Full Title: Real-time genomic and epidemiological investigation of a multi-institution outbreak of KPC-producing Enterobacteriaceae: a translational study

Short Title: Real-time genomic and epidemiological investigation of a KPC outbreak

Kwong JC [1,2,3], Lane C [4,5], Romanes F [5], Gonçalves da Silva A [1,4], Easton M [5], Cronin K [6], Waters MJ [6], Tomita T [4], Stevens K [4], Schultz MB [1,4], Baines SL [1,2], Sherry NL [1,2,3], Carter G [1,2], Mu A [1,2], Sait M [4], Ballard SA [1,4], Seemann T [1,7], Stinear TP [1,2] & Howden BP [1,2,3,4].

[1] Doherty Applied Microbial Genomics, and [2] Department of Microbiology and Immunology, The University of Melbourne at The Peter Doherty Institute for Infection & Immunity, Melbourne, Australia

[3] Department of Infectious Diseases, Austin Health, Heidelberg, Australia

[4] Antimicrobial Reference and Research Unit, Microbiological Diagnostic Unit Public Health Laboratory, The University of Melbourne at The Peter Doherty Institute for Infection & Immunity, Melbourne, Australia

[5] Health Protection Branch, Department of Health and Human Services, Victoria, Australia

[6] Department of Microbiology, St Vincent's Hospital Melbourne, Fitzroy, Australia

[7] Melbourne Bioinformatics, The University of Melbourne, Carlton, Australia

Keywords:

Klebsiella pneumoniae carbapenemase, KPC, whole-genome sequencing, outbreak investigation

Correspondence:

Prof Benjamin P Howden

Microbiological Diagnostic Unit Public Health Laboratory

Doherty Institute for Infection & Immunity

792 Elizabeth Street

Melbourne, Victoria

Australia 3000

Email: bhowden@unimelb.edu.au

Phone: +61 3 8344 5701

Word count:

Abstract 296; Main text 6288

ABSTRACT

Background: Until recently, KPC-producing Enterobacteriaceae were rarely identified in Australia. Following an increase in the number of incident cases across the state of Victoria, we undertook a real-time combined genomic and epidemiological investigation. The scope of this study included identifying risk factors and routes of transmission, and investigating the utility of genomics to enhance traditional field epidemiology for informing management of established widespread outbreaks.

Methods and Findings: All KPC-producing Enterobacteriaceae isolates referred to the state reference laboratory from 2012 onwards were included. Whole-genome sequencing (WGS) was performed in parallel with a detailed descriptive epidemiological investigation of each case, using Illumina sequencing on each isolate. This was complemented with PacBio long-read sequencing on selected isolates to establish high-quality reference sequences and interrogate characteristics of KPC-encoding plasmids. Initial investigations indicated the outbreak was widespread, with 86 KPC-producing Enterobacteriaceae isolates (*K. pneumoniae* 92%) identified from 35 different locations across metropolitan and rural Victoria between 2012-2015. Initial combined analyses of the epidemiological and genomic data resolved the outbreak into distinct nosocomial transmission networks, and identified healthcare facilities at the epicentre of KPC transmission. New cases were assigned to transmission networks in real-time, allowing focussed infection control efforts. PacBio sequencing confirmed a secondary transmission network arising from inter-species plasmid transmission. Insights from Bayesian transmission inference and analyses of within-host diversity informed the development of state-wide public health and infection control guidelines, including interventions such as an intensive approach to screening contacts following new case detection to minimise unrecognised colonisation.

63 **Conclusions:** A real-time combined epidemiological and genomic investigation proved
64 critical to identifying and defining multiple transmission networks of KPC
65 Enterobacteriaceae, while data from either investigation alone were inconclusive. The
66 investigation was fundamental to informing infection control measures in real-time and the
67 development of state-wide public health guidelines on carbapenemase producing
68 Enterobacteriaceae management.
69

BACKGROUND

Carbapenemase-producing Enterobacteriaceae (CPE) are among the most urgent antimicrobial resistance threats worldwide [1]. In addition to producing the carbapenemase, capable of inactivating almost all beta-lactam antibiotics, including penicillins, cephalosporins and carbapenems, these organisms frequently harbour multiple other antibiotic resistance genes and mutations [2]. Few therapeutic options exist, and the available options are often limited by tolerability and efficacy [3, 4].

In Australia, carbapenemases have been rarely identified in Enterobacteriaceae, apart from the weakly carbapenem-hydrolysing $bla_{\text{IMP-4}}$ which has established low level endemicity [5, 6]. Although small outbreaks and limited patient-to-patient transmission of CPE in Australia have been described [7-11], the majority of patients identified with CPE are thought to have acquired these organisms during international travel to endemic countries [12-16]. Limited transmission in the community has also been reported [17].

Klebsiella pneumoniae carbapenemases (KPCs) were first identified in the United States of America in 1996, but have since spread worldwide to be endemic in several countries including the USA, Israel, Italy, Greece, Brazil and China [18]. The first reported KPC-producing organism in Australia was isolated in 2010 from a patient who had returned to Sydney, New South Wales, after being hospitalised in Greece [19]. In 2012, KPC was first identified in the state of Victoria in a patient who had been repatriated to a metropolitan hospital also after a prolonged admission in a Greek hospital [12].

Whole-genome sequencing (WGS) has emerged as a powerful tool for bacterial strain typing and outbreak investigation [20], and has been used in public health to assess transmission of

95 *Listeria monocytogenes* and other foodborne pathogens at jurisdictional, national and
96 international levels [21]. It has also been used at a single institution level to investigate small-
97 to-medium sized outbreaks of KPC-producing Enterobacteriaceae [22-28]. While the details
98 of who infected whom can be traced among a small group of individuals in conjunction with
99 a detailed epidemiological investigation, the transmission dynamics of larger established
100 outbreaks across multiple institutions are more difficult to resolve [29].
101
102 Due to the increasing incidence of KPC-producing Enterobacteriaceae during 2012–2014
103 from multiple sources across the state of Victoria (approximate population 6 million), we
104 undertook an outbreak investigation employing WGS in parallel with traditional outbreak
105 epidemiology. Here we report the utility of advanced genomic approaches including
106 sampling for within-host clonal diversity, Bayesian transmission modelling, and plasmid
107 genome reconstruction to assess the transmission dynamics of the outbreak and inform
108 infection control management in real-time during the course of the outbreak.
109
110

METHODS

Laboratory and Genomic Methods

Isolate selection and antimicrobial susceptibility assays. From 2012, following the first KPC isolate in the state, all Victorian diagnostic microbiology laboratories were asked to refer suspected carbapenemase-producing isolates to the Microbiological Diagnostic Unit Public Health Laboratory (MDU) for further testing. Suspected isolates were defined as Enterobacteriaceae with a meropenem minimum inhibitory concentration (MIC) ≥0.5 mg/L or zone diameter (ZD) ≤23 mm, or an imipenem MIC ≥2 mg/L or ZD ≤23 mm. Isolates with a positive colorimetric (e.g. Rapidec® CarbaNP [30]) or molecular (e.g. Cepheid GeneXpert® [31]) test were also requested. Duplicate KPC-producing Enterobacteriaceae isolates of the same species from a patient during a single hospital admission were usually excluded by referring laboratories. However, for some patients, multiple isolates were referred to MDU if they originated from different laboratories, and were subsequently included in the analyses.

Species identification was confirmed using matrix-assisted laser desorption/ionisation time-of-flight (MALDI-TOF) mass spectrometry (VITEK® MS, bioMérieux, France) with routine susceptibilities tested on selected isolates using VITEK® 2 Compact (AST-N246 cards, bioMérieux, France). All suspected CPE isolates meeting the above criteria underwent testing for carbapenemase genes using polymerase chain reaction (PCR) (Supplementary Table S1). Isolates confirmed as KPC-producing organisms underwent WGS and were included in the genomic analyses, with the respective patients included in the epidemiological investigation. Isolates obtained prior to 2014 were sequenced retrospectively, while WGS was performed prospectively during the outbreak on isolates collected from 2014 onwards.

136 ***Multiple colony sampling.*** To understand the genomic diversity present within a single
137 individual and assess the influence on reconstructing transmission networks, primary faecal
138 specimens were obtained from one patient. After overnight culture on Brilliance™ CRE
139 (Thermo Fisher, Waltham, Massachusetts, US) selective media, 10-15 colonies, including
140 any that differed in colony morphology, were selected from the plates for DNA extraction
141 and WGS. Multiple colony sampling and sequencing for other patient samples was not
142 performed due to cost limitations.
143
144 ***DNA extraction and whole genome sequencing***. Bacterial cultures were purified for DNA
145 extraction by two successive single colony selections after streaking onto horse blood agar
146 incubated overnight at 37°C. DNA was extracted from a liquid suspension of the purified
147 cultures using the QIAmp DNA Mini Kit (Qiagen, Hilden, Germany) or the JANUS
148 Chemagic Workstation with the Chemagic Viral DNA/RNA kit (CMG-1033; PerkinElmer,
149 Waltham, Massachusetts, US).
150
151 WGS was performed on the Illumina MiSeq or NextSeq platforms using Nextera XT libraries
152 and protocols (Illumina, San Diego, California, US) with a minimum average quality score of
153 30 and a target sequencing depth of ≥50x as previously described [32]. Isolates not meeting
154 these metrics were resequenced. Raw sequence data has been uploaded to the Sequence Read
155 Archive under BioProject PRJNA397262.
156
157 Single molecule real-time (SMRT) sequencing of one *Klebsiella pneumoniae* and one
158 *Citrobacter farmeri* isolate was also performed on the PacBio RS II platform (Pacific
159 Biosciences, Menlo Park, California, US) using previously reported methods [32]. In brief,
160 genomic DNA was extracted using a GenElute™ Bacterial Genomic DNA kit (Sigma-

Aldrich, St. Louis, Missouri, USA) and SMRTbell libraries were prepared using manufacturer protocols (Pacific Biosciences, Menlo Park, California). Libraries underwent an additional size-selection step using a 20-kb template BluePippin size-selection protocol (Sage Science, Beverly, Massachusetts, USA).

Bioinformatic analyses. Illumina raw sequencing reads were trimmed to clip Nextera adapters and low-quality sequence (Phred score <10) using *Trimmomatic* v0.36 [33]. The trimmed reads were assembled *de novo* with the *SPAdes* v3.7.1 assembler [34] and auto-annotated with *Prokka* v1.12-beta [35]. The SMRT analysis portal v2.3.0.140936 (Pacific Biosciences) was used for isolates sequenced on the PacBio RS II, with raw sequence reads assembled *de novo* using the HGAP3 protocol, and error correction and polishing with *Quiver* v1. Further error correction was performed by mapping Illumina short reads from the same isolate to the PacBio assembly with *Pilon* v1.21 [36] and *Snippy* v3.2 (https://github.com/tseemann/snippy). The completed genome assemblies have been uploaded to GenBank under BioProject PRJNA397262.

From the annotated assemblies, the multi-locus sequence type (MLST) was determined *in silico* as was the presence or absence of antimicrobial resistance ("resistome") and other genes (pan-genome) using BLAST-based tools (https://github.com/tseemann/mlst) (https://github.com/tseemann/abricate) (https://github.com/sanger-pathogens/Roary) [37]. The pan-genome data was imported into *FriPan* (https://github.com/drpowell/FriPan) for web-browser visualisation using a Python script (https://github.com/kwongj/roary2fripan), and annotated genomes were visualised in *Geneious* v7.1.5 (http://www.geneious.com/).

