Klebsiella quasipneumoniae provides a window into carbapenemase gene transfer, plasmid rearrangements and nosocomial acquisition from 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 probable 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.

41 Introduction:

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

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

61 Whole-genome sequencing studies have demonstrated that our understanding of the interplay between
 62 antimicrobial resistance plasmids and their host strains/species is limited(8). The host range of a plasmid is
 63 critical for acquisition and persistence in specific species, but it appears that some bacterial strains are better
 64 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).

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, but did not stay in rooms H or I. 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 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 Tn4401 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 Tn4401 (Fig. 1a). Two sites (positions 8015 and 9663 in the Tn4401b 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 Tn4401 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 Tn4401. On the contrary, there was significant diversity in Tn4401 flanking regions, with eight and seven different 5 bp sequences on the left and right sides of Tn4401 respectively, suggesting active transposition of Tn4401 within KpIIA and/or multiple plasmid acquisitions.

To better understand the origin of the genetic diversity within and surrounding Tn4401, 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 47,095 bp “RepA” *bla*_{KPC-3} plasmid, and the *S. marcescens* isolate had a 69,158 bp IncU/IncX5 *bla*_{KPC-2} plasmid (16). Both plasmids contained Tn440Ib, however there were two SNV differences within the Tn440Ib 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 Tn440I, presumably as a result of intramolecular transposition, 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 Tn440I sequence (Fig. 3h, 4a).

Both isolates also harboured the ancestral RepA *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 Tn440I, such that the CAV2018 RepA 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 plasmid 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 Tn440I 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 Tn440I 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**

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 Tn4401 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 colonised with *bla*_{KPC}-*S. marcescens* carrying Tn4401b with a T9663C mutation and TTTT/TTTT flanking sequences. Four months later, *bla*_{KPC}-KpIIB was identified with the same Tn4401 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 potential reservoir in the transmission of carbapenemase-producing *Enterobacteriaceae* (CPE), but delineating transmission chains is often challenging(17, 18). 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. Understanding the dynamics and natural history of colonization of premise plumbing with CPE will be important in designing effective interventions to limit transmission(19).

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, 20, 21). As seen here, it is not readily distinguished from *K. pneumoniae* with current clinical microbiology techniques and thus the true prevalence is unknown(2, 22). 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, 23). 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. 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). Specifically, there were multiple independent *bla*_{KPC} plasmid acquisitions, homologous recombination between different *bla*_{KPC} plasmids, transposition of Tn4401 into new plasmids, intramolecular transposition of Tn4401, a deletion within Tn4401 and a deletion truncating Tn4401. This high degree of genetic mobility has been similarly observed in other small studies(25, 26), 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 Tn4401 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 Tn4401 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(24).

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(27), 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(28). Screening was performed as previously described(28). 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

Illumina sequencing was performed as described previously(29). PacBio long-read sequencing and assembly were performed as previously described(13).

Broad level species classification was performed using Kraken(30). 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(31) 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 (32). A phylogeny was generated using IQ-TREE v1.3.13 (33) 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 Tn4401 variation and flanking sequences from Illumina data, we used TETyper with published parameters(34).

Plasmid Inc typing was performed using the February 2018 version of the PlasmidFinder Enterobacteriaceae database(16), with an identity threshold of 95% and minimum length 60%.

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280 **Figures**

281 **Fig. 1. Maximum likelihood phylogeny for KpIIA (a) and KpIIB (b) isolates, with Tn4401 variation and**
 282 **flanking genetic contexts.** Branch lengths are shown as SNVs per genome.

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

288 **Fig. 3. Plasmid structures determined from long-read sequencing of four isolates and inferred intermediate**
 289 ***bla*_{KPC} plasmid structures.** a-d. Sequenced isolates. e-j. Inferred intermediate plasmid structures. Note that the
 290 ordering of deletion, homologous recombination, transposition and plasmid loss events is arbitrarily represented as
 291 the actual order of events is unknown. k. Examples of crossover events leading to the generation of new
 292 combinations of SNVs within Tn4401 (top) or the complete swapping of Tn4401 variants between different
 293 plasmids (bottom). Black boxes indicate products of homologous recombination that were observed in long-read
 294 data (top) or Illumina data (bottom).

