J Antimicrob Chemother 2021; **76**: 380–384
doi:10.1093/jac/dkaa466 Advance Access publication 17 November 2020

Journal of Antimicrobial Chemotherapy

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

Angela Gomez-Simmonds[1], Medini K. Annavajhala[1], Thomas H. McConville[1], Donald E. Dietz[1], Sherif M. Shoucri[1], Justin C. Laracy[1], Felix D. Rozenberg[1], Brian Nelson[1], William G. Greendyke[1], E. Yoko Furuya[1], Susan Whittier[2] and Anne-Catrin Uhlemann[1]*

[1]*Division of Infectious Diseases, Department of Medicine, Columbia University Irving Medical Center, 630 W 168th St, New York City, NY 10032, USA;* [2]*Department of Pathology and Cell Biology, Columbia University Irving Medical Center, 630 W 168th St, New York City, NY 10032, USA*

*Corresponding author. E-mail: au2110@cumc.columbia.edu

Received 6 August 2020; accepted 19 October 2020

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 bacteraemia; 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 bla_{KPC} and bla_{NDM} were detected using the Xpert Carba-R assay (Cepheid) for meropenem-resistant isolates. We also performed broth microdilution (BMD) assays for meropenem and Etests (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 MinION (Oxford Nanopore Technologies). To identify the isolate MLST, plasmid *rep* genes and antibiotic resistance genes (ARGs), reads were aligned against established databases.[6–9] *De novo* assemblies were generated using Canu.[10] 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.[11] 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[12] and visualized using the R 'ggtree' library.[13] 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). Carbapenemase genes were not detected in carbapenem-resistant Enterobacterales isolated from an additional seven patients and were not included in the analysis. Most patients in this cohort were elderly (median age 67 years, IQR 50–72) and all but one had documented chronic comorbidities. The most common source of infection was the respiratory tract (11/13); bacteraemia developed in seven (54%) patients. All patients had prolonged, complex hospitalizations, with 12/13 patients requiring intubation and ICU-level care, and 10 requiring renal replacement therapy (RRT). Prior to CPE detection, 10/13 patients received immunomodulators including corticosteroids (9/10) and/or IL-6 receptor antagonists (3/10). All patients also received broad-spectrum β-lactam antibiotics including piperacillin/tazobactam, cefepime and/or meropenem. Initial treatment of KPC-producing *K. pneumoniae* infections consisted primarily of ceftazidime/avibactam (10/11; one patient died before initiation of appropriate antibiotics), given in combination with other agents (polymyxin B, eravacycline or levofloxacin) in three patients. Importantly, 8/13 patients had persistently positive cultures ≥7 days after initial CPE detection, in some cases with subsequent development of bacteraemia or infection at a different body site. Three patients initially treated with ceftazidime/avibactam monotherapy subsequently required additional or alternative antibiotics

Table 1. Cohort of patients with COVID-19 and isolates causing secondary CPE infections

Patient	Age, sex	Isolate	CPE organism	Culture site	Isolate genotype, carbapenemase gene	CPE treatment[a]	Outcome at discharge
1	67, M	KP1826	*K. pneumoniae*	blood	ST258, KPC-2	none	died
		NK1608	*K. pneumoniae*	blood (post-mortem)	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	ST70, 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*	urine	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[b]	71, M	N/A	*K. pneumoniae*	respiratory	KPC (subtype unavailable)	CZA, LVX	alive
11	48, M	NK1321	*E. cloacae* complex	respiratory	ST270, NDM-1	ERV	alive
		NK1396	*E. cloacae* complex	respiratory	ST270, NDM-1		
12	23, M	NK1513	*K. pneumoniae*	urine	ST258, KPC-3	CZA	alive
13	86, F	NK1644	*E. cloacae* complex	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.

[a]Antibiotics added or substituted in the setting of initial treatment failure are denoted in bold.

[b]Patient 10 isolates were unavailable for further analysis or WGS; for all other patients, only available isolates are shown.

(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 bla_{KPC-2} (15/17). In ST258 isolates, bla_{KPC-2} was found within the Tn*4401a* transposon but was encoded by two different plasmids belonging to the IncN/R 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 bla_{KPC-2} on an IncFIB/FII plasmid. Only one ST258 isolate harboured bla_{KPC-3}. Lastly, three *E. cloacae* ST270 isolates from two patients harboured bla_{NDM-1}, putatively encoded by an IncHI2 plasmid.