Sequencing reads were also aligned to a reference genome to produce a reference-based whole-genome alignment including single nucleotide polymorphism (SNP), invariant and missing sites (https://github.com/tseemann/snippy). This alignment was then trimmed to exclude plasmid sites (https://github.com/kwongj/trim-aln). Putative regions of recombination were predicted using *ClonalFrameML* v1.0 [38], and masked in the alignment (https://github.com/kwongj/cfml-maskrc). Core genome SNP sites were extracted from the recombination-filtered alignment [39] and a maximum likelihood phylogenetic tree inferred from the resulting SNP alignment in *RAxML* v8.2.4 [40], using a General Time Reversible model of nucleotide substitution with a Γ model of rate heterogeneity and four rate categories, with 1000 bootstrap replicates to determine branch support. Hierarchical Bayesian Analysis of Population Structure (*hierBAPS*) [41] was used to provide further support for identifying phylogenetic clades, with clustering performed using 8 levels in the hierarchy (L) and the prior maximum number of clusters (maxK) set at 10. Publicly available sequencing data was retrieved from the National Center for Biotechnology Information (NCBI) Sequence Read Archive and GenBank for comparison with local genomes (Supplementary Tables S2 and S3). Three reference genomes were used. The initial genomic analysis was performed using KPNIH24 (GenBank accession CP008797), with PacBio sequences of internal isolates (*K. pneumoniae* AUSMDU00008079; GenBank accession SAMN07452764 and *C. farmeri* AUSMDU00008141; GenBank accession SAMN07452765) used for subsequent analyses to minimise inaccuracies and biases from using distant genomes and draft genome assemblies as references (Fig S1).

The genomic context of the bla_{KPC} gene was investigated using a custom Python script (https://github.com/kwongj/contig-puller) to extract and align *de novo* assembled contigs carrying the gene. The flanking *Tn*4401 transposon regions were compared to previously

described isoforms [42-45], using *BLAST*+ [46]. Plasmid replicons were identified in genome assemblies using *BLAST*+ against the *PlasmidFinder* database [47]. Plasmids were presumed to carry KPC if bla_{KPC} was identified on the same contiguous sequence of DNA as the plasmid replicon.

Bayesian evolutionary analyses were also conducted to ascertain if the genomic signal could estimate the date of emergence of any outbreak clusters, and if any additional data could help inform where and when transmission events were taking place. A recombination-filtered chromosomal alignment was obtained as described above. The subsequent alignment was used as input into *BEAST2* v2.4.3 [48] with collection dates entered for each isolate. The relationship between root-to-tip distance and date of isolation was assessed in *TempEst* v1.5 [49]. We fitted a model with a relaxed log-normal clock [50] to the alignment to account for inter-clade variation, assuming a HKY model for nucleotide substitution with Γ distributed among site rate variation [51], and used a constant population size coalescent prior on the genealogy. Nodes were selected for logging the likely time of the most recent common ancestor (MRCA) for major clades identified in the maximum likelihood tree. We used eight Markov chain Monte Carlo (MCMC) runs of 100 million states, with sampling every 5000 states, and a burn-in of 50 percent. The posterior samples from each chain were checked in *Tracer* v1.6 (http://tree.bio.ed.ac.uk/software/tracer/) for convergence, and then grouped into a single chain. The posterior samples for the dates of the nodes of interest were annotated on a maximum clade credibility tree and exported.

The maximum clade credibility tree was used as input into *TransPhylo* v1.0 [52] to reconstruct the transmission chain. We based our prior for generation time (the time from primary infection of an individual to any subsequent secondary infection) on a previous study

on the duration of carriage of KPC-producing Enterobacteriaceae [53], assuming detection of KPC as an indicator of likelihood for onwards transmission. We therefore used a Gamma distribution with shape parameter 1.2 and scale 1.0 for the generation time prior, with a distribution mean of 1.2 years (438 days), standard deviation 1.096 years (400 days), and mode 0.2 years (73 days). The MCMC was run for 100000 iterations, with transmissions inferred from a consensus transmission tree.

Outbreak genomic investigation. An initial analysis using the bioinformatic methods described above was performed in 2014 on 41 KPC-producing *K. pneumoniae* isolates that had undergone WGS, including *de novo* genome assembly, *in silico* multi-locus sequence typing (MLST), resistance gene detection and phylogenetic analysis. Subsequently, a customised, in-house pipeline was developed to streamline and automate handling of sequence data for ongoing assessment and analysis (https://github.com/tseemann/nullarbor). A summary report was generated for each set of isolates run through Nullarbor including quality control metrics, MLST, resistome and pan-genome comparison, and a maximum likelihood phylogeny inferred from the core SNP alignment [54].

From this initial report, clonal complex (CC) 258 isolates underwent further analysis using a PacBio-assembled reference genome of a local isolate for a higher resolution core genome SNP alignment and phylogenetic comparison. As additional KPC-positive isolates were identified over time, the *Nullarbor* pipeline was repeated in an iterative process to establish the genomic relationship of new isolates to existing isolates, and where new CC258 isolates were identified, the CC258-specific analysis was also repeated with the new isolates. These analyses were interpreted together with epidemiological data in real-time as new cases emerged (see below – “Real-time outbreak investigation, analysis and reporting”). Additional

detailed analyses, including recombination filtering and Bayesian temporal analyses, were performed *ad hoc* to gain an overall understanding of the outbreak, and although were not used to directly inform infection control of individual cases, were used to inform overall outbreak management and guideline development.

Epidemiological Methods

Following the results of the initial genomic analysis and concern regarding local transmission, the ongoing genomic investigation was accompanied by collection of detailed epidemiological data, retrospectively for cases detected prior to 2014 and in real-time from 2014.

Ethics. Data were collected as part of an outbreak investigation through the Victorian Department of Health and Human Services under the Public Health and Wellbeing Act 2008 (https://www2.health.vic.gov.au/about/legislation/public-health-and-wellbeing-act).

Data Collection. All patients from whom KPC-producing Enterobacteriaceae were identified from the first isolate in 2012 to 31 December 2015 were included in the epidemiological investigation. A case report form was developed to collect detailed epidemiological data, especially regarding patient hospitalisation details. Patients, or their next of kin, were interviewed by phone to ascertain demographic information on age, sex, country of birth, and risk factors for CPE such as hospitalisation and medical procedures, overseas travel, and comorbidities. Where a patient was in a healthcare facility at the time of specimen collection, or reported hospitalisation in the 12 months prior to initial CPE identification, medical records from each hospitalisation were examined to obtain data on specimen collection, clinical details of infection or colonisation, procedures and patient movements both before

and after CPE identification, where available. Treating doctors, general practitioners and infection control personnel were contacted to obtain additional and/or missing information. Patient hospital admission data were collated and used to identify putative transmission networks of patients linked by proximity in time and space.

Once identified with KPC, patients were assumed to be colonised indefinitely, thus subsequent KPC isolates were deemed to constitute the same patient episode and epidemiological data were not re-collected. KPC-producing Enterobacteriaceae isolated from normally sterile sample sites *e.g.* blood, cerebrospinal fluid, pleural fluid, were considered to represent "infections". Isolates obtained from non-sterile sites (*e.g.* urine, wound swab, aspirate from intra-abdominal collection) where clinical evidence of infection was present and the patient was treated with antibiotics with activity against KPC-producing organisms, or where the treating clinician identified infection but elected to palliate the patient, were also considered to represent "infections". Other isolates were considered to represent "colonisations", unless no epidemiological data were available ("unknown").

Infection control investigation

For patients where local transmission of KPC-producing Enterobacteriaceae was suspected, interviews were conducted with infection control practitioners (ICPs) at the facility with putative transmission. Data were collected on environmental and contact screening activities, patient follow up and patient management alerts, isolation and collection of subsequent screening specimens.

Outbreak reporting and oversight

Data from both genomic and epidemiological investigations were analysed together in real-time, and reported back to the Victorian Department of Health and Human Services. Where transmission events were recognised, the healthcare institutions involved were also informed. An incident management team was established through the Department of Health and Human Services to oversee the investigation and management of the outbreak.

RESULTS

A total of 86 KPC-producing Enterobacteriaceae were referred to MDU between 2012-2015 from 69 patients in 26 different healthcare facilities, six general practices and three aged care facilities across both metropolitan and rural Victoria (Fig 1) (Supplementary Table S4). Specimen source was reported for 85 of the isolates, with most specimens being urine (n = 42; 49%), faeces or rectal swabs (n = 20; 23%), or blood cultures (n = 10; 12%). Almost all isolates were KPC-2 producing Enterobacteriaceae, apart from two KPC-3 producing *K. pneumoniae* isolates from the one patient. Overall, 79 of the isolates were *K. pneumoniae*, five were *Citrobacter farmeri*, and one isolate each of were *Klebsiella oxytoca* and *Citrobacter freundii*.

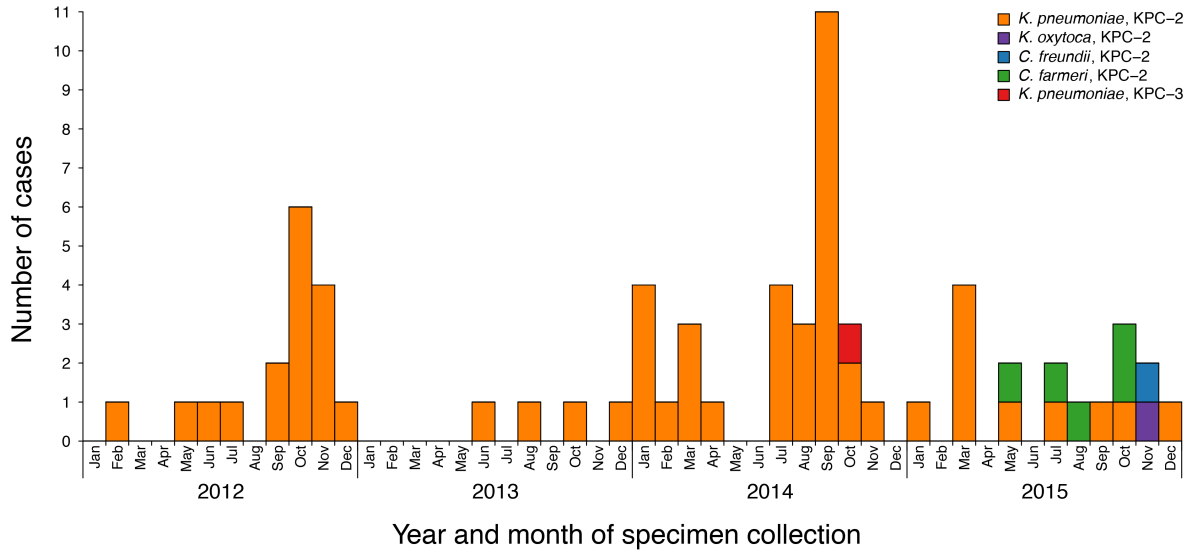


Fig 1: Incidence of new KPC-producing Enterobacteriaceae cases referred to MDU PHL, 2012-2015.
Blocks are coloured by the species and KPC allele of the referred isolates. Repeated detections of KPC-producing isolates from the same patient have been excluded.

