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Klebsiella quasipneumoniae provides a window into carbapenemase gene transfer, plasmid rearrangements and patient interactions with the hospital environment

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Running Title: Nosocomial KPC-*K. quasipneumoniae*

Abstract

Several emerging pathogens have arisen as a result of selection pressures exerted by modern healthcare. *Klebsiella quasipneumoniae* was recently defined as a new species, yet its prevalence, niche, and propensity to acquire antimicrobial resistance genes are not fully described. We have been tracking inter- and intra-species transmission of the *Klebsiella pneumoniae* carbapenemase (KPC) gene, bla_{KPC}, between bacteria isolated from a single institution. We applied a combination of Illumina and PacBio whole-genome sequencing to identify and compare *K. quasipneumoniae* from patients and the hospital environment over 10 and five-year periods respectively. There were 32 bla_{KPC}-positive *K. quasipneumoniae* isolates, all of which were identified as *K. pneumoniae* in the clinical microbiology laboratory, from eight patients and 11 sink drains, with evidence for seven separate bla_{KPC} plasmid acquisitions. Analysis of a single subclade of *K. quasipneumoniae* subspecies *quasipneumoniae* (n=23 isolates) from three patients and six rooms demonstrated seeding of a sink by a patient, subsequent persistence of the strain in the hospital environment, and then possible transmission to another patient. Longitudinal analysis of this strain demonstrated the acquisition of two unique bla_{KPC} plasmids and then subsequent within-strain genetic rearrangement through transposition and homologous recombination. Our analysis highlights the apparent molecular propensity of *K. quasipneumoniae* to persist in the environment as well as acquire carbapenemase plasmids from other species and enabled an assessment of the genetic rearrangements which may facilitate horizontal transmission of carbapenemases.

Introduction:

In the last 50 years transformations in healthcare have created new niches for microorganisms such as *Acinetobacter baumannii* complex and *Candida auris* to arise from obscurity and emerge as important pathogens. Similarly, we have seen an increasing number of highly resistant *Klebsiella pneumoniae* strains which have been successfully transmitted worldwide(1). *Klebsiella pneumoniae* has proven to be an important contributor to the modern antibiotic resistance epidemic with its ability to acquire and carry antimicrobial resistance plasmids, as well as being successful a human pathogen. More recently, whole-genome sequencing has revealed that many isolates classified as *K. pneumoniae* actually encompass three related but distinct species – *K. pneumoniae, K. variicola* and *K. quasipneumoniae*(1, 2). *K. quasipneumoniae* was originally thought to be largely confined to agriculture and the environment, however it appears that it may also be prominent in human disease(3), and several recent reports have demonstrated that it harbors virulence factors and acquires clinically relevant genes of antimicrobial resistance(4, 5). Although there have been relatively few reports of *K. quasipneumoniae* to date, the true prevalence of this organism is likely underestimated as it is not generally distinguished from *K. pneumoniae* in routine testing of clinical laboratories(2).

Bacterial evolution via horizontal gene transfer is central to the ongoing crisis of antimicrobial resistance among clinically relevant bacteria. Hospital wastewater is being increasingly recognized as an ideal reservoir for resistance gene exchange and amplification, with ongoing antimicrobial selection pressure exerted through antimicrobials excreted in patient waste(6). Premise plumbing can be seeded by antimicrobial resistance genes in diverse bacterial strains and species, and represents a difficult-to-treat reservoir for ongoing gene exchange, creating successful drug-resistant bacteria that can thrive in both the environmental and human niches(7).

Whole-genome sequencing studies have demonstrated that our understanding of the interplay between antimicrobial resistance plasmids and their host strains/species is limited(8). The host range of a plasmid is critical for acquisition and persistence in specific species, but it appears that some bacterial strains are better equipped than others to prevent acquisition of or destroy foreign plasmid DNA(9). The durability of plasmid

acquisition events and the creation of new highly resistant strains reflects complex dynamics which depend on the characteristics of the plasmid in question as well as host strain tolerance(10, 11). Seldom do we have the opportunity to witness strains acquiring plasmids *in vivo* or in the environment and inferences about genetic re-arrangements are often highly speculative. However, understanding the mechanisms and frequency of resistance gene transfer events occurring in real world contexts can provide important insights into the wider evolutionary landscape creating modern multidrug resistant bacteria which cannot be effectively modeled in lab experiments(12).

Within our institution we have seen ongoing transmission of diverse carbapenemase-producing organisms for the last decade, driven by genetic exchange of the *Klebsiella pneumoniae* carbapenemase (KPC) gene (bla_{KPC}) in patients and the environment(13, 14). This has enabled us to understand specific pathways of genetic mobility involving numerous different mobile genetic elements and host bacterial species(13, 15). Herein we examine bla_{KPC} acquisition and associated genetic rearrangements within *K. quasipneumoniae* as a real-life representation of an emerging pathogen associated with the hospital wastewater environment.

