	9	0
Bacillus clausii	1	0	0	0	0	0	1	0	1
Citrobacter spp	10	1	4	0	5	0	0	1	9
Clostridioides difficile	2	0	1	0	0	0	1	0	2
coagulase negative Staphylococcus	20	4	2	10	1	2	1	16	4
Corynebacterium spp	7	0	1	0	5	0	1	0	7
Delftia acidovorans	3	0	2	0	0	0	1	0	3
Enterobacter spp	31	0	15	1	11	0	4	1	30
Enterococcus faecalis	62	1	29	1	22	0	9	2	60
Enterococcus faecium	16	2	6	1	5	1	1	4	12
Enterococcus spp	1	1	0	0	0	0	0	1	0
Escherichia coli	26	3	11	2	7	1	2	6	20
Fusobacterium necrophorum	1	0	0	0	1	0	0	0	1
Haemophilus influenzae	3	0	3	0	0	0	0	0	3
Hafnia alvei	8	0	3	0	3	1	1	1	7
Klebsiella spp	87	19	24	14	17	1	12	34	53
Legionella pneumophila	1	0	1	0	0	0	0	0	1
Morganella morganii	5	0	5	0	0	0	0	0	5
Proteus mirabilis	9	3	5	0	1	0	0	3	6
Providencia stuartii	1	0	0	1	0	0	0	1	0
Pseudomonas aeruginosa	101	11	33	7	35	4	11	22	79
Pseudomonas spp	1	0	1	0	0	0	0	0	1
Serratia marcescens	7	0	0	0	1	0	6	0	7
Staphylococcus aureus	102	13	39	12	20	3	15	28	74
Stenotrophomonas maltophilia	21	0	12	0	6	0	3	0	21
Streptococcus agalactiae	2	0	1	1	0	0	0	1	1
Streptococcus anginosus	2	0	1	0	1	0	0	0	2
Streptococcus pneumoniae	7	0	6	0	1	0	0	0	7

Appendix Table 7. Steroid administration before MDROs events. Both administration before and during ICU stay are considered. Comparison between no-MDR, $MDR_{COL>48h}$ and $MDR_{INF>48h}$ groups. $MDR_{INF>48h}$ patients are divided in subgroups based on the diagnosis of prior MDRO colonization.

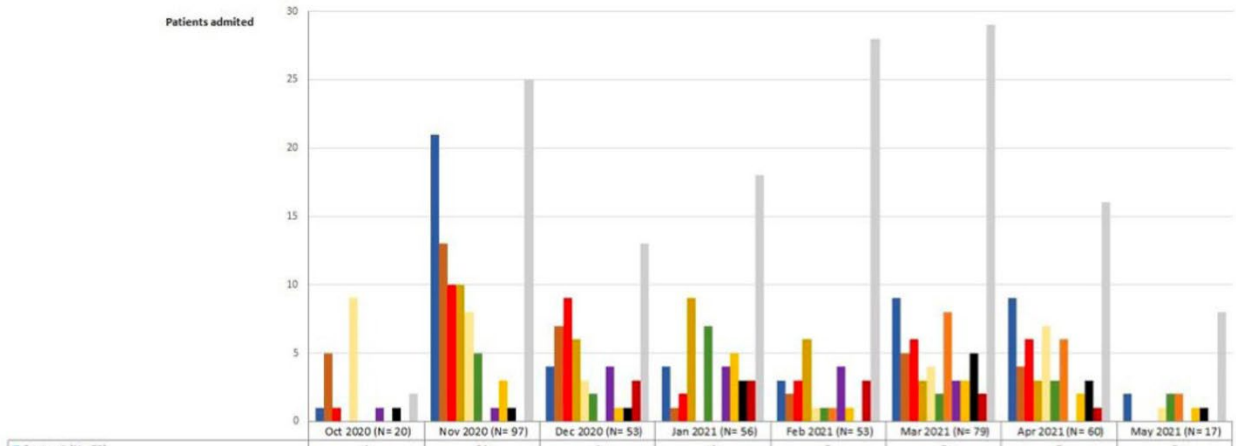
	Steroid administration before MDRO occurrence*		
	YES	NO	Total
no-MDR	132 (94.3)	8 (5.7)	140 (41.4)
$MDR_{COL>48h}$	98 (95.1)	5 (4.9)	103 (30.5)
$MDRO_{INF>48h}$	83 (87.4)	12 (12.6)	95 (28.1)
MDRO Infection only**	40 (85.1)	7 (14.9)	47 (13.9)
MDR colonization and subsequent MDRO infection (different species)	19 (90.5)	2 (9.5)	21 (6.2)
MDR colonization and subsequent MDRO infection (same species)	24 (88.9)	3 (11.1)	27 (8.0)
Total	251 (74.3)	87 (25.7)	338

Numbers are expressed as frequency (percentages). * administration during ICU stay for no-MDR patients; ** 23 patients with subsequent MDRO colonization by different species. Nine patients are not reported for missing information on steroid therapy

Appendix Table 8. Antibiotic administration before MDROs events, comparison between no-MDR and $MDR_{>48h}$ groups. To balance intakes between groups, only administration occurred during the first 10 d of ICU is considered (which represents the upper quartile (Q3) of the time from ICU admission to first MDROs isolation).