333 **Initial genomic analysis**

334 In the initial analysis, 40/41 (98%) *K. pneumoniae* isolates collected from 29 patients prior to
335 June 2014 were clonal complex (CC) 258 by *in silico* MLST, with all of these belonging to
336 clade 1 of sequence type (ST) 258 *K. pneumoniae* described by Deleo *et al*. [55], based on
337 analysis of the capsular polysaccharide gene island. Phylogenetically, these isolates clustered
338 together in comparison to other previously reported international ST258 *K. pneumoniae*
339 isolates (Supplementary Fig S2 & Fig S3). Analysis of the local ST258 isolates revealed three
340 distinct phylogenetic clades involving patients in 20 different healthcare locations, supported
341 by pairwise SNP distributions and Bayesian Analysis of the Population Structure (Fig 2 &
342 Supplementary Fig S4).
343

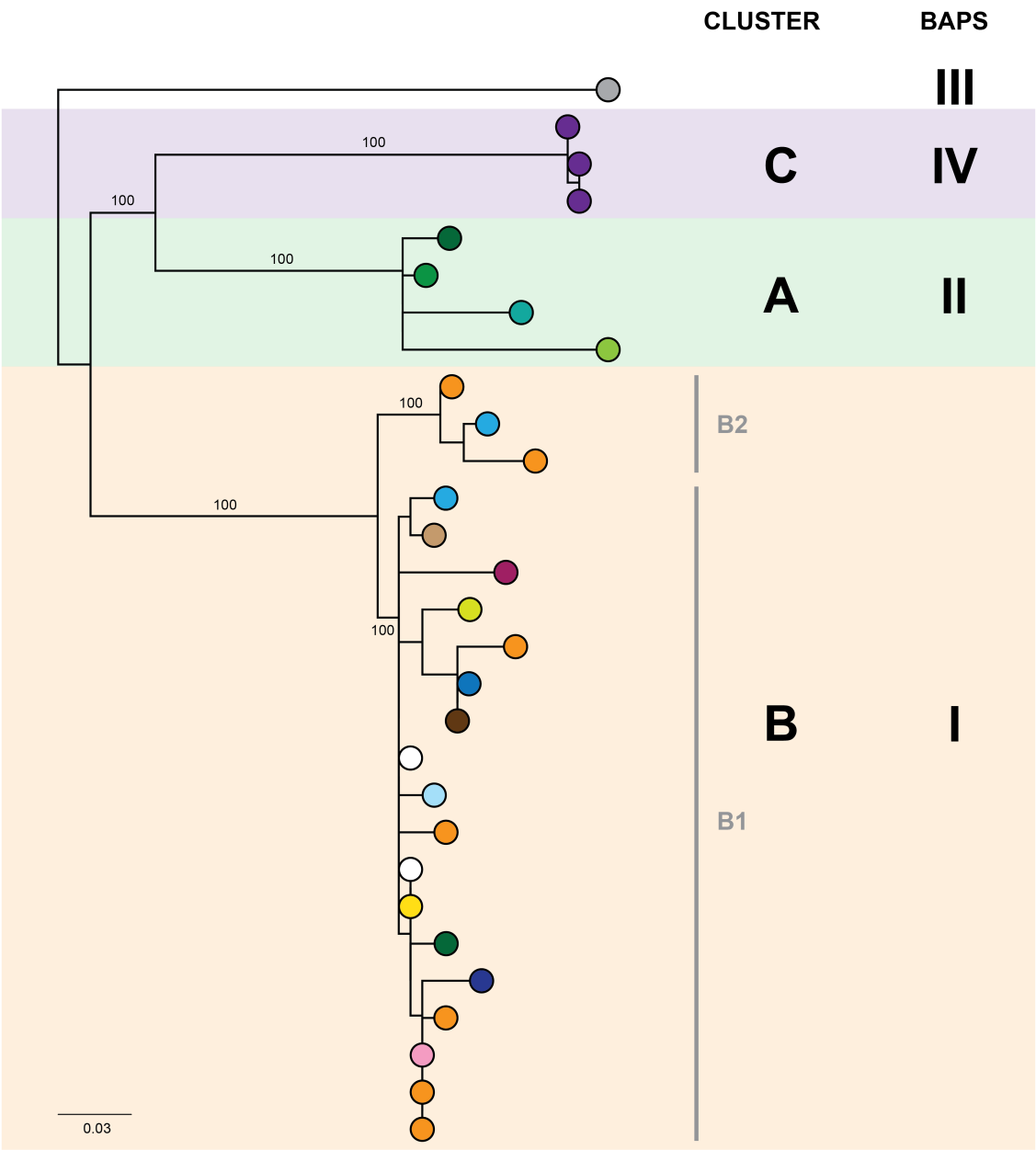


Fig 2: The initial maximum likelihood phylogenetic tree comprised three dominant clades. The tree includes 29 ST258 *K. pneumoniae* isolates collected from 29 patients prior to June 2014, with external nodes coloured according to healthcare facility at time of sample collection. Recurrent isolates from each patient have been excluded. Support values (%) from 1000 bootstrap replicates are shown for major branches. Major phylogenetic clades have been labelled cluster A (green shading), B (orange shading), and C (purple shading) in the order that the clades emerged, with the larger clade B comprising two subclades, B1 and B2. Corresponding clusters identified through Bayesian Analysis of the Population Structure (BAPS) are also shown. The tree was rooted using an outgroup isolate (*K. pneumoniae* NJST258_1, GenBank accession: CP006923.1; not shown in the tree) from a different ST258 clade.

Epidemiological analysis

A total of 57 patients were found to harbour isolates belonging to CC258 from 2012-2015. Most patients presented to the Emergency Department (n=7) or were admitted to hospital (n=44) at the time of initial specimen collection. Thirty-three (58%) were male, and the median age of the affected patients was 74 (range 20 – 94; IQR 62-83) (Supplementary Table S5). Clinical infection was suspected in 61% of the cases, with the most common presentation being urinary tract infection (n=24; 42%). Sepsis was reported in 63% of those with infection due to KPC-producing *K. pneumoniae*. KPC-producing isolates were obtained from patients with clinical infection more commonly during 2012-2014 than 2015, where >50% of the KPC-producing isolates identified were colonising or screening isolates (Supplementary Fig S5).

Of patients from whom a complete travel history was able to be obtained (n=46), 22 (48%) reported overseas travel since 1996, and only eight (14%) reported travel in the 12 months prior to hospitalisation, strongly reinforcing the suspicion of local transmission of KPC. All 57 patients were found to have been hospitalised in Australian healthcare facilities in the 12 months prior to initial positive specimen collection, with a median length of stay of 61 inpatient days (range 0-181 days; IQR 34-101). Four patients had fewer than ten inpatient days in an Australian healthcare facility during this period, three of whom had recent overseas hospitalisation, for which complete hospitalisation data could not be collected.

A detailed analysis of patient admission data was undertaken in attempt to identify location or source of acquisition. Forty-one patients (72%) had previously attended one facility (Facility F) in the 12 months prior to initial identification of a KPC-producing isolate. However, the remaining 16 patients reported no hospitalisation in this facility, and 18

382 additional facilities were identified in which two or more patients had been admitted. Three
383 putative transmission networks were identified based on overlapping admissions to the same
384 hospital ward at the same time among patients detected with KPC. It became apparent that
385 due to the sheer number of hospitalisations and the complexity of patient movements,
386 drawing firm conclusions on who transmitted to whom and where transmission had occurred
387 solely from epidemiological data would be difficult (Fig 3 & Supplementary Fig S6).
388

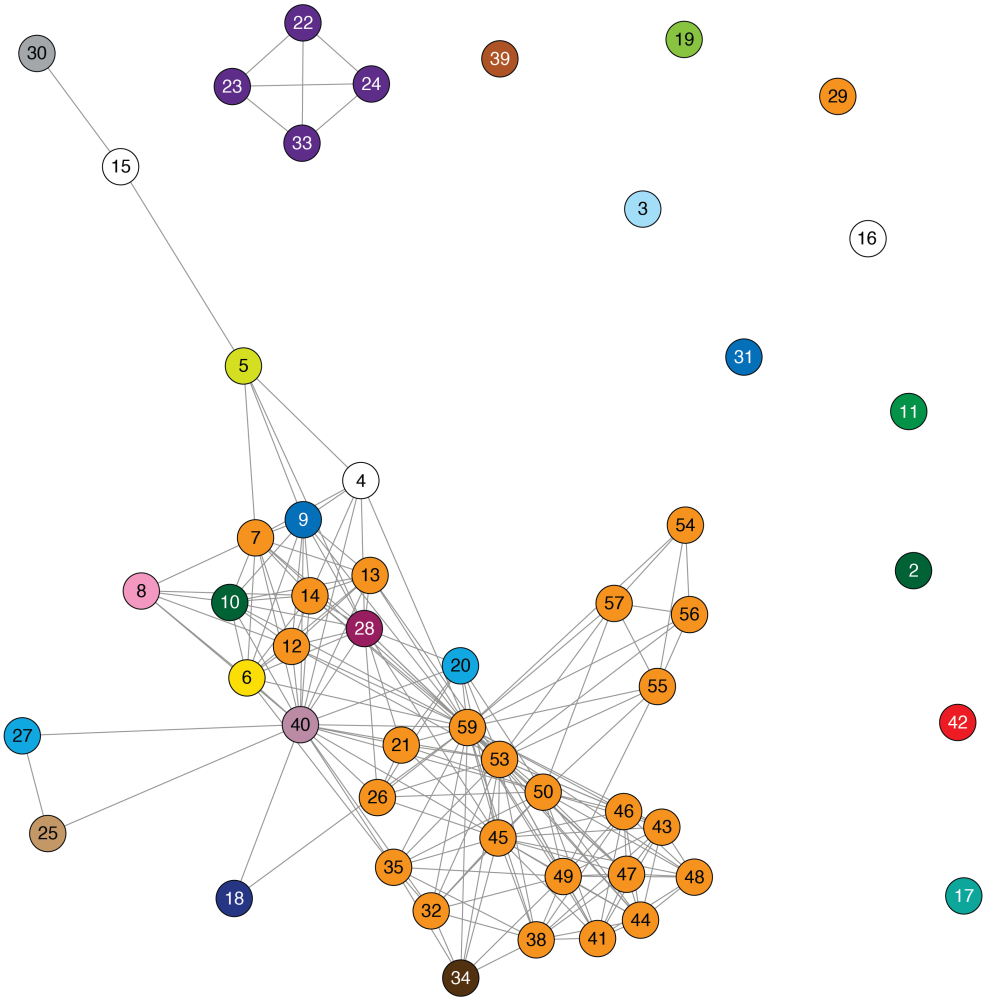


Fig 3: Epidemiological links based on overlapping patient admissions were unable to resolve where and when transmission was occurring for most isolates.

Links in the above network connect patients based on overlapping patient admissions in the same hospital ward at the same time (minimum of one day overlap). Links that occurred after detection of *bla*$_{KPC}$ in both patients have been excluded. Nodes are numbered by patient, and coloured by healthcare facility (as in Fig 2) at the time of sample collection. The epidemiological network connecting patients 22, 23, 24 and 33 correlates with the closely related genomes in Cluster C (Fig 2).

401 **Combined analysis of CC258 KPC-2-producing *K. pneumoniae* isolates**

402 Despite identification of three distinct phylogenetic clades in the initial genomic analysis (Fig
403 2), we were unable to exactly define where transmission was occurring as the samples were
404 distributed across multiple locations. Of the three clades (designated as genomic cluster A, B
405 and C), cluster C had the best correlation with an epidemiologically-defined transmission
406 network, comprising isolates from patients in the same healthcare facility at the same time
407 (Fig 3). However, other correlations were not immediately apparent. Cluster A included
408 isolates from three patients in three different facilities that were within the same healthcare
409 network and/or were geographically located in the same region within a 20 km radius, though
410 were 30 km away from the fourth patient in a different healthcare network in the cluster.
411 Cluster B included isolates from patients in 12 different locations across the state of Victoria,
412 separated by up to 500 km. Seven isolates were obtained from patients attending local general
413 practice (primary care) clinics.
414
415 Using the genomic data to enhance and allow flexibility in the epidemiological data (*e.g.*
416 including admissions to adjacent wards or admissions separated by a few days as
417 "overlapping"), it became apparent that each of the genomic clusters corresponded to a
418 separate transmission network (Fig 4). The largest of these (cluster B) was located at a single
419 institution (Facility F), and as further isolates were identified, two subclusters (B1, B2) were
420 defined. Cluster B2 included isolates arising from suspected transmission in mid-2014 in an
421 inpatient aged care ward, while cluster included isolates from a number of patients who were
422 admitted to facility F in 2012, though to several different wards. A second transmission
423 network in cluster B1 was identified in another aged care ward in 2015. The predicted
424 transmission network corresponding with cluster C was also confirmed, with the isolate
425 genomes differing by <5 SNPs, and the corresponding epidemiological data for the respective

426 patients showing overlapping hospital admissions to a single hospital ward in late 2013 (Fig
427 5).
428

429

430 **Fig 4: Combined analysis, with genomic relationships between isolates overlaid upon**
431 **epidemiological data, delineated multiple transmission networks.**
432 A maximum likelihood phylogenetic tree is shown on the left, labelled with major genomic clusters
433 and supporting branch bootstrap values (%) from 1000 replicates for major branches. Nodes of the
434 tree are coloured by healthcare facility at the time of sample collection. Coloured horizontal bars on
435 the right indicate healthcare facility admissions over time (x axis), with different colours representing
436 different healthcare networks. Black diamonds (◆) indicate first detection of KPC for each patient.
437

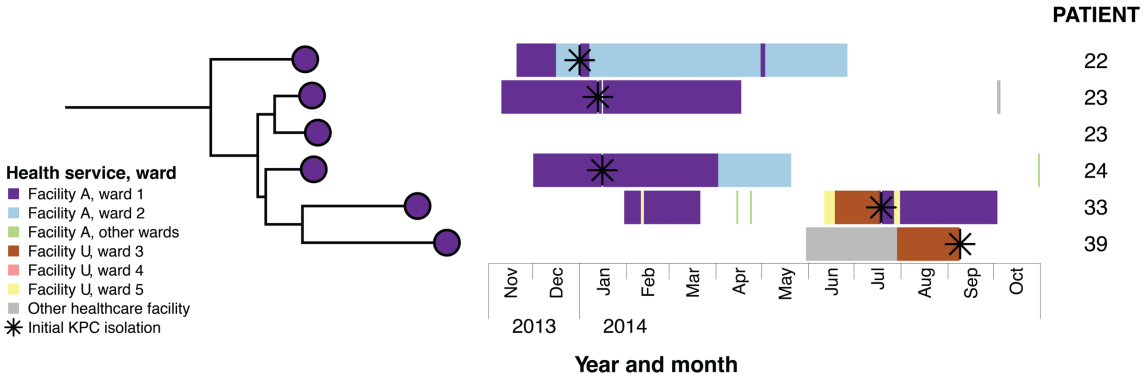


Fig 5: Combined analysis of genomic and epidemiological data of the cluster C network identified secondary transmission.

