Carbapenemase-producing Enterobacterales causing secondary infections during the COVID-19 crisis at a New York City hospital

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Background: Patients with COVID-19 may be at increased risk for secondary bacterial infections with MDR pathogens, including carbapenemase-producing Enterobacterales (CPE).

Objectives: We sought to rapidly investigate the clinical characteristics, population structure and mechanisms of resistance of CPE causing secondary infections in patients with COVID-19.

Methods: We retrospectively identified CPE clinical isolates collected from patients testing positive for SARS-CoV-2 between March and April 2020 at our medical centre in New York City. Available isolates underwent nanopore sequencing for rapid genotyping, antibiotic resistance gene detection and phylogenetic analysis.

Results: We identified 31 CPE isolates from 13 patients, including 27 Klebsiella pneumoniae and 4 Enterobacter cloacae complex isolates. Most patients (11/13) had a positive respiratory culture and 7/13 developed bacteremia; treatment failure was common. Twenty isolates were available for WGS. Most K. pneumoniae (16/17) belonged to ST258 and encoded KPC (15 KPC-2; 1 KPC-3); one ST70 isolate encoded KPC-2. E. cloacae isolates belonged to ST270 and encoded NDM-1. Nanopore sequencing enabled identification of at least four distinct ST258 lineages in COVID-19 patients, which were validated by Illumina sequencing data.

Conclusions: While CPE prevalence has declined substantially in New York City in recent years, increased detection in patients with COVID-19 may signal a re-emergence of these highly resistant pathogens in the wake of the global pandemic. Increased surveillance and antimicrobial stewardship efforts, as well as identification of optimal treatment approaches for CPE, will be needed to mitigate their future impact.

Introduction
The COVID-19 pandemic, caused by the novel respiratory tract pathogen SARS-CoV-2, seems likely to be accompanied by a wave of secondary bacterial infections. Available evidence indicates that culture-proven secondary infections occur in 4%-15% of hospitalized patients and are significantly associated with mortality.1-4 However, few studies further characterize corresponding clinical and microbiological data. Moreover, antibiotic use has been widespread in patients with COVID-19,5 raising concerns about the emergence of MDR organisms. Here we describe 13 patients with severe COVID-19 who subsequently developed hospital-associated infections with carbapenemase-producing Enterobacterales (CPE) at our medical centre in New York City.

Materials and methods
All patients included in this cohort had positive tests for SARS-CoV-2 between 10 March and 30 April 2020 and had a clinical culture positive for carbapenemase gene-positive Enterobacterales identified by retrospective chart review up to 25 May 2020. Surveillance for MDR Gram-negative organisms was not routinely performed on hospital or ICU admission during the study period. Clinical isolates underwent routine species identification and susceptibility testing by MicroScan (Beckman Coulter) in our clinical microbiology laboratory. Carbapenemase genes including blaKPC and blaNDM were detected using the Xpert Carba-R assay (Cepheid) for meropenem-resistant isolates. We also performed broth microdilution (BMD) assays for meropenem and Ettests (bioMérieux) for ceftazidime/avibactam and meropenem/vaborbactam on available isolates, with MIC > 2 mg/L used to define meropenem, ceftazidime/avibactam and meropenem/vaborbactam resistance. Finally, we used nanopore long-read WGS to rapidly derive isolate
genotypes and perform phylogenetic analysis. Briefly, genomic DNA libraries were prepared using the Rapid Barcoding Kit and sequenced on a MiniION (Oxford Nanopore Technologies). To identify the isolate MLST, plasmid rep genes and antibiotic resistance genes (ARGs), reads were aligned against established databases. De novo assemblies were generated using Canu. For isolates belonging to *Klebsiella pneumoniae* ST258, we constructed phylogenetic trees from isolates with available nanopore sequencing data. Isolates derived from patients with COVID-19 and historical ST258 clinical isolates collected at our hospital between 2011 and 2017 were included. Here, core-genome SNPs were identified by mapping ical ST258 clinical isolates collected at our hospital between 2011 and 2017 sequencing data. Isolates derived from patients with COVID-19 and constructed phylogenetic trees from isolates with available nanopore sequencing data. Isolates derived from patients with COVID-19 and historically ST258 clinical isolates collected at our hospital between 2011 and 2017 were included. Here, core-genome SNPs were identified by mapping Canu-assembled nanopore genomes against an internal ST258 reference genome using Snippy (https://github.com/tseemann/snippy). To validate phylogenetic inferences made from nanopore-sequenced genomes, isolates were also sequenced on a MiSeq platform using the Nextera Flex Library Preparation Kit (Illumina). Phylogenetic trees were generated using RAxML and visualized using the R ‘ggtree’ library. Study procedures were approved by the Columbia University Institutional Review Board (Protocol AAAR7701). Genomes were deposited in Genbank (https://www.ncbi.nlm.nih.gov/genbank/) under BioProject number PRJNA645930.

