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Whole Genome Sequencing detects Inter-Facility Transmission of Carbapenem-resistant *Klebsiella pneumoniae*

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SUMMARY

Objectives: To identify transmission patterns of Carbapenem-resistant *Klebsiella pneumoniae* infection during an outbreak at a large, tertiary care hospital and to detect whether the outbreak organisms spread to other facilities in the integrated healthcare network.

Methods: We analyzed 71 *K. pneumoniae* whole genome sequences collected from clinical specimens before, during and after the outbreak and reviewed corresponding patient medical records. Sequence and patient data were used to model probable transmissions and assess factors associated with the outbreak.

Results: We identified close genetic relationships among carbapenem-resistant *K. pneumoniae* isolates sampled during the study period. Transmission tree analysis combined with patient records uncovered extended periods of silent colonization in many study patients and transmission routes that were likely the result of asymptomatic patients transitioning between facilities.

Conclusions: Detecting how and where Carbapenem-resistant *K. pneumoniae* infections spread is challenging in an environment of rising prevalence, asymptomatic carriage and mobility of patients. Whole genome sequencing improved the precision of investigating inter-facility

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Conflicts of interest
None.

transmissions. Our results emphasize that containment of Carbapenem-resistant *K. pneumoniae* infections requires coordinated efforts between healthcare networks and settings of care that acknowledge and mitigate transmission risk conferred by undetected carriage and by patient transfers between facilities.

Keywords

Klebsiella pneumoniae; Epidemiology; Carbapenem; Whole genome sequencing; transmission; Healthcare; Infection control

Introduction

Carbapenem Resistant *Enterobacteriaceae* (CRE) infections have become endemic in more than 20% of U.S. hospitals.[1,2] CRE are resistant to most antibiotics, resulting in few effective treatment options and mortality rates up to 50% for bloodstream and deep seated infections.[3–5] Resistance to colistin, one of the treatments of last resort, has been identified in CRE organisms cultured from hospital infections, exacerbating the threat to patients and the public.[6–8]

The natural history of CRE infection challenges standard infection prevention and control efforts. Studies have shown persistence of CRE in asymptomatic carriers for long periods,[9] with one study reporting a mean time to negative culture of 387 days.[10] Undetected, endemic CRE colonization further complicates infection management in hospitals and other facilities.[11] Carriage has been reported as high as 30% in long-term care facilities[12,13] where extended use of antibiotics is high.[14] CRE infections are a particular risk for the sickest, most vulnerable patients who have frequent, lengthy hospitalizations that increase the chance of exposure. Long periods of asymptomatic colonization and frequent patient transfers between facilities[15] make it difficult to pinpoint transmissions and establish effective infection control measures. Infection outbreaks, poor patient outcomes and repeated readmissions are the result.[16]

Recent studies have demonstrated that Whole Genome Sequencing (WGS) can provide a high-resolution tool to identify infection sources and augment efforts to prevent and eradicate infections.[17–20] A pivotal 2012 study was among the first to combine analysis of CRE genomes with epidemiological data to infer probable transmission routes between patients, which could not be determined from contact tracing alone.[21] This work suggested that real-time WGS can play an important role in prevention and management of CRE infections. Further CRE WGS studies extended these methods to demonstrate how CRE antimicrobial resistance mechanisms can inform genomic inference of transmission.[18,22,23]

Between 2010 and 2013, Atrium Health, one of the largest public healthcare systems in the United States, experienced increasing numbers of CRE infections in acute care facilities, consistent with rising carbapenem resistance worldwide.[24] *Klebsiella pneumoniae* (KP) was identified as the species causing the majority of infections, and internal analysis showed steady escalation in KP drug resistance over a period of years. When an outbreak occurred at one hospital, simultaneous infections at other facilities raised concerns that Carbapenem Resistant *Klebsiella pneumoniae* (CRKP) had been transmitted between facilities. An initial

epidemiologic investigation was inconclusive, leading Atrium Health to initiate a CRE WGS study to investigate the outbreak and assess the likelihood of inter-facility spread. Eighty CRE isolates from clinical specimens were selected for sequencing. Transmissions investigation combined phylogenetic analysis with detailed patient healthcare histories and epidemiological data. These efforts improved the resolution to detect probable inter-facility transmissions, to recognize long-term asymptomatic colonization as an undetected contributor to the outbreak and to distinguish outbreak isolates from other unrelated CRKP infections that occurred during the same time period.

Materials and methods

Study setting

This study was conducted as part of an infection prevention program across the Atrium Health primary enterprise network and included acute care, rehabilitation and outpatient facilities in North Carolina. The use of all clinical isolates and patient data were governed by research protocols #08–13-05E, #04–14-13E and 03–17-08E, approved by the Atrium Health Institutional Review Board and by the Atrium Health Data Governance Committee.

Sample selection

Carbapenem non-susceptible *Enterobacteriaceae* sp. isolates collected from patients with confirmed infections were routinely frozen and stored by the clinical microbiology laboratories at Atrium Health. Eighty isolates were chosen from these frozen stocks to investigate and characterize a CRKP outbreak identified by Infectious Disease specialists and epidemiologists. We selected isolates without regard to CLSI breakpoints to avoid any deliberate selection bias. Isolate 21 was the only isolate where we could not identify clear carbapenem resistance mechanisms, even though it exhibited phenotypic carbapenem resistance. Timing and location of isolate specimen collection were primary criteria for inclusion. Selection prioritized CRKP isolates obtained during and immediately after the outbreak, as well as those collected >100 days before the outbreak began ($N = 25$). Study isolates were also selected to include clinical specimens collected across Atrium Health facilities where CRE infections had occurred. Of the 80 isolates, 76 were successfully sequenced. Five organisms that were not *Klebsiella* sp. were removed from the study, leaving 71 isolates for analysis. No active CRE surveillance was ongoing during the study period.

Sample phenotype assessment and preparation

All isolates were previously tested for antibiotic susceptibility during routine clinical care and, specifically, for ertapenem, meropenem, and imipenem, according to FDA breakpoints on automated instruments (MicroScan). Manual e-tests were conducted to evaluate susceptibility based on CLSI breakpoints (M100), which changed over the course of the study.[25] Mueller Hinton medium was used in susceptibility testing. Any isolate that showed resistance to one or more carbapenems and had a positive Modified Hodge test (MHT) was classified as CRE and the resistance profile was documented as resistant to all carbapenems, regardless of automated Minimum Inhibitory Concentrations (MIC) results. Per laboratory

protocol, confirmed CRE isolates were labeled, frozen on dry ice and immediately transferred to −80° freezer.

All isolates selected for the study demonstrated confirmed carbapenem resistance and MIC ≥1 as determined by methods described. Stored, frozen isolates were quick-thawed on ice and streaked for isolation onto a Blood agar plate and a MacConkey agar plate. Four to five colonies of similar morphology were selected and placed on a t-soy agar slant, then shipped on dry ice to The Broad Institute for DNA extraction and sequencing.

DNA extraction

Bacterial DNA was extracted using BioRobot EZ1 following the EZ1 DNA Tissue Kit protocol with EZ1 DNA Bacterial Card specifications or using QiaSymphony AS (Qiagen) as previously described.[23]

Library preparation, genome sequencing and assembly

Illumina sequencing libraries were prepared as previously described in Cerqueira *et al.*, using Illumina HiSeq2000 platform with 101 bp, paired-end sequences to provide 100–200-fold genome coverage.[23,26] Sequence reads were assembled using ALLPATHSLG[27] with Pilon[28] for base call correction, BWA read alignment[29] and manual assembly correction.[23] Post assembly scaffolding was completed using SSPACE[30] with Pilon for additional assembly improvement for paired end reads, as previously described.[23] All sequence reads and assemblies were submitted to the Sequence Read Archive and Genbank, respectively, at the National Center for Biotechnology Information (Bioproject “Carbapenem Resistance”, accession PRJNA202876[31]; Supplemental Table S1 for individual genome sequencing and assembly accession numbers). A subset of study organisms was deposited at BEI Resources (www.beiresources.org; Supplemental Table S2).