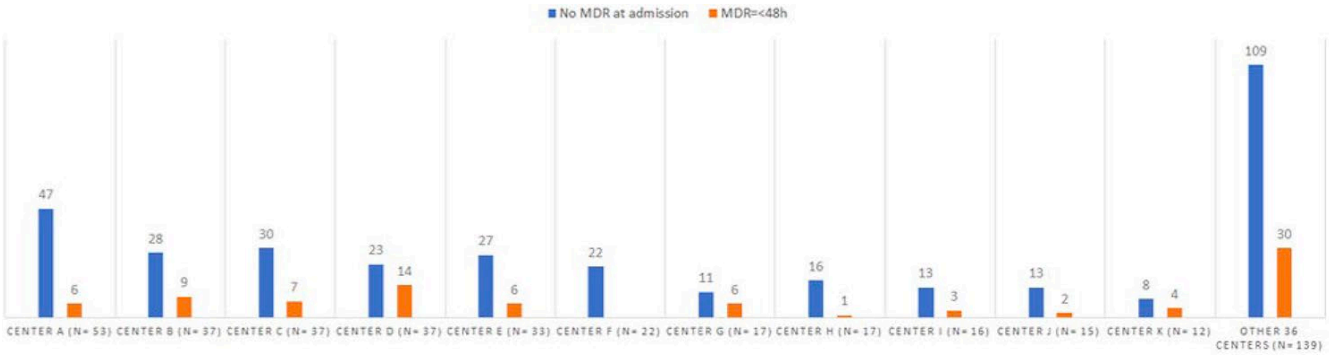
	Antibiotic administration before MDRO occurrence or within 10 d from ICU admission, whichever comes first*		
Patients group	YES	NO	Total
no-MDR	18 (12.9)	122 (87.1)	140 (41.5)
$MDR_{>48h}$	116 (58.9)	81 (41.1)	197 (58.5)
Total	134 (39.8)	203 (60.2)	337
Numbers are expressed as frequency (percentages). * chi-square test p-value <0.001. Ten patients are not reported for missing information on antibiotic therapy			

	mean (95% CI)	median (Q1-Q3)
Length of ICU stay, days • no-MDR • $MDR_{>48h}$	 20.5 (17.8–23.3) 28.5 (26.1–30.8)	 15.5 (10–24) 25 (16–37)
Days from ICU admission to MDROs event • no-MDR • $MDR_{>48h}$	 - 8.2 (7.1–9.2)	 - 7 (4–10)