Results

From our collection of bla_{KPC}-positive isolates from patients (2007-2017) and the hospital environment (2013-2017), there were a total of 32 bla_{KPC}-positive *K. quasipneumoniae* isolates, all of which were identified as *K. pneumoniae* in the clinical microbiology laboratory (Table 1). Twenty-three of these were *K. quasipneumoniae* subspecies *quasipneumoniae* (KpIIA) (ten patient isolates from four patients and 13 environmental isolates from seven rooms) and nine were *K. quasipneumoniae* subspecies *similipneumoniae* (KpIIB) (five patient isolates from four patients and four environmental isolates from four rooms). The KpIIA and KpIIB isolates were separated by >100,000 single nucleotide variants (SNVs). We identified a single strain of KpIIA and four strains of KpIIB differing from each other by >20,000 SNVs (Fig. 1). Many isolates have multiple virulence factors (Supplemental data) including several genes involved in capsule production (16) and several fimbrial elements. A type VI secretion system was present in all KpIIA but not all KpIIB. From a resistance gene standpoint, in addition to bla_{KPC} all isolates harbored a *fosA*, bla_{OKP} as well as a multidrug efflux transporter (*oqxA/oqxB*)(17).

Within the KpIIA strain, there were two subclades separated by ~150 SNVs (Fig. 1a). The first subclade contained two isolates separated by 10 SNVs (Fig. 1a). CAV1360 was from patient 1 in November 2009 and CAV2279 was identified in early 2014 (shortly after environmental sampling began) from room B that patient 1 had occupied in May 2009.

The second subclade of KpIIA contained isolates from three patients (patients 2-4) and six rooms (rooms A, C-G). The earliest of these was from patient 2 in November 2013. Patient 2 was in the hospital with a prolonged stay in the Surgical Trauma and Burn Intensive Care Unit (STBICU) following complications of a liver transplant (Figure 2). Patient 2 was noted to be first colonized with *bla*$_{KPC}$-positive KpIIA in November 2013. KpIIA was not found in the STBICU environment prior to closure for remediation of KPC-contamination of the drains in December 2013. Following drain exchange and unit re-opening patient 2 was immediately moved back into the STBICU and subsequently occupied rooms C, D, E and G in the STBICU, suggesting that the KpIIA isolates in these rooms originated from patient 2 (Figure 2). Patient 3 was admitted to the STBICU at the same time as patient 2 and thus could have acquired KPC-KpIIA directly from patient 2 without environmental transmission. Patient 4 was later admitted to STBICU room E for 28 days and discharged before he was found to have KpIIA. He was never on a ward at the same time as any other patients known to carry KpIIA, suggesting acquisition from the hospital environment.

There were four patients (patients 5-8) carrying four distinct strains of *bla*$_{KPC}$-KpIIB seen over a five year period (Fig. 1b, Table 1). For patient 7, the same KpIIB strain (~80 SNV differences) was also seen in sinks from two rooms in the Medical Intensive Care Unit (MICU) (rooms H-I) and two rooms in the STBICU (rooms J-K) in December 2013 when environmental sampling first began; this preceded detection in the patient in February 2014. Patient 7 was admitted to the MICU (location of rooms H and I), but did not stay any of the rooms where the isolates within the same KpIIB were identified. The other three patients with KpIIB each had a unique *bla*$_{KPC}$ strain, none of which were identified in another patient or the environment. Patient 6 also with a unique strain had a prolonged hospital stay and was also colonized/infected with another *bla*$_{KPC}$-positive species (*K. pneumoniae*).

Three patients developed infections with KPC-KpIIA (Table 1). Patient 1 died of ventilator-associated pneumonia with KPC-KpIIA following a complicated heart transplant. Patient 2 had both ventilator-acquired pneumonia, which was successfully treated, and a subsequent untreatable intraabdominal infection with KPC-KpIIA bacteremia, which contributed to the patient's death after a long hospital stay with a complicated liver transplant. Patient 4 had a successfully treated complicated KPC-KpIIA urinary tract infection. Patient 3 did not develop an infection with KpIIA. None of the patients with KpIIB developed *K. quasipneumoniae* infections, however two of the patients did develop infections with other species carrying bla_{KPC} (*K. pneumoniae* for patient 6 and *Serratia marcescens* for patient 8) (Table 2).

Genetic variation and rearrangements within KpIIA

All KpIIA isolates were closely related at the core chromosome level, with a maximum divergence of <180 SNVs. If bla_{KPC} were acquired only once in this lineage then any sequence variation within the 10 kb bla_{KPC} transposon Tn*4401* would be the result of mutational change, which is expected to be rare. Surprisingly, the Illumina sequence data revealed a great deal of sequence variation within Tn*4401* (Fig. 1a). Two sites (positions 8015 and 9663 in the Tn*4401*b reference) showed variation at the single-nucleotide level, and one isolate had a deletion at positions 7075-7153. Interestingly, several isolates showed mixtures at one or both of the variable sites, indicating two or more different versions of Tn*4401* in the same isolate. This included mixtures at position 8015, which is located within the bla_{KPC} gene and differentiates bla_{KPC-2} and bla_{KPC-3}, indicating that there were isolates with both bla_{KPC} alleles.

Similarly, if a single bla_{KPC} plasmid were acquired and stably maintained within KpIIA, then we would expect to see a single flanking sequence context for Tn*4401*. On the contrary, there was significant diversity in Tn*4401* flanking regions, with eight and seven different 5 bp sequences on the left and right sides of Tn*4401* respectively, suggesting active transposition of Tn*4401* within KpIIA and/or multiple plasmid acquisitions.

To better understand the origin of the genetic diversity within and surrounding Tn*4401*, we performed long-read PacBio sequencing on three of the KpIIA isolates (CAV2013 from patient 2, CAV1947 from room C and

CAV2018 from room C), as well as a *S. marcescens* isolate from patient 2 (CAV1761). The room C isolates were chosen because this room only became positive after admission of patient 2 following sink trap exchange in the STBICU, hence they are expected to be descended from the patient 2 KpIIA.