295 **Fig 4. Alignments of IncU/IncX5 (a), RepA (b) and non-typeable (c) *bla*_{KPC} plasmid structures determined**
 296 **from long-read sequencing.** Tn4401 is indicated by a grey arrow. Light pink shading indicates regions of identity,
 297 light blue shading shows inverted regions, SNVs are indicated by red lines and short indels by blue lines.

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

label	Isolate	Subspecies of <i>K. quasipneumoniae</i>	Date	Source	Infection/outcome
1	CAV1360	<i>KpIIA</i>	Nov-09	Sputum	Ventilator associated pneumonia. in complicated heart transplant recipient/Expired
2	CAV2013	<i>KpIIA</i>	Nov-13	Perirectal surveillance	N/A
2	CAVp203	<i>KpIIA</i>	Dec-13	Bronchoscopy	Ventilator associated pneumonia //Successful treatment
2	CAVp26	<i>KpIIA</i>	Apr-14	Blood	Intraabdominal infection/Expired
2	CAVp20	<i>KpIIA</i>	Mar-14	Perirectal surveillance	N/A
2	CAVp11	<i>KpIIA</i>	Feb-14	Abdominal fluid	Intraabdominal infection//Successful treatment
2	CAVp64	<i>KpIIA</i>	Aug-14	Perirectal surveillance	N/A
2	CAVp72	<i>KpIIA</i>	Sep-14	Perirectal surveillance	N/A
2	CAVp103	<i>KpIIA</i>	Nov-14	Blood	Successful treatment of ...
3	CAVp50	<i>KpIIA</i>	Jul-14	Perirectal surveillance	N/A
3	CAVp57	<i>KpIIA</i>	Jul-14	Perirectal surveillance	N/A
3	CAVp67	<i>KpIIA</i>	Aug-14	Perirectal surveillance	N/A
3	CAVp71	<i>KpIIA</i>	Aug-14	Perirectal surveillance	N/A
3	CAVp104	<i>KpIIA</i>	Dec-14	Perirectal surveillance	N/A
4	CAVp275	<i>KpIIA</i>	Jul-15	Urine	Complicated urinary tract infection/ Successful treatment
5	CAV1142	<i>KpIIB</i>	Aug-09	Perirectal surveillance	N/A

6	CAVp186	<i>KpIIB</i>	Dec-13	Perirectal surveillance	N/A
7	CAV2009	<i>KpIIB</i>	Feb-14	Perirectal surveillance	N/A
8	CAVp296	<i>KpIIB</i>	Oct-15	Perirectal surveillance	N/A
8	CAVp360	<i>KpIIB</i>	Dec-16	Perirectal surveillance	N/A
Room A (MICU)	CAV2244	<i>KpIIA</i>	Jan-14	Shower	
Room B (CTA)	CAV2279	<i>KpIIA</i>	Jan-14	Shower	
Room C (STBICU)	CAV1945	<i>KpIIA</i>	Feb-14	Drain swab (First sample after replacement)	
Room C (STBICU)	CAV1947	<i>KpIIA</i>	Feb-14	p-trap water -(First sample after replacement)	
Room C (STBICU)	CAV1964	<i>KpIIA</i>	Mar-14	Drain swab	
Room C (STBICU)	CAV2018	<i>KpIIA</i>	Apr-14	p-trap water	
Room D (STBICU)	CAV2019	<i>KpIIA</i>	Apr-14	p-trap water	
Room C (STBICU)	CAV2397	<i>KpIIA</i>	May-14	Drain swab	
Room E (STBICU)	CAV2697	<i>KpIIA</i>	Jul-14	Drain swab	
Room F (MICU)	CAV2957	<i>KpIIA</i>	Sep-15	Drain swab	
Room G (STBICU)	CAV2983	<i>KpIIA</i>	Oct-15	p-trap water	
Room G (STBICU)	CAV2984	<i>KpIIA</i>	Oct-15	Drain swab	
Room G (STBICU)	CAV3444	<i>KpIIA</i>	Feb-16	p-trap water	
Room H (MICU)	CAV1880	<i>KpIIB</i>	Dec-13	Drain swab	
Room I (MICU)	CAV1895	<i>KpIIB</i>	Dec-13	Drain swab	
Room J (STBICU)	CAV1832	<i>KpIIB</i>	Dec-13	p-trap water	
Room K (STBICU)	CAV1887	<i>KpIIB</i>	Dec-13	p-trap water	