### Results and discussion

Of 3152 patients with laboratory-confirmed COVID-19 who were admitted to the hospital during the study period, we identified 13 patients who had 31 positive cultures for CPE (Table 1).

| Patient | Age, sex | Isolate | CPE organism | Culture site | Isolate genotype, carbapenemase gene | CPE treatment | Outcome at discharge |
|---------|----------|---------|--------------|--------------|--------------------------------------|--------------|---------------------|
| 1       | 67, M    | KP1826  | *K. pneumoniae* | blood        | ST258, KPC-2                          | none         | died                |
|         |          | NK1608  | *K. pneumoniae* | blood        | ST258, KPC-2                          |              |                     |
| 2       | 50, M    | NK1590  | *K. pneumoniae* | respiratory  | ST258, KPC-2                          | CZA, PMB     | died                |
|         |          | KP1827  | *K. pneumoniae* | blood        | ST258, KPC-2                          |              |                     |
| 3       | 70, M    | NK1596  | *K. pneumoniae* | respiratory  | ST258, KPC-2                          | CZA, PMB     | died                |
|         |          | KP1828  | *K. pneumoniae* | blood        | ST258, KPC-2                          |              |                     |
|         |          | NK1661  | *K. pneumoniae* | respiratory  | ST258, KPC-2                          |              |                     |
| 4       | 72, M    | NK1597  | *K. pneumoniae* | respiratory  | ST258, KPC-2                          | CZA, PMB, MEM| died                |
|         |          | NK1677  | *K. pneumoniae* | respiratory  | ST258, KPC-2                          |              |                     |
| 5       | 39, F    | NK1593  | *K. pneumoniae* | respiratory  | ST258, KPC-2                          | CZA          | alive               |
|         |          | NK1607  | *K. pneumoniae* | respiratory  | ST258, KPC-2                          |              |                     |
| 6       | 72, M    | NK1580  | *K. pneumoniae* | respiratory  | ST258, KPC-2                          | CZA, ERV     | alive               |
| 7       | 59, M    | KP1829  | *K. pneumoniae* | blood        | ST258, KPC-2                          | CZA          | alive               |
|         |          | NK1586  | *K. pneumoniae* | respiratory  | ST258, KPC-2                          |              |                     |
| 8       | 65, M    | NK1594  | *K. pneumoniae* | respiratory  | ST258, KPC-2                          | CZA          | alive               |
| 9       | 74, M    | NK1595  | *K. pneumoniae* | respiratory  | ST258, KPC-2                          | CZA, MVB     | alive               |
| 10      | 71, M    | N/A     | *K. pneumoniae* | respiratory  | KPC (subtype unavailable)             | CZA, LVX     | alive               |
|         |          |         | *E. cloacae*    |              |                                       |              |                     |
| 11      | 48, M    | NK1321  | *E. cloacae*    | respiratory  | ST270, NDM-1                          | ERV          | alive               |
|         |          | NK1396  | *E. cloacae*    | respiratory  | ST270, NDM-1                          |              |                     |
| 12      | 23, M    | NK1513  | *K. pneumoniae* | urine        | ST258, KPC-3                          | CZA          | alive               |
| 13      | 86, F    | NK1644  | *E. cloacae*    | respiratory  | ST270, NDM-1                          | LVX          | died                |

MEM, meropenem; MVB, meropenem/vaborbactam; CZA, ceftazidime/avibactam; PMB, polymyxin B; ERV, eravacycline; LVX, levofloxacin; M, male; F, female; N/A, not available.