Both patient 2 isolates had a single bla_{KPC} plasmid each (Figure 3a-b). The KpIIA isolate had a 447,095 bp "RepA_CP011611" bla_{KPC-3} plasmid, and the *S. marcescens* isolate had a 69,158 bp IncU/IncX5 bla_{KPC-2} plasmid (18). Both plasmids contained Tn*4401*b, however there were two SNV differences within the Tn*4401*b sequence, one at position 8015 (differentiating bla_{KPC-2} and bla_{KPC-3}) and one at position 9663.

The KpIIA isolates from room C (CAV1947 and CAV2018) had three and two bla_{KPC} plasmids respectively (Fig. 3c-d). Both isolates harbored the IncU/IncX5 bla_{KPC} plasmid from the patient 2 *S. marcescens* isolate, indicating likely bla_{KPC} plasmid transfer from *S. marcescens* to *K. quasipneumoniae* (Fig. 3e). In CAV1947, the plasmid sequence was identical to the patient isolate, CAV1761, with the exception of two large indels (Fig. 4a). One of these was a 16,315 bp deletion immediately adjacent to Tn*4401*, presumably as a result of intramolecular transposition in cis, that converted the left flanking sequence from TTTTT to ACAAT and removed the IncU replicon sequence (Fig. 3g). In CAV2018, the plasmid sequence was identical to CAV1761, except for a single 5,923 bp deletion that truncated part of the Tn*4401* sequence (Fig. 3h, 4a).

Both isolates also harboured the ancestral RepA_CP011611 bla_{KPC} plasmid from the patient 2 KpIIA isolate, with several SNVs and large indels (Fig. 4b). Interestingly, in CAV2018, one of the SNVs was located within Tn*4401*, such that the CAV2018 RepA_CP011611 plasmid contained bla_{KPC-2} rather than bla_{KPC-3}. Given that there was plasmid transfer of the IncU/IncX5 bla_{KPC-2} plasmid from *S. marcescens*, we infer that the bla_{KPC-2}-containing RepA_CP011611plasmid most likely arose as a result of homologous recombination between these two different plasmids flanking the bla_{KPC} region (Fig. 3f, k). The Illumina data also revealed similar patterns of homologous recombination in other isolates (notably CAV2983, CAV2984, CAV3444, CAVp64 and CAVp275, which all have the TTTTT IncU/IncX5 plasmid flanking sequences, but with the C8015T bla_{KPC-3} mutation and without the

T9663C mutation), suggesting frequent exchange of Tn*4401* variants between different *bla*$_{KPC}$ plasmids within the same host bacterium (Fig. 1, 3k).

CAV1947 also harboured a third *bla*$_{KPC}$ plasmid, representing transposition of Tn*4401* into a 4,095 bp non-typeable plasmid that was present in the CAV2013 ancestor from patient 2 (Fig. 3i, 4c).

K. quasipneumoniae has acquired *bla*$_{KPC}$ on multiple occasions

The average unique plasmid Inc types per isolate was over four according to plasmid finder (Supplemental data). Within KpIIB, there were four divergent strains separated by >20,000 SNVs, suggesting four separate acquisitions of *bla*$_{KPC}$ in this subspecies. Within KpIIA, there were two subclades separated by ~180 SNVs. Given that Tn*4401* variation and flanking sequences were different between the two subclades (apart from the GTTCT flanking sequence which is known to be present in many different *bla*$_{KPC}$ plasmids)(13); and that there was no epidemiological overlap, it is most likely that the subclades acquired *bla*$_{KPC}$ independently. Additionally, as described above, the second subclade likely acquired *bla*$_{KPC}$ on two occasions, with the second acquisition originating from *S. marcescens*. Therefore, overall there were likely seven acquisitions of *bla*$_{KPC}$ by *K. quasipneumoniae*, three in KpIIA and four in KpIIB.

Interestingly, there was evidence that one of the acquisitions in KpIIB also originated from *S. marcescens*, indicating the compatibility of these two species in exchanging plasmids. This was in the patient 8 KpIIB lineage. Patient 8 was first colonized with *bla*$_{KPC}$-*S. marcescens* carrying Tn*4401*b with a T9663C mutation and TTTTT/TTTTT flanking sequences. Four months later, *bla*$_{KPC}$-KpIIB was identified with the same Tn*4401* mutation and flanking sequences, suggesting plasmid transfer from *S. marcescens* to *K. quasipneumoniae* within this patient.

Discussion

We describe the behaviour of nosocomial *bla*$_{KPC}$-positive *K. quasipneumoniae* strains within a single-hospital setting, observing their propensity to uptake multiple carbapenemase plasmids from other species, and

disseminate between patients and sink drains. Our study also suggests that rapid genetic rearrangement occurs in the mobile genetic elements carrying bla_{KPC} in KpIIA.

