Since January 2020 Elsevier has created a COVID-19 resource centre with free information in English and Mandarin on the novel coronavirus COVID-19. The COVID-19 resource centre is hosted on Elsevier Connect, the company's public news and information website.

International Journal of Antimicrobial Agents 57 (2021) 106245

Contents lists available at ScienceDirect

International Journal of Antimicrobial Agents

journal homepage: www.elsevier.com/locate/ijantimicag

Letter to the Editor

Klebsiella pneumoniae infections in COVID-19 patients: a 2-month retrospective analysis in an Italian hospital

Italy has experienced one of the harshest and earliest COVID-19 (coronavirus disease 2019) epidemics, with the number of patients infected following, from the end of February up to the end of March 2020, an exponential trend [1]. Between the 6 March 2020 and 2 May 2020, a total of 394 patients were confirmed positive for SARS-CoV-2 (severe acute respiratory syndrome coronavirus 2) at the University Hospital of Rome Policlinico Umberto I (PUI) (Rome, Italy) [2]. At the PUI, 5 COVID-19-devoted wards were organised, including two brand-new intensive care units (ICUs), counting 32 dedicated to COVID-19 patients: the first was the old general ICU converted into a dedicated COVID-19 ICU, while the second was created in the spaces of four operating rooms (new ICU).

In the period of this study, a total of 80 COVID-19-affected patients were hospitalised in the two ICUs at PUI. Among them, 65 patients were screened for colonisation by carbapenemase-producing Enterobacterales (CPE) (Brilliance^{TM} CRE medium plates; Oxoid Ltd., Basingstoke, UK), including 41 of 47 SARS-CoV-2 patients hospitalised in the old ICU and 24 of 33 in the new ICU. Carbapenemase-producing *Klebsiella pneumoniae* were detected in 14/41 patients (34%) only in the old ICU. No CPE were detected from rectal swabs tested in patients hospitalised in the new ICU. In the same period, 11 CPE were identified from 39 rectal swabs of 48 SARS-CoV-2-negative patients (28%) hospitalised in the non-COVID ICU of the same hospital. Seven COVID-19 patients developed CPE co-infection (five bronchoalveolar lavages and two blood cultures tested positive for carbapenemase-producing *K. pneumoniae*), whilst in the non-COVID-19 ICU seven bloodstream infections (BSIs) also occurred (Table 1). Symptomatic patients were successfully treated with ceftazidime/avibactam.

By preliminary PCR testing (performed on one strain per patient by GeneXpert®; Cepheid, Sunnyvale, CA, USA), 10/14 *K. pneumoniae* strains (71%) from COVID-19 patients were positive for the bla_{OXA-48} gene (5 from bronchoalveolar lavage, 1 from blood culture and 4 from rectal swabs) and 4 (29%) for bla_{KPC} (1 from blood culture and 3 from rectal swabs). The 11 strains from non-COVID-19 patients were all KPC carbapenemase-producing *K. pneumoniae*.

Whole-genome sequencing was performed using an Illumina MiSeq instrument (Illumina Inc., San Diego, CA, USA) on four OXA-48- and the four KPC-producers from COVID-19 patients and on four KPC-producers from non-COVID patients. By multilocus sequence typing (MLST), all *K. pneumoniae* from COVID patients were ST101 (https://bigsdb.pasteur.fr/klebsiella/klebsiella.html). Two strains from non-COVID-19 patients were ST512 and two were ST101.

Despite MLST suggesting the spread of an outbreak clone through the hospital, there were significant differences among the isolates. ST101-OXA-48 were positive for *wzi* allele 64, capsular type K14.K64, while the ST101-KPC-3 had *wzi* allele 137, capsular type 17. Furthermore, ST101-OXA-48 were also positive for the *aac(6')-Ib3, aac(3)-IIa, aadA1, dfrA14, tet*(D) *and* bla_{TEM-1A} resistance genes and two of them carried the $bla_{CTX-M-15}$ extended-spectrum β-lactamase gene (Supplementary Fig. S1), while ST101-KPC-3 were characterised by the 16S rRNA methylase *armA* and the macrolide *mph(E)* resistance genes.

Phylogenetic analysis performed on single nucleotide polymorphisms (SNPs) using kSNP (Galaxy version 3.1) software on 20 *K. pneumoniae* ST101 genomes (10 downloaded from public databases, 8 COVID-19 and 2 non-COVID-19) showed that ST101-OXA-48 from COVID-19 patients were a unique clone. The ST101-KPC-3 from COVID-19 and non-COVID patients were all related but differed from the ST101-OXA-48 clone (>1500 SNPs; Supplementary Fig. S1). These data indicated the contemporary circulation of two independent clones in the same COVID-19 ICU. Both clones belonged to the high-risk sequence type ST101 [3] but were substantially different. OXA-48-producers have been rarely reported in Italy, while KPC-producers are endemic in our country (http://atlas.ecdc.europa.eu/public/index.aspx).

This study aims to provide a starting point of reflection about the great risk of CPE colonisation and hospital-acquired infection (HAI) in COVID-19 ICUs.