Sequence analysis methods

Genome annotations were performed using the Broad Institute’s prokaryotic annotation pipeline.[32] Assignment of sequence type (ST) was made by BLAST alignment of all assembly contigs to a curated multilocus sequence typing (MLST) database (across 96 species obtained from http://pubmlst.org and http://pubmlst.org/mlst/) and identifying the combination of the best matching MLST allele from all MLST schemes.[33] In order to construct a core genome alignment, orthologous genes were identified in all 76 genomes using Synergy2 (https://sourceforge.net/projects/synergytwo/). Orthogroups contain orthologs, which are vertically inherited genes that likely have the same function, and also possibly paralogs, which are duplicated genes that may have different function. There were 2,342 single copy core orthogroups across our set of 76 strains. Sequences in each core orthogroup were aligned using MUSCLE.[34] These alignments were concatenated, and a single copy core phylogenetic tree was generated using RAxML.[35] Pairwise SNP distances from the concatenated core alignment were calculated as the number of non-identical characters between each pair of core genomes.

Generation of transmission trees

The SeqTrack algorithm was used to construct probable transmission pathways based on isolate genomic relatedness and dates of specimen collection.[36] A SNP distance cut-off was used to identify isolates likely to be related to the outbreak and to remove novel organism introductions that were inconsistent with Seq-Track's outbreak structure. For SeqTrack analysis, we empirically determined that an 11 SNP threshold (Supplemental Fig. S1) offered a reasonable trade-off between tree inclusiveness and exclusiveness, which was in line with other studies.[21,37–43] This cut-off was also consistent with previous literature for transmission analysis for other organisms.[43,44]

Detection and annotation of antibiotic resistance genes

Nucleotide BLAST (ncbi-blast-2.2.29 +)[45] was used to compare the gene predictions from the assemblies to the Comprehensive Antibiotic Resistance Database (CARD) protein homolog database, version 1.0.4.[46] A hit was defined as any BLAST result that aligned > 90% of the length of the CARD gene with > 80% identity. Resistance genes present in > 75% of the genomes are shown in this paper. The variant reported for each gene is the BLAST result with the highest bit score. MAFFT[47] was used to align the nucleotide sequences of genes identified within gene families. To confirm that no genes were missing from the gene predictions, a nucleotide BLAST[45] of the scaffolds was also performed and produced similar results. The scaffold results were not used further to identify plasmids due to limitations of assemblies created using short Illumina sequences.[48,49] Further confirmation of the absence of *bla*$_{KPC}$ from isolates 21 and 66 was performed by using BWA[29] to align the reads to KPC-2 from the CARD database. We examined other resistance mechanisms[23] and searched for disruptions in porin genes and their upstream promoter regions using ISfinder (https://www-is.biotoul.fr/index.php).

Identification of virulence factors

Each isolate genome was compared, using BLAST, against a downloaded copy of the Virulence Factor Database (VFDB)[50] "core" dataset, which contains genes associated with experimentally-verified virulence factors. Hits of length >100 with e-value <1e−10 were retained and tabulated.

Patient data and analysis

Patients associated with the 71 CRKP isolates defined the cohort for retrospective review of medical record data over the study period. Patient history prior to the specimen collection date of each isolate was included to inform epidemiological analysis. Medical record data included patient and isolate characteristics, details of patient healthcare encounters, clinical and surveillance cultures, clinical laboratory data, patient demographics and other patient health information. Numbers of healthcare encounter days were defined by identifying each day on which a patient had an encounter at any Atrium Health facility. For any day on which a patient had encounters at more than one facility, each was counted separately. These data were accessed through the Atrium Health Enterprise Data Warehouse (EDW). SunQuest® laboratory data and results from genomic sequencing were integrated with EDW data to identify all CRKP positive cultures associated with each patient during the study period,

whether or not the isolates were sequenced. Patient records were quality checked, de-identified and assigned a patient-specific numeric identifier. Isolates were labeled to correspond to patient record identifiers. In the case of four patients with two samples each, isolates retained the patient numeric identifier plus an “a” or “b” to distinguish between the isolates from the same patient.

Analysis methods

Data for historical analysis of antibiotic resistance was collected from clinical laboratory records. The percentage of *K. pneumoniae* organisms that were resistant to each selected antibiotic was calculated using a denominator based on the total number of *K. pneumoniae* cultures from laboratory records for that year. Two-way hierarchical clustering was conducted using Ward’s method for analysis of antibiotic resistance by year. Virulence patterns were compared between sequence types using multivariate methods. For subgroup analyses related to the outbreak, we defined an outbreak cohort which included only those isolates with collection dates within a three-month time period surrounding the outbreak (study days 2019–2118). The Wilcoxon Rank Sum test was used for pairwise comparisons of SNP distance data that were not normally distributed. Bivariate linear fit models were used to test associations between continuous variables and logistic models or Student’s *T*-tests for relationships between numeric and categorical variables that were normally distributed. All statistical analyses were conducted using JMP® 12.1.0 for Microsoft Windows (Copyright © 2015 SAS Institute Inc.).

Results

Increasing antibiotic resistance across an integrated healthcare network

Between 2010 and 2013, clinical testing identified increasing CRKP cultures in Atrium Health acute care facilities. Laboratory data for *K. pneumoniae* isolates showed growing antibiotic resistance with increasing carbapenem-resistance between 2011 and 2013 (Fig. 1A). A 2-month spike in CRKP infection rates at Hospital 2 (Fig. 1B) was identified as an outbreak. Examination of medical records uncovered increased incidence of CRKP clinical cultures in other hospitals and outpatient sites during the same time period, which raised concerns about inter-facility transmission and led to an investigation of transmissions using WGS.

Clinical characteristics of sequenced CRKP isolates

Seventy-one CRKP organisms were among the 76 clinical specimens successfully sequenced. These isolates were collected from 67 unique patients across 9 Atrium Health facilities. Hospital 2 provided the majority of CRKP isolates with others collected from different facilities (Table 1, Supplemental Table S3). Most isolates came from urine specimens, which is typical for CRKP in healthcare settings,[51] and the remainder were collected from other body sites. More than half of study patients had additional un-sequenced CRKP + cultures collected during the study period. A subset of these were mixed cultures ($N = 67$) with CRKP as the dominant organism.

Patient attributes and epidemiology

Health histories of cohort patients (Table 2, Supplemental Table S4) identified significant exposure to inpatient and outpatient Atrium Health facilities and wide variation between patients (mean hospital days and visits: 204 [SD 264], 18 [SD 17]; mean rehab days and visits: 56 [SD 151], 1 [SD 2]; mean outpatient days = 18 [SD 42]. Many patients had hospital and rehab healthcare visits at more than one facility (patients = 61, mean facilities/patient 3.27 [SD 1.41]). Patient medical histories were plotted to show the study date for all healthcare visits and CRKP + cultures in order of collection date (Fig. 2). To understand patients' health burdens, Charlson Comorbidity Indices (CCI)[52] were calculated on the day prior to collection of the sequenced CRKP isolate.[53] Results showed 45 patients with CCI ≥ 6, indicating serious health conditions with high risk of one-year mortality and need for extended acute care. CCI was positively associated with healthcare days ($P = 0.015$) and patient death during the study ($P = 0.004$) (data not shown). Most patients had more than one CRKP + culture (Table 2). Of these, 19 had CRKP + cultures from different facilities, and 11 were collected over more than a year, suggesting long-term CRKP colonization (Supplemental Table S4 and Supplemental Fig. S2).