There is increasing recognition that the hospital environment is an important reservoir in the transmission of carbapenemase-producing *Enterobacteriaceae* (CPE), but delineating transmission chains is often challenging(19, 20). Through our *K. quasipneumoniae* example we provide compelling evidence for patient-to-drain and drain-to-patient transmission, as has been observed in other studies(7). We also provide evidence supporting the ability of *K. quasipneumoniae* to be maintained in the environment for a long period of time, with the first subclade of KpIIA detected in the environment on initial sampling, even though it had not been seen in a patient nor had that patient been in the room for over three years. The costly closure of the STBICU and exchange of all the sink drain plumbing pipes had a limited effect on environmental contamination with CPE; instead it appears to have provided an environment for immediate new seeding and establishment of previously unobserved carbapenem-resistant strains. There are potential other reservoirs to consider but health care workers have not been identified as a source of CPE. We have a fairly robust screening program in place and have sequenced all patient isolates and included all *K. quasipneumoniae* in this series making silent colonization less likely(21, 22). We were not sampling the toilets or hoppers during most of the study and we have only sequenced a portion of environmental isolate which could provide an unidentified environmental source of *K. quasipneumoniae*(14). Understanding the dynamics and natural history of colonization of premise plumbing with CPE will be important in designing effective interventions to limit transmission(23).

Although there have only been a handful of reports of *K. quasipneumoniae* since its definition as a species in 2014, it does appear that this organism is widespread(2, 5, 24, 25). As seen here, it is not readily distinguished from *K. pneumoniae* with current clinical microbiology techniques and thus the true prevalence is unknown(2, 26). On the evolutionary time scale, modern medicine has provided a novel ecology with immunocompromised patients, widespread antimicrobial use, newly circulating antimicrobial resistance genes and the design of the modern hospital providing new microbiologic niche for organisms to emerge(7, 27). We found several virulence factors in our collection some of which have been identified in other *K. pneumoniae* or *K. quasipneumoniae*;

capsule, fimbrial adhesion proteins, and a type VI secretion system (5, 16). As seen here we provide evidence for *K. quasipneumoniae* to be sustained in both a human host and the environment encountering several different species which may be relatively new in the evolutionary tree of *Klebsiella* sp(1). As a consequence of these encounters transfer of mobile DNA occurs via traceable carbapenemase plasmids. We found evidence for seven independent acquisitions of bla_{KPC} by *K. quasipneumoniae*, suggesting that this species is amenable to plasmid uptake from other species of Enterobacteriaceae. Given the difficulties in accurately identifying *K. quasipneumoniae*, this species may therefore be more significant in the context of bla_{KPC} dissemination than has previously been recognised.

Within *K. quasipneumoniae*, there was surprising variability in mobile elements carrying bla_{KPC}, which was the result of several different processes observed amongst a limited number of highly related isolates(n=23). We also found multiple acquired antimicrobial resistance genes and every isolate had more than one plasmid incompatibility type(18). Specifically, there were multiple independent bla_{KPC} plasmid acquisitions, homologous recombination between different bla_{KPC} plasmids, transposition of Tn*4401* into new plasmids, intramolecular transposition in cis of Tn*4401*, a deletion within Tn*4401* and a deletion truncating Tn*4401*. This high degree of genetic mobility has been similarly observed in other small studies(28, 29), and highlights the difficulty in developing an accurate understanding of the transmission epidemiology of important drug resistance genes which can be rapidly mobilized by multiple independent genetic modalities.

Within KpIIA, there were multiple acquisitions of bla_{KPC} within the same lineage, such that a bla_{KPC}-positive KpIIA strain acquired a second, unrelated bla_{KPC} plasmid from *S. marcescens*. Consequently, there were then two different bla_{KPC} plasmids, with different Tn*4401* sequences and different bla_{KPC} alleles, within the same host bacterium. This situation facilitated multiple rearrangements via homologous recombination between the different plasmids, resulting in the generation of new combinations of Tn*4401* SNVs and host plasmids. Multiple acquisition of resistance plasmids followed by rearrangements between those plasmids is likely to be important in the generation of adaptive allelic combinations which contribute to the amplification of cross-class antimicrobial

resistance within strains. High-risk clones with a propensity for uptake of antimicrobial resistance plasmids may represent important targets for intervention(30).

This study has several limitations. Most notably, it is a small retrospective series, preventing a full understanding of the role of the environment. Also, the order of genetic rearrangements is also not completely known given the limited number of long-read sequenced isolates and inability to capture all isolates from the environment over time. We offer however, that this is higher resolution than seen in many studies, and the analysis does contribute to the greater understanding of rapid rearrangement and mechanisms at play around mobility of genetic elements harbouring genes of antibiotic resistance in *Enterobacteriaceae*.

In summary, we demonstrate the relevance of *K. quasipneumoniae* as a species fit for nosocomial transmission in the modern era that is capable of acquiring and maintaining relevant resistance elements.

Methods:

Setting

Isolates were collected at the University of Virginia, a 619-bed tertiary care hospital, from August 2007- May 2017. A robust *K. pneumoniae* carbapenemase-producing organism (KPCO) prevention program existed throughout the study period as previously described(31), and included perirectal screening beginning in April 2009 in the medical intensive care unit (MICU) and surgical intensive care unit (STBICU), and weekly screening of all patients in the MICU and STBICU as well as units where any known KPCO-colonized patient was present(32). Screening was performed as previously described(32). Clinical Enterobacteriales and Aeromonadaceae isolates, as identified by MALDI-TOF or VITEK2 (Biomerieux, Durham, NC), with an elevated ertapenem or meropenem minimum inhibitory concentration (MIC) by VITEK2 (Biomerieux, Durham, NC) immediately underwent CarbaR (Cepheid Sunnyvale, CA) carbapenemase PCR testing. All species identification was performed using a combination of VITEK2, VITEK-MS (Biomerieux, Durham, NC). Clinical data was

gathered by retrospective electronic medical record review under University of Virginia Health Sciences IRB#13558 with waiver of consent.