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

Pat	Isolate	Species	Date	Source	Infection	Genetic	Flank
ien						information	
t						Tn4401	Right/left
						isoform	
2	CAVp202	<i>S. marcescens</i>	Dec-13	Urine		Tn4401b-8	TTTTT/TTTTT
2	CAVp12	<i>S. marcescens</i>	Feb-14	Respiratory		Tn4401b-8	TTTTT/TTTTT
2	CAV1761*	<i>S. marcescens</i>	Mar-14	Perirectal surveillance		Tn4401b-8	TTTTT/TTTTT
6	CAV1750	<i>Klebsiella pneumoniae</i>	Dec-12	Perirectal surveillance	N/A	Tn4401b-1	GTTCT/GTTCT
6	CAVp127	<i>Klebsiella pneumoniae</i>	Feb-13	Perirectal surveillance	N/A	Tn4401b-1	GTTCT/GTTCT
6	CAVp130	<i>Klebsiella pneumoniae</i>	Mar-13	Urine	Yes	Tn4401b-1	GTTCT/GTTCT
6	CAVp139	<i>Klebsiella pneumoniae</i>	Apr-13	Perirectal surveillance	N/A	Tn4401b-1	GTTCT/GTTCT
6	CAVp151	<i>Klebsiella pneumoniae</i>	Jul-13	Perirectal surveillance	N/A	Tn4401b-1	GTTCT/GTTCT
6	CAVp152	<i>Klebsiella pneumoniae</i>	Jul-13	Perirectal surveillance	N/A	Tn4401b-1	GTTCT/GTTCT
6	CAVp177	<i>Klebsiella pneumoniae</i>	Sep-13	Perirectal surveillance	N/A	Tn4401b-1	GTTCT/GTTCT
6	CAVp180	<i>Klebsiella pneumoniae</i>	Nov-13	Perirectal surveillance	N/A	Tn4401b-1	GTTCT TACCT/AGCAT GTTCT
6	CAVp183	<i>Klebsiella pneumoniae</i>	Nov-13	Intraabdominal abscess	Yes	Tn4401b-1	GTTCT/GTTCT
6	CAVp184	<i>Klebsiella pneumoniae</i>	Nov-13	Perirectal surveillance	N/A	Tn4401b-1	GTTCT/GTTCT
6	CAVp185	<i>Klebsiella pneumoniae</i>	Nov-13	Perirectal surveillance	N/A	Tn4401b-1	ATATT GTTCT/ATATT GTTCT
6	CAVp3	<i>Klebsiella pneumoniae</i>	Jan-14	Biliary drain	Yes	Tn4401b-1	GTTCT/GTTCT
8	CAVp269	<i>Serratia marcescens</i>	Jun-15	Blood	Yes	Tn4401b-8	TTTTT/TTTTT

8	CAVp270	<i>Serratia marcescens</i>	Jun-15	Perirectal surveillance	N/A	Tn440Ib-8	TTTTT/TTTTT
8	CAVp361	<i>Escherichia coli</i>	Dec-16	Perirectal surveillance	N/A	Tn440Ib-8	TTTTT/TTTTT
8	CAVp374	<i>Citrobacter freundii</i>	Mar-17	Perirectal surveillance	N/A	Tn440Ib-8	TTTTT/TTTTT