Genomic analysis identifies closely-related isolates

SNP distances between CRKP core genomes demonstrated that many organisms were closely related (Table 1, Supplemental Table S5) (median distance 24 SNPs; range 0–13,900 SNPs), and 28 isolate pairs had identical core genomes. Most isolates ($N = 66$) were ST258 (Table 1, Supplemental Table S3). Phylogenetic analysis (Fig. 3) was consistent with SNP distance and strain assignment. Among 4 patients (8, 26, 38 and 47) who had 2 different isolates sequenced, the core genomes of 3 isolate pairs were nearly identical (47a-b: 0 SNPs; 26a-b 1 SNP; 38a-b: 1 SNP) despite several days difference between cultures. Isolates 8a-b were distinct strains (ST258 and ST25; 12,804 SNPs) collected 174 days apart. For isolate pairs where SNP distances were 11 or fewer SNPS (19.3%), a comparison of pairwise SNP distances and days between cultures demonstrated strong positive correlation (Fig. 4A; $p < 0.0001$).

Transmission analysis identifies outbreak characteristics and probable inter-facility spread

A primary research objective was to evaluate outbreak transmission patterns to investigate transmission between facilities. Using only isolates collected during the outbreak period that were fewer than 800 SNPs from any other isolate, we analyzed paired SNP distances between two groups of isolates, those collected from the same facility versus from different facilities. Results showed same facility SNP distances were smaller than different facility distances (Fig. 4B; $P < 0.0001$), suggesting limited direct transmission between facilities, even though 242 different facility pairs were less than 11 SNPs apart. We then compared SNP distances between pairs of isolates only at Hospital 2 versus pairs within the same facilities that were not Hospital 2. The pairs collected within Hospital 2 were more closely related than those obtained from other facilities (Fig. 4C; $P < 0.0001$), indicating the outbreak was concentrated in Hospital 2.[23]

To investigate transmission routes, we used SeqTrack[36] to combine CRKP SNP distances from the core genome alignment with isolate culture dates to generate a probable

transmission tree (Fig. 5). The resulting tree confirmed that primary outbreak intensity was centered in Hospital 2, with isolates 38a and 39 as likely donors. Information on unsequenced CRKP + cultures, which were added to the tree, uncovered patients with possible long-term, latent CRKP carriage that obscured donor and recipient roles in transmission. Examples include outbreak patients (30, 32, 26, 42, 38, and 75) who had several CRKP + cultures collected prior to the sequenced isolate.

Within the Hospital 2 outbreak, 4 sequenced isolates were collected in facilities other than Hospital 2 (38b, 62, 71 and 42). Three had core genome distances of 0–1 SNPs, indicative of direct transmission. Examination of medical records provided clues for how these transmissions could have occurred.

- Isolate 38b and isolate 38a were collected from the same patient 16 days apart at Hospital 2 and Rehab.
- Patient 38 (isolate 38b) had a CRKP + culture in Hospital 2 prior to an overlapping Rehab visit with patient 62, identifying a possible Rehab transmission route between Hospital 2 and Rehab.
- Patient 71 had a Hospital 2 visit over study days 2069–2081, which overlapped with visits of patients 25, 32, 34, 38, 39, and 72 and occurred before patient 71's Outpatient visit, where the sequenced isolate was collected on study day 2110.
- Although isolate 42 was collected within the outbreak period, there were no overlapping Hospital 2 or Rehab visits with patients in the outbreak, consistent with this isolate's more distant (8 SNPs) relationship. It is noteworthy that patient 42 had 3 previous, un-sequenced CRKP + cultures. The earliest was collected on study day 697, suggesting long-term colonization that obscured accurate assessment of transmission.

The SeqTrack tree identified two other isolate clusters that were closely related and collected from different facilities (Fig. 5). One group of isolates (50, 60, 61 and 63) were from Hospital 5, Rehab and Outpatient facilities (Fig. 6A). Overlapping visits between patients 63 and 61 at Rehab, 63 and 60 at Hospital 1 and 61 and 50 at Rehab provided opportunities for transmissions across facilities. In addition, patient 63 had earlier overlapping visits at Hospital 2 with patient 30, who had a long history of CRKP + cultures. The second cluster (57, 58, 59 and 65) included overlapping visits to the same rural Skilled Nursing Facility for patients 58 and 59), additional CRKP + cultures (Isolate 58) at Hospital 4 for patient 58, and subsequent overlapping stays at Hospital 4 (patients 57, 58 and 59) (Fig. 6B). The transmission path between patient 65 and other cluster patients was not clear.

Study isolates contain multiple antibiotic resistance genes

All isolates contained at least one β-lactamase gene (Supplemental Fig.S3). Most (96%) had one or more carbapenamase genes ($bla_{\text{KPC-2}}$ or $bla_{\text{KPC-3}}$), which have been associated with clonal expansion in ST258 clades I and II.[24] Two isolates (24 and 31) had $bla_{\text{KPC-3}}$ which resulted from a single nucleotide change in $bla_{\text{KPC-2}}$ (Supplemental Fig. S4A). Although the core genomes of isolates 31 and 49 were very closely related, isolate 49 did not have a $bla_{\text{KPC-3}}$ gene. Fifty-seven isolates contained the $bla_{\text{OXA-9}}$ gene, 12 had no bla_{OXA} variants

and isolates 24 and 66 had only *bla*$_{OXA-1}$ genes. Two of the most distinct isolates, 21 and 66, lacked *bla*$_{KPC}$.[24] None of their sequence reads mapped to *bla*$_{KPC}$ in the CARD database, confirming that missing *bla*$_{KPC}$ genes did not result from assembly errors (Supplemental Fig. S5A-B). While isolate 66 contained *bla*$_{OXA-1}$, providing an explanation for its carbapenem resistance,[54,55] isolate 21 had neither *bla*$_{KPC}$ nor *bla*$_{OXA}$ genes (Supplemental Fig. S4A-B), which raised the question of whether porin inactivation along with the presence of extended spectrum β-lactamase (ESBL) activity was responsible for carbapenem resistance.[22,56] Further analysis did not identify porin inactivation or other known mechanisms to explain isolate 21's carbapenem resistance.

Isolateswith *bla*$_{TEM}$ and *bla*$_{SHV}$ genes aligned to multiple variants in the CARD database (Supplemental Fig. S4, C-D). All but one isolate contained the *bla*$_{SHV}$ gene, and several had more than one. Most isolates (83%) had *bla*$_{TEM}$ genes. Other antibiotic resistance genes that confer multidrug efflux, aminoglycoside resistance and sulfonamide resistance functions (Supplemental Fig. S3) were identified through sequence alignment.

Although most isolate core genomes were closely related, 8b and 48 were the only pair with identical antibiotic resistance profiles; however, the general presence/absence pattern of β-lactamase genes showed no correlation with the overall pattern of SNP distances between isolates, consistent with mobility of genes on plasmids compared to those on core genomes.