In September 2013 sink trap sampling for KPCO began using previously described techniques(14) with a swab for drain collection and p-trap water. Following identification of KPCO in the hospital environment, the STBICU was closed to patient care in December 2013. Over the following 9 weeks all sink drain pipes were removed and replaced with sink traps that eliminated overflows on the sink bowl. Patients were readmitted to the surgical intensive care unit in February 2014. Bleach, hydrogen peroxide and ozone impregnated water (2ppm) were applied weekly from February-May 2014 in the STBICU (following drain exchange and sink bowl overflow closure and removal) and from March-May 2014 in the MICU (without drain exchange or sink bowl overflow removal).

Whole-genome sequencing and Bioinformatics Analysis

Illumina sequencing was performed as described previously(33). PacBio long-read sequencing and assembly were performed as previously described(13).

Broad level species classification was performed using Kraken(34). To identify *K. quasipneumoniae* isolates, we queried all isolates initially classified as *K. pneumoniae* against reference sequences representing each of the four clades in Holt et al(1). We arbitrarily selected a single reference sequence for each clade; these were: ERR025521 (KpI), ERR025986 (KpIIA), ERR025528 (KpIIB) and ERR025573 (KpIII). We used mash v1.1.1(35) with parameters "-r -m 5" to compare Illumina data for each of our isolates to these reference sequences. Each isolate was then assigned to one of the four Kp clades according to the reference with the lowest distance value. All isolates assigned to KpIIA or KpIIB were included in the analysis. In addition, we also included any other KPCO isolates from patients carrying *K. quasipneumoniae*.

To identify chromosomal single-nucleotide variants (SNVs), Illumina reads for each *K. quasipneumoniae* isolate were mapped to the CAV2013 chromosome sequence (derived from long-read sequencing), with mapping and variant calling performed as described previously (36). A phylogeny was generated using IQ-TREE v1.3.13 (37)

from an alignment of variable sites where at least 70% of samples had a high-quality reference/variant call (i.e. we excluded sites where >30% of samples had an "N" call). This was run with parameters "-blmin 0.000000001 -nt 4 -m GTR", with -fconst used to specify the number of invariant sites.

To identify Tn*4401* variation and flanking sequences from Illumina data, we used TETyper with published parameters(38).

The Illumina paired-end short reads were *de-novo* assembled using SPAdes assembler v 3.10.1 (35). Assembly statistics were evaluated using QUAST v4.0. (36) Plasmid Inc typing was performed using PlasmidFinder v2.0.1 against the Feb 2018 version of *Enterobacteriaceae* database (16), with minimum identity of 80% and minimum coverage of 50%. Acquired antimicrobial resistance genes were screened from the assemblies using NCBI's AMRFinder tool v1.0, which relies on a curated AMR protein database and a collection of Hidden Markov Models, with 90% minimum identity to translated amino-acid residues and 50% minimum coverage of reference protein sequence. (37) Identification of bacterial virulence genes was performed using ABRicate v0.8.11 (https://github.com/tseemann/abricate), against the Virulence Factors Database (accessed on Feb 2019), with 80% minimum identity and 50% minimum coverage.

Data availability

Illumina paired-end sequence data can be accessed from NCBI BioProject ID PRJNA411762. The accession numbers for completed closed genomes from hybrid assembly of PacBio and Illumina are - GCA_003146655.1 (CAV2013), GCA_003146685.1 (CAV1947), GCA_003146635.1 (CAV2018), and GCA_003146705.1 (CAV1761). All other relevant data for the manuscript are within supplementary data tables.

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310 **Figures**

311 **Fig. 1. Maximum likelihood phylogeny for KpIIA (a) and KpIIB (b) isolates, with Tn*4401* variation and**
312 **flanking genetic contexts.** Branch lengths are shown as SNVs per genome.

313 **Fig. 2. Patient movements and positive environmental samples with a single strain of *K. quasipneumoniae***
314 **(KpIIA) in the STBICU.** Colored bars for patients match rooms where environmental isolates were identified.
315 Black bars represent rooms with no KpIIA identified. The dotted lines indicate STBICU closure with removal and
316 new installation of sink drains and exposed sink plumbing. Patient 1 is not depicted as there was no admission to
317 the STBICU and no overlap in time or space with other patients carrying KpIIA.

318 **Fig. 3. Plasmid structures determined from long-read sequencing of four isolates and inferred intermediate**
319 ***bla*$_{KPC}$ plasmid structures.** a-d. Sequenced isolates. e-j. Inferred intermediate plasmid structures. Note that the
320 ordering of deletion, homologous recombination, transposition and plasmid loss events is arbitrarily represented
321 as the actual order of events is unknown. k. Examples of crossover events leading to the generation of new
322 combinations of SNVs within Tn*4401* (top) or the complete swapping of Tn*4401* variants between different
323 plasmids (bottom). Black boxes indicate products of homologous recombination that were observed in long-read
324 data (top) or Illumina data (bottom).

325 **Fig 4. Alignments of IncU/IncX5 (a), RepA_CP011611 (b) and non-typeable (c) *bla*$_{KPC}$ plasmid structures**
326 **determined from long-read sequencing.** Tn*4401* is indicated by a grey arrow. Light pink shading indicates
327 regions of identity, light blue shading shows inverted regions, SNVs are indicated by red lines and short indels by
328 blue lines.