*Antibiotics added or substituted in the setting of initial treatment failure are denoted in bold.

**Patient 10 isolates were unavailable for further analysis or WGS; for all other patients, only available isolates are shown.**
CPE co-infections in COVID-19 patients

(meropenem/vaborbactam or polymyxin B). Five patients in this cohort expired, two of whom had positive post-mortem cultures, and the other eight survived to hospital discharge.

Twenty isolates from 12 patients were available for nanopore sequencing (Table 1). CPE isolates consisted of KPC-producing K. pneumoniae (27/31) and NDM-producing Enterobacter cloacae complex (4/31). The majority of sequenced K. pneumoniae isolates belonged to ST258 harbouring blαKPC-2 (15/17). In ST258 isolates, blαKPC-2 was found within the Tn4401a transposon but was encoded by two different plasmids belonging to the IncIV/K and IncFIB/FII families. One patient who developed ventilator-associated pneumonia (VAP) with K. pneumoniae ST258 and had persistently positive cultures was subsequently found to have K. pneumoniae ST70 harbouring blαKPC-2 on an IncFIB/FII plasmid. Only one ST258 isolate harbour blαKPC-3. Lastly, three E. cloacae ST270 isolates from two patients harboured blαNDM-1, putatively encoded by an IncHI2 plasmid.

Most of the available isolates in this collection (18/20) demonstrated high-level meropenem resistance (meropenem MIC ≥ 16 mg/L by BMD), with MIC50 and MIC90 values of 64 and 128 mg/L for all isolates, respectively. Most KPC-producing K. pneumoniae isolates were susceptible to ceftazidime/avibactam (MIC50 and MIC90 of 2 and 4 mg/L, respectively). However, one patient with K. pneumoniae VAP developed ceftazidime/avibactam treatment failure attributable to development of resistance, also with reversion of meropenem MIC to the susceptible range (MIC 0.094 mg/L). blαKPC-2 Sequencing of the ceftazidime/avibactam-resistant isolate (NK1677) confirmed an aspartic acid-to-tyrosine substitution at Ambler amino acid position 179 (D179Y). All NDM-producing E. cloacae isolates were highly resistant to ceftazidime/avibactam. CPE isolates also encoded a large number of additional ARGs (median 18, IQR 16.5–18.5) conferring resistance across a broad range of antibiotic classes, including aminoglycosides, fluoroquinolones and tetracyclines.

In a phylogenetic analysis of ST258 genomes performed to further investigate isolate clustering, we found evidence for several distinct lineages in patients with COVID-19, each with ≥80% bootstrap support (Figure 1). Most of these belonged to two branches corresponding to isolates harbouring blαKPC-2 on IncN/R versus IncFIB/FII plasmids (Figure 1a–c). These two branches also largely consisted of isolates derived from patients admitted to two separate hospital units (Units 1 and 2). Two isolates from one patient with COVID-19 from a different hospital unit (Unit 3), which also harboured blαKPC-2 on IncN/R plasmids, comprised a separate branch, as did one isolate encoding blαKPC-3 from a patient who was also hospitalized on a different unit (Unit 4). Phylogenetic tree topology was similar and evidence of clustering was evident using both sequencing approaches, albeit with larger branch lengths for nanopore-sequenced (Figure 1a) compared with Illumina-sequenced genomes (Figure 1b). Notably, each lineage of isolates from COVID-19 patients was closely related to at least one K. pneumoniae ST258 isolate from respiratory cultures collected at our hospital between 2011 and 2016 (100% bootstrap support) (Figure 1c).

