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Tracking a Hospital Outbreak of Carbapenem-Resistant *Klebsiella pneumoniae* with Whole-Genome Sequencing

Evan S. Snitkin *et al.*

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Editor's Summary

A Detective Story

Some infections are largely a thing of the past—plague, syphilis. The unfortunate result of these antibiotic-driven successes is the emergence of drug-resistant pathogens. And, ironically enough, hospitals are at the center of the problem. An example of this occurred in 2011 at the Clinical Center of the U.S. National Institutes of Health (NIH), in which an outbreak of drug-resistant *Klebsiella pneumoniae* infected 18 patients, causing the death of 6 of them. Using a combination of whole-genome sequencing and patient tracking, Snitkin and his colleagues examined how the bacteria was spreading through the hospital. The results outline a complicated path of transmission within the hospital that defied standard containment methods, yielding lessons for the future.

A patient known to be infected with a drug-resistant form of *K. pneumoniae* was admitted to the NIH Clinical Center on 13 June 2011. Enhanced isolation procedures were immediately implemented, and no spread of the bacteria was seen for the month she was in the hospital. Although all seemed well, a few weeks later on August 5th, a second infected patient was discovered, followed by a series of other patients with infection or colonization —about 1 a week to a total of 18 by the end of 2011. Six people ultimately died as a result of the bacteria. The outbreak was finally contained by rigorous control procedures.

A careful survey of the bed locations of each patient did not shed much light on how the bacteria traveled on its deadly path: The first patient did not even come into contact with any of the others. So the authors performed whole-genome sequencing on all of the bacteria that were found, determining the most likely evolutionary relationships among them by comparing the variations at single nucleotides that arise as bacteria grow. Combining this evolutionary information with the physical tracking of the patients pointed to the most likely transmission scenario.

The authors concluded that all of the *K. pneumoniae* cases likely originated with the index patient, from at least two different sites on her body, rather than by independently introduced bacteria. There were at least three different initial transmission events. Particularly disturbing was the fact that one of the infections could be linked to contamination of a ventilator that had been cleaned by thorough methods.

Sophisticated deployment of whole-genome sequencing revealed the weaknesses in this medical who-done-it, informing improvements in hospital preventive measures. If applied rapidly, such analysis can even expose the causes of nosocomial infections in real time.

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NOSOCOMIAL INFECTION

Tracking a Hospital Outbreak of Carbapenem-Resistant *Klebsiella pneumoniae* with Whole-Genome Sequencing

Evan S. Snitkin,[1] Adrian M. Zelazny,[2] Pamela J. Thomas,[1] Frida Stock,[2] NISC Comparative Sequencing Program,[3] David K. Henderson,[2] Tara N. Palmore,[2]* Julia A. Segre[1]*

The Gram-negative bacteria *Klebsiella pneumoniae* is a major cause of nosocomial infections, primarily among immunocompromised patients. The emergence of strains resistant to carbapenems has left few treatment options, making infection containment critical. In 2011, the U.S. National Institutes of Health Clinical Center experienced an outbreak of carbapenem-resistant *K. pneumoniae* that affected 18 patients, 11 of whom died. Whole-genome sequencing was performed on *K. pneumoniae* isolates to gain insight into why the outbreak progressed despite early implementation of infection control procedures. Integrated genomic and epidemiological analysis traced the outbreak to three independent transmissions from a single patient who was discharged 3 weeks before the next case became clinically apparent. Additional genomic comparisons provided evidence for unexpected transmission routes, with subsequent mining of epidemiological data pointing to possible explanations for these transmissions. Our analysis demonstrates that integration of genomic and epidemiological data can yield actionable insights and facilitate the control of nosocomial transmission.

INTRODUCTION

The bacterial pathogen *Klebsiella pneumoniae* is responsible for roughly 15% of Gram-negative infections in hospital intensive care units (ICUs) (*1*), primarily affecting immunocompromised patients (*2*). In recent years, the threat posed by *K. pneumoniae* has markedly increased with the emergence of strains resistant to carbapenem antibiotics (*3*) and their worldwide dissemination (*4*, *5*). Infections caused by carbapenem-resistant strains have few treatment options (*6*, *7*) and are associated with mortality rates upwards of 50% (*8*, *9*). Although multiple resistance mechanisms have been identified (*10*), carbapenem resistance in the United States is primarily caused by the plasmid-encoded *K. pneumoniae* carbapenemase (KPC) gene (*5*).

Exacerbating the problems associated with the emergence of these highly resistant strains of *K. pneumoniae* is their propensity to cause outbreaks in health care institutions (*11*–*13*). As with most nosocomial pathogens, multiple-drug resistance offers inherent selective advantage (*11*), which allows such organisms to persist both in the flora of hospitalized patients and in the hospital environment, in which antibiotic usage is widespread. More specific to *K. pneumoniae* is its capacity to silently colonize patients or hospital personnel (*14*), that is, by establishing residence in the gastrointestinal tract without causing any signs of infection. Individuals can be silently colonized or asymptomatic carriers for long periods of time, with detection of these carriers often proving difficult (*12*). These silent carriers act as reservoirs for continued transmission that make spread difficult to control and outbreaks difficult to stop (*13*). In addition, *K. pneumoniae* can survive for several hours on the hands of hospital personnel, which likely facilitates nosocomial spread (*15*).

Effective control of *K. pneumoniae* outbreaks requires a detailed understanding of how transmission occurs. Molecular typing approaches, such as pulsed-field gel electrophoresis and multi-locus sequence typing, have been used to classify *K. pneumoniae* and thus understand its local and global dissemination (*16*, *17*). However, the high *K. pneumoniae* clonality (*16*, *18*) creates difficulty in tracking outbreaks because available methods may not provide sufficient resolution to distinguish intra-institutional spread from introduction of closely related strains from other health care facilities. In the United States, KPC–*K. pneumoniae* isolates are highly clonal, with 70% belonging to sequence type (ST) 258 (*16*).

With rapid technological advances, whole-genome sequencing is emerging as the gold standard in bacterial typing (*19*, *20*). Success in tracking worldwide epidemics (*21*–*24*), regional outbreaks (*23*, *25*), food-borne outbreaks (*26*, *27*), and bioterrorism agents (*28*) has demonstrated that the fine resolution provided by whole-genome sequencing facilitates our understanding of the spread of infectious agents. The continued improvements in turnaround time and accessibility of DNA sequencing technologies are now approaching a point where genomic data can be generated in a clinically relevant time frame. Genome sequencing has been applied recently to nosocomial strains (*29*–*32*) but with limited study size or scope in reconstructing transmission links during the course of the outbreak.

Here, we applied whole-genome sequencing to track an outbreak of carbapenem-resistant *K. pneumoniae* at the U.S. National Institutes of Health (NIH) Clinical Center that colonized 18 patients, with 6 deaths attributable to *K. pneumoniae* infection. We developed an algorithm to reconstruct the outbreak transmissions based on whole-genome sequencing of isolates and epidemiological data that tracked the location of patients throughout their hospital stays.

RESULTS

Overview of outbreak

On 13 June 2011, patient 1 was transferred to our ICU from a hospital in New York City and was discharged on 15 July. She was known to

[1]National Human Genome Research Institute, Bethesda, MD 20892, USA. [2]National Institutes of Health Clinical Center, Bethesda, MD 20892, USA. [3]National Institutes of Health Intramural Sequencing Center (NISC), Bethesda, MD 20892, USA.
*To whom correspondence should be addressed. E-mail: tpalmore@mail.nih.gov (T.N.P.); jsegre@nhgri.nih.gov (J.A.S.)

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be colonized with carbapenem-resistant *K. pneumoniae* and was immediately placed in enhanced contact isolation in a private room in which staff and visitors are required to don gloves and gowns for entry. During her stay, she spent two 24-hour stints in the ICU. Another instance of a KPC–*K. pneumoniae* isolate was not observed in clinical or surveillance cultures until August 5 when it was cultured from the tracheal aspirate of patient 2. Whether the two KPC–*K. pneumoniae* infections were related was unclear, especially because the two patients were never housed in the same ward at the same time (Fig. 1, A and B). Repetitive polymerase chain reaction (PCR) and pulsed-field gel electrophoresis (fig. S1) identified both patient isolates as belonging to the epidemic ST 258 *K. pneumoniae* lineage. However, because of the ubiquity of ST 258 in U.S. hospitals (*16*) and the time lag between patient presentations, both nosocomial transmission and independent introductions were deemed possible.

In the following weeks, an average of 1 new case of colonization or active infection with KPC–*K. pneumoniae* was detected each week in the NIH Clinical Center, with a total of 17 cases as of 1 January 2012 (table S2). During the outbreak, 10 of the 17 KPC–*K. pneumoniae*–colonized patients died, with 6 deaths attributable to *K. pneumoniae* infection and 4 to the underlying disease (Table 1). The outbreak was ultimately contained by implementing strict cohorting of colonized patients to minimize sharing of hospital equipment and of care providers between outbreak patients and the other patients in the hospital (see Materials and Methods for details). To identify asymptomatically colonized patients, three rounds of rectal surveillance cultures were performed on all hospital patients, augmented with more frequent rectal surveillance cultures performed on patients in selected hospital wards (see Materials and Methods). Although implementation of rigorous infection control procedures ultimately halted the outbreak, we sought to understand better how the outbreak progressed to learn how to control future outbreaks of *K. pneumonia* more effectively.

Genome sequencing and comparison of outbreak isolates

Deciphering the transmission events between patients solely on the basis of the epidemiologic data proved extremely difficult because extensive overlap of patients within the hospital wards, particularly the ICU, supported numerous outbreak scenarios (Fig. 1C). To learn more about carbapenem-resistant *K. pneumoniae* transmission, first we performed whole-genome sequencing of an isolate from the index patient. An additional six isolates were cultured from four body sites over the index patient's 4-week stay at the NIH Clinical Center. Because the index patient had been colonized for several months, we examined potential genetic heterogeneity among these seven isolates to reconstruct putative transmission events more accurately. Sequencing of variants of the index patient's isolates revealed a total of seven single nucleotide variants (SNVs) among the seven isolates (Fig. 2A). The four urine isolates matched the NTUH-K2044 *K. pneumoniae* reference strain at each of the seven variant sites, supporting this as the "ancestral" genotype. By contrast, the groin and bronchoalveolar lavage (BAL) isolates both exhibited three SNVs compared to the urine isolates, and the throat isolate exhibited three different SNVs compared to the urine, distinct from the groin and BAL isolates. Note that the groin isolate also contained one SNV in addition to the BAL isolate. Identification of this genetic heterogeneity within the isolates recovered from the index patient proved immensely valuable in reconstructing transmission and demonstrated the importance of culturing and sequencing isolates from multiple body sites over time from long-term colonized patients.

We next performed whole-genome sequencing of a single KPC–*K. pneumoniae* isolate from each of the 18 affected patients, in hopes of using the SNVs found in their genomes to infer transmission links. Genomic comparisons revealed a total of 41 SNVs among the 18 outbreak patient isolates, that is, the isolates varied at a total of 41 sites distributed over their 6 million base pair (bp) genomes (table S4).

To address the central question of whether patient 1 initiated the outbreak and, if so, how she was linked to other patients, we compared the 18 isolates' genomes. Grouping KPC–*K. pneumoniae* outbreak patient isolates on the basis of the patterns of shared variants partitioned them into two large clusters and a third cluster consisting only of patient 8 (Fig. 2B). Inspection of the variants common among these clusters revealed that members of clusters 1 and 3 share the three variants present in patient 1's BAL and groin isolates, whereas the isolates

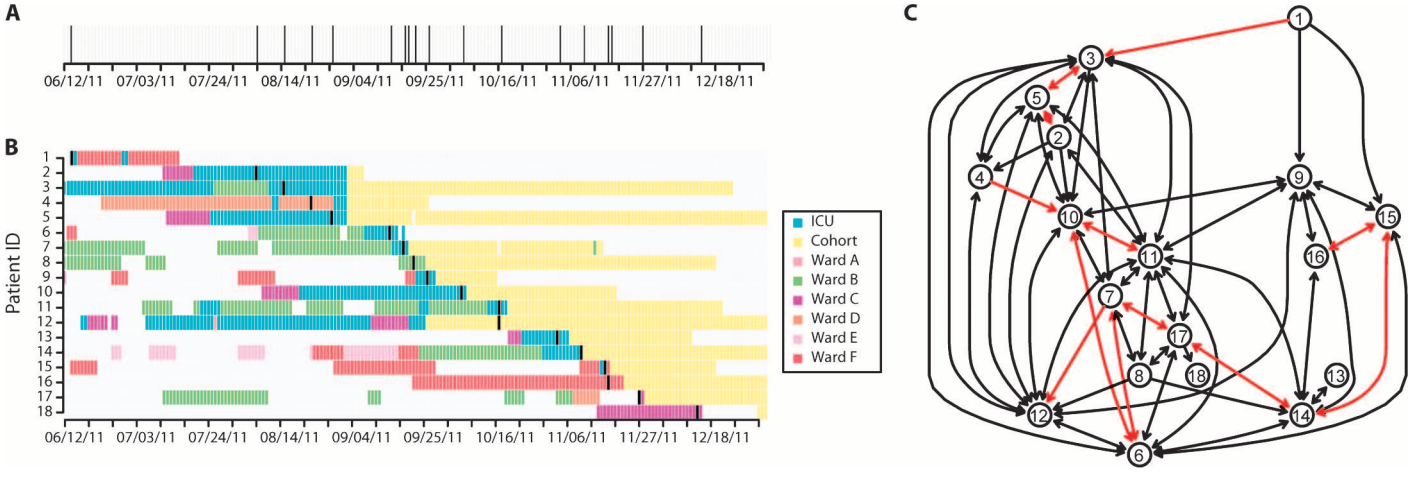

Fig. 1. Patient location and overlap during the outbreak. (**A**) Timeline of first positive cultures of the outbreak strain for the 18 affected patients. (**B**) Patient traces for each of the 18 patients shown in (A). Black lines, first positive culture; blue lines, medical ICU; yellow lines, cohorted areas; other colors represent specific wards at the NIH Clinical Center. (**C**) Graph of possible transmission links among patients. Patient IDs are within the circles. An arrow is present from one patient to another if the two patients overlapped in the same unit before the potential recipient culturing positive. Red links, the transmission event is predicted by the analysis reported here (see Fig. 3).

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Table 1. Clinical histories of patients colonized with the outbreak of KPC-producing *K. pneumonia* strain. KPC, died of KPC infection; underlying, died of underlying disease; MUD, matched unrelated donor; MRD, matched related donor; NA, not applicable.

Patient ID	Underlying	Gender	Age (years)	Date of positive culture	Culture type	Location of acquisition	Transplantation	Developed bloodstream infection	Outcome
1	Pulmonary alveolar proteinosis	F	43	6/14/11	Sputum, urine	ICU	Lung	No	Alive
2	Solid tumor	M	34	8/5/11	Tracheal aspirate	ICU	NA	Yes	Died (KPC)
3	Primary immunodeficiency	F	27	8/15/11	Throat, groin surveillance	ICU	MUD	Yes	Died (KPC)
4	Lymphoma	M	29	8/23/11	Peritoneal fluid	ICU	NA	Yes	Died (underlying)
5	Solid tumor	M	54	8/29/11	Groin surveillance	ICU	NA	No	Died (underlying)
6	Lymphoma	M	65	9/15/11	Rectal surveillance	ICU	MUD	Yes	Died (KPC)
7	Aplastic anemia	M	29	9/19/11	Rectal surveillance	ICU	MRD	Yes	Died (KPC)
8	Lymphoma	M	71	9/22/11	Rectal surveillance	Floor	MRD	No	Alive
9	Bronchiectasis	F	59	9/26/11	Rectal surveillance	ICU	NA	No	Alive
10	Solid tumor	F	72	10/6/11	Rectal surveillance	ICU	NA	Yes	Died (KPC)
11	Lymphoma	M	50	10/17/11	Throat, groin surveillance	ICU	MRD	No	Died (underlying)
12	Solid tumor	M	45	10/17/11	Rectal surveillance	ICU	NA	No	Died (underlying)
13	Solid tumor	M	39	11/3/11	Throat, groin surveillance	ICU	NA	No	Alive
14	Primary immunodeficiency	M	19	11/10/11	Throat, groin, rectal surveillance	ICU	MUD	Yes	Died (KPC)
15	Bronchiectasis	F	60	11/17/11	Rectal surveillance	Floor	NA	No	Alive
16	Undefined immunodeficiency	M	59	11/18/11	Rectal surveillance	Floor	NA	No	Alive
17	Aplastic anemia	M	34	11/27/11	Rectal surveillance	Floor	NA	Yes	Died (underlying)
18	Sickle cell disease	F	37	12/14/11	Rectal surveillance	Floor	NA	No	Alive

in cluster 2 share the three variants present in patient 1's throat isolate (Fig. 2B). These data therefore suggest that genetically distinct isolates from patient 1 were transmitted to other patients. Moreover, the data indicate that not only is patient 1 linked to the outbreak but also three independent transmission events from this patient led to hospital-wide dissemination of the outbreak strain.

Inference of most likely transmission route

Next, we sought to extract insights from the genetic data more rigorously and to reconstruct a putative transmission map. To distinguish among scenarios that were equally likely based on the genetic data, we used patient overlap in hospital wards and deemed the most probable transmission sequence as the one requiring the minimal length of undetected silent colonization (see Materials and Methods for details of algorithm implementation). The transmission map generated by our approach (Fig. 3) unravels the complexity of Fig. 1C and represents an outbreak progression that is most parsimonious with respect to both genetic and patient overlap data. Comparisons of this map to transmission maps generated on the basis of either genetic (fig. S4) or patient trace data (fig. S5) alone revealed a marked effect of incorporating both types of data.

Consistent with our observations above, the integrated map identified three transmissions stemming from the index patient. However, the first transmission was predicted to go through patient 3, and not patient 2, despite patient 2's presentation with infection 10 days earlier than patient 3's. This scenario was supported by the genetic information (patient 2's isolate contains an SNV not found in patient 1's or 3's) and also by the patient trace, which indicates that patient 3, but not patient 2, overlapped with patient 1 in the ICU (Fig. 1B).

A second transmission from patient 1 was predicted to go through patient 4 and is based entirely on genetic data. Because no direct contact occurred between patients 1 and 4, we looked for evidence of patients acting as asymptomatic intermediates in a transmission between these two patients. Knowledge of patient locations in the hospital allowed us to identify patients whose movements within the hospital make them candidates for being silent transmission vectors. Specifically, we identified all patients who overlapped with the index patient and then with patient 4 before he cultured positive. Among the 1115

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patients at the NIH Clinical Center during the outbreak, there were only 5 patients who overlapped with patients 1 and 4 and could have acted as vectors for transmission (Fig. 4). Patients B and D are especially compelling because of their extensive overlap with patients 1 and 4, but neither one cultured positive with surveillance cultures. Although the asymptomatic carrier could have been colonized below detection level or could have been an untested health care provider, this type of mining of epidemiological data has the potential to identify a handful of candidates who merit additional surveillance cultures and/or placement in contact isolation. As genome sequencing becomes even faster, such insights could be obtained in real time to perform targeted, thorough surveillance of patients of interest.

Four other links in the patient transmission chart cannot be explained by geographic overlap in the same ward (Fig. 1C). These also suggest more complicated transmission modes, perhaps through asymptomatic patients who were never detected or via health care personnel or equipment. Our epidemiologic investigation revealed that no single staff member, group of health care personnel, or procedure was a likely source of transmission to the nonoverlapping patients. However, the possibility of transmission through inanimate objects could not be ruled out and was in fact bolstered by culturing the outbreak strain from six sink drains and from a ventilator. The ventilator had been thoroughly cleaned after being used on patient 6, twice with a quaternary ammonium compound and once with bleach. The ventilator isolate had only one SNV distinguishing it from patient 6's original isolate, confirming that the isolate likely survived the cleaning process. All other environmental cultures were negative, with the exception of those taken from the room of patient 8 before decontamination.

Identification of mutations associated with colistin resistance

Finally, we compared genome sequences of outbreak isolates to gain insight into evolution of antibiotic resistance during the course of the outbreak. The index patient's isolate was highly resistant to multiple antibiotics, with minimum inhibitory concentration (MIC) values in the susceptible range for only gentamicin, tigecycline, and colistin (table S3). During the course of the outbreak, isolates with resistant MICs for all of these drugs were observed, leaving no effective therapeutic options for some patients. We focused on colistin resistance because it appeared to evolve independently in all three (only two are described) patient clusters, indicating that the outbreak strain was on the verge of becoming colistin-resistant upon arrival at the NIH Clinical Center. Patient 8's initial isolate was colistin-resistant, and patient 2's isolate acquired colistin resistance during the course of treatment. The resistant isolate from patient 2 had two mutations distinguishing it from its susceptible ancestor, and the isolate from patient 8 had six unique mutations relative to that of patient 1's (Table 2). Patient 2's colistin-resistant isolate had a 2-bp insertion in the coding region of a putative microcin transporter, resulting in a truncated protein. In *Salmonella enterica* (*33*) and *Escherichia coli* (*34*), mutations disabling the microcin transporter are sufficient to confer resistance to antimicrobial peptides

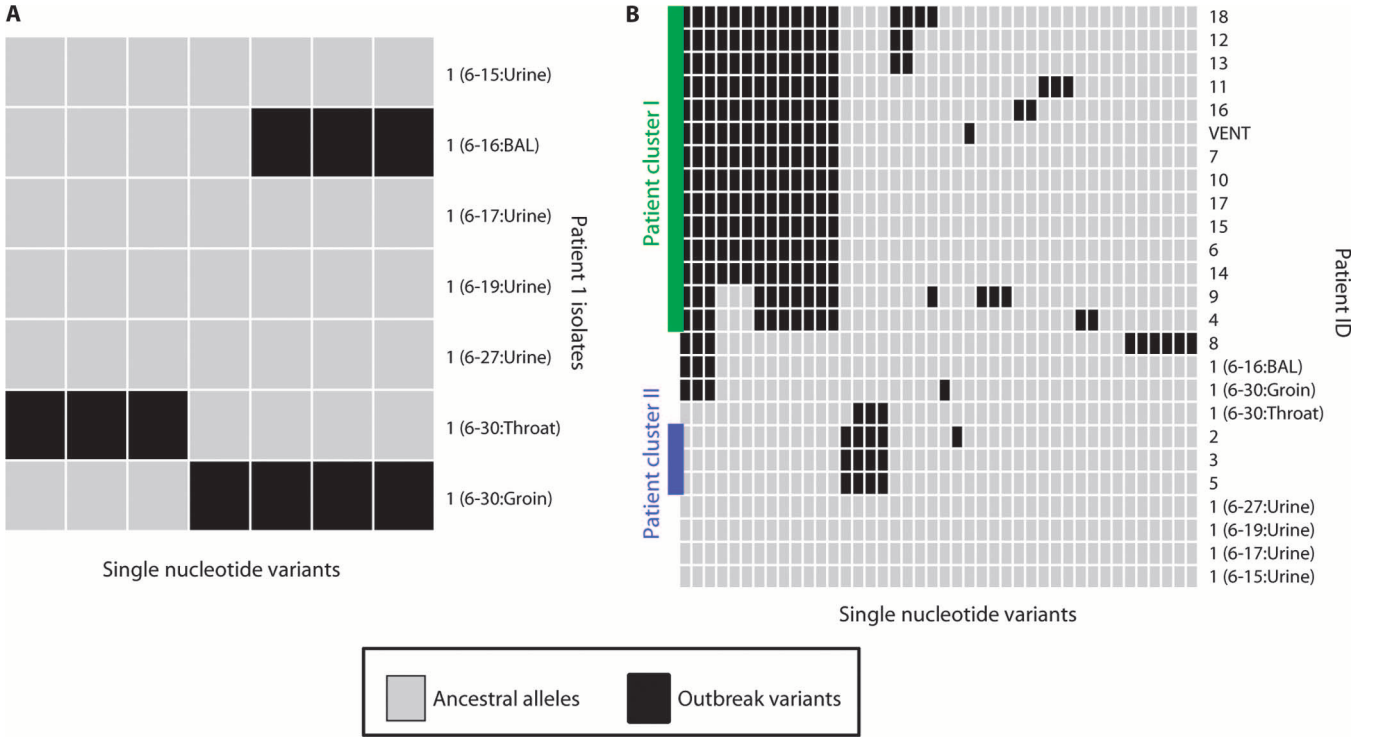

Fig. 2. SNVs identified in *K. pneumoniae* genomes. (**A**) DNA sequence variation among isolates taken from patient 1 while at the NIH Clinical Center is shown as a heat map, with isolates in chronological order on the *y* axis and variants on the *x* axis. Gray, ancestral *K. pneumoniae* alleles present in the previously sequenced NTUH-K2044 strain; black, variants found in at least one of the isolates. (**B**) Variants among all outbreak genomes are shown in a clustered heat map, with patients shown on the *y* axis and variable positions in their respective genomes on the *x* axis. Blue and green, the two major patient clusters identified on the basis of shared variants.

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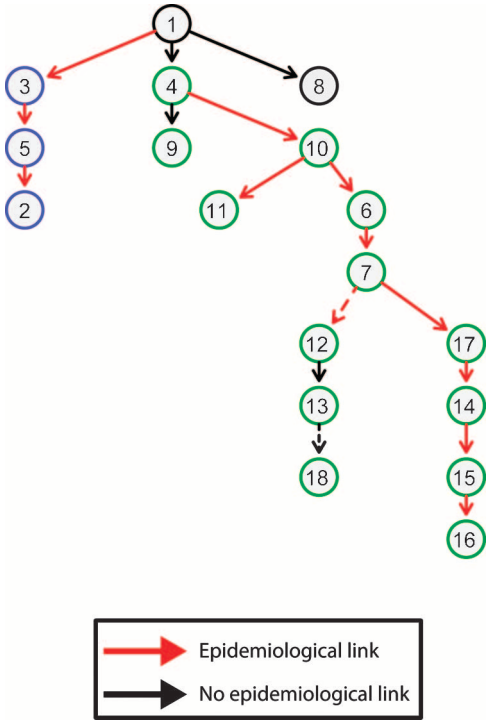

Fig. 3. Putative map of *K. pneumoniae* transmission during outbreak. The transmission map was constructed with genetic and patient trace data, as detailed in Materials and Methods. Nodes represent patients, and arrows indicate a transmission event directly or indirectly from one patient to another. Blue, cluster I; green, cluster II (from Fig. 2B). Red arrows, opportunity for a direct transmission event from patients overlapping in the same ward before the recipient culturing positive; black arrows, transmission events that cannot be explained by patient overlap (may result from a more complicated transmission route or an intermediate patient or environmental source); dashed lines, at least one other equally parsimonious transmission link exists leading to the given patient.

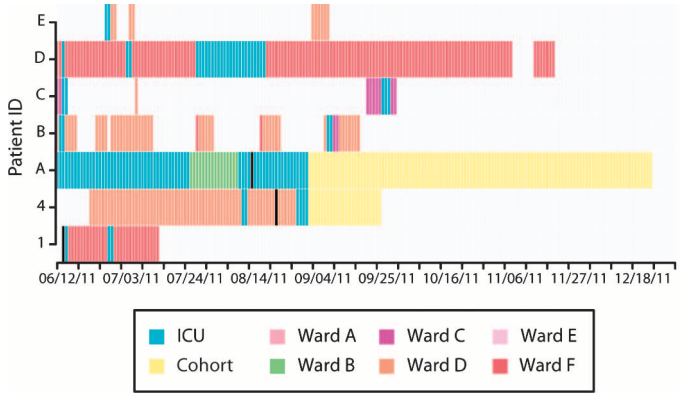

Fig. 4. Patient trace for patients 1 and 4 and all patients who could have acted as transmission intermediates between them. Patient traces for the 1115 patients present in the hospital during the outbreak were mined to identify those patients whose location in the hospital could have allowed them to act as an intermediary in a transmission event between patients 1 and 4. Patients who overlapped with patient 1 after her arrival to the hospital and overlapped with patient 4 before his first positive culture were selected. The patient traces for patients 1 and 4 and the five patients (patients A to E) fitting the criteria of potential transmission intermediate are shown. Locations of patients in different hospital wards are indicated by different colors.

in vitro, although not to colistin. Of the six mutations unique to patient 8's isolate, four result in amino acid changes, three of which are in putative membrane proteins. Thus, in both cases, mutations altering membrane function, possibly reducing drug influx, are associated with colistin resistance.

DISCUSSION

The increasing speed and decreasing cost of high-throughput DNA sequencing technologies are enabling its application to the practice of medicine (*35*). Here, we tested whether genome sequencing could help to unravel a nosocomial outbreak and affect hospital infection control decisions. We sequenced patient and environmental isolates within a clinically relevant turnaround time during a hospital KPC–*K. pneumoniae* outbreak. Among the key insights provided by sequencing were that (i) the outbreak was monoclonal, despite a 3-week interval between the index case and the identification of subsequent cases, (ii) transmission likely occurred from at least two different sites of the index patient, (iii) at least three independent transmission events from the index patient led to two major clusters of colonized patients, (iv) one patient could be linked to a contaminated ventilator, and (v) a small number of putative resistance mutations could be identified in newly colistin-resistant isolates. Moreover, by combining the genetic data with patient trace data and by implementing an algorithm that accounted for *K. pneumoniae*'s capacity for silent colonization, we reconstructed the most likely outbreak transmission route.

Our results point to ways in which carbapenem-resistant *K. pneumoniae* outbreaks may be controlled more effectively in the future. First, although additional sequencing studies capturing the genetic diversity of clinical strains of *K. pneumoniae* will be necessary to verify that this outbreak did not stem from multiple introductions, the genetic diversity that accumulated just during the course of the outbreak suggests that a monoclonal outbreak is most likely. The finding that the outbreak likely stemmed from an index patient who left the hospital 3 weeks before the strain was isolated from another patient provides direct evidence that *K. pneumoniae*'s ability to silently colonize patients allows an outbreak to develop stealthily over an extended period of time (*14*). The potential for undetected transmission provides support for extensive and frequent surveillance during and after discharge of a carbapenem-resistant *K. pneumoniae*–colonized patient. Second, we think it is likely that groin and throat surveillance cultures performed on patients residing in the ICU concurrent with the index patient failed to detect that one or more of these patients had indeed become colonized. Whether these early cultures were ineffective because of the site of culturing (groin and throat instead of rectum) or because of less sensitive culturing techniques used early in the outbreak (MacConkey agar instead of CHROMagar) is unclear. Nevertheless, our results demonstrate the importance of having ongoing, effective surveillance protocols in place before outbreaks occur. Third, the finding that *K. pneumoniae* could survive on hospital equipment that had undergone rigorous cleaning suggests that *K. pneumoniae* has a high degree of environmental stability

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Table 2. Mutations unique to colistin-resistant isolates.

Strain	Unique mutations
Patient 2 (resistant)	Coding indel in query: GA at position 321 of 1221 in KPNIH1_08595 (microcin B17 transporter)
	Noncoding SNP: G→A, between KPNIH1_06013 (hypothetical protein) and KPNIH1_06008 (putative acid phosphatase)
Patient 8 (initial)	Coding SNP: CTG→ATG (L→M) at 130 of 309 in KPNIH1_18808 (putative membrane protein)
	Coding SNP: ACC→ATC (T→I) at 1106 of 1119 in KPNIH1_07189 (L-Ala-D/L-Glu epimerase) (methyl viologen resistance protein SmvA)
	Noncoding SNP: G→T, between KPNIH1_24168 (putative phosphatase) and KPNIH1_24173 (hypothetical protein)
	Coding SNP: GGC→TGC (G→C) at 811 of 1110 in KPNIH1_05438 (putative transport protein)
	Coding SNP: CTT→CCT (L→P) at 941 of 1476 in KPNIH1_07179
	Noncoding SNP: G→T, between KPNIH1_15985 (aspartate kinase III) and KPNIH1_15980 (glucose-6-phosphate isomerase)

under some circumstances (*13*) and indicates that decontamination should be verified after the cleaning process.

In addition to supporting the implementation of these specific infection control procedures, our results suggest several ways in which whole-genome sequencing of outbreak isolates in real time could guide future infection control efforts. First, genetic data can allow for the identification of unexpected modes of transmission. For instance, it was initially assumed that patient 4 was colonized during a 24-hour stay in the ICU, during which he overlapped with patients 2 and 3. However, sequence analysis demonstrated that patient 4's isolate could not have come from patients 2 or 3 and must have derived from an independent transmission chain from patient 1. This finding motivated the search for an intermediate patient to explain the transmission from patient 1 to patient 4, ultimately revealing four highly plausible candidates. If we had such knowledge in real time, these putative intermediate patients could have undergone more rigorous surveillance culturing to identify KPC–*K. pneumoniae* colonization and/or been placed on contact isolation, potentially terminating a silent transmission chain. Second, genomic sequencing may distinguish between alternate transmission scenarios, which may be critical for surmising the scope of an outbreak within the hospital. For example, patients 15 and 16 cultured positive for KPC-producing *K. pneumoniae* within 1 day of each other while residing in the same non-ICU ward. There was no obvious epidemiological link connecting these patients to the outbreak, which raised the possibility that patient 16 had brought a new strain to the hospital from a long-term care facility in which he had recently had a prolonged stay. Genomic sequencing revealed that patient 15's and 16's isolates both matched the dominant ICU strain, suggesting that transmission had occurred within our hospital to a non-ICU ward. Finally, genetic data can link patients directly to environmental or infrastructure isolates. Such findings can motivate refinement in cleaning and decontamination procedures by providing insight as to how and when contamination occurred.

Beyond applications to outbreak containment, we foresee that future applications of real-time sequencing have the potential to transform both the control and the treatment of hospital infection. In both epidemic and endemic settings, real-time genomic sequencing can ultimately provide a powerful tool to define the nosocomial epidemiology of important health care–associated pathogens with heretofore unprecedented precision. Sequencing serial isolates in patients undergoing antibiotic therapy can allow unprecedented insight into how bacterial populations respond to treatments and the evolution of resistance (*36*, *37*). Furthermore, by observing how bacteria evolve in different patient populations and in response to different treatments, optimal therapeutic regimes may be tailored, eliminating those associated with the development of resistance. Finally, genetic variation underlying changes in bacterial virulence or tissue specificity may be identified. With the identification of markers of these key phenotypic attributes may come the ability for infection control to anticipate and outpace the evolution of infectious agents.

MATERIALS AND METHODS

Description of outbreak

In June 2011, a patient, known to be colonized with KPC–*K. pneumoniae* (hereafter called the index patient), was admitted to the NIH Clinical Center, a 243-bed clinical research hospital. After this patient's two overnight stays in the ICU, the hospital experienced a largely ICU-based outbreak of carbapenem-resistant *K. pneumoniae* despite early implementation of extensive infection control measures described below.

Active surveillance cultures

Immediately after index patient's second ICU stay, we collected two sets of throat and groin cultures (BBL CultureSwab with Stuart's Transport Medium, Becton-Dickinson) on all contemporaneous ICU patients. After detection of the second KPC–*K. pneumoniae* case, surveillance groin and throat cultures were performed on all ICU patients at admission and twice weekly thereafter. We believe that at least in the instance of patient 3, these cultures failed to identify a colonization. Surveillance culture–negative patients transferred to other units remained in enhanced contact isolation (described below) until they had additional negative surveillance cultures. Although rectal cultures are more sensitive for KPC–*K. pneumoniae* detection, our clinical microbiology laboratory was only able to validate this methodology 4 weeks into the outbreak when appropriate selective medium was acquired. At this time, we implemented rectal surveillance cultures in addition to groin and throat cultures for patients in the ICU. Rectal screening was performed in high-risk wards as cases were detected and three times in the entire hospital patient population between August 2011 and January 2012.

Infection control interventions

The index patient was placed in enhanced contact isolation upon arrival at our hospital and throughout her stay. During the outbreak, all ICU patients, regardless of colonization status, were placed on enhanced contact isolation, including strict enforcement of fastidious hand hygiene before entering and upon leaving patient rooms, universal use of gowns and gloves for all staff and visitors entering patient rooms,

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restrictions on patient activity outside rooms, restrictions on staff and visitor traffic, dedicating equipment for single-patient use (when feasible), extensive cleaning of shared equipment, and double-cleaning of vacated rooms with bleach. A private company was hired to decontaminate the ICU and all rooms and equipment used by KPC–*K. pneumoniae* patients with hydrogen peroxide vapor (*38*).

ICU patients positive for KPC–*K. pneumoniae* were transferred to a cohorted ICU after we recognized the fourth case in the outbreak. Patient cohorting included geographic separation of KPC–*K. pneumoniae*–colonized patients from other hospital patients. Staff cohorting included the use of a dedicated cadre of staff, including nursing, respiratory therapy, housekeeping, and physical therapy staff, to care only for KPC–*K. pneumoniae*–colonized patients. This cohorted staff did not provide care to noncohorted patients during a given shift. Health care personnel were assigned around the clock to monitor and enforce adherence to infection control precautions (*39*). A second non-ICU cohorted area, as described above, was established for patients who were not critically ill. Physicians were not cohorted and were allowed to care for both KPC–*K. pneumoniae*–colonized and other patients.

Outbreak investigation

Data were extracted from clinical charts detailing patients' clinical history, contact with staff members or ancillary departments, and movements throughout the hospital. Patient traces were conducted with TheraDoc (Hospira) to establish temporal and geographic overlap among patients. Throughout the outbreak, 164 environmental surveillance cultures were collected with extensive sampling on high-touch surfaces in patient rooms, the ICU, inpatient units with detected transmission, and ancillary departments of the hospital. Sink drain cultures were collected with swabs inserted through the sieve.

Detection of KPC-positive *K. pneumoniae*

Early screening cultures for *K. pneumoniae* were performed with MacConkey agar (Remel), and then starting in September 2011 with more sensitive CHROMagar KPC. Species level identification was done with matrix-assisted laser desorption/ionization–time-of flight (MALDI-TOF) microflex LT mass spectrometer (Bruker Daltonics Inc.), and carbapenemase production was assessed by the modified Hodge test. Antibiotic susceptibility testing was performed with the BD Phoenix (Becton-Dickinson) and Etest (bioMérieux) systems.

Genome sequencing, assembly, and annotation

Genomic libraries were constructed with the Roche 454 Titanium Kit (Roche 454 Life Sciences). Sequencing was performed on the Roche/454 XLR instrument with an average N50 contig size of 154,336 bp and an average of 135 large contigs. Sequencing assembly statistics for each isolate are provided in table S1. Contig assembly was executed with the gsAssembler v.2.3. Genome annotation was performed with the publicly available Prokaryotic Genomes Automatic Annotation Pipeline.

Detection and filtering of nucleotide differences

Large contigs from each genome assembly were ordered and oriented relative to a finished reference *K. pneumoniae* genome (NTUH-K2044) and then stitched together to form a pseudo-chromosome with the Mauve contig mover (*40*). An initial list of SNVs among the outbreak isolates was generated from the Mauve alignments with the export single-nucleotide polymorphism (SNP) function (*41*). SNVs were filtered to retain only high-confidence SNVs as detailed in the Supplementary Materials.

Constructing the putative transmission map

To construct the most probable transmission map, we integrated both genetic and epidemiological information, building on an approach reported in (*42*). The most parsimonious transmission map was generated by finding the set of links, spanning all patients with the minimum total genetic distance, with patient trace information being used to differentiate between transmission scenarios that were equally probable based on the genetic data. We incorporated epidemiological data quantitatively by taking into consideration how *K. pneumoniae* nosocomial outbreaks manifest. Specifically, we accounted for these aspects: (i) outbreaks are thought to spread primarily through patient-to-patient transmission via hospital personnel, equipment, and infrastructure (*13*); (ii) silent colonization of patients may result in detection of colonization in a recipient well after the actual transmission event (*14*); and (iii) silent colonization of patients may result in instances in which a source patient cultures positive after another patient to whom s/he transmitted. To account for patient-to-patient transmission as the most likely route, we penalized links with patient A transmitting to patient B if they did not overlap in the same ward before patient B grew the outbreak organism. While allowing for silent colonization, we down-weighted links based on the total number of days of silent colonization required (fig. S6). Despite the reported sensitivity of rectal surveillance (*43*), for the purpose of constructing the transmission map, we chose to not preclude patients from being carriers based on negative rectal surveillance. This decision was made because the true sensitivity of rectal surveillance remains unknown. Although previous studies show that rectal surveillance is the most sensitive assay, there is no way to accurately quantify false negatives. Evidence for sensitivity not being 100% comes from instances in which rectal surveillance cultures within single patients fluctuate between positive and negative (for example, patient 9 in fig. S2).

Despite the possible limitations of rectal surveillance, it is of value to determine to what degree an effective surveillance approach can simplify outbreak reconstruction. To this end, we performed a parallel analysis in which we assumed that a negative rectal surveillance precluded silent colonization. Incorporating this constraint greatly reduced the number of possible transmission links between patients (fig. S3). However, this reconstruction is not a priori more accurate, underscoring the imperative to determine the true sensitivity and specificity of different surveillance approaches. See Supplementary Materials for details of algorithm implementation.

SUPPLEMENTARY MATERIALS

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Methods

Fig. S1. Repetitive element PCR and pulsed-field gels of representative outbreak isolates.

Fig. S2. Surveillance cultures for outbreak patients.

Fig. S3. Transmission opportunities between patients when using negative rectal surveillance to exclude patient colonization

Fig. S4. Predicted transmission chart based only on genetic data.

Fig. S5. Predicted transmission chart based only on epidemiological data.

Fig. S6. Computing epidemiological distances between patients.

Table S1. Genome sequencing statistics.

Table S2. Characteristics of patients who acquired outbreak strain.

Table S3. MICs for antibiotic susceptibility of outbreak isolates.

Table S4. Mutations identified among outbreak genomes.

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