Most of the available isolates in this collection (18/20) demonstrated high-level meropenem resistance (meropenem MIC $\geq$ 16 mg/L by BMD), with MIC_{50} and MIC_{90} values of 64 and 128 mg/L for all isolates, respectively. Most KPC-producing *K. pneumoniae* isolates were susceptible to ceftazidime/avibactam (MIC_{50} and MIC_{90} 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). bla_{KPC-2} sequencing of the ceftazidime/avibactam-resistant isolate (NK1677) confirmed an aspartic acid-to-tyrosine substitution at Ambler amino acid position 179 (D179Y).[14,15] 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 $\geq$80% bootstrap support (Figure 1). Most of these belonged to two branches corresponding to isolates harbouring bla_{KPC-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 bla_{KPC-2} on IncN/R plasmids, comprised a separate branch, as did one isolate encoding bla_{KPC-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 Enterobacterales 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.[16] According to hospital antibiogram data, the proportion of inpatient Enterobacterales 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 bla_{KPC-2}. Most patients in this cohort developed respiratory tract infections and many also required RRT, both risk factors for ceftazidime/avibactam failure.[17] 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

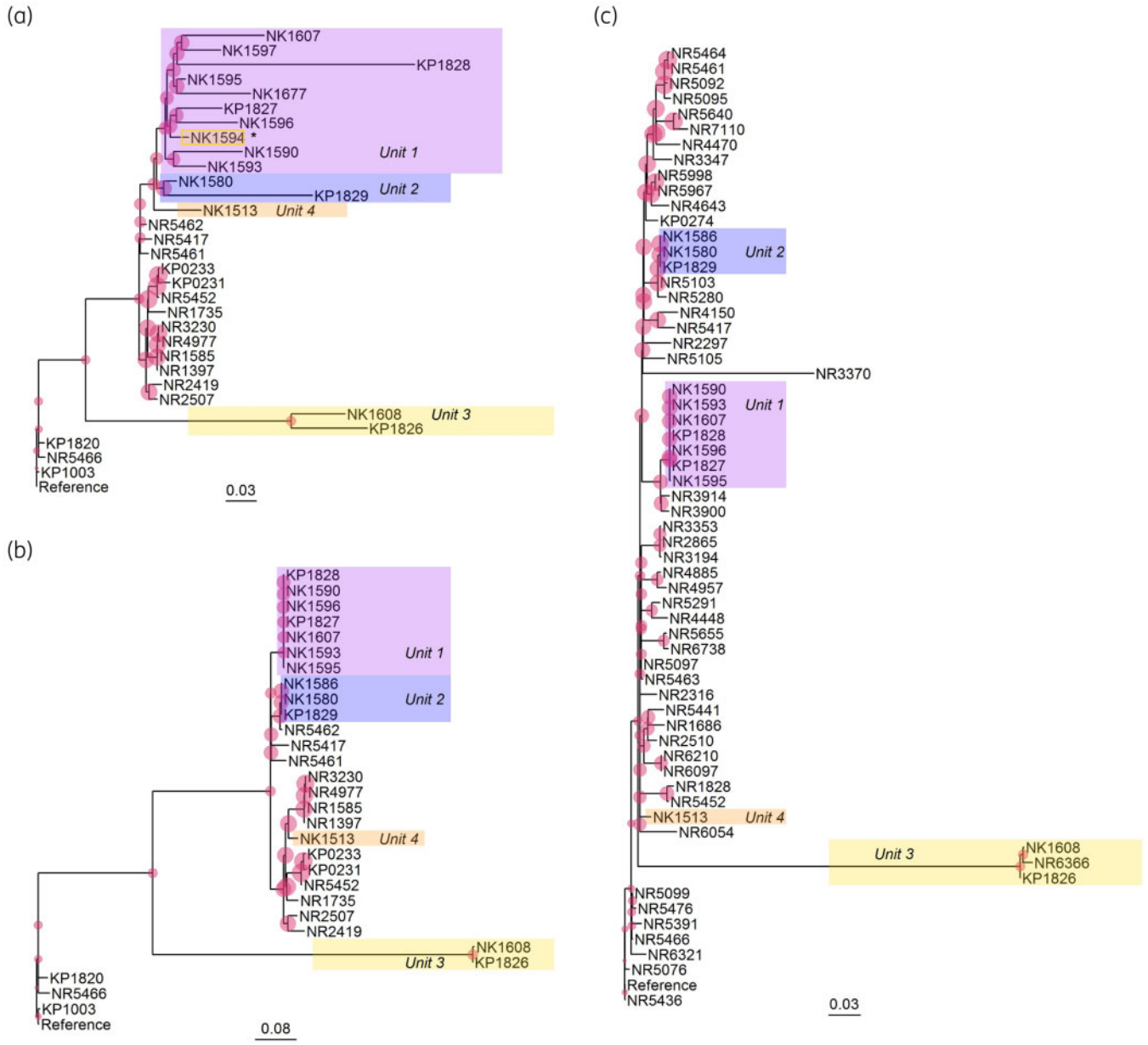

Figure 1. Maximum-likelihood phylogenetic trees generated using nanopore and Illumina-sequenced genomes of *K. pneumoniae* ST258 historical isolates and isolates derived from patients with COVID-19. Maximum-likelihood trees of *de novo* assembled *K. pneumoniae* ST258 isolates were constructed based 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*.

in this patient population should be a necessary component of management strategies for patients with COVID-19.