Conclusions

A broad range of factors likely contributed to the development of CPE infections in this patient population. Underlying disease manifestations such as severe lung injury leading to prolonged mechanical ventilation, coupled with immune dysregulation in the setting of critical illness and use of immunomodulatory therapies, such as corticosteroids and IL-6 inhibitors, almost certainly increased the risk of bacterial superinfection. Receipt of broad-spectrum antibiotics may have generated antibiotic pressure for the emergence of MDR pathogens. Beginning in March 2020, our hospital underwent a massive escalation of inpatient and critical care capacity to accommodate the rapid influx of patients with COVID-19. While clearly necessary to address the heavy burden of disease in our area, measures such as ICU patient cohorting and extended use of gowns and other personal protective equipment in COVID-19 units may have enabled transmission of other pathogens including MDR bacteria within hospital units. Given that we observed several different strains of KPC- and NDM-producing Enterobacteriales in this cohort, however, we suspect that prolonged critical illness and other clinical factors produced an optimal host environment supporting the emergence of diverse CPE in this setting.

CPE infections are associated with increased mortality in the face of multi-class antibiotic resistance and could place additional burdens on already overstretched health systems. While CPE are endemic in New York City, the appearance of CPE in this patient population is notable for occurring in the setting of a recent decrease in the incidence at our institution and citywide. According to hospital antibiogram data, the proportion of inpatient Enterobacteriales isolates resistant to meropenem decreased from a peak of 7.5% in 2010 to 1.5% in 2018; 27 meropenem-resistant K. pneumoniae or E. cloacae isolates were identified in 2018. Moreover, optimal treatment regimens for CPE in this patient population have not yet been determined. A large proportion of patients with KPC-producing K. pneumoniae infections treated with ceftazidime/avibactam demonstrated persistently positive cultures and one patient was found to have treatment failure due to development of a resistance-conferring mutation in blαKPC-2. Most patients in this cohort developed respiratory tract infections and many also required RRT, both risk factors for ceftazidime/avibactam failure. As the pandemic continues, it will be critical to devise active surveillance and prevention strategies and optimize treatment approaches to limit the impact of these MDR pathogens.

Use of nanopore sequencing facilitated rapid genotyping and ARG profiling, including data availability within several hours of initiating sequencing. We also successfully carried out phylogenetic analysis using long reads only. Although within-patient SNP distances were much higher than with corresponding Illumina sequencing data, results were consistent and corroborated available epidemiological and genotyping data. We were able to use this approach to demonstrate the presence of multiple different ST258 lineages driving the emergence of CPE in this patient population and link these lineages to historical isolates. Taken together, this approach could be integrated into infection control approaches to rapidly identify ARGs and putative transmission clusters in patients with COVID-19.

In conclusion, increased detection of CPE in patients with COVID-19 supports recent concerns regarding the emergence of MDR bacterial co-infections in the wake of the global pandemic. Developing approaches for mitigating the impact of MDR infections...
in this patient population should be a necessary component of management strategies for patients with COVID-19.

Figure 1. Maximum-likelihood phylogenetic trees generated using nanopore and Illumina-sequenced genomes of K. pneumoniae ST258 historical isolates and isolates derived from patients with COVID-19. Maximum-likelihood trees of de novo assembled K. pneumoniae ST258 isolates were constructed based on core-genome concatenated SNPs, including all available isolates from patients with COVID-19 and historical isolates with available nanopore sequencing data (Figure 1a). The resulting tree topology was similar to that derived using Illumina sequencing (Figure 1b), although with shorter branch length and improved within-cluster resolution, and confirmed the presence of at least four different distinct ST258 lineages within this cohort. Finally, inclusion of all historical respiratory ST258 isolates with available Illumina sequencing data demonstrated links within each COVID-19-associated lineage (Figure 1c). In all tree topologies, isolates derived from patients with COVID-19 largely corresponded to four separate hospital units (Units 1–4), designated by shaded boxes in each tree, whereas historical isolates are unmarked. One isolate (KP1594), which clustered with Unit 1 genomes, was derived from a separate hospital unit and is marked with an asterisk (Figure 1a). This figure appears in colour in the online version of JAC and in black and white in the print version of JAC.

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