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- 308 1. Holt KE, Wertheim H, Zadoks RN, Baker S, Whitehouse CA, Dance D, Jenney A, Connor TR, Hsu LY,
309 Severin J, Brisse S, Cao H, Wilksch J, Gorrie C, Schultz MB, Edwards DJ, Nguyen KV, Nguyen TV, Dao
310 TT, Mensink M, Minh VL, Nhu NT, Schultsz C, Kuntaman K, Newton PN, Moore CE, Strugnell RA,
311 Thomson NR. 2015. Genomic analysis of diversity, population structure, virulence, and antimicrobial
312 resistance in *Klebsiella pneumoniae*, an urgent threat to public health. *Proc Natl Acad Sci U S A*
313 112:E3574-81.
- 314 2. Long SW, Linson SE, Ojeda Saavedra M, Cantu C, Davis JJ, Brettin T, Olsen RJ. 2017. Whole-Genome
315 Sequencing of Human Clinical *Klebsiella pneumoniae* Isolates Reveals Misidentification and
316 Misunderstandings of *Klebsiella pneumoniae*, *Klebsiella variicola*, and *Klebsiella quasipneumoniae*.
317 *mSphere* 2.
- 318 3. Brisse S, Passet V, Grimont PA. 2014. Description of *Klebsiella quasipneumoniae* sp. nov., isolated from
319 human infections, with two subspecies, *Klebsiella quasipneumoniae* subsp. *quasipneumoniae* subsp. nov.
320 and *Klebsiella quasipneumoniae* subsp. *similipneumoniae* subsp. nov., and demonstration that *Klebsiella*
321 *singaporensis* is a junior heterotypic synonym of *Klebsiella variicola*. *Int J Syst Evol Microbiol* 64:3146-
322 52.
- 323 4. Becker L, Fuchs S, Pfeifer Y, Semmler T, Eckmanns T, Korr G, Sissolak D, Friedrichs M, Zill E, Tung
324 ML, Dohle C, Kaase M, Gatermann S, Russmann H, Steglich M, Haller S, Werner G. 2018. Whole

Genome Sequence Analysis of CTX-M-15 Producing *Klebsiella* Isolates Allowed Dissecting a Polyclonal Outbreak Scenario. *Front Microbiol* 9:322.

5. Nicolas MF, Ramos PIP, Marques de Carvalho F, Camargo DRA, de Fatima Morais Alves C, Loss de Morais G, Almeida LGP, Souza RC, Ciapina LP, Vicente ACP, Coimbra RS, Ribeiro de Vasconcelos AT. 2018. Comparative Genomic Analysis of a Clinical Isolate of *Klebsiella quasipneumoniae* subsp. *similipneumoniae*, a KPC-2 and OKP-B-6 Beta-Lactamases Producer Harboring Two Drug-Resistance Plasmids from Southeast Brazil. *Front Microbiol* 9:220.
6. Hocquet D, Muller A, Bertrand X. 2016. What happens in hospitals does not stay in hospitals: antibiotic-resistant bacteria in hospital wastewater systems. *J Hosp Infect* 93:395-402.
7. Weingarten RA, Johnson RC, Conlan S, Ramsburg AM, Dekker JP, Lau AF, Khil P, Odom RT, Deming C, Park M, Thomas PJ, Henderson DK, Palmore TN, Segre JA, Frank KM. 2018. Genomic Analysis of Hospital Plumbing Reveals Diverse Reservoir of Bacterial Plasmids Conferring Carbapenem Resistance. *MBio* 9.
8. Suzuki H, Yano H, Brown CJ, Top EM. 2010. Predicting plasmid promiscuity based on genomic signature. *J Bacteriol* 192:6045-55.
9. Price VJ, Huo W, Sharifi A, Palmer KL. 2016. CRISPR-Cas and Restriction-Modification Act Additively against Conjugative Antibiotic Resistance Plasmid Transfer in *Enterococcus faecalis*. *mSphere* 1.
10. Loftie-Eaton W, Yano H, Burleigh S, Simmons RS, Hughes JM, Rogers LM, Hunter SS, Settles ML, Forney LJ, Ponciano JM, Top EM. 2016. Evolutionary Paths That Expand Plasmid Host-Range: Implications for Spread of Antibiotic Resistance. *Mol Biol Evol* 33:885-97.
11. Loftie-Eaton W, Bashford K, Quinn H, Dong K, Millstein J, Hunter S, Thomason MK, Merrikh H, Ponciano JM, Top EM. 2017. Compensatory mutations improve general permissiveness to antibiotic resistance plasmids. *Nat Ecol Evol* 1:1354-1363.
12. Hardiman CA, Weingarten RA, Conlan S, Khil P, Dekker JP, Mathers AJ, Sheppard AE, Segre JA, Frank KM. 2016. Horizontal Transfer of Carbapenemase-Encoding Plasmids and Comparison with Hospital Epidemiology Data. *Antimicrob Agents Chemother* 60:4910-9.