Virulence factors strongly associated with MLST

Analysis of VFDB gene presence showed that 85% of ST258 isolates had identical virulence profiles that included 30 genes (Supplemental Table S6). The 10 remaining ST258 isolates segregated into two clusters. The first (N = 4) had 1 gene missing from the dominant pattern. Three of these isolates (58, 59 and 65) belonged to the Hospital 4 cluster; however, the other Hospital 4 cluster isolate (57) retained the dominant ST258 pattern. The second ST258 group (N = 6) had 11 genes absent from the dominant pattern, as did ST11, ST14 and ST45 isolates. The ST45 isolate was missing one additional virulence gene. The ST14 isolate had 2 missing and 2 additional genes. ST11 had one additional gene, *wbaP/rfbP*, involved in lipopolysaccharide synthesis. The patient corresponding to this isolate had a bloodstream infection and died in hospital, consistent with, but not proof of, a more virulent strain. Both ST25 isolates had the dominant ST258 pattern, plus the same 9 additional genes, which are involved in siderophore biosynthesis and iron use and known to contribute to a hypervirulent phenotype.[57,58]

Discussion

This study was initiated to investigate the origins and transmission dynamics of a CRKP outbreak that appeared to be concentrated in a single facility. Determining whether inter-facility CRKP transmissions had occurred was an institutional patient care priority that demanded precision. WGS provided a proven, high resolution tool to augment classical epidemiology in outbreak investigation and transmission tracking.[18–20] By combining CRKP genomic analysis and detailed patient medical records, our efforts untangled a web of interactions between patients that identified previously undetected CRKP transmission routes and highlighted the importance of asymptomatic colonization in disease spread.

[18,20,59] Our results demonstrated that closely related CRKP organisms were shared between facilities, which catalyzed systemwide changes to address gaps in traditional, facility-based infection prevention and control (Table 3).

Core genome analysis identified most study isolates as ST258, the primary sequence type associated with nosocomial transmission in the U.S.[51] Many isolates had closely related core genomes and displayed identical virulence factor gene profiles that underscored the likelihood of a common infection source.[42] Of the pairs of isolates with 0 SNP distances, 80% were collected within 31 days of one another, indicating direct transmissions between patients during the outbreak event. The concentrated network of highly related genomes within the outbreak made sources and recipients virtually indistinguishable.[60] Using WGS, our SeqTrack analysis (Fig. 5) inferred a transmission tree and visual representation that separated outbreak isolates from sub-trees beyond the outbreak and provided convincing evidence of inter-facility transmission.

The extent of repeated CRKP cultures we identified among many patients over long periods of time was an unexpected finding. Almost half of patients had 4 or more CRKP + cultures during the study. Several patients had CRKP infection identified early in the study period and additional positive cultures much later, adding to evidence showing that colonization can persist for years.[9,10] Patterns of repeated cultures and accompanying medical records indicated that discharges into the community or transfers between facilities occurred during extended asymptomatic CRKP colonization.[20,59,60] Long-term CRKP carriage contributed to an endemic level of unrecognized CRKP and increased transmission opportunities in the absence of precautions that are normally in place when CRKP presence is known. Once long-term colonization was recognized, Atrium Health adopted contact precautions, patient cohorting and dedicated equipment for infected patients, in addition to hygiene education and reinforcement, to decrease silent, unrecognized transmissions (Table 3).[61] To reduce transmission risk from colonization, an Electronic Medical Record (EMR) flag was developed to identify Atrium Health patients with a known history of CRE and to alert providers to conduct active surveillance on admission and to take special precautions when caring for these patients.

Identifying a clear source of inter-facility transmissions is a complicated and imprecise exercise, particularly when asymptomatic carriage is suspected and isolate sampling may be incomplete.[59] Within the outbreak period, where sampling was most robust, WGS analyses identified probable inter-facility transmissions but also indicated that direct inter-facility transmission was not widespread. Of the four outbreak isolates collected from facilities other than Hospital 2, three had likely transmission sources within the outbreak. Beyond the outbreak, two separate clusters with closely related core genomes identified probable, but limited, inter-facility spread.

As a large public healthcare system, Atrium Health provides comprehensive healthcare services to a diverse set of patients using a common, integrated EMR system that allows in-depth review of patient healthcare histories across all facilities in the system. Initial observations revealed long-term CRKP infections and a burden of co-morbidities among patients in the study. Patient sharing naturally led to repeated healthcare visits across Atrium

Health facilities that increased opportunities for exposure and transmission. Our hypothesis of inter-facility spread was supported by the presence of closely related pairs of isolates that had identifiable routes of transmission between different facilities and overlapping patient healthcare visits. Patients' existing comorbidities increased susceptibility to infection.[11,62] Our finding that CCI scores were strongly associated with mortality during the study is consistent with disease burden that also reduced patients' ability to fight infection. With endemic CRKP reported in U.S. hospitals, public health risk can only increase without coordinated action to protect vulnerable patients and the community.[18,20]

Immediate application of our results to improve infection prevention and control across our integrated health system was a major strength of this work. We uncovered a pattern of repeated CRKP cultures in individual patients collected from different types of facilities in different locations. These findings demonstrated apparent asymptomatic carriage that increased inter-facility transmission risk.[63] SeqTrack analysis provided an easily interpreted transmission tree that visually identified probable interfacility transmissions and revealed the potential implications of long-term colonization on endemic CRKP spread. SeqTrack software also had limitations. The resulting CRKP tree conveyed a level of precision that does not reflect actual uncertainty in the direction, timing and sources of transmissions. For complex infections with extended periods of asymptomatic colonization, like CRKP, transmission events identified in SeqTrack analysis may be misleading and should therefore be interpreted with due caution.

Further limitations were the result of study design decisions and constraints. As a retrospective investigation, we used frozen clinical isolates that prevented assessment of within-patient strain diversity and may have reduced precision in transmission detection. Alternatively, analyzing a set of potentially divergent strains multiplied by tens or hundreds of patients creates computational and interpretation challenges.[18,64] Using core genome alignment for phylogenetic analysis, rather than the entire genome, may have lowered resolution to distinguish closely-related isolates but reduced computational complexity and cost. We also recognize that incomplete sampling of isolates during the study period limited our ability to determine potentially important sources of infection that were invisible in our analysis. This limitation introduced further uncertainty regarding the extent and sources of inter-facility transmissions.[20] With the continuing decrease in the cost of sequencing, future studies will be able to sample more patients within an outbreak, which will lead to increased confidence in the resulting transmission models.

Finally, use of short-read sequencing, which was what was available and affordable at the time, limited our options for plasmid analysis. We did not attempt plasmid reconstruction from short-read sequence data due to our inability to completely assemble or confidently assign assembled plasmid fragments to specific plasmids.[18,65] Future studies should prioritize the acquisition of long-read data to allow for accurate plasmid reconstruction.

Despite these limitations, our work provides a comprehensive analysis of CRKP transmission within an integrated healthcare system that included physically separated, diverse facilities across the care continuum. For patients with a history of CRKP, this research exposed vulnerabilities and patient safety risks of managing care across a network

where long-term CRKP colonization may go unrecognized. While this study exists in the context of a single outbreak in a geographically-bound healthcare system, the results are applicable beyond any single geography or organization.