Example case study: Patients 22 and 23 both reported overseas hospitalisation in the 12 months prior to first detection of KPC – patient 22 in Vietnam for spinal surgery following a motor vehicle accident, and patient 23 in Greece for stem cell therapy. Both patients had undergone rectal screening on admission to Ward 1 in Facility A, with patient 22 being placed in intensive contact precautions for the duration of his hospital admissions after isolating another multidrug-resistant organism, though was required to use shared bathroom facilities with patients in the adjacent room. Having required treatment with meropenem for both hospital-acquired pneumonia and a surgical wound infection, patient 22 was later diagnosed with a KPC-producing *K. pneumoniae* indwelling catheter-associated urinary infection in January 2014. Twelve days later, patient 23 was subsequently found to have a polymicrobial sacral wound infection, with cultures including KPC-producing *K. pneumoniae* from sacral tissue. In response to this, all patients on the ward who had been admitted to the same room and/or shared bathroom facilities with patients 22 and 23 were screened, with the subsequent identification of patient 24. Alerts were placed on the records of patients meeting the criteria who had been previously discharged. Environmental screening of the rooms and bathrooms was conducted, with no KPC-producing organisms identified, and extended bleach cleaning with changes of curtains, chairs and other furnishings was conducted for the entire ward.

Patient 33 was also admitted to Ward 1 in Facility A in February 2014, subsequent to the identification of KPC-2 in patients 22, 23 and 24. This patient was not screened as he had not been admitted to the same room, nor had he shared a bathroom with the identified cases. He also reported no recent history of overseas travel. However, he was identified in July 2014 through routine screening at Facility A following transfer from Ward 3, Facility U, located 25 km away. A KPC-producing isolate from Patient 39 was identified in September 2014, and although the isolate genomically clustered with isolates from Patients 22, 23, 24 and 33 identified at Facility A, she had no previous presentations to that healthcare facility. However, immediately prior to identification of KPC, she had also been in Facility U on Ward 3, though she was admitted there 13 days after Patient 33's discharge. Given this was the only plausible epidemiological link to the other cluster C patients, secondary transmission was presumed to have occurred in Ward 3, Facility U.

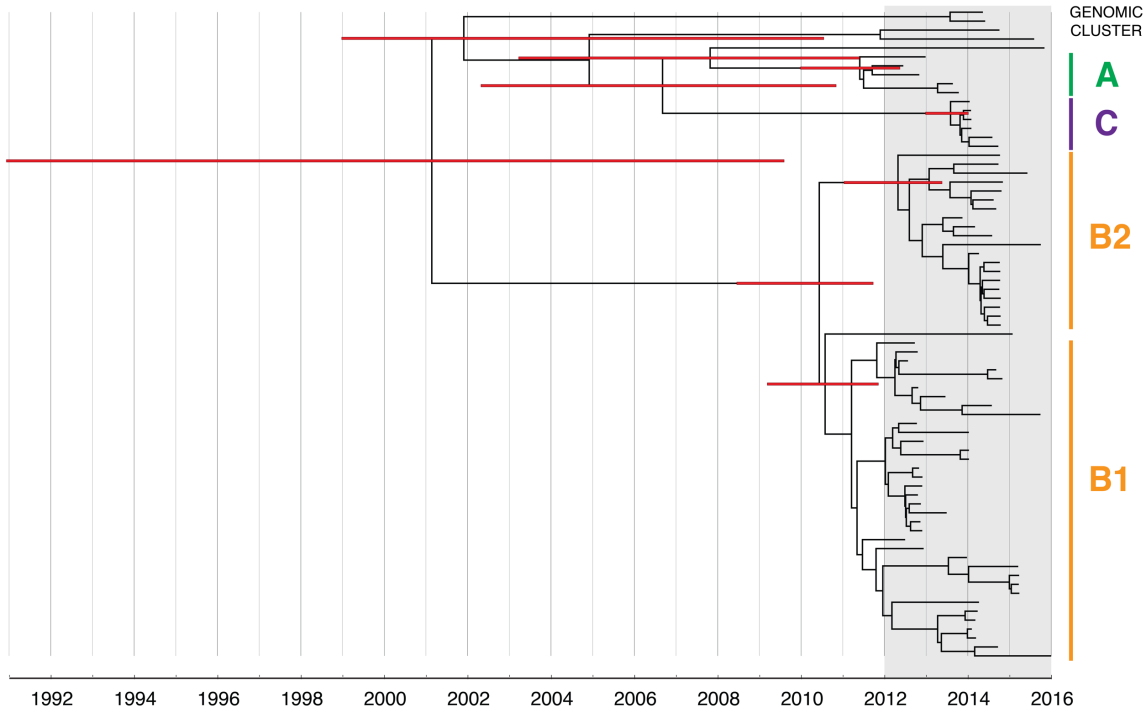
Combining epidemiological and genomic data also revealed secondary transmission events, where patients with undetected colonisation at one hospital were transferred to a second healthcare service, with subsequent diagnosis and onwards transmission. Patients whose isolates were phylogenetically clustered with other isolates from a known transmission network, but who had not previously been admitted to the hospital where transmission was occurring, were flagged as potentially having acquired KPC through a secondary transmission event. Through this, two putative secondary transmission events were later identified from these main transmission networks (see example case study in Fig 5).

Bayesian evolutionary and transmission analysis

Although from the combined genomic and epidemiological analyses, it appeared that local transmission within separate hospitals was driving the outbreak, local isolates from each cluster were closer to other local isolates (median pairwise SNP distance = 16; IQR 9-38) than to international ST258 isolates from GenBank (median pairwise SNP distance = 243; IQR 80-258). To investigate the possibility of initial inter-hospital transmission leading to subsequent transmission networks within each hospital, we explored whether Bayesian evolutionary and transmission modelling could be used to provide additional insight.

From these analyses, genomic clusters A and C each appeared to be derived from separate overseas importations of KPC-producing *K. pneumoniae* with subsequent spread within a single hospital or healthcare network rather than inter-hospital transmission, with a most recent common ancestor (MRCA) predicted to have occurred prior to 2010 (median 2007.78; 95% HPD 2003.30–2011.09) when the first KPC-producing organism was reported in Australia [19], and 2012, when the first KPC-producing organism was reported in Victoria [12] (Fig 6). Similarly, the unclustered isolates represented separate individual importations

497 in these analyses, supporting the known history of recent overseas travel for these patients
498 (Supplementary Table S5).
499



500

501 **Fig 6: Bayesian evolutionary analysis indicates each of the phylogenetic clades corresponding to**
502 **the genomic clusters emerged prior to the detection of KPC in Victoria.**
503 A maximum clade credibility timed phylogeny from Bayesian evolutionary analysis of local CC258 *K.*
504 *pneumoniae* isolate genomes is shown, with median node heights displayed. The thin red bars indicate
505 95% highest posterior density (HPD) intervals for the most recent common ancestor (MRCA) for major
506 clades and defined genomic clusters (indicated on the right). The shaded grey region indicates the recent
507 period when KPC isolates were detected in Victoria.
508

509

510 Cluster B likely arose from another overseas importation (MRCA cluster A and B: median
511 2002.52; 95% HPD 1991.72–2008.79), though the timed phylogeny (Fig 6) suggested strains
512 of KPC-producing *K. pneumoniae* from cluster B may have been circulating in Victoria since
513 2011 (MRCA cluster B1 and B2: median 2010.59; 95% HPD 2008.67–2011.86). A number
514 of internal nodes within cluster B1 were dated to 2012 (Supplementary Fig S8),
515 corresponding with overlapping patient admissions at Facility F at that time, which affirmed

the putative transmission network in Facility F in 2012. In both clusters B1 and B2, many isolates were linked by transmission events that were predicted to have occurred during 2013, despite a decline in new cases detected during this period. Notably, many of the isolates derived from these transmission events were not detected until later, in 2014 or 2015. This supported the epidemiological hypothesis that detection of previously unrecognised colonisation was driving a large proportion of the new cases in 2014 and 2015, rather than new transmission events (Fig 7). For each of the suspected transmission networks identified in the combined outbreak investigation, posterior probability distributions for the MRCA were generally consistent with the epidemiological data (Supplementary Fig S8).

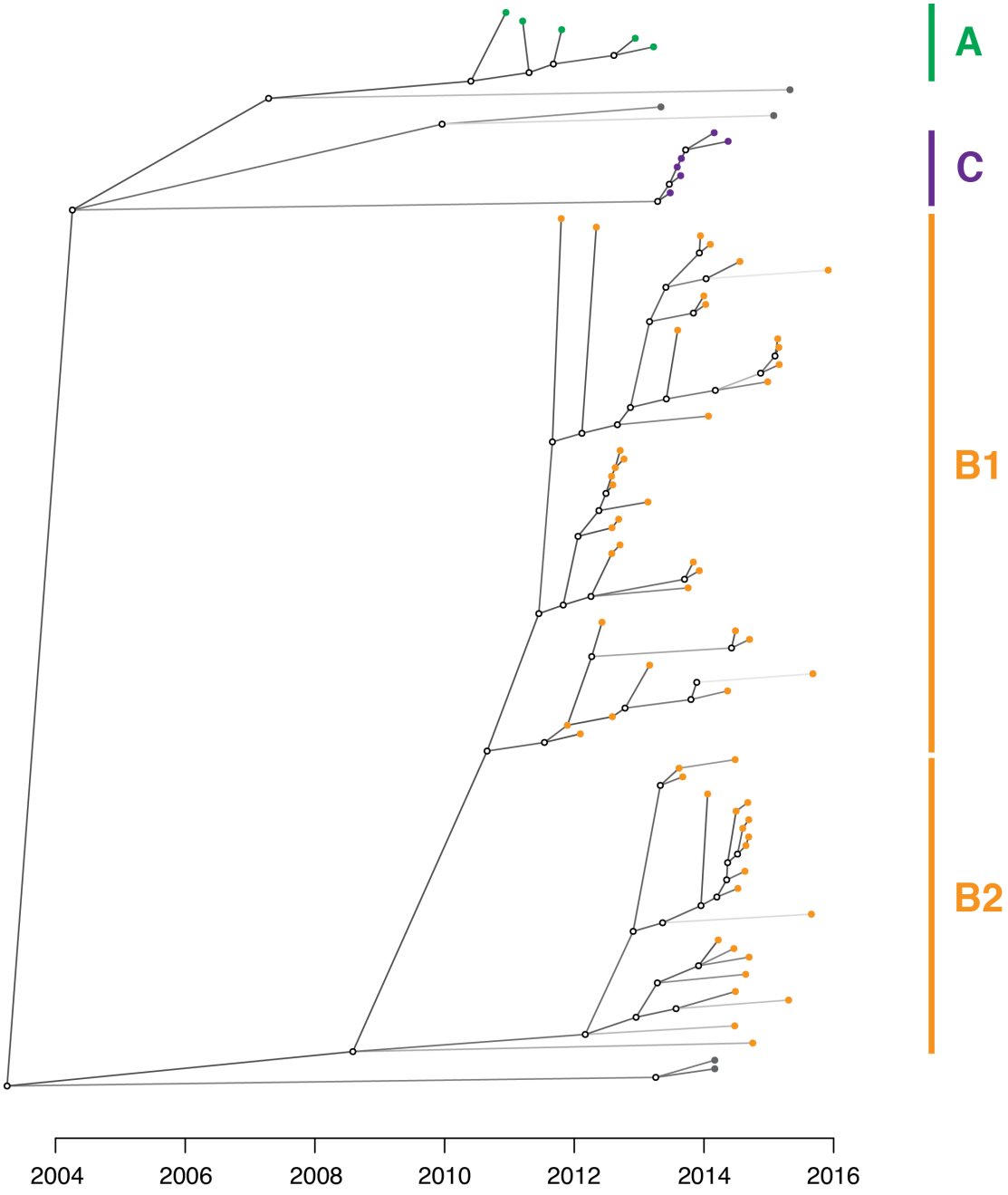


Fig 7: An inferred transmission tree shows that undetected colonisation was significant in propagating the outbreak.
Solid nodes represent the posterior mean time of KPC acquisition by individuals and are coloured by the corresponding genomic cluster, with empty circles representing inferred unsampled individuals contributing to the transmission tree. Braches are shaded by number of missing links in the transmission tree, with lighter branches representing increasing numbers of missing links implicated.