- 351 13. Sheppard AE, Stoesser N, Wilson DJ, Sebra R, Kasarskis A, Anson LW, Giess A, Pankhurst LJ, Vaughan
352 A, Grim CJ, Cox HL, Yeh AJ, Sifri CD, Walker AS, Peto TE, Crook DW, Mathers AJ, Group MMMMI.
353 2016. Nested Russian Doll-like Genetic Mobility Drives Rapid Dissemination of the Carbapenem
354 Resistance Gene blaKPC. Antimicrob Agents Chemother.
- 355 14. Mathers AJ, Vegesana K, German Mesner I, Barry KE, Pannone A, Baumann J, Crook DW, Stoesser N,
356 Kotay S, Carroll J, Sifri CD. 2018. Intensive Care Unit Wastewater Interventions to Prevent Transmission
357 of Multi-species *Klebsiella pneumoniae* Carbapenemase (KPC) Producing Organisms. Clin Infect Dis.
- 358 15. Mathers AJ, Stoesser N, Chai W, Carroll J, Barry K, Cherunvanky A, Sebra R, Kasarskis A, Peto TE,
359 Walker AS, Sifri CD, Crook DW, Sheppard AE. 2017. Chromosomal Integration of the *Klebsiella*
360 *pneumoniae* Carbapenemase Gene, blaKPC, in *Klebsiella* Species Is Elusive but Not Rare. Antimicrob
361 Agents Chemother 61.
- 362 16. Carattoli A, Zankari E, Garcia-Fernandez A, Voldby Larsen M, Lund O, Villa L, Moller Aarestrup F,
363 Hasman H. 2014. In silico detection and typing of plasmids using PlasmidFinder and plasmid multilocus
364 sequence typing. Antimicrob Agents Chemother 58:3895-903.
- 365 17. Kotsanas D, Wijesooriya WR, Korman TM, Gillespie EE, Wright L, Snook K, Williams N, Bell JM, Li
366 HY, Stuart RL. 2013. "Down the drain": carbapenem-resistant bacteria in intensive care unit patients and
367 handwashing sinks. Med J Aust 198:267-9.
- 368 18. De Geyter D, Blommaert L, Verbraeken N, Sevenois M, Huyghens L, Martini H, Covens L, Piérard D,
369 Wybo I. 2017. The sink as a potential source of transmission of carbapenemase-producing
370 Enterobacteriaceae in the intensive care unit. Antimicrob Resist Infect Control 6:24.
- 371 19. Hopman J, Tostmann A, Wertheim H, Bos M, Kolwijck E, Akkermans R, Sturm P, Voss A, Pickkers P,
372 Vd Hoeven H. 2017. Reduced rate of intensive care unit acquired gram-negative bacilli after removal of
373 sinks and introduction of 'water-free' patient care. Antimicrob Resist Infect Control 6:59.
- 374 20. Gan HM, Rajasekaram G, Eng WWH, Kaniappan P, Dhanoa A. 2017. Whole-Genome Sequences of Two
375 Carbapenem-Resistant *Klebsiella quasipneumoniae* Strains Isolated from a Tertiary Hospital in Johor,
376 Malaysia. Genome Announc 5.