During the first months of the COVID-19 pandemic, antimicrobial stewardship programmes have been put to the test; although greater attention to hand hygiene and attempts to limit patient contact could have led to a reduction in the transmission of HAIs, at the same time other risky behaviours took place. Giving the priority of isolation chambers to quarantine COVID-19-affected patients and gathering them together in devoted wards or ICUs without the chance to contain patients colonised with CPE may have led to the introduction of colonised patients into the ICU, followed by potential propagation and hospital transmission of the introduced CPE [4]. Gloves and gowns, which were rare goods at that time, have been primarily delivered and, in that emergency situation, utilised more for personal protection of healthcare workers from COVID-19 than as tools to protect patients from HAIs. In parallel, the potential propagation of CPE may also be worsened by an increased rate of antimicrobial prescription in the absence of clear guidelines [5]. As a matter of fact, there are limited data and no controlled clinical trials evaluating the use of empirical antimicrobials in COVID-19 patients. The experience of those months

https://doi.org/10.1016/j.ijantimicag.2020.106245

Table 1
Carbapenemase-producing Enterobacterales (CPE) in COVID-19 and non-COVID-19 patients (6 March–2 May 2020) in the general ICU (non-COVID-19) and in the COVID-19-devoted ICUs.

ICU type	Blood cultures			Rectal swabs			No. of patients tested/total no. of patients in the ward (%)	Blood culture/colonisation rate (%)
	Pos.	Neg.	% CPE-pos.	Pos.	Neg.	% CPE-pos.		
Non-COVID-19	7[a]	29	19.4%	11[b]	28	28.2%	39/48 (81.3%)	7/11 (63.6%)
COVID-19	2[c]	23	8.0%	14[d]	27	34.1%	65/80 (81.3%)	2/14 (14.3%)

COVID-19, coronavirus disease 2019; ICU, intensive care unit.
[a–d]Number of isolates per bacterial species and carbapenemase type:
[a] 7 *Klebsiella pneumoniae* KPC-positive
[b] 11 *K. pneumoniae* KPC-positive
[c] 1 *K. pneumoniae* KPC-positive and 1 *K. pneumoniae* OXA-48-positive.
[d] 4 *K. pneumoniae* KPC-positive and 10 *K. pneumoniae* OXA-48-positive.

should be used to improve COVID-19 patient management, maintaining strict surveillance for CPE colonisation and co-infection.

Declarations

Funding: This work was supported by Sapienza University of Rome, Top Scientist Funds 2019 to A. Carattoli and by Ministero della Salute [Bando Ricerca COVID-19 grant no. COVID-2020-12371817] to G. Antonelli.

Competing interests: None declared.

Ethical approval: Procedures performed in the study were in accordance with the ethical standards of the Institutional and National Research Committee and with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards.

Data availability

The Whole Genome Project has been deposited at DDBJ/ENA/GenBank with the accession no. **PRJNA648931** [https://www.ncbi.nlm.nih.gov/bioproject/PRJNA648931].

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.ijantimicag.2020.106245.

References

[1] Rosenbaum L. Facing Covid-19 in Italy—ethics, logistics, and therapeutics on the epidemic's front line. N Engl J Med 2020;382:1873–5. doi:10.1056/NEJMp2005492.
[2] Turriziani O, Sciandra I, Mazzuti L, Di Carlo D, Bitossi C, Calabretto M, et al. SARS-CoV-2 diagnostics in the virology laboratory of a university hospital in Rome during the lockdown period. J Med Virol 2020 Jul 22 [Epub ahead of print]. doi:10.1002/jmv.26332.
[3] Roe CC, Vazquez AJ, Esposito EP, Zarrilli R, Sahl JW. Diversity, virulence, and antimicrobial resistance in isolates from the newly emerging *Klebsiella pneumoniae* ST101 lineage. Front Microbiol 2019;10:542. doi:10.3389/fmicb.2019.00542.
[4] Huttner BD, Catho G, Pano-Pardo JR, Pulcini C, Schouten J. COVID-19: don't neglect antimicrobial stewardship principles!. Clin Microbiol Infect 2020;26:808–10. doi:10.1016/j.cmi.2020.04.024.
[5] Chen N, Zhou M, Dong X, Qu J, Gong FG, Han Y, et al. Epidemiological and clinical characteristics of 99 cases of 2019 novel coronavirus pneumonia in Wuhan. China: a descriptive study. Lancet 2020;395:507–13.

Gabriele Arcari
Department of Molecular Medicine, Sapienza University of Rome, Rome, Italy

Giammarco Raponi
Department of Public Health and Infectious Diseases, Sapienza University of Rome, Rome, Italy
Sapienza University Hospital 'Policlinico Umberto I', Rome, Italy

Federica Sacco
Department of Public Health and Infectious Diseases, Sapienza University of Rome, Rome, Italy

Giulia Bibbolino
Department of Molecular Medicine and Medical Biotechnologies, University of Naples Federico II, Naples, Italy

Federica Maria Di Lella
Department of Experimental Medicine, University of Campania Luigi Vanvitelli, Naples, Italy

Francesco Alessandri
Sapienza University of Rome, Department of Anesthesia and Critical Care Medicine, Policlinico Umberto I, Rome, Italy

Monica Coletti, Maria Trancassini
Department of Public Health and Infectious Diseases, Sapienza University of Rome, Rome, Italy
Sapienza University Hospital 'Policlinico Umberto I', Rome, Italy

Alberto Deales
Sapienza University Hospital 'Policlinico Umberto I', Rome, Italy

Francesco Pugliese
Sapienza University of Rome, Department of Anesthesia and Critical Care Medicine, Policlinico Umberto I, Rome, Italy

Guido Antonelli*
Department of Molecular Medicine, Sapienza University of Rome, Rome, Italy
Sapienza University Hospital 'Policlinico Umberto I', Rome, Italy

Alessandra Carattoli
Department of Molecular Medicine, Sapienza University of Rome, Rome, Italy

*Corresponding author. Present address: Department of Molecular Medicine, Sapienza University of Rome, Rome, Italy.
E-mail address: guido.antonelli@uniroma1.it (G. Antonelli)