From the *TransPhylo* analysis, the mean reproductive number (R_0) across the outbreak was calculated to be 1.63 (SD = 0.13), accounting for an average estimated sampling proportion of 0.27 (SD = 0.05).

Plasmid analysis

In late 2015, other Enterobacteriaceae carrying *bla*$_{KPC-2}$ were detected, including *Citrobacter farmeri*, *Citrobacter freundii*, and *Klebsiella oxytoca* (Fig 1), which raised concern about KPC-plasmid spread. Analysis of the *de novo* assembled draft genomes indicated the *bla*$_{KPC-2}$ carrying plasmids in these organisms matched a *bla*$_{KPC-2}$ carrying plasmid found in the locally circulating *K. pneumoniae* isolates, suggesting inter-species plasmid movement, and subsequent organism transmission. All *K. pneumoniae* ST258 isolates were found to harbour *bla*$_{KPC-2}$ within the "a" isoform of the characteristic Tn*4401* transposon, and usually on a contig containing an IncFIB(pQil)-type plasmid replicon. However, cluster C isolates lacked the pQil-type plasmid, and instead carried *bla*$_{KPC-2}$ on an IncFIB(K)-type plasmid, while a few isolates within clusters B1 also lacked the pQil-type plasmid and probably carried *bla*$_{KPC-2}$ on a X3-type plasmid, though the assemblies were inadequate to confirm this. Surprisingly, one *K. pneumoniae* isolate cluster B1 that underwent PacBio sequencing was found to harbour both IncFIB(pQil) and IncFIB(K) plasmids, as did several other *K. pneumoniae* isolates based on replicons identified from their assemblies of Illumina sequencing data, though there was no prior contact identified between patients harbouring these isolates and cluster C patients. Chromosomal integration of the Tn*4401* within the putative diguanylate cyclase gene, *ycdT*, was found in two epidemiologically linked ST258 *K. pneumoniae* isolates. One ST258 *K. pneumoniae* isolate lacked *bla*$_{KPC-2}$, and subsequent repeat susceptibility testing confirmed the isolate to be susceptible to carbapenems. Re-testing of 10 colonies subcultured from the stored glycerol stock for that patient sample found only 7/10

560 displayed carbapenemase hydrolytic acitivity with the CarbaNP test, indicating *in vivo* or *in*
561 *vitro* loss of the bla_{KPC} gene.
562
563 One *C. farmeri* isolate also underwent PacBio sequencing. Sequence comparisons between
564 the KPC-plasmid genome from this isolate and the KPC-plasmid genome from a *K.*
565 *pneumoniae* isolate indicated almost identical plasmids. The *C. farmeri* plasmid carried an
566 IncR-type replicon and lacked a Type I restriction modification system, but otherwise
567 comprised an identical sequence to 85% of the *K. pneumoniae* KPC plasmid following a
568 recombination and inversion event (Fig 8). The *C. freundii* isolate was also found to have an
569 IncR-type plasmid, but lacked other gene content found in the *C. farmeri* IncR plasmids,
570 suggesting mobilisation of the Tn*4401* transposon. The exact location of bla_{KPC-2} and plasmid
571 type of the *C. freundii* and *K. oxytoca* isolates were unable to be determined due to the
572 limitations of the assembled short-read sequencing data.
573

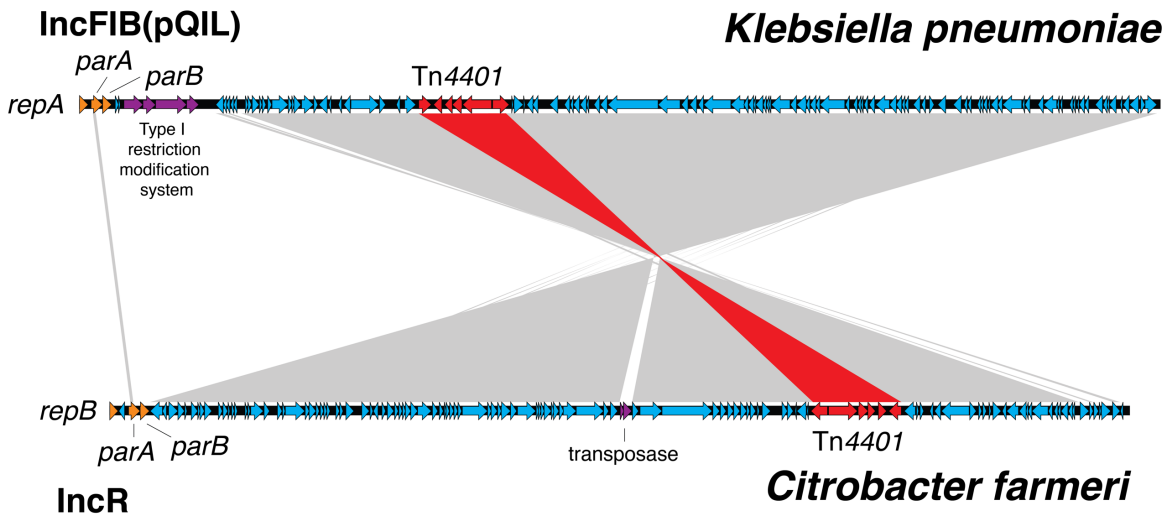


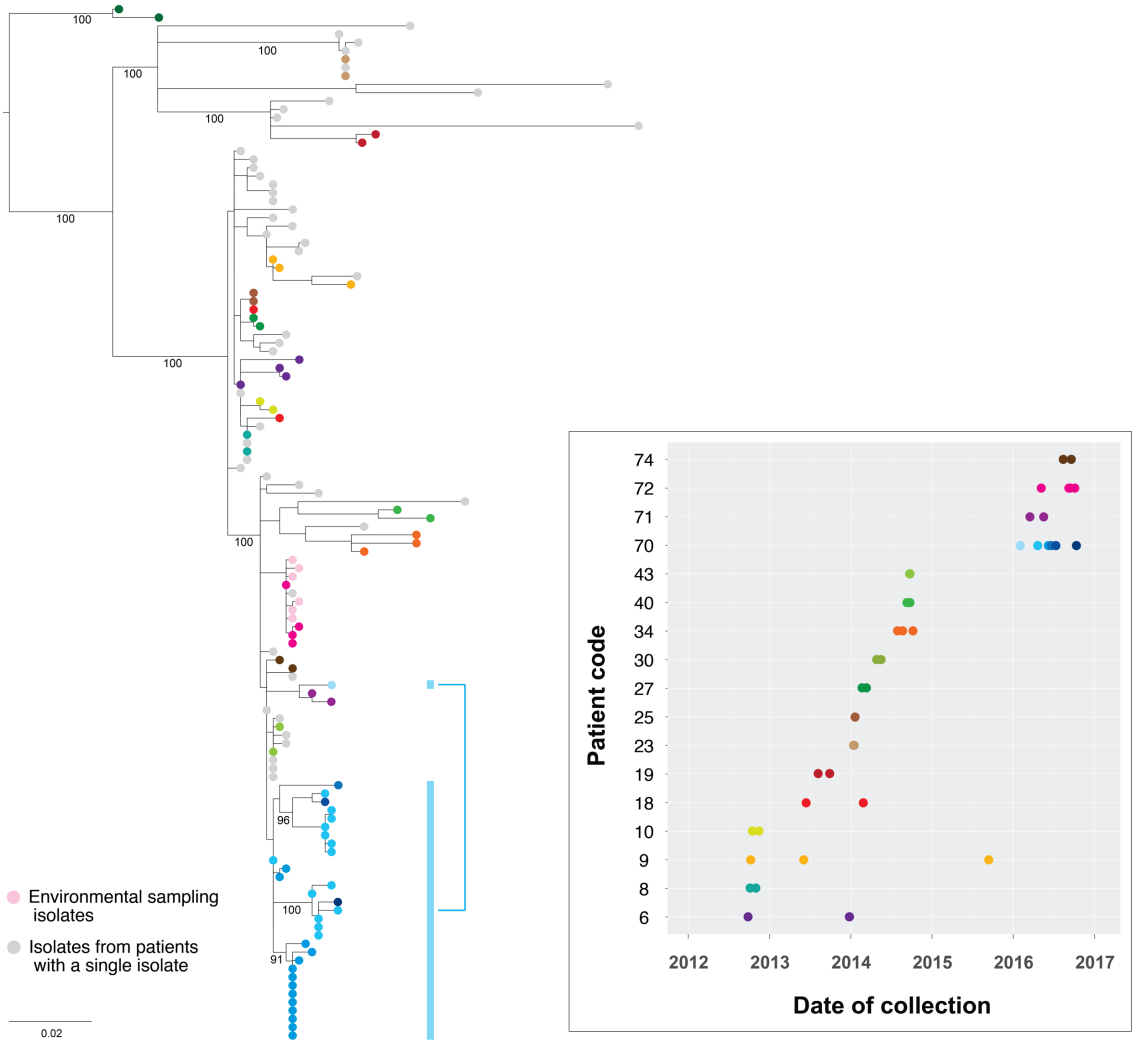
Fig 8: KPC plasmids from the *C. farmeri* isolates were almost identical to KPC plasmids from *K. pneumoniae* outbreak isolates, despite differing replication proteins.
BLAST comparison between an IncFIB (pQIL-like) plasmid genome from a ST258 *Klebsiella pneumoniae* isolate, AUSMDU00008079 (above), and an IncR plasmid genome from a *Citrobacter farmeri* isolate, AUSMDU00008141 (below), from the outbreak. The grey shading indicates corresponding DNA regions of high nucleotide identity transcribed in opposing directions, with the Tn*4401* transposon harbouring bla_{KPC-2} highlighted in red. The plasmid genomes have been orientated to their respective replicons and downstream plasmid partitioning genes, *parA* and *parB*.

Phylogenetic analysis of the *C. farmeri* isolates inferred from core genome (chromosomal) SNPs confirmed the isolates were closely related (Supplementary Fig S9).

Within-host diversity

To assess the within-host genomic diversity of KPC-producing Enterobacteriaceae, the isolate genomes from 17 patients who had multiple isolates obtained and sequenced were compared. Of these, one patient had 32 isolates collected from seven samples over an 8-month period from February 2016 to October 2016, including multiple colony sampling from

595 two faecal samples. The other 16 patients had multiple (median = 2; range 2-4) isolates
596 referred to MDU, obtained through recurrent presentations at different healthcare facilities.
597
598 Genomic comparisons indicated isolates from the same patient differed by up to 21 SNPs in
599 the core genome. This included the 32 isolates obtained from patient 70, with several internal
600 lineages emerging from the same common ancestor within that individual (Fig 9).
601 Pangenome analyses of these isolates also demonstrated changes in the accessory genome
602 correlating with the emerging internal lineages in the core genome (chromosomal)
603 phylogeny, and lineage-specific plasmid carriage. There was a poor molecular clock signal
604 for these isolates (root-to-tip divergence $R^2 = 0.026$), and no further temporal analyses were
605 undertaken.