- 377 21. Shankar C, Nabarro LEB, Muthuirulandi Sethuvel DP, Raj A, Devanga Ragupathi NK, Doss GP,
378 Veeraraghavan B. 2017. Draft genome of a hypervirulent *Klebsiella quasipneumoniae* subsp.
379 *similipneumoniae* with novel sequence type ST2320 isolated from a chronic liver disease patient. *J Glob*
380 *Antimicrob Resist* 9:30-31.
- 381 22. Martinez-Romero E, Rodriguez-Medina N, Beltran-Rojel M, Silva-Sanchez J, Barrios-Camacho H,
382 Perez-Rueda E, Garza-Ramos U. 2018. Genome misclassification of *Klebsiella variicola* and *Klebsiella*
383 *quasipneumoniae* isolated from plants, animals and humans. *Salud Publica Mex* 60:56-62.
- 384 23. Gomi R, Matsuda T, Yamamoto M, Chou PH, Tanaka M, Ichiyama S, Yoneda M, Matsumura Y. 2018.
385 Characteristics of Carbapenemase-Producing Enterobacteriaceae in Wastewater Revealed by Genomic
386 Analysis. *Antimicrob Agents Chemother*.
- 387 24. Mathers AJ, Peirano G, Pitout JD. 2015. The Role of Epidemic Resistance Plasmids and International
388 High-Risk Clones in the Spread of Multidrug-Resistant Enterobacteriaceae. *Clin Microbiol Rev* 28:565-
389 591.
- 390 25. Wailan AM, Sartor AL, Zowawi HM, Perry JD, Paterson DL, Sidjabat HE. 2015. Genetic Contexts of
391 blaNDM-1 in Patients Carrying Multiple NDM-Producing Strains. *Antimicrob Agents Chemother*
392 59:7405-10.
- 393 26. Snesrud E, Ong AC, Corey B, Kwak YI, Clifford R, Gleeson T, Wood S, Whitman TJ, Lesho EP, Hinkle
394 M, McGann P. 2017. Analysis of Serial Isolates of mcr-1-Positive *Escherichia coli* Reveals a Highly
395 Active ISApl1 Transposon. *Antimicrob Agents Chemother* 61.
- 396 27. Grabowski ME, Kang H, Wells KM, Sifri CD, Mathers AJ, Lobo JM. 2017. Provider Role in
397 Transmission of Carbapenem-Resistant Enterobacteriaceae. *Infect Control Hosp Epidemiol* 38:1329-
398 1334.
- 399 28. Mathers AJ, Poulter M, Dirks D, Carroll J, Sifri CD, Hazen KC. 2014. Clinical Microbiology Costs for
400 Methods of Active Surveillance for *Klebsiella pneumoniae* Carbapenemase-Producing
401 Enterobacteriaceae. *Infect Control Hosp Epidemiol* 35:350-5.

- Mathers AJ, Stoesser N, Sheppard AE, Pankhurst L, Giess A, Yeh AJ, Didelot X, Turner SD, Sebra R, Kasarskis A, Peto T, Crook D, Sifri CD. 2015. *Klebsiella pneumoniae* carbapenemase (KPC)-producing *K. pneumoniae* at a single institution: insights into endemicity from whole-genome sequencing. Antimicrob Agents Chemother 59:1656-63.
- Wood DE, Salzberg SL. 2014. Kraken: ultrafast metagenomic sequence classification using exact alignments. Genome Biol 15:R46.
- Ondov BD, Treangen TJ, Melsted P, Mallonee AB, Bergman NH, Koren S, Phillippy AM. 2016. Mash: fast genome and metagenome distance estimation using MinHash. Genome Biol 17:132.
- Stoesser N, Sheppard AE, Peirano G, Anson LW, Pankhurst L, Sebra R, Phan HTT, Kasarskis A, Mathers AJ, Peto TEA, Bradford P, Motyl MR, Walker AS, Crook DW, Pitout JD. 2017. Genomic epidemiology of global *Klebsiella pneumoniae* carbapenemase (KPC)-producing *Escherichia coli*. Sci Rep 7:5917.
- Nguyen LT, Schmidt HA, von Haeseler A, Minh BQ. 2015. IQ-TREE: a fast and effective stochastic algorithm for estimating maximum-likelihood phylogenies. Mol Biol Evol 32:268-74.
- Sheppard AE SN, German-Mesner I , Vegesana K , Walker AS , Crook DW. 2018. TETyper: a bioinformatic pipeline for classifying variation and genetic contexts of transposable elements from short-read whole-genome sequencing data, vol 288001. Cold Spring Harbor, bioRxiv (Accepted in Microbial Genomics).

Tn4401 SNVs:

- Reference nucleotide
- Variant nucleotide
- Both alleles

Flanking sequences:

- Both ends of Tn4401
- Left side only
- Right side only

50.98

Tn4401 SNVs:

□ Reference nucleotide

■ Variant nucleotide

Flanking sequences:

● Both ends of Tn4401

◐ Left side only

◑ Right side only

7417.28

CAV2018

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30000

40000

50000

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CAV1761

CAV1947

CAV2013

CAV1947