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333 **Table 1. All Sequenced *bla*$_{KPC}$-*Klebsiella quasipneumoniae* isolates from patients and the hospital**
334 **environment**

label	Isolate	Subspecies of *K. quasipneumoniae*	Date	Source	Infection/outcome
1	CAV1360	*KpIIA*	Nov-09	Sputum	Ventilator associated pneumonia. in complicated heart transplant recipient/Expired
2	CAV2013	*KpIIA*	Nov-13	Perirectal surveillance	N/A
2	CAVp203	*KpIIA*	Dec-13	Bronchoscopy	Ventilator associated pneumonia //Successful treatment
2	CAVp26	*KpIIA*	Apr-14	Blood	Intraabdominal infection/Expired
2	CAVp20	*KpIIA*	Mar-14	Perirectal surveillance	N/A
2	CAVp64	*KpIIA*	Aug-14	Perirectal surveillance	N/A
2	CAVp72	*KpIIA*	Sep-14	Perirectal surveillance	N/A
2	CAVp103	*KpIIA*	Nov-14	Blood	Successful treatment of …
3	CAVp67	*KpIIA*	Aug-14	Perirectal surveillance	N/A
4	CAVp275	*KpIIA*	Jul-15	Urine	Complicated urinary tract infection/ Successful treatment
5	CAV1142	*KpIIB*	Aug-09	Perirectal surveillance	N/A
6	CAVp186	*KpIIB*	Dec-13	Perirectal surveillance	N/A
7	CAV2009	*KpIIB*	Feb-14	Perirectal surveillance	N/A
8	CAVp296	*KpIIB*	Oct-15	Perirectal surveillance	N/A

8	CAVp360	*KpIIB*	Dec-16	Perirectal surveillance	N/A
Room A (MICU)	CAV2244	*KpIIA*	Jan-14	Shower	
Room B (CTA)	CAV2279	*KpIIA*	Jan-14	Shower	
Room C (STBICU)	CAV1945	*KpIIA*	Feb-14	Drain swab (First sample after replacement)	
Room C (STBICU)	CAV1947	*KpIIA*	Feb-14	p-trap water -(First sample after replacement)	
Room C (STBICU)	CAV1964	*KpIIA*	Mar-14	Drain swab	
Room C (STBICU)	CAV2018	*KpIIA*	Apr-14	p-trap water	
Room D (STBICU)	CAV2019	*KpIIA*	Apr-14	p-trap water	
Room C (STBICU)	CAV2397	*KpIIA*	May-14	Drain swab	
Room E (STBICU)	CAV2697	*KpIIA*	Jul-14	Drain swab	
Room F (MICU)	CAV2957	*KpIIA*	Sep-15	Drain swab	
Room G (STBICU)	CAV2983	*KpIIA*	Oct-15	p-trap water	
Room G (STBICU)	CAV2984	*KpIIA*	Oct-15	Drain swab	
Room G (STBICU)	CAV3444	*KpIIA*	Feb-16	p-trap water	
Room H (MICU)	CAV1880	*KpIIB*	Dec-13	Drain swab	
Room I (MICU)	CAV1895	*KpIIB*	Dec-13	Drain swab	
Room J (STBICU)	CAV1832	*KpIIB*	Dec-13	p-trap water	
Room K (STBICU)	CAV1887	*KpIIB*	Dec-13	p-trap water	

335 Medical Intensive Care Unit (MICU), Surgical Trauma and Burn ICU (STBICU)

336 **Table 2. All additional *bla*$_{KPC}$-positive isolates from patients with *K. quasipneumoniae***

Patient	Isolate	Species	Date	Source	Infection	Genetic information	Flank
						Tn*4401* isoform	Right/left
2	CAVp202	*S. marcescens*	Dec-13	Urine	No	Tn*4401*b-8	TTTTT/TTTTT
2	CAVp11	*S. marcescens*	Feb-14	Intraabdominal abscess	Yes	Tn*4401*b-8	TTTTT/TTTTT
2	CAV1761*	*S. marcescens*	Mar-14	Perirectal surveillance		Tn*4401*b-8	TTTTT/TTTTT
3	CAVp50	*Klebsiella pneumoniae*	Jul-14	Perirectal surveillance	N/A	Tn*4401*b-truncated (deletion 9299-10006)	-/TTGCA
3	CAVp57	*Klebsiella pneumoniae*	Jul-14	Perirectal surveillance	N/A	Tn*4401*b-truncated	-/TTGCA
3	CAVp71	*Klebsiella pneumoniae*	Aug-14	Perirectal surveillance	N/A	Tn*4401*b-truncated	-/TTGCA
3	CAVp104	*Klebsiella pneumoniae*	Dec-14	Perirectal surveillance	N/A	Tn*4401*b-truncated	-/TTGCA
6	CAV1750	*Klebsiella pneumoniae*	Dec-12	Perirectal surveillance	N/A	Tn*4401*b-1	GTTCT/GTTCT
6	CAVp127	*Klebsiella pneumoniae*	Feb-13	Perirectal surveillance	N/A	Tn*4401*b-1	GTTCT/GTTCT
6	CAVp130	*Klebsiella pneumoniae*	Mar-13	Urine	Yes	Tn*4401*b-1	GTTCT/GTTCT
6	CAVp139	*Klebsiella pneumoniae*	Apr-13	Perirectal surveillance	N/A	Tn*4401*b-1	GTTCT/GTTCT
6	CAVp151	*Klebsiella pneumoniae*	Jul-13	Perirectal surveillance	N/A	Tn*4401*b-1	GTTCT/GTTCT
6	CAVp152	*Klebsiella pneumoniae*	Jul-13	Perirectal surveillance	N/A	Tn*4401*b-1	GTTCT/GTTCT
6	CAVp177	*Klebsiella pneumoniae*	Sep-13	Perirectal surveillance	N/A	Tn*4401*b-1	GTTCT/GTTCT
6	CAVp180	*Klebsiella pneumoniae*	Nov-13	Perirectal surveillance	N/A	Tn*4401*b-1	GTTCT|TACCT / AGCAT|GTTCT