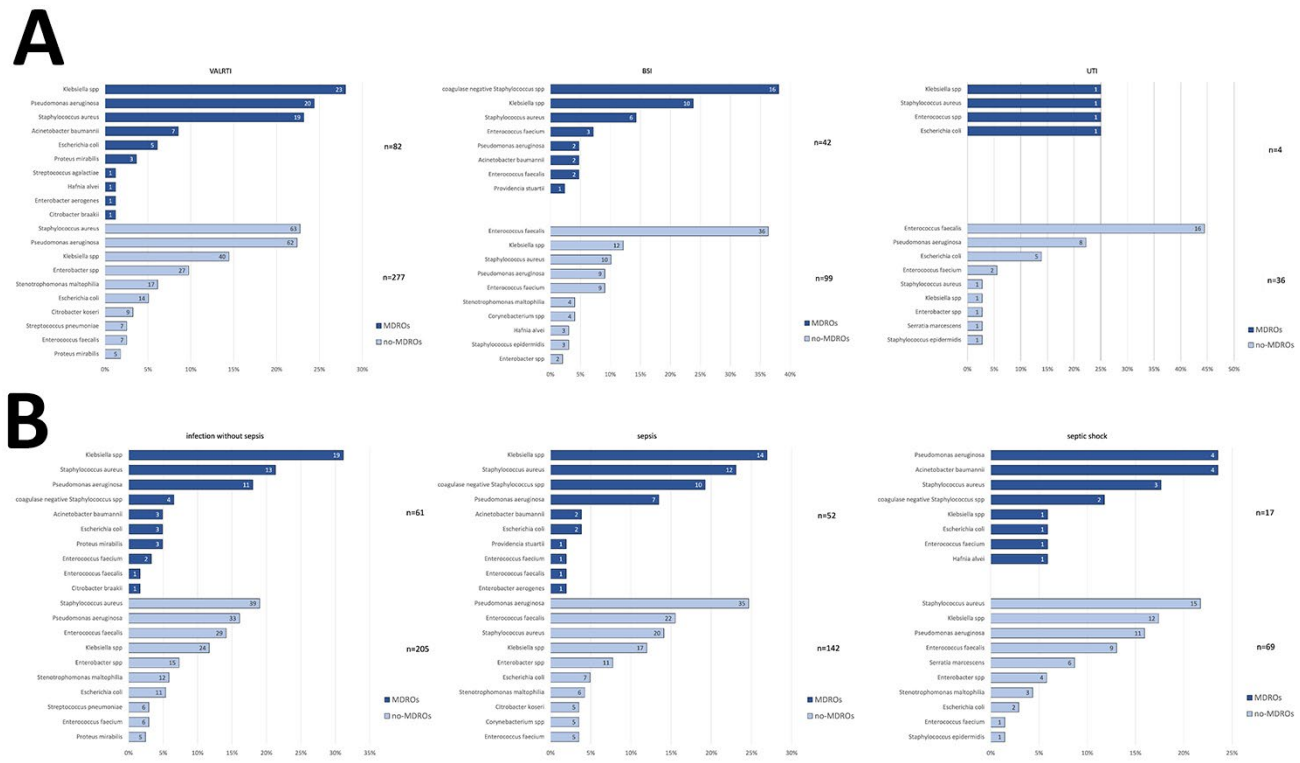


	Oct 2020 (N= 20)	Nov 2020 (N= 97)	Dec 2020 (N= 53)	Jan 2021 (N= 56)	Feb 2021 (N= 53)	Mar 2021 (N= 79)	Apr 2021 (N= 60)	May 2021 (N= 17)
Center A (N= 53)	1	21	4	4	3	9	9	2
Center B (N= 37)	5	13	7	1	2	5	4	0
Center C (N= 37)	1	10	9	2	3	6	6	0
Center D (N= 37)	0	10	6	9	6	3	3	0
Center E (N= 33)	9	8	3	0	1	4	7	1
Center F (N= 22)	0	5	2	7	1	2	3	2
Center G (N= 17)	0	0	0	0	1	8	6	2
Center H (N= 17)	1	1	4	4	4	3	0	0
Center I (N= 16)	0	3	1	5	1	3	2	1
Center J (N= 15)	1	1	1	3	0	5	3	1
Center K (N= 12)	0	0	3	3	3	2	1	0
Other 36 centers with 1 to 9 patients admitted (N= 139)	2	25	13	18	28	29	16	8

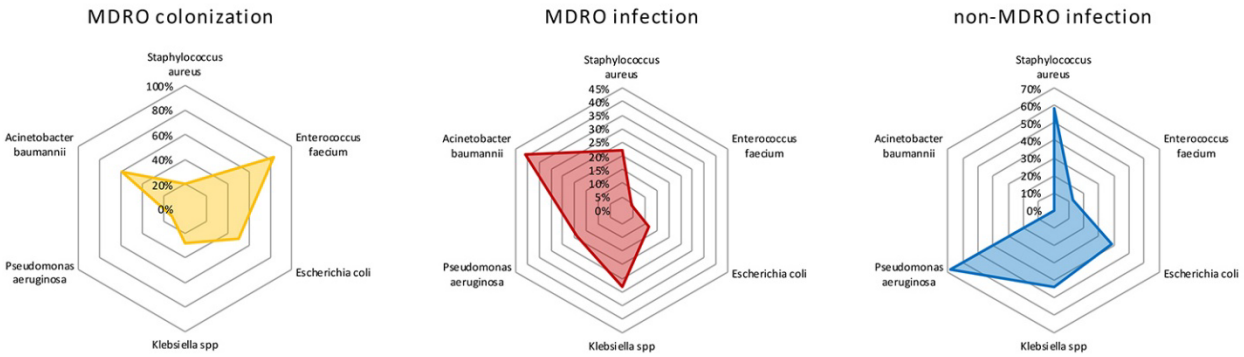
Appendix Figure 1. Trend of patient enrollment by referring hospital per month.



Appendix Figure 2. Number of patients with MDRO isolated in the first 48 hours from admission by referring hospital.



Appendix Figure 3. Multidrug-resistant bacterial colonization and infections in large retrospective cohort of COVID-19 mechanically ventilated patients. Etiology of microbiologically confirmed infections according to infection site (A) and disease severity (B).The 10 most frequent pathogens are reported as a percentage of all positive samples of that type. The number at the end of each bar indicates the total number of positive samples for the pathogen. Numbers annotated on the plots indicate the total number of organisms for each subgroup. Pathogen identification is stratified into MDRO (blue) and non-MDRO (light blue) isolates. Details with absolute numbers for each bacterial species are reported in Appendix Tables 5, 6 (https://wwwnc.cdc.gov/EID/article/29/8/23-0115-App1.pdf). MDRO, multidrug-resistant organism



bacterial species	n° total isolates	n° MDRO colonization	% MDRO colonization	n° MDRO infection	% MDRO infection	n° non-MDRO infection	% non-MDRO infection
Staphylococcus aureus	127	25	20%	28	22%	74	58%
Enterococcus faecium	96	80	83%	4	4%	12	13%
Escherichia coli	52	26	50%	6	12%	20	38%
Klebsiella spp	121	34	28%	34	28%	53	44%
Pseudomonas aeruginosa	116	15	13%	22	19%	79	68%
Acinetobacter baumannii	22	13	59%	9	41%	0	0%

Appendix Figure 4. Radar chart and table of the most frequently isolated bacterial species. Proportions within species of MDRO colonization, MDRO infection and non-MDRO infection among the most frequently isolated bacteria of the WHO priority pathogens list are reported.