606

607 **Fig 9: Genomes of isolates from the same host group together in the phylogeny.**
608 Maximum likelihood tree of the study isolates with additional isolates obtained up to 1 November 2016
609 included. Multiple isolates from the same patient have been coloured by patient, with the accompanying
610 graph indicating the collection dates for the corresponding isolates. Thirty-two isolates from six clinical
611 samples obtained from patient 70 (blue) over 8 months have been highlighted to illustrate the within-
612 host lineages emerging in this patient. Bootstrap values (%) from 1000 replicates have been displayed
613 for major branches in the tree.
614

615

When compared to other outbreak isolates, multiple isolates from the same host clustered together within a single subclade in the phylogenetic tree. This supported the concept of a "transmission bottleneck", where despite an assumed diverse within-host population of KPC-producing Enterobacteriaceae, transmission only involved a single isolate, with subsequent within-host evolution following the initial transmission event. This also suggested multiple acquisitions of different strains of KPC-producing *K. pneumoniae* by the same patient were uncommon among the patient cohort. Of patients with multiple isolates spanning a six-month period, only patients 18 and 70 appeared to have isolates from differing subclades, though bootstrap branch support for these subclades was marginal (0.70-0.75).

Infection control investigation

Qualitative interviews were conducted with infection control practitioners at three facilities regarding five transmission networks (four primary clusters of KPC-producing ST258 *K. pneumoniae* including two secondary transmission events at different locations, in addition to one cluster of KPC-producing *C. farmeri*) of suspected local transmission involving 17 patients. The majority of transmission events were thought to have occurred due to unrecognised colonisation, and prior to the implementation of contact precautions – single room isolation with *en-suite* bathroom, with requirements for use of personal protective equipment (gloves and gown) by staff and visitors entering the room. However, two instances of apparent transmission to and from patients under contact precautions were reported. In each of these situations, after being admitted directly into contact precautions due to known colonisation with other multidrug-resistant organisms, a patient acquired a KPC-producing *K. pneumoniae* isolate that was highly related to other isolates in a genomic cluster (<5 SNPs in the core genome).

Due to the lack of local guidelines at the time of the outbreak, the management of wards where transmission was found to have occurred was at the discretion of the individual healthcare facilities involved. A retrospective review of the methods used to interrupt transmission identified a number of bundled approaches, with the common measures being contact screening, enhanced cleaning (in contrast to standard cleaning), and isolation of identified cases under contact precautions (Supplementary Table S6).

Impact of whole-genome sequencing on the outbreak investigation and development of local containment guidelines

In response to the genomic and epidemiological evidence of an evolving outbreak with local transmission, a state-wide management guideline was developed for the surveillance and containment of carbapenemase-producing Enterobacteriaceae incorporating the routine use of WGS to determine relatedness between isolates [56].

Several insights were gleaned through the use of genomics that were fundamental to the outbreak investigation and informed development of the guidelines and their focus on hospitals. For example, through the epidemiological investigation, the identification of KPC in previously hospitalised patients suggested nosocomial rather than community transmission, though which hospital transmission was occurring within was not immediately clear, given the number of hospitals and prior overlapping hospitalisations involved. The resolution of the genomic data refined epidemiological hypotheses to pinpoint transmission areas in individual hospitals to focus infection control efforts, with Bayesian analyses of the genomic data also supporting the epidemiological findings. By determining when and where transmission was occurring, it was evident that many new cases were readmissions of patients with previously unrecognised colonisation, rather than new transmission events,

666 making it difficult to "control" the outbreak. This highlighted the need to intensively screen
667 at-risk patient contacts, including flagging those that had been discharged from an affected
668 ward prior to recognition of transmission for screening and pre-emptive isolation upon
669 subsequent hospital presentations. This real-time change-in-practice was reflected by the
670 increasing proportion of cases identified as "colonisations" rather than "infections" due to
671 intensified screening practices over time (Supplementary Fig S5).
672
673

674 **DISCUSSION**

675 The emergence of carbapenemase producing Enterobacteriaceae (CPE) is a major threat to
676 human health [1, 5, 57], with significant interventions required at state and national levels to
677 contain the spread once established [58]. Here, we describe the largest outbreak of CPE
678 reported in Australia, and demonstrate how a combined genomics and epidemiological
679 investigation delineated the outbreak into five separate nosocomial transmission networks
680 (four clusters of *K. pneumoniae* and one cluster of *C. farmeri*) across the state over four
681 years, resulting in targeted interventions for each transmission area. The use of WGS for
682 outbreak analysis has been well established, including for transmission of multidrug resistant
683 hospital pathogens [27, 59], but these studies have been predominantly small scale, single
684 institution studies, and often retrospective. Genomics has rarely been used prospectively and
685 in real-time during a complex multi-institutional outbreak requiring a coordinated state-wide
686 public health response. Due to prolonged colonisation, many CPE cases linked to hospitals
687 with suspected transmission were identified through other healthcare facilities, and some
688 through general practice. With the number of potential transmission opportunities in
689 retrospective hospitalisation data from all facilities, the epidemiological investigation would
690 have been difficult to interpret and translate into focussed interventions without the resolution
691 offered by genomics. Similarly, accurate interpretation of the genomic data would have been
692 difficult without the supporting epidemiologic data. As others have pointed out, the two must
693 go hand-in-hand [60]. By integrating our detailed epidemiological investigation with genomic
694 analyses, we were able to refine our hypotheses, and coordinate an effective public health
695 response to target areas with ongoing transmission, emphasising the ability of WGS to
696 enhance surveillance systems and outbreak investigations.
697

Despite integrating genomic data with detailed epidemiological information, we still encountered several challenges. Accurate inference of plasmid transmission can be challenging from short-read Illumina sequencing data, with repetitive elements such as insertion sequences that accompany mobile resistance elements frequently confounding short-read assemblers and read-mappers [61]. As we demonstrated here and as reported by others [61], long-read sequencing technology such as Pacific Biosciences single-molecule sequencing can be highly useful in resolving and tracking the diversity of plasmids carrying carbapenemases and other resistance genes, although we could only sequence a limited representation of the outbreak isolates due to cost limitations. The data gained from these completed genomes were invaluable. The use of a local internal reference provided additional confidence in SNP calling among highly clonal isolates. We were able to compare completely assembled plasmids between different species, which otherwise would not have been possible. Many isolates in the outbreak carried $bla_{\text{KPC-2}}$ on either an IncFIB(K) or IncFIB(pQil) plasmid, with long-read sequencing showing it was possible for a patient to be colonised with an isolate carrying multiple plasmids of the same incompatibility group. As we found in two isolates and as others have recently reported [62], chromosomal integration of Tn*4401* also occasionally occurs. The movement of these resistance elements adds complexity to understanding transmission dynamics using genomics, and highlights the limitations of short-read sequencing data.

It is increasingly evident that the subtly diverse populations of a single clonal type due to within-host evolution [63], and more diverse populations from transmission of mixed infections [64, 65], can impair accurate reconstruction of transmission pathways from genomic data [66]. In our study, we found some individuals not only had different plasmid variants, but also had distinct within-host evolutionary lineages, indicating a complex

723 evolutionary history of transmission, within-host evolution, and plasmid movement,
724 mirroring recent reports in other patients with prolonged KPC colonisation [67]. However,
725 measuring this diversity is difficult in outbreak investigations. Although single-colony
726 sampling and sequencing arguably provides the most informative data, it may be subject to
727 colony selection bias, so some uncertainty remains. This can be offset by sampling more
728 colonies, but the time and cost of sequencing each colony can quickly become prohibitive in
729 an outbreak investigation and real-world public health environment. Although some have
730 attempted to detect the presence of mixed infections in short-read datasets through analysis of
731 short-read mapping from deep sequencing [64], this relies on WGS performed on a sweep of
732 a primary culture plate, introducing the potential for exogenous DNA in the dataset, and
733 becomes considerably more complicated with fluxes in accessory genome content. As we
734 found, *bla*$_{KPC}$-plasmids can be lost even through laboratory passage of stored isolates. Even if
735 the within-host genomic diversity can be captured, incorporating these data into models can
736 be difficult. Recent attempts to account for elements such as within-host diversity and
737 unsampled data in reconstructing transmission trees have proved successful in some
738 situations [52, 68-70]. However, each of these make several assumptions, such as the
739 presence of a complete transmission bottleneck that does not allow for repeated acquisition, a
740 constant reproduction number across the outbreak, few or no unsampled cases, and that
741 genetic variation is accumulated in pathogens in a clock-like fashion. The within-host
742 diversity and horizontal transmission of mobile genetic elements in outbreak investigations
743 complicates analyses further, and consequently, accurate reconstruction of exact transmission
744 routes remains difficult for large and moderate-sized CPE outbreaks.
745
746 Our study has some limitations and points of note. Firstly, although most patient isolates had
747 a clear epidemiological link to other isolates within the defined genomic clusters, not every

748 isolate was able to be linked to another patient with KPC or a history of recent overseas
749 travel, indicating a larger pool of unrecognised colonisation, though influenced in part by
750 differing stringency in contact screening and data collection. For example, no patient in
751 cluster A reported overseas travel since 1996, when KPC was first detected. Furthermore,
752 although these patients were located at healthcare facilities in the same geographical region
753 of Victoria and part of the same healthcare service, they did not have any overlapping
754 inpatient admissions. Thus, it is likely that there are other individuals or environmental
755 sources that serve as intermediary reservoirs of isolates not captured in our sampling, that
756 facilitate ongoing transmission. Although environmental and contact screening was initially
757 performed using methods published by others [71], based on our data, the optimal approaches
758 are still not well understood. Secondly, although the outbreak has slowed, cases are still
759 emerging despite the extensive investigation and interventions implemented by the individual
760 healthcare services. However, as shown in our analyses, many of these may be unrecognised
761 colonisation following previous exposure rather than new transmission events. As a result of
762 this knowledge, through the development of our guidelines, we have redirected infection
763 control resources into intensive screening to identify individuals with asymptomatic
764 colonisation, focussing efforts on healthcare facilities with ongoing transmission.
765
766 Our study reports a real-world prospective utilisation of WGS, including the difficulties
767 encountered, to enhance a complex epidemiological investigation in real-time for an
768 important pathogen. In contrast to most reported outbreaks of CPE within a single institution,
769 our outbreak demanded a coordinated public health response. Previously the domain and
770 responsibility of individual hospitals and healthcare facilities, the spread of CPE has become
771 a public health issue. Through our experience, WGS has since been incorporated into our
772 state guidelines for the management of CPE [56]. Developed during the outbreak

773 investigation, this system entails routine WGS of all suspected CPE isolates with concurrent
774 epidemiological investigation to allow prospective, centralised comparison of isolates and
775 epidemiological data from multiple health services. In turn, this enables identification of
776 potential transmission events between patients geographically and temporally dispersed at
777 identification, translating into focussed interventions at the designated transmission locations.
778 For such extensively drug-resistant organisms with limited treatment options, all feasible
779 interventions towards reducing the early burden of disease are warranted.
780
781
782 **FUNDING STATEMENT**
783 MDU PHL is funded by the Victorian Government, Australia. JCK (GNT1074824), NLS
784 (GNT1093468), TPS (GNT1105525) and BPH (GNT1105905) are supported by the National
785 Health and Medical Research Council (NHMRC) of Australia. SLB is supported by an
786 Australian Postgraduate Award. JCK, SLB and NLS are also supported by Australian
787 Government Research Training Program Scholarships.
788
789 The funders had no role in study design, data collection and analysis, decision to publish, or
790 preparation of the manuscript.
791

REFERENCES

1. Centers for Disease Control and Prevention, US Department of Health and Human Services. Antibiotic resistance threats in the United States, 2013. 2013. Available from: https://www.cdc.gov/drugresistance/threat-report-2013/index.html

2. Queenan AM, Bush K. Carbapenemases: the versatile beta-lactamases. Clin Microbiol Rev. 2007;20(3):440-58, table of contents. doi: 10.1128/CMR.00001-07. PubMed PMID: 17630334; PubMed Central PMCID: PMC1932750.