6	CAVp183	*Klebsiella pneumoniae*	Nov-13	Intraabdominal abscess	Yes	Tn*4401*b-1	GTTCT/GTTCT
6	CAVp184	*Klebsiella pneumoniae*	Nov-13	Perirectal surveillance	N/A	Tn*4401*b-1	GTTCT/GTTCT
6	CAVp185	*Klebsiella pneumoniae*	Nov-13	Perirectal surveillance	N/A	Tn*4401*b-1	ATATT|GTTCT /ATATT|GTTC T
6	CAVp3	*Klebsiella pneumoniae*	Jan-14	Biliary drain	Yes	Tn*4401*b-1	GTTCT/GTTCT
8	CAVp269	*Serratia marcescens*	Jun-15	Blood	Yes	Tn*4401*b-8	TTTTT/TTTTT
8	CAVp270	*Serratia marcescens*	Jun-15	Perirectal surveillance	N/A	Tn*4401*b-8	TTTTT/TTTTT
8	CAVp361	*Escherichia coli*	Dec-16	Perirectal surveillance	N/A	Tn*4401*b-8	TTTTT/TTTTT
8	CAVp374	*Citrobacter freundii*	Mar-17	Perirectal surveillance	N/A	Tn*4401*b-8	TTTTT/TTTTT

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Fig 1a.

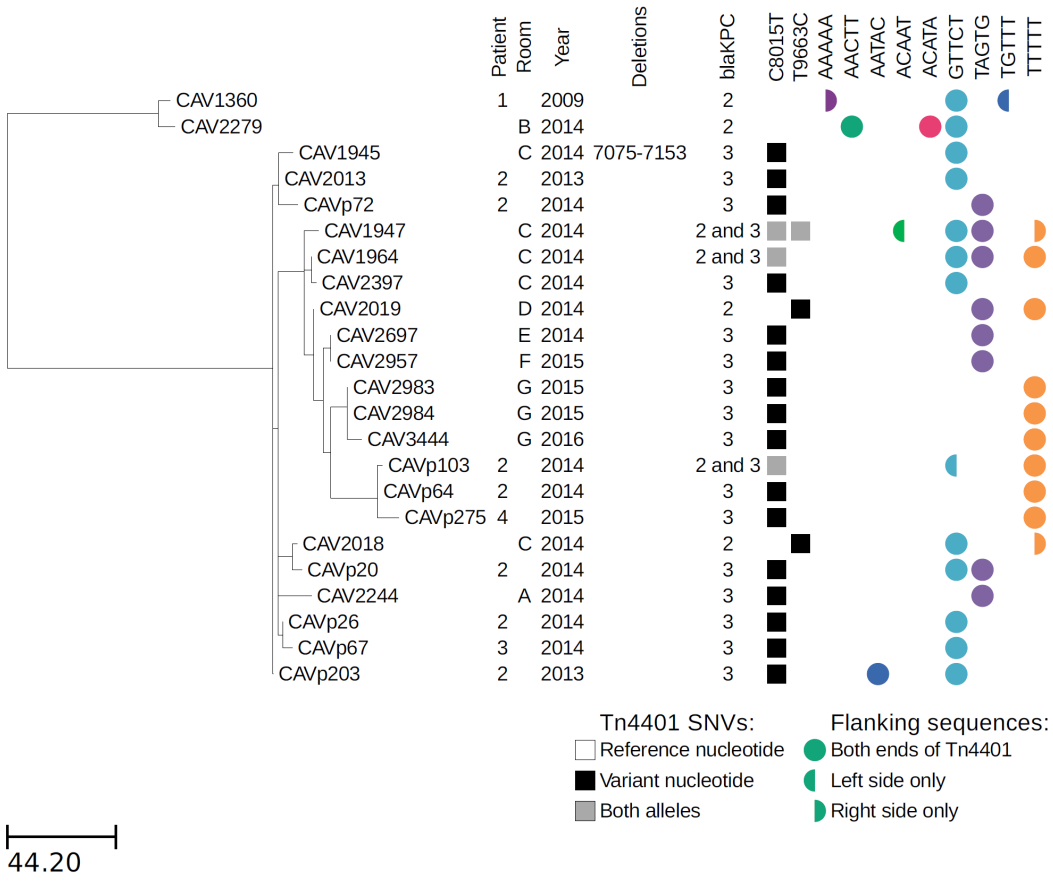

Fig 1b.