Our evolving understanding of transmission dynamics, endemicity, long-term asymptomatic carriage and resistance mechanisms compel us to reconsider how we better manage multidrug resistant infections such as CRKP. Our study is part of a suite of studies that have found transmission across a wide region of space and time.[20,23,42,59,66] One recent study by Kwong *et al.* demonstrates not only the feasibility of real-time WGS for infection prevention and management across a sizable geography but also suggests a model that identified the requirements and challenges of implementing a WGS-based infection control system. Since multiple studies have now established that resistant bacteria are spreading across healthcare facilities and geographic boundaries, adopting such a model will require breakthroughs in securing and sharing data and resources needed for isolate and data collection, sequencing, construction of bioinformatics pipelines and analytical expertise to apply WGS to widespread infection management.[18,49] Better integration of the different responsibilities of public health services, government agencies, academic institutions and healthcare entities will be essential to realize individual patient benefits that have always been the promise of the WGS frontier.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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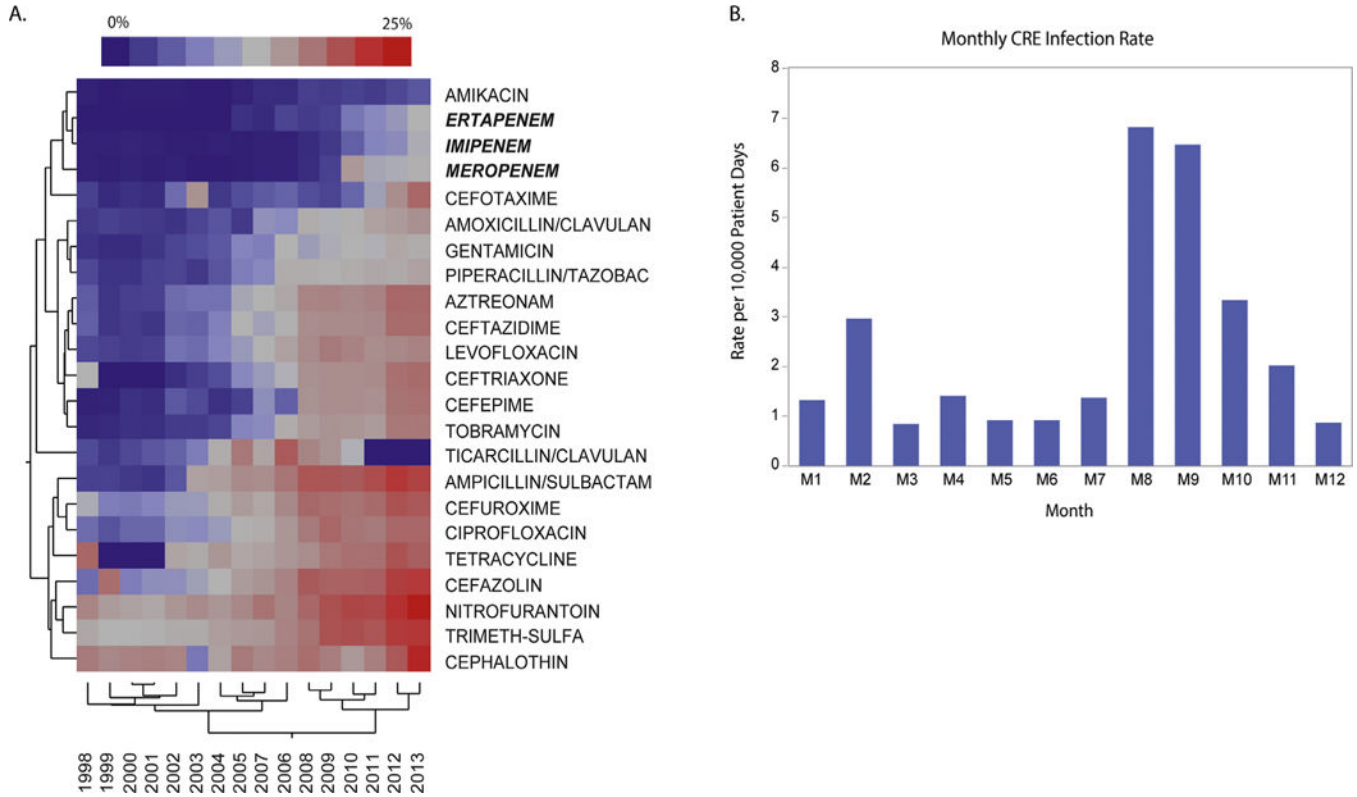

Fig. 1. Increasing K. pneumoniae antibiotic resistance and CRKP outbreak.

(A). Heat map showing two-way hierarchical clustering of antibiotic resistance. Colors indicate the percentage of *K.* pneumoniae cultures collected at Atrium Health acute care hospitals that were resistant to specific antibiotics. Carbapenems are indicated in bold and italics. (B). Plot of CRKP infection rates over 12 months including the Hospital 2 outbreak. Each point represents the CRKP infection rate per 10,000 patient days for each month (M1–M12). Rates include all CRKP infection or colonization identified by clinical culture.

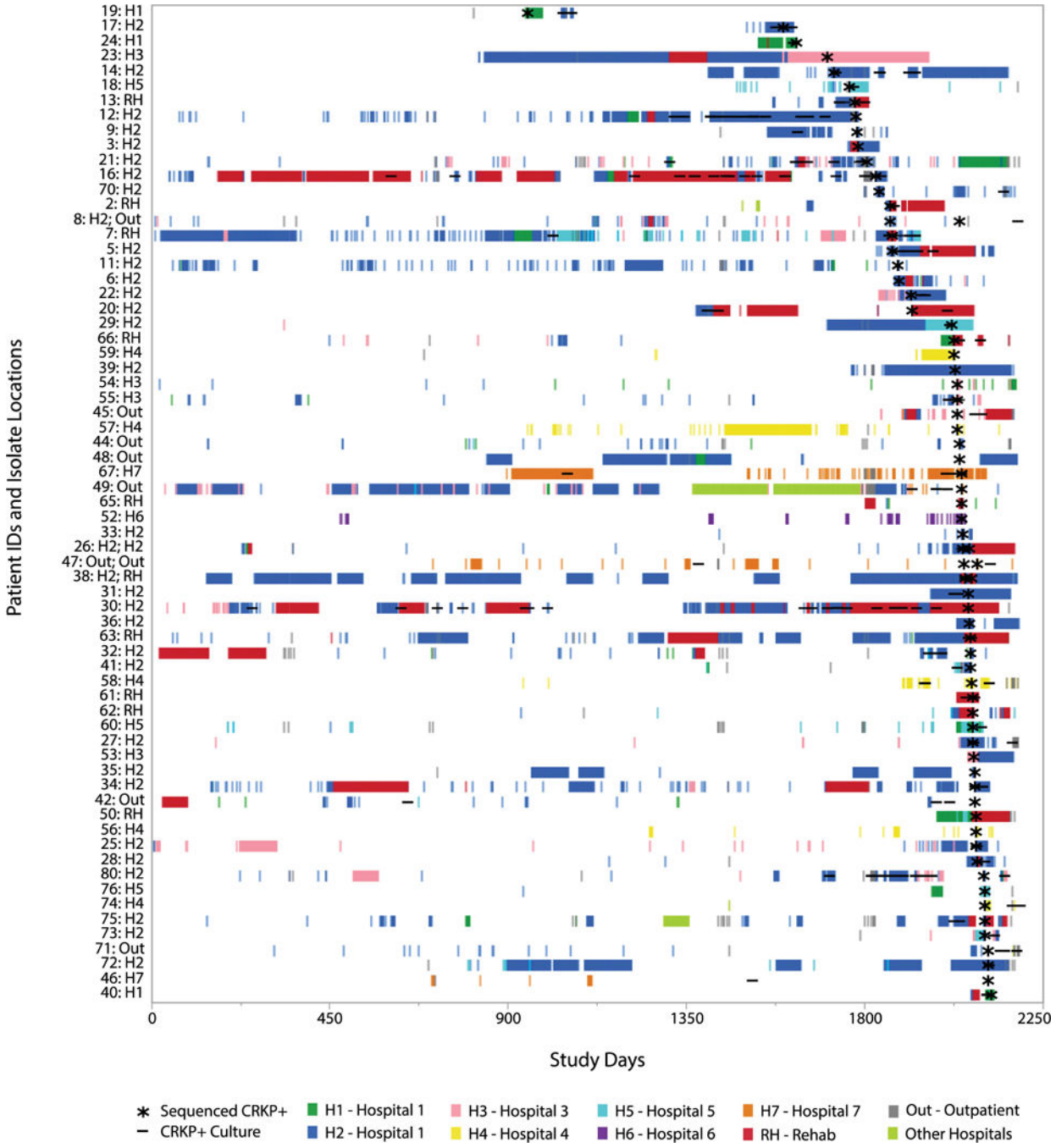

Fig. 2. Plot identifying all healthcare visits for study patients with CRKP over the 2200-day study period.