3. Falagas ME, Lourida P, Poulikakos P, Rafailidis PI, Tansarli GS. Antibiotic treatment of infections due to carbapenem-resistant Enterobacteriaceae: systematic evaluation of the available evidence. Antimicrob Agents Chemother. 2014;58(2):654-63. doi: 10.1128/AAC.01222-13. PubMed PMID: 24080646; PubMed Central PMCID: PMC3910850.

4. Tzouvelekis LS, Markogiannakis A, Psichogiou M, Tassios PT, Daikos GL. Carbapenemases in Klebsiella pneumoniae and other Enterobacteriaceae: an evolving crisis of global dimensions. Clin Microbiol Rev. 2012;25(4):682-707. doi: 10.1128/CMR.05035-11. PubMed PMID: 23034326; PubMed Central PMCID: PMC3485753.

5. Australian Commission on Safety and Quality in Health Care. Recommendations for the control of multi-drug resistant Gram-negatives: carbapenem resistant Enterobacteriaceae. Sydney: Australian Commission on Safety and Quality in Health Care, 2013. Available from: https://www.safetyandquality.gov.au/publications/mrgn-guidelines-enterobacteriacea/

6. Australian Group on Antimicrobial Resistance. The evolution of carbapenemases in Enterobacteriaceae in Australia. 2014. Available from: http://www.agargroup.org/files/AGAR_Carbapenemase_evolution_JTv24.pdf

7. Espedido BA, Steen JA, Ziochos H, Grimmond SM, Cooper MA, Gosbell IB, et al. Whole genome sequence analysis of the first Australian OXA-48-producing outbreak-associated Klebsiella pneumoniae isolates: the resistome and in vivo evolution. PLoS One.

2013;8(3):e59920. doi: 10.1371/journal.pone.0059920. PubMed PMID: 23555831; PubMed Central PMCID: PMC3612081.

8. Kotsanas D, Wijesooriya WR, Korman TM, Gillespie EE, Wright L, Snook K, et al. "Down the drain": carbapenem-resistant bacteria in intensive care unit patients and handwashing sinks. Med J Aust. 2013;198(5):267-9. PubMed PMID: 23496403.

9. Peleg AY, Franklin C, Bell JM, Spelman DW. Dissemination of the metallo-beta-lactamase gene blaIMP-4 among gram-negative pathogens in a clinical setting in Australia. Clin Infect Dis. 2005;41(11):1549-56. doi: 10.1086/497831. PubMed PMID: 16267725.

10. Tai AY, Stuart RL, Sidjabat HE, Lemoh CN, Rogers BA, Graham M, et al. Local acquisition and nosocomial transmission of Klebsiella pneumoniae harbouring the blaNDM-1 gene in Australia. Med J Aust. 2015;202(5):270-2. PubMed PMID: 25758700.

11. Chang LW, Buising KL, Jeremiah CJ, Cronin K, Poy Lorenzo YS, Howden BP, et al. Managing a nosocomial outbreak of carbapenem-resistant Klebsiella pneumoniae: an early Australian hospital experience. Intern Med J. 2015;45(10):1037-43. doi: 10.1111/imj.12863. PubMed PMID: 26178306.

12. Chua KY, Grayson ML, Burgess AN, Lee JY, Howden BP. The growing burden of multidrug-resistant infections among returned Australian travellers. Med J Aust. 2014;200(2):116-8. PubMed PMID: 24484117.

13. Fernando GA, Collignon PJ, Bell JM. A risk for returned travellers: the "post-antibiotic era". Med J Aust. 2010;193(1):59. PubMed PMID: 20618119.

14. Poirel L, Lagrutta E, Taylor P, Pham J, Nordmann P. Emergence of metallo-beta-lactamase NDM-1-producing multidrug-resistant Escherichia coli in Australia. Antimicrob Agents Chemother. 2010;54(11):4914-6. doi: 10.1128/AAC.00878-10. PubMed PMID: 20823289; PubMed Central PMCID: PMC2976126.

15. Sidjabat H, Nimmo GR, Walsh TR, Binotto E, Htin A, Hayashi Y, et al. Carbapenem resistance in Klebsiella pneumoniae due to the New Delhi Metallo-beta-lactamase. Clin Infect Dis. 2011;52(4):481-4. doi: 10.1093/cid/ciq178. PubMed PMID: 21258100; PubMed Central PMCID: PMC3106237.

16. Sidjabat HE, Kennedy K, Silvey A, Collignon P, Paterson DL. Emergence of bla(OXA-181)-carrying ColE plasmid in Klebsiella pneumoniae in Australia. Int J Antimicrob Agents. 2013;41(3):294-6. doi: 10.1016/j.ijantimicag.2012.11.003. PubMed PMID: 23313398.

17. Blyth CC, Pereira L, Goire N. New Delhi metallo-beta-lactamase-producing enterobacteriaceae in an Australian child who had not travelled overseas. Med J Aust. 2014;200(7):386. PubMed PMID: 24794661.

18. Munoz-Price LS, Poirel L, Bonomo RA, Schwaber MJ, Daikos GL, Cormican M, et al. Clinical epidemiology of the global expansion of Klebsiella pneumoniae carbapenemases. Lancet Infect Dis. 2013;13(9):785-96. doi: 10.1016/S1473-3099(13)70190-7. PubMed PMID: 23969216; PubMed Central PMCID: PMC4673667.

19. Coatsworth NR, Huntington PG, Hardiman RP, Hudson BJ, Fernandes CJ. A case of carbapenemase-producing Klebsiella pneumoniae in Australia. Pathology. 2012;44(1):42-4. doi: 10.1097/PAT.0b013e32834e4228. PubMed PMID: 22157689.

20. Kwong JC, McCallum N, Sintchenko V, Howden BP. Whole genome sequencing in clinical and public health microbiology. Pathology. 2015;47(3):199-210. doi: 10.1097/PAT.0000000000000235. PubMed PMID: 25730631; PubMed Central PMCID: PMC4389090.

21. Kwong JC, Mercoulia K, Tomita T, Easton M, Li HY, Bulach DM, et al. Prospective Whole-Genome Sequencing Enhances National Surveillance of Listeria monocytogenes. J Clin Microbiol. 2016;54(2):333-42. doi: 10.1128/JCM.02344-15. PubMed PMID: 26607978; PubMed Central PMCID: PMC4733179.

22. Jiang Y, Wei Z, Wang Y, Hua X, Feng Y, Yu Y. Tracking a hospital outbreak of KPC-producing ST11 Klebsiella pneumoniae with whole genome sequencing. Clin Microbiol Infect. 2015;21(11):1001-7. doi: 10.1016/j.cmi.2015.07.001. PubMed PMID: 26166545.

23. Kanamori H, Parobek CM, Juliano JJ, van Duin D, Cairns BA, Weber DJ, et al. A Prolonged Outbreak of KPC-3-Producing Enterobacter cloacae and Klebsiella pneumoniae Driven by Multiple Mechanisms of Resistance Transmission at a Large Academic Burn Center.

Antimicrob Agents Chemother. 2017;61(2). doi: 10.1128/AAC.01516-16. PubMed PMID: 27919898; PubMed Central PMCID: PMC5278681.

24. Marsh JW, Krauland MG, Nelson JS, Schlackman JL, Brooks AM, Pasculle AW, et al. Genomic Epidemiology of an Endoscope-Associated Outbreak of Klebsiella pneumoniae Carbapenemase (KPC)-Producing K. pneumoniae. PLoS One. 2015;10(12):e0144310. doi: 10.1371/journal.pone.0144310. PubMed PMID: 26637170; PubMed Central PMCID: PMC4670079.

25. Mathers AJ, Stoesser N, Sheppard AE, Pankhurst L, Giess A, Yeh AJ, et al. Klebsiella pneumoniae carbapenemase (KPC)-producing K. pneumoniae at a single institution: insights into endemicity from whole-genome sequencing. Antimicrob Agents Chemother. 2015;59(3):1656-63. doi: 10.1128/AAC.04292-14. PubMed PMID: 25561339; PubMed Central PMCID: PMC4325807.

26. Ruppe E, Olearo F, Pires D, Baud D, Renzi G, Cherkaoui A, et al. Clonal or not clonal? Investigating hospital outbreaks of KPC-producing Klebsiella pneumoniae with whole-genome sequencing. Clin Microbiol Infect. 2017. doi: 10.1016/j.cmi.2017.01.015. PubMed PMID: 28143787.

27. Snitkin ES, Zelazny AM, Thomas PJ, Stock F, Group NCSP, Henderson DK, et al. Tracking a hospital outbreak of carbapenem-resistant Klebsiella pneumoniae with whole-genome sequencing. Sci Transl Med. 2012;4(148):148ra16. doi: 10.1126/scitranslmed.3004129. PubMed PMID: 22914622; PubMed Central PMCID: PMC3521604.

28. Weterings V, Zhou K, Rossen JW, van Steenis D, Thewessen E, Kluytmans J, et al. An outbreak of colistin-resistant Klebsiella pneumoniae carbapenemase-producing Klebsiella pneumoniae in the Netherlands (July to December 2013), with inter-institutional spread. Eur J Clin Microbiol Infect Dis. 2015;34(8):1647-55. doi: 10.1007/s10096-015-2401-2. PubMed PMID: 26067658.

29. Gilchrist CA, Turner SD, Riley MF, Petri WA, Jr., Hewlett EL. Whole-genome sequencing in outbreak analysis. Clin Microbiol Rev. 2015;28(3):541-63. doi: 10.1128/CMR.00075-13. PubMed PMID: 25876885; PubMed Central PMCID: PMC4399107.

902 30. Nordmann P, Poirel L, Dortet L. Rapid detection of carbapenemase-producing
903 Enterobacteriaceae. Emerg Infect Dis. 2012;18(9):1503-7. doi: 10.3201/eid1809.120355.
904 PubMed PMID: 22932472; PubMed Central PMCID: PMC3437707.
905 31. Tenover FC, Canton R, Kop J, Chan R, Ryan J, Weir F, et al. Detection of colonization by
906 carbapenemase-producing Gram-negative Bacilli in patients by use of the Xpert MDRO
907 assay. J Clin Microbiol. 2013;51(11):3780-7. doi: 10.1128/JCM.01092-13. PubMed PMID:
908 24006011; PubMed Central PMCID: PMC3889767.
909 32. Carter GP, Buultjens AH, Ballard SA, Baines SL, Tomita T, Strachan J, et al. Emergence of
910 endemic MLST non-typeable vancomycin-resistant Enterococcus faecium. J Antimicrob
911 Chemother. 2016;71(12):3367-71. doi: 10.1093/jac/dkw314. PubMed PMID: 27530751.
912 33. Bolger AM, Lohse M, Usadel B. Trimmomatic: a flexible trimmer for Illumina sequence
913 data. Bioinformatics. 2014;30(15):2114-20. doi: 10.1093/bioinformatics/btu170. PubMed
914 PMID: 24695404; PubMed Central PMCID: PMC4103590.
915 34. Bankevich A, Nurk S, Antipov D, Gurevich AA, Dvorkin M, Kulikov AS, et al. SPAdes: a
916 new genome assembly algorithm and its applications to single-cell sequencing. J Comput
917 Biol. 2012;19(5):455-77. doi: 10.1089/cmb.2012.0021. PubMed PMID: 22506599; PubMed
918 Central PMCID: PMC3342519.
919 35. Seemann T. Prokka: rapid prokaryotic genome annotation. Bioinformatics.
920 2014;30(14):2068-9. doi: 10.1093/bioinformatics/btu153. PubMed PMID: 24642063.
921 36. Walker BJ, Abeel T, Shea T, Priest M, Abouelliel A, Sakthikumar S, et al. Pilon: an
922 integrated tool for comprehensive microbial variant detection and genome assembly
923 improvement. PLoS One. 2014;9(11):e112963. doi: 10.1371/journal.pone.0112963. PubMed
924 PMID: 25409509; PubMed Central PMCID: PMC4237348.
925 37. Page AJ, Cummins CA, Hunt M, Wong VK, Reuter S, Holden MT, et al. Roary: rapid large-
926 scale prokaryote pan genome analysis. Bioinformatics. 2015;31(22):3691-3. doi:
927 10.1093/bioinformatics/btv421. PubMed PMID: 26198102; PubMed Central PMCID:
928 PMC4817141.