Funding

This study was supported by the NIAID at the NIH (RO1 AI116939 to A.C.U. and K23 AI137316 to A.G.S.). The funders of the study had no role in the study design, data collection, data analysis, data interpretation or writing of the report.

Transparency declarations

A.-C.U. has received research funding from Merck, GlaxoSmithKline and Allergan. All other authors have no potential conflicts to disclose.

References

1 Huang C, Wang Y, Li X *et al*. Clinical features of patients infected with 2019 novel coronavirus in Wuhan, China. *Lancet* 2020; **395**: 497–506.

2 Zhou F, Yu T, Du R *et al*. Clinical course and risk factors for mortality of adult inpatients with COVID-19 in Wuhan, China: a retrospective cohort study. *Lancet* 2020; **395**: 1054–62.

3 Hughes S, Troise O, Donaldson H *et al*. Bacterial and fungal coinfection among hospitalized patients with COVID-19: a retrospective cohort study in a UK secondary-care setting. *Clin Microbiol Infect* 2020; **26**: 1395–9.

4 Nori P, Cowman K, Chen V *et al*. Bacterial and fungal co-infections in COVID-19 patients hospitalized during the New York City pandemic surge. *Infect Control Hosp Epidemiol* 2020; doi: 10.1017/ice.2020.368.

5 Rawson TM, Moore LSP, Zhu N *et al*. Bacterial and fungal co-infection in individuals with coronavirus: a rapid review to support COVID-19 antimicrobial prescribing. *Clin Infect Dis* 2020; doi: 10.1093/cid/ciaa530.

6 Diancourt L, Passet V, Verhoef J *et al*. Multilocus sequence typing of *Klebsiella pneumoniae* nosocomial isolates. *J Clin Microbiol* 2005; **43**: 4178–82.

7 Miyoshi-Akiyama T, Hayakawa K, Ohmagari N *et al*. Multilocus sequence typing (MLST) for characterization of *Enterobacter cloacae*. *PLoS One* 2013; **8**: e66358.

8 Gupta SK, Padmanabhan BR, Diene SM *et al*. ARG-ANNOT, a new bioinformatic tool to discover antibiotic resistance genes in bacterial genomes. *Antimicrob Agents Chemother* 2014; **58**: 212–20.

9 Carattoli A, Zankari E, García-Fernández A *et al*. *In silico* detection and typing of plasmids using PlasmidFinder and plasmid multilocus sequence typing. *Antimicrob Agents Chemother* 2014; **58**: 3895–903.

10 Koren S, Walenz BP, Berlin K *et al*. Canu: scalable and accurate long-read assembly via adaptive k-mer weighting and repeat separation. *Genome Res* 2017; **27**: 722–36.

11 Macesic N, Nelson B, McConville TH *et al*. Emergence of polymyxin resistance in clinical *Klebsiella pneumoniae* through diverse genetic adaptations: a genomic, retrospective cohort study. *Clin Infect Dis* 2020; **70**: 2084–91.

12 Stamatakis A. RAxML version 8: a tool for phylogenetic analysis and post-analysis of large phylogenies. *Bioinformatics* 2014; **30**: 1312–3.

13 Yu G. Using ggtree to visualize data on tree-like structures. *Curr Protoc Bioinformatics* 2020; **69**: e96.

14 Shields RK, Nguyen MH, Press EG *et al*. Emergence of ceftazidime-avibactam resistance and restoration of carbapenem susceptibility in *Klebsiella pneumoniae* carbapenemase-producing *K. pneumoniae*: a case report and review of literature. *Open Forum Infect Dis* 2017; **4**: ofx101.

15 Livermore DM, Warner M, Jamrozy D *et al*. In vitro selection of ceftazidime-avibactam resistance in Enterobacteriaceae with KPC-3 carbapenemase. *Antimicrob Agents Chemother* 2015; **59**: 5324–30.

16 Park SO, Liu J, Furuya EY *et al*. Carbapenem-resistant *Klebsiella pneumoniae* infection in three New York City hospitals trended downwards from 2006 to 2014. *Open Forum Infect Dis* 2016; **3**: ofw222.

17 Shields RK, Nguyen MH, Chen L *et al*. Pneumonia and renal replacement therapy are risk factors for ceftazidime-avibactam treatment failures and resistance among patients with carbapenem-resistant Enterobacteriaceae infections. *Antimicrob Agents Chemother* 2018; **62**: e02497-17.