Colors indicate the facility where the visit occurred and included 11 hospitals, 3 inpatient facilities and 29 outpatient facilities. Sequenced CRKP + cultures are represented by a black asterisk. Additional unsequenced CRKP + cultures are shown by a black dash.

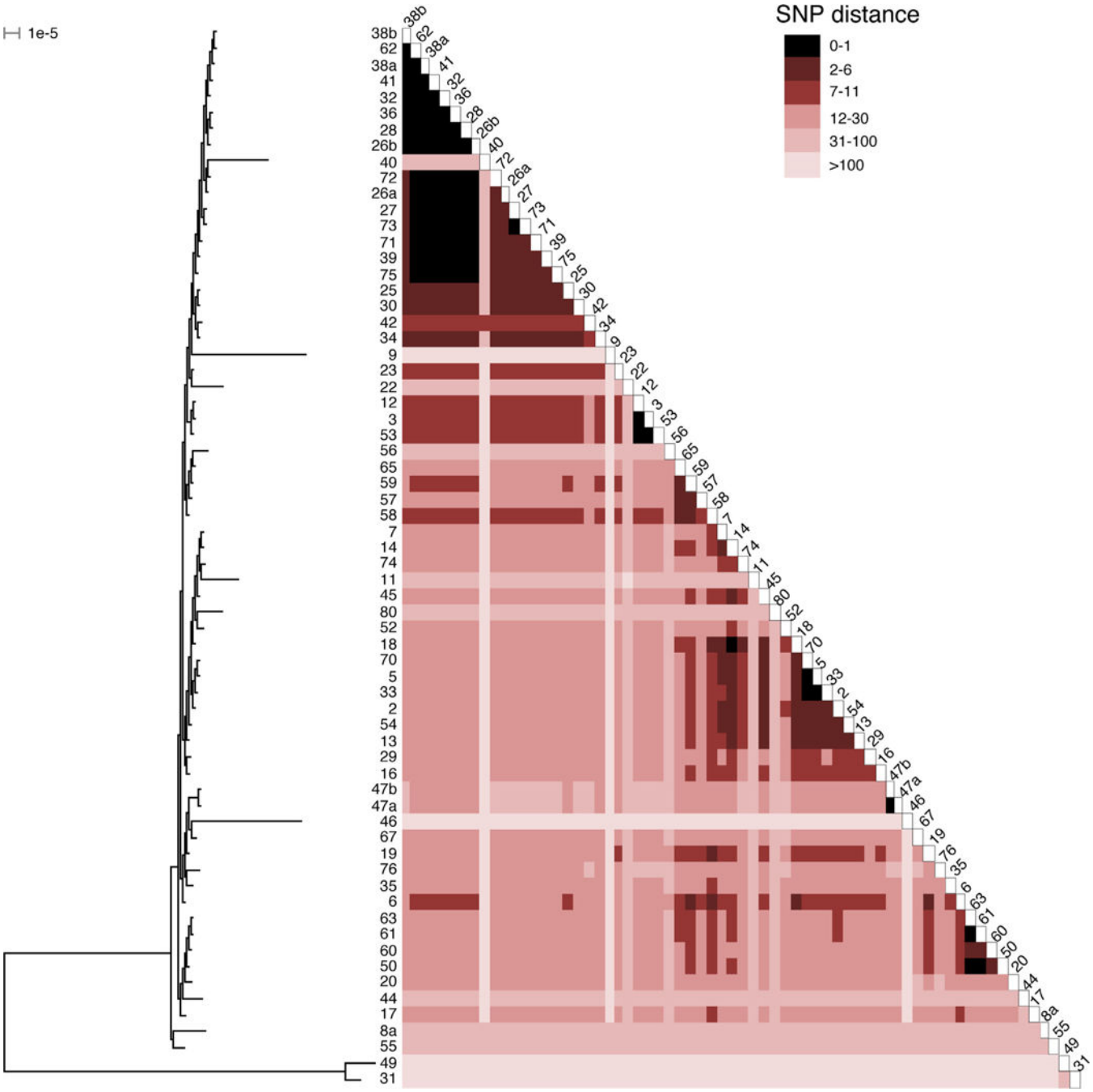

Fig. 3. Phylogenetic Tree identifying genomic relationships between CRKP ST258 isolates.

The tree was constructed using the core genome alignment of the 76 CRE genomes included in SNP distance calculation. Only the CRKP ST258 isolates are included in the tree construction to allow visualization of the primary endemic strain. Color gradient shows SNP distance ranges between individual isolate pairs.