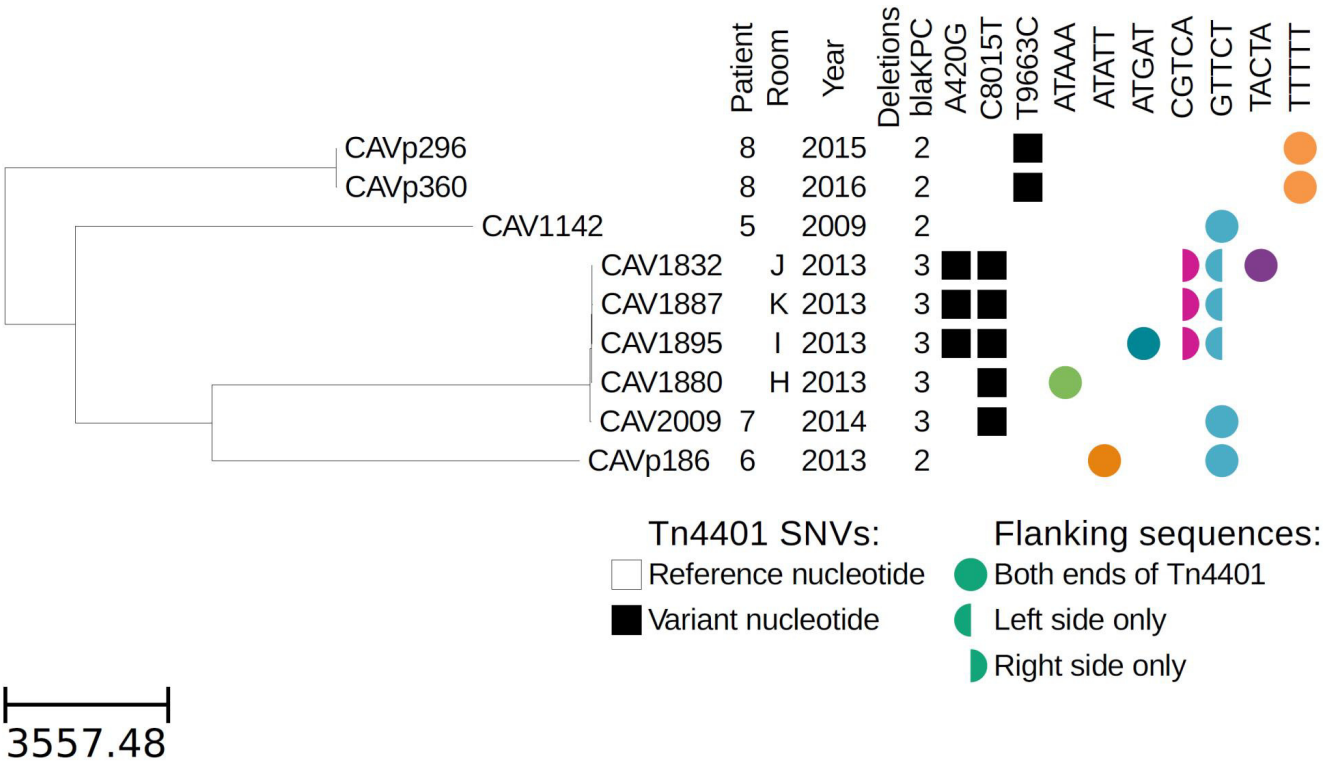

Fig 2.

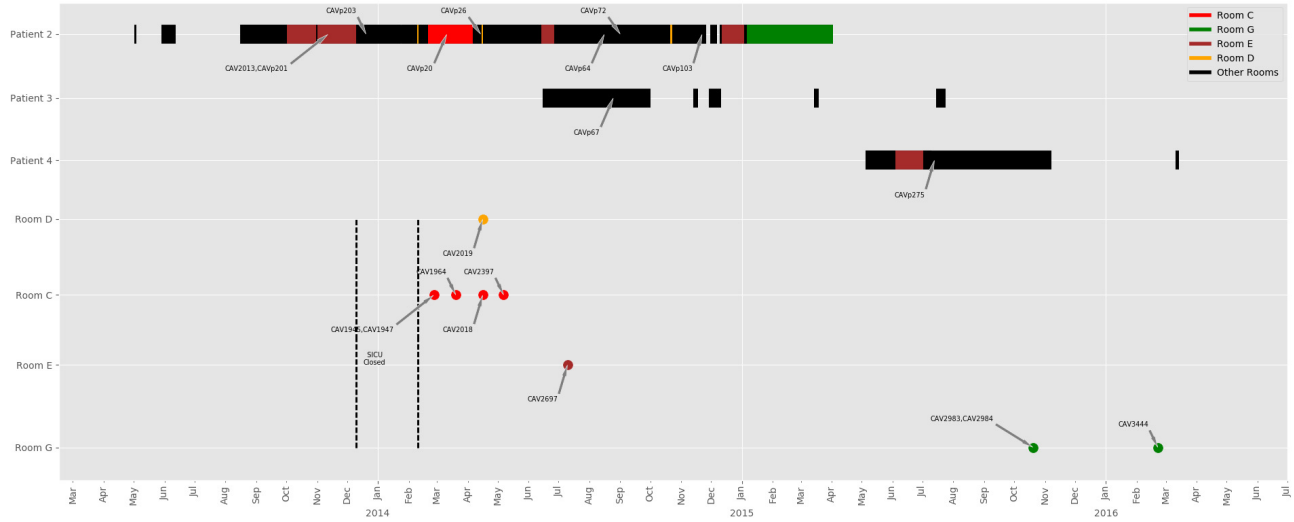

Fig 3.