929 38. Didelot X, Wilson DJ. ClonalFrameML: efficient inference of recombination in whole
930 bacterial genomes. PLoS Comput Biol. 2015;11(2):e1004041. doi:
931 10.1371/journal.pcbi.1004041. PubMed PMID: 25675341; PubMed Central PMCID:
932 PMC4326465.
933 39. Page AJ, Taylor B, Delaney AJ, Soares J, Seemann T, Keane JA, et al. SNP-sites: rapid
934 efficient extraction of SNPs from multi-FASTA alignments. Microb Genom.
935 2016;2(4):e000056. doi: 10.1099/mgen.0.000056. PubMed PMID: 28348851; PubMed
936 Central PMCID: PMC5320690.
937 40. Stamatakis A. RAxML version 8: a tool for phylogenetic analysis and post-analysis of large
938 phylogenies. Bioinformatics. 2014;30(9):1312-3. doi: 10.1093/bioinformatics/btu033.
939 PubMed PMID: 24451623; PubMed Central PMCID: PMC3998144.
940 41. Cheng L, Connor TR, Siren J, Aanensen DM, Corander J. Hierarchical and spatially explicit
941 clustering of DNA sequences with BAPS software. Mol Biol Evol. 2013;30(5):1224-8. doi:
942 10.1093/molbev/mst028. PubMed PMID: 23408797; PubMed Central PMCID:
943 PMC3670731.
944 42. Chen L, Chavda KD, Mediavilla JR, Jacobs MR, Levi MH, Bonomo RA, et al. Partial
945 excision of blaKPC from Tn4401 in carbapenem-resistant Klebsiella pneumoniae. Antimicrob
946 Agents Chemother. 2012;56(3):1635-8. doi: 10.1128/AAC.06182-11. PubMed PMID:
947 22203593; PubMed Central PMCID: PMC3294926.
948 43. Kitchel B, Rasheed JK, Patel JB, Srinivasan A, Navon-Venezia S, Carmeli Y, et al. Molecular
949 epidemiology of KPC-producing Klebsiella pneumoniae isolates in the United States: clonal
950 expansion of multilocus sequence type 258. Antimicrob Agents Chemother. 2009;53(8):3365-
951 70. doi: 10.1128/AAC.00126-09. PubMed PMID: 19506063; PubMed Central PMCID:
952 PMC2715580.
953 44. Naas T, Cuzon G, Truong HV, Nordmann P. Role of ISKpn7 and deletions in blaKPC gene
954 expression. Antimicrob Agents Chemother. 2012;56(9):4753-9. doi: 10.1128/AAC.00334-12.
955 PubMed PMID: 22733068; PubMed Central PMCID: PMC3421896.

956 45. Naas T, Cuzon G, Villegas MV, Lartigue MF, Quinn JP, Nordmann P. Genetic structures at
957 the origin of acquisition of the beta-lactamase bla KPC gene. Antimicrob Agents Chemother.
958 2008;52(4):1257-63. doi: 10.1128/AAC.01451-07. PubMed PMID: 18227185; PubMed
959 Central PMCID: PMC2292522.
960 46. Camacho C, Coulouris G, Avagyan V, Ma N, Papadopoulos J, Bealer K, et al. BLAST+:
961 architecture and applications. BMC Bioinformatics. 2009;10:421. doi: 10.1186/1471-2105-
962 10-421. PubMed PMID: 20003500; PubMed Central PMCID: PMC2803857.
963 47. Carattoli A, Zankari E, Garcia-Fernandez A, Voldby Larsen M, Lund O, Villa L, et al. In
964 silico detection and typing of plasmids using PlasmidFinder and plasmid multilocus sequence
965 typing. Antimicrob Agents Chemother. 2014;58(7):3895-903. doi: 10.1128/AAC.02412-14.
966 PubMed PMID: 24777092; PubMed Central PMCID: PMC4068535.
967 48. Bouckaert R, Heled J, Kuhnert D, Vaughan T, Wu CH, Xie D, et al. BEAST 2: a software
968 platform for Bayesian evolutionary analysis. PLoS Comput Biol. 2014;10(4):e1003537. doi:
969 10.1371/journal.pcbi.1003537. PubMed PMID: 24722319; PubMed Central PMCID:
970 PMC3985171.
971 49. Rambaut A, Lam TT, Max Carvalho L, Pybus OG. Exploring the temporal structure of
972 heterochronous sequences using TempEst (formerly Path-O-Gen). Virus Evol.
973 2016;2(1):vew007. doi: 10.1093/ve/vew007. PubMed PMID: 27774300; PubMed Central
974 PMCID: PMC4989882.
975 50. Drummond AJ, Ho SY, Phillips MJ, Rambaut A. Relaxed phylogenetics and dating with
976 confidence. PLoS Biol. 2006;4(5):e88. doi: 10.1371/journal.pbio.0040088. PubMed PMID:
977 16683862; PubMed Central PMCID: PMC1395354.
978 51. Hasegawa M, Kishino H, Yano T. Dating of the human-ape splitting by a molecular clock of
979 mitochondrial DNA. J Mol Evol. 1985;22(2):160-74. PubMed PMID: 3934395.
980 52. Didelot X, Fraser C, Gardy J, Colijn C. Genomic Infectious Disease Epidemiology in
981 Partially Sampled and Ongoing Outbreaks. Mol Biol Evol. 2017;34(4):997-1007. doi:
982 10.1093/molbev/msw275. PubMed PMID: 28100788.

983 53. Zimmerman FS, Assous MV, Bdolah-Abram T, Lachish T, Yinnon AM, Wiener-Well Y.
984 Duration of carriage of carbapenem-resistant Enterobacteriaceae following hospital discharge.
985 Am J Infect Control. 2013;41(3):190-4. doi: 10.1016/j.ajic.2012.09.020. PubMed PMID:
986 23449280.
987 54. Price MN, Dehal PS, Arkin AP. FastTree 2--approximately maximum-likelihood trees for
988 large alignments. PLoS One. 2010;5(3):e9490. doi: 10.1371/journal.pone.0009490. PubMed
989 PMID: 20224823; PubMed Central PMCID: PMC2835736.
990 55. Deleo FR, Chen L, Porcella SF, Martens CA, Kobayashi SD, Porter AR, et al. Molecular
991 dissection of the evolution of carbapenem-resistant multilocus sequence type 258 Klebsiella
992 pneumoniae. Proc Natl Acad Sci U S A. 2014;111(13):4988-93. doi:
993 10.1073/pnas.1321364111. PubMed PMID: 24639510; PubMed Central PMCID:
994 PMC3977278.
995 56. Department of Health & Human Services, Victoria State Government. Victorian guidelines
996 on carbapenemase-producing Enterobacteriaceae. Melbourne: Victoria State Government,
997 2015. Available from:
998 https://www2.health.vic.gov.au/about/publications/policiesandguidelines/carbapenemase-
999 producing-enterobacteriaceae-guidelines
1000 57. World Health Organization. Antimicrobial resistance: global report on surveillance. 2014.
1001 Available from: http://www.who.int/drugresistance/documents/surveillancereport/en/
1002 58. Schwaber MJ, Carmeli Y. An ongoing national intervention to contain the spread of
1003 carbapenem-resistant enterobacteriaceae. Clin Infect Dis. 2014;58(5):697-703. doi:
1004 10.1093/cid/cit795. PubMed PMID: 24304707.
1005 59. Palmore TN, Henderson DK. Managing transmission of carbapenem-resistant
1006 enterobacteriaceae in healthcare settings: a view from the trenches. Clin Infect Dis.
1007 2013;57(11):1593-9. doi: 10.1093/cid/cit531. PubMed PMID: 23934166; PubMed Central
1008 PMCID: PMC3888298.
1009 60. Tong SY. Genomic polish for shoe-leather epidemiology. Nat Rev Microbiol. 2013;11(1):8.
1010 doi: 10.1038/nrmicro2935. PubMed PMID: 23202526.

1011 61. Conlan S, Thomas PJ, Deming C, Park M, Lau AF, Dekker JP, et al. Single-molecule
1012 sequencing to track plasmid diversity of hospital-associated carbapenemase-producing
1013 Enterobacteriaceae. Sci Transl Med. 2014;6(254):254ra126. doi:
1014 10.1126/scitranslmed.3009845. PubMed PMID: 25232178; PubMed Central PMCID:
1015 PMC4203314.
1016 62. Mathers AJ, Stoesser N, Chai W, Carroll J, Barry K, Cherunvanky A, et al. Chromosomal
1017 Integration of the Klebsiella pneumoniae Carbapenemase Gene, blaKPC, in Klebsiella
1018 Species Is Elusive but Not Rare. Antimicrob Agents Chemother. 2017;61(3). doi:
1019 10.1128/AAC.01823-16. PubMed PMID: 28031204; PubMed Central PMCID:
1020 PMC5328509.
1021 63. Golubchik T, Batty EM, Miller RR, Farr H, Young BC, Larner-Svensson H, et al. Within-
1022 host evolution of Staphylococcus aureus during asymptomatic carriage. PLoS One.
1023 2013;8(5):e61319. doi: 10.1371/journal.pone.0061319. PubMed PMID: 23658690; PubMed
1024 Central PMCID: PMC3641031.
1025 64. Eyre DW, Cule ML, Griffiths D, Crook DW, Peto TE, Walker AS, et al. Detection of mixed
1026 infection from bacterial whole genome sequence data allows assessment of its role in
1027 Clostridium difficile transmission. PLoS Comput Biol. 2013;9(5):e1003059. doi:
1028 10.1371/journal.pcbi.1003059. PubMed PMID: 23658511; PubMed Central PMCID:
1029 PMC3642043.
1030 65. Hatherell HA, Colijn C, Stagg HR, Jackson C, Winter JR, Abubakar I. Interpreting whole
1031 genome sequencing for investigating tuberculosis transmission: a systematic review. BMC
1032 Med. 2016;14:21. doi: 10.1186/s12916-016-0566-x. PubMed PMID: 27005433; PubMed
1033 Central PMCID: PMC4804562.
1034 66. Worby CJ, Lipsitch M, Hanage WP. Within-host bacterial diversity hinders accurate
1035 reconstruction of transmission networks from genomic distance data. PLoS Comput Biol.
1036 2014;10(3):e1003549. doi: 10.1371/journal.pcbi.1003549. PubMed PMID: 24675511;
1037 PubMed Central PMCID: PMC3967931.

67. Conlan S, Park M, Deming C, Thomas PJ, Young AC, Coleman H, et al. Plasmid Dynamics in KPC-Positive Klebsiella pneumoniae during Long-Term Patient Colonization. MBio. 2016;7(3). doi: 10.1128/mBio.00742-16. PubMed PMID: 27353756.

68. Jombart T, Cori A, Didelot X, Cauchemez S, Fraser C, Ferguson N. Bayesian reconstruction of disease outbreaks by combining epidemiologic and genomic data. PLoS Comput Biol. 2014;10(1):e1003457. doi: 10.1371/journal.pcbi.1003457. PubMed PMID: 24465202; PubMed Central PMCID: PMC3900386.

69. Klinkenberg D, Backer JA, Didelot X, Colijn C, Wallinga J. Simultaneous inference of phylogenetic and transmission trees in infectious disease outbreaks. PLoS Comput Biol. 2017;13(5):e1005495. doi: 10.1371/journal.pcbi.1005495. PubMed PMID: 28545083; PubMed Central PMCID: PMC5436636.

70. Ypma RJ, van Ballegooijen WM, Wallinga J. Relating phylogenetic trees to transmission trees of infectious disease outbreaks. Genetics. 2013;195(3):1055-62. doi: 10.1534/genetics.113.154856. PubMed PMID: 24037268; PubMed Central PMCID: PMC3813836.

71. Centers for Disease Control and Prevention, US Department of Health and Human Services. Facility Guidance for Control of Carbapenem-resistant Enterobacteriaceae (CRE). 2015. Available from: https://www.cdc.gov/hai/organisms/cre/cre-toolkit/index.html