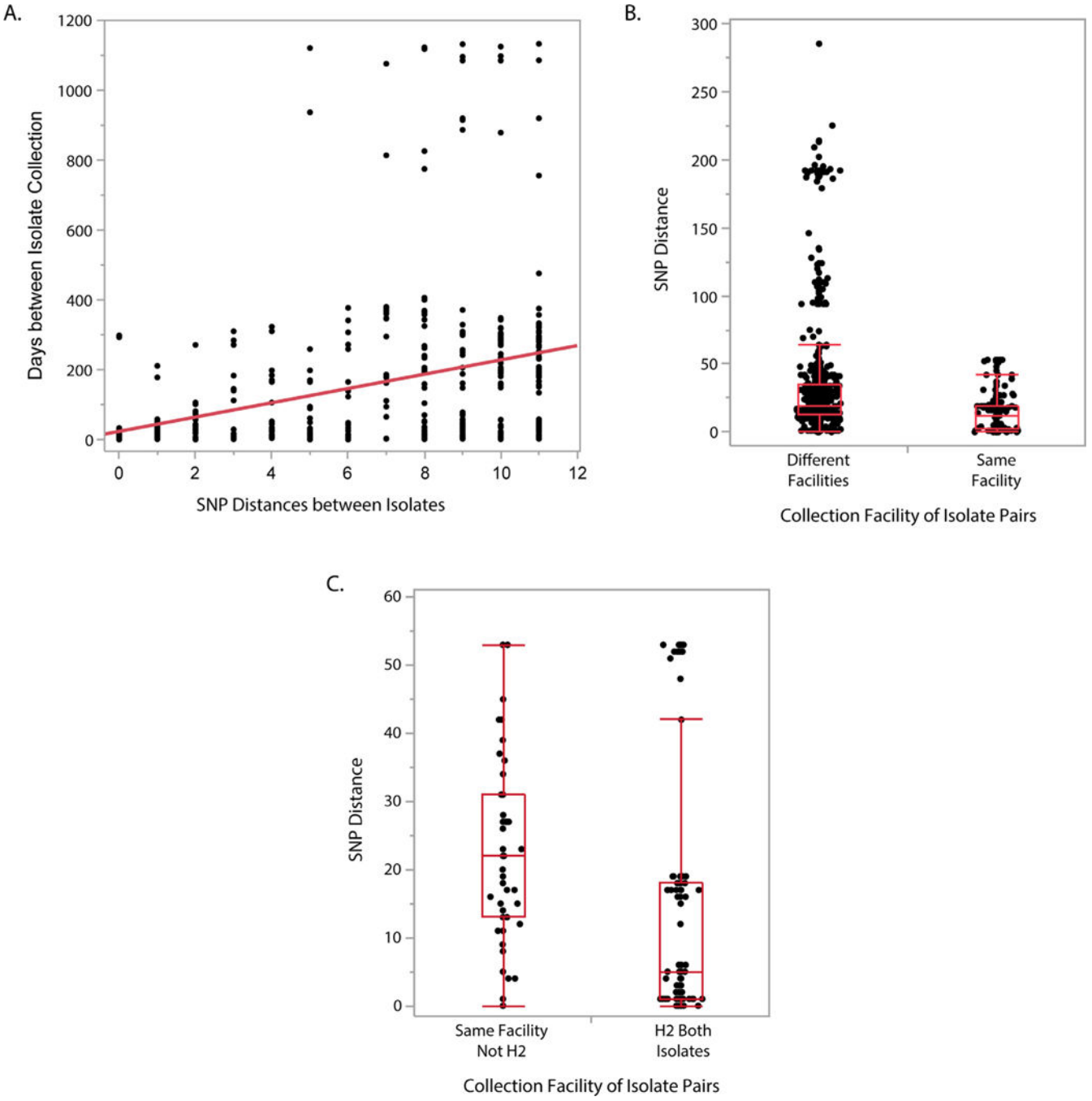

Fig. 4. Analysis of SNP distance relationships to collection time and facility reveals correspondence between genomics and transmissions.

(A). Comparison of collection times and SNP distances between pairs of isolates with 11 or fewer SNPs demonstrated a strong positive correlation ($R2 = 0.125$; $P < 0.0001$) despite variation. (B). Comparison of SNP distances between pairs collected during the outbreak period at the same facility (Mean 14.71; 10%ile 12.91, 90%ile 16.52) versus those collected at different facilities (Mean 35.74; 10%ile 33.08, 90%ile 38.41) showed that isolates from the same facility were much more closely related than those from different facilities ($P <$

0.0001). (C). Comparison of SNP distances between isolate pairs collected during the outbreak period where both were collected at Hospital 2 (Mean 12.22; 10%ile 9.72, 90%ile 14.71) versus isolate pairs where both were collected at the same facility, but not Hospital 2, (Mean 22.20, 10%ile 18.52, 90%ile 25.88) demonstrated that direct or close transmissions (defined by short core genome SNP differences) occurred significantly more frequently within Hospital 2 than at other facilities during the outbreak period.

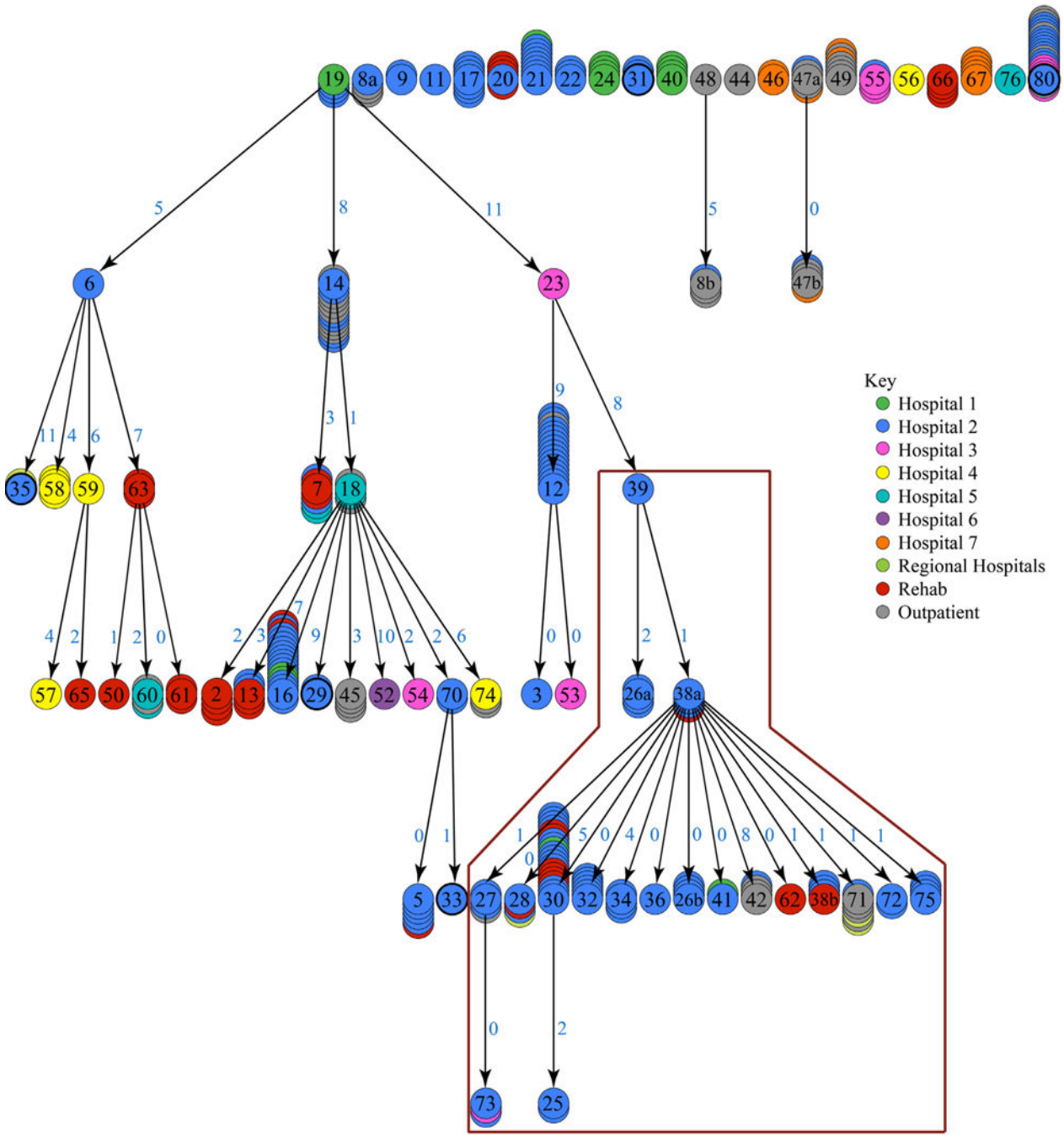

Fig. 5. Transmission tree combining genomics and culture dates indicates potential transmission between facilities.

SeqTrack analysis was conducted using culture date and core genome SNP distance to infer a probable transmission tree. Nodes represent sequenced isolates with each isolate ID displayed. Edges are likely transmission paths with the SNP distance between isolates shown in blue. Isolates without edges are >11 SNPs from ancestor isolate predicted by SeqTrack analysis. Node colors indicate the facility where the specimen was collected. Stacks of nodes above each numbered node were added to the tree to show additional CRKP + cultures collected during the study prior to the collection date of the sequenced isolate.

Those below the main node are isolates that were collected after. Isolates contained within the red polygon were designated by SeqTrack as part of the primary outbreak at Hospital 2. Isolates determined to be outside the primary outbreak that were collected at Hospital 2 during the outbreak period have a heavy black circle surrounding the node.

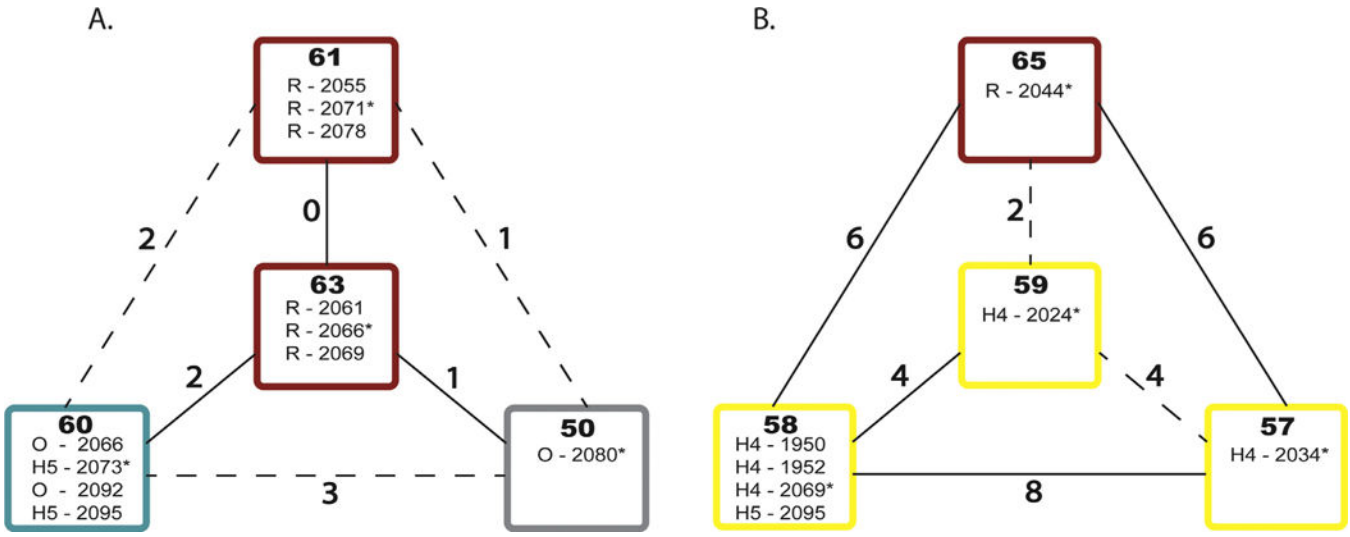

Fig. 6. Analysis of two isolate clusters beyond the outbreak identifies evidence that closely related isolates were shared between facilities.

Study dates of all CRKP + cultures for each patient in the 2 clusters are shown within the nodes with each sequenced culture indicated by an asterisk. The facilities where the cultures were collected are: Red = Rehab, Turquoise = Hospital 5, Gray = Outpatient and Yellow = Hospital 4, consistent with other figures. Edges show genetic connections between isolates with each the SNP distance between isolates displayed. Solid edges display the direct connections and SNP distances on the SeqTrack transmissions tree. Dashed edges show SNP distances and indirect connections on the tree. (A). Isolates 61, 63, 60, and 50 were closely related on the SeqTrack tree (0–3 SNPs), yet were collected at 3 different facilities. (B). Three of the isolates in the second cluster were collected at Hospital 4, as were their other unsequenced CRKP + cultures. Isolate 65 had the closest genetic relationship to 59 but was collected at Rehab.

Table 1

Isolate summary statistics.

CRKP sequenced isolate specimen source	
Urine	49 (69%)
Blood	12 (17%)
Trachea	3 (4%)
Abcess	3 (4%)
Wound	2 (3%)
Rectal Swab	2 (3%)
CRKP sequenced isolate collection location	
H1	3 (4%)
H2	34 (48%)
H3	4 (6%)
H4	5 (7%)
H5	3 (4%)
H6	1 (1%)
H7	2 (3%)
Rehab	10 (14%)
Outpatient	9 (13%)
CRKP isolate sequence types	
ST 258	65
ST1199 (sub ST258)	1
ST25	2
ST45	1
ST11	1
ST14	1
Total	71
Shortest SNP distance to any other isolate	
0	18 (25%)
1–5	27 (38%)
6–11	8 (11%)
>11	18 (25%)
CRKP cultures collected from patients during study period	
Total CRKP cultures	280 (100%)
Sequenced CRKP isolates	71 (25%)
CRKP only	213 (76%)
CRKP in Mixed Cultures	67 (24%)

Table 2

Patient summary statistics.

Patient age ranges	
≤40	8 (12%)
41–50	8 (12%)
51–60	13 (19%)
61–70	17 (25%)
>70	21 (31%)
CRKP cultures per patient	
1	19 (28%)
2–4	33 (49%)
5–9	10 (15%)
10 or more	5 (8%)
CRKP different facilities	19 (28%)
CRKP collected >1 year	11 (16%)
Atrium Health Facilities Visited During Study	
1–2	23 (34%)
3–4	30 (45%)
5 or more	14 (21%)
Healthcare Days During Study	
<50	12 (18%)
50–99	18 (27%)
100–199	10 (15%)
200–499	17 (25%)
500–999	5 (7%)
>1000	5 (7%)
Patient Charleson Comorbidity Score	
<6	22 (33%)
6–9	22 (33%)
10 or more	23 (24%)

Table 3

Infection prevention actions in response to CRKP outbreak and increasing prevalence.

Actions taken	Implementation	Locations
Hand hygiene program and training	Always in place	All acute care facilities
Surveillance for incidence and prevalence of MRDO infection	Always in place	All acute care facilities
Contact precautions for patients with MDRO infections	Always in place	All acute care facilities
Chlorohexidine bathing for ICU patients	2009	Hospital 2
Surveillance for incidence and prevalence of MRDO infection	2010	Hospital 2
Electronic medical record alert for patients with MDRO infection	2010	System-wide
Consistent process for surveillance for CRE incidence and prevalence	2010	Staged roll-out to facilities
Contact isolation for patients with CRE	2010	All acute care facilities
Chlorohexidine gluconate bathing for patients with CRE	2012	Hospital 2
Secret shoppers hand hygiene program and performance reporting	2012	Staged roll-out to larger facilities
Patients with CRE cohorted on dedicated hospital floors	2012	Hospital 2
Adenosine triphosphate bioluminescence assessment of environmental cleanliness	2013	Staged roll-out to facilities
Patients with CRE provided with dedicated equipment and nursing staff	2013	Hospital 2
Increased clinical and non-clinical CRE education and resources	2013	Hospital 2
CRE surveillance on high-risk wards and rotation on all units	2013	Hospital 2
Ultraviolet light disinfection (Tru-D)	2013	Hospitals 2, 7
Antibiotic stewardship program with antibiotic selection guidance	2013–2017	Staged roll-out to facilities
Chlorohexidine gluconate bathing for patients with central lines, MRSA	2014	All acute care facilities
Standardization of Environmental Services training and cleaning procedures	2014	Ongoing improvements and roll-out
Active CRE rectal swab surveillance program for high-risk patients	2015	Hospital 2
Chlorohexidine gluconate bathing for patients in preop for specific surgeries	2015	All acute care facilities
Electronic medical record alert for patients with history of CRE